Flooding and *Phytophthora cinnamomi*: Effects on photosynthesis and chlorophyll fluorescence in shoots of non-grafted *Persea americana* (Mill.) rootstocks differing in tolerance to Phytophthora root rot

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A R T I C L E   I N F O

A B S T R A C T

Losses in the production of avocado (*Persea americana* (Mill.)) are incurred due to Phytophthora root rot (PRR), a disease of the feeder roots that results in tree-dieback and eventual tree death. Avocado is also a flood-sensitive species and flooding exacerbates the effects of PRR. The avocado industry relies on the use of rootstocks tolerant to PRR to minimise losses. The present study compared the gas exchange and chlorophyll fluorescence responses of avocado rootstock plants of 'Dusa™', the current South African industry standard, with 'Duke 7', and the selections R0.12 and R0.06 which show reduced and superior tolerance to PRR, respectively. A decline in stomatal conductance (g_s) and net CO_2 assimilation (P_N) over the 30 day evaluation period were early responses to flooding. 'Dusa™', the more tolerant rootstock plants, demonstrated a better recovery in P_N and g_s in response to inoculation; however, both rootstocks performed poorly under flooded conditions. A decline in P_N in infected 'Duke 7' plants appeared to be associated with stomatal limitations due to reduced stomatal conductance. The decline in P_N and g_s was not apparent in infected 'Dusa™' plants. Non-stomatal limitations to P_N in rootstock plants exposed to flooding were also evident as indicated by increases in the ratio of internal to atmospheric CO_2 concentrations (C_i/C_a). Impaired photosynthetic capacity in flooded rootstock plants was reflected by reduced photosystem II efficiency and photochemical quenching. In comparison to 'Dusa™', R0.12 rootstock plants showed reduced P_N and g_s following inoculation with *Phytophthora cinnamomi* whereas the more tolerant R0.06 rootstock plants revealed sustained photosynthetic activity. Interestingly R0.06 was the only rootstock able to maintain P_N and g_s in non-inoculated, flooded plants.

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1. Introduction

Avocado (*Persea americana* Mill.) is a commercially valuable tropical and subtropical fruit tree belonging to the Lauraceae family (Bergh and Ellstrand, 1986). World production of avocado was estimated at over 4.36 million tonnes in 2012, with South Africa contributing substantially as an exporter (http://faostat.fao.org). Phytophthora root rot (PRR) is a disease of the fine feeder roots of avocado and is the most limiting disease to avocado production worldwide (Coffey, 1987; Pegg et al.,

2002; Zentmeyer, 1984). The soil-borne oomycete, *Phytophthora cinnamomi*, is the causal agent of PRR, and infection with this pathogen results in the feeder roots becoming brittle and turning black, as the root tissue decays. This restricts water and nutrient uptake by the trees and leads to branch-dieback and eventual tree death. *P. cinnamomi* occurs globally and has a broad host range exceeding 1000 plant species (Hardham, 2005; Zentmeyer, 1980), which along with the production of resilient oospores, contributes to its persistence in soils. Control strategies include phosphonate trunk injections, development and use of tolerant rootstocks, and proper orchard management practices (Coffey, 1987), including use of pathogen-free material and prudent irrigation scheduling. Irrigation and soil water content are particularly important factors to consider when avocados are grown in the presence of *P. cinnamomi*, as the effects of PRR can be exacerbated in wet soils (Ploetz and Schaffer, 1989).

Waterlogged or flooded soils may result from high rainfall, river overflow, elevated water tables, inadequate drainage and improper irrigation management (Colmer and Voesenek, 2009; Pandey et al., 2010). Avocado trees are sensitive to flooding and decreases in growth and

yield, nutrient deficiencies, branch-dieback, and tree death may result in flooded or poorly drained soils (Schaffer et al., 1992). Other effects include growth reductions, premature senescence and leaf abscission, root decay, reduced photosynthetic ability, and lowered enzyme efficiencies (Davies and Flore, 1986; Fleischmann et al., 2002). Insufficient oxygen availability to the roots under waterlogged conditions is partly responsible for reductions in growth and yield (Davies and Flore, 1986; Oosterhuis et al., 1990) and the amplified effects of many diseases (Stolzy and Sojka, 1984). The increased severity of root rots, caused by *Phytophthora* spp. in particular, has been noted in flooded plants (Ploetz and Schaffer, 1989).

The effects of flooding on infection of avocados with *P. cinnamomi* have been described by Wager (1942) who noted that even transient flooding results in root rot that causes plants to wilt and die. Avocado trees that are flooded in the presence of *P. cinnamomi* have been observed to succumb much more rapidly than trees that are flooded in the absence of *P. cinnamomi*, with significant reductions in CO_2 assimilation (P_N), stomatal conductance (g_s), and transpiration (E) (Ploetz and Schaffer, 1989; Schaffer and Ploetz, 1989; Wager, 1942). However, this is also dependent on both the physical and chemical properties of the soil, as in fine textured soils, which are poorly drained and have a greater proportion of micropores, avocados can succumb so rapidly to flooding that the presence of PRR has limited impact. The increased damage caused by PRR under flood conditions has been ascribed to an increase in zoospore motility, which leads to an enhanced ability of the oomycete to infect roots (Kenerley et al., 1984; Robin et al., 2001). It may also be due to an increase in the susceptibility of the plant to infection under conditions of low oxygen caused by flooding (Schoeneweiss, 1975), changes in soil chemistry, enhanced pathogen activity, or a combination of these factors.

Development and selection of rootstocks showing tolerance to PRR are integral parts of managing the disease and are on-going processes. Selections assessing additional traits, such as tolerance to flooding, will be an important aspect in improving tree performance and longevity in areas prone to waterlogging. At present, flood tolerance is not assessed when selecting new rootstocks. Rootstock selection is a lengthy and tedious process and the use of physiological markers for desirable traits could improve this process. An understanding of the physiological response of avocado rootstocks to flooding and infection will aid in the development of such markers for tolerance to PRR and flooding. These markers will make the selection process more efficient and possibly result in selection of rootstocks showing tolerance to both traits. In this study we assessed the phenotypic response of the industry standard 'Dusa™' rootstock to inoculation with *P. cinnamomi* and flooding by comparing it first to the previous industry standard 'Duke 7' rootstock in a glasshouse and subsequently by comparing it in a shadehouse trial to a rootstock less tolerant to PRR (R0.12) and a rootstock recently selected for superior tolerance to PRR (R0.06). To date there have been no studies assessing the response of 'Dusa™' rootstocks to infection and flooding. The aim was to investigate whether rootstocks showing high tolerance to PRR would maintain this tolerance when infection was experienced in combination with flooding. In addition the tolerance to flooding of PRR tolerant rootstocks was also assessed.

Two trials evaluating a number of physiological parameters, including leaf gas exchange, stomatal conductance, and chlorophyll fluorescence parameters, were carried out to determine the onset of stress and the impact of flooding and infection by *P. cinnamomi* on these parameters. Whilst reduced P_N and g_s are known early responses to flooding there is still some uncertainty as to whether P_N is reduced as a result of stomatal closure or due to non-stomatal limitations that are related to the biochemical reactions of photosynthesis (Gimeno et al., 2012; Schaffer et al., 1992). In addition, it is important to determine if these changes in photosynthetic parameters occur prior to the onset of visible symptoms of stress, as this would be important when developing physiological markers to be used in selection programmes.

2. Materials and methods

2.1. P. cinnamomi isolates and inoculation

P. cinnamomi was isolated from declining avocado orchards in Tzaneen, South Africa. Isolation was performed using the method described by Christie (2012); however, nystatin was used instead of pimaricin. Long-term stocks were stored in autoclaved, distilled H_2O (dH_2O) with a blade of grass. Cultures were grown on V8 agar (20% V8 juice (v:v), 0.25% $CaCO_3$, agar 17 g l^{-1}) and kept in the dark at 20 °C. Plants in the first trial were inoculated using only zoospores, whilst plants in the second trial were inoculated using both zoospores and mycelia. Zoospore production was carried out according to the method described in Christie (2012) and involved placing blocks of colonised V8 into 2% V8 broth until sufficient mycelial growth was evident (usually 3 days). Mycelial blocks were then rinsed three times with dH_2O to remove all V8 broth. Stream water was used to aid in the induction of sporangia as it provides both minerals and other microorganisms, both of which are known to aid sporangia development (Chee and Newhook, 1966; Chen and Zentmyer, 1970). Stream water was filtered twice and poured into 90 mm petri dishes. Mycelial plugs were then placed in plates and left under UV light for 2–3 days to induce sporangia formation. Once sufficient sporangia formation was observed cultures were cold-shocked at 4 °C for 45 min. Cultures were then left on the bench at room temperature for 1 h to allow zoospore release. Infection was carried out as soon as sufficient release was observed to ensure motility of zoospores. Plants were inoculated with 50 ml/plant of a zoospore suspension (2.5×10^4 zoospores/ml and 3×10^4 zoospores/ml for the glasshouse and shadehouse trials, respectively) by pouring the suspension directly into the potting medium alongside the stem. Mycelia, used in the shadehouse trial, were homogenised using a blender and poured into the potting medium (25 ml/plant). Infection was confirmed by re-isolation of the pathogen and subsequent use of the *P. cinnamomi* specific LPV3 forward (5′-GAA CCA CAA CAG GCA CGT-3′) and LPV3 reverse (5′-GTG CAG ACT GTC CAT GTG-3′) primers (Kong et al., 2003) in a polymerase chain reaction (PCR).

2.2. Plant material

No scions were grafted onto any of the rootstocks and shoots were derived from the respective rootstocks. Measurements thus reflect the photosynthetic and chlorophyll fluorescence responses in shoots of non-grafted avocado rootstocks. Plants of each rootstock were divided into four treatments: control plants, infected plants, flooded plants, and plants that were both infected and flooded (combined stress). Control plants were neither infected nor flooded. At the end of both trials *P. cinnamomi* was successfully re-isolated from inoculated plants and was found to be absent in non-inoculated plants, confirming that there was no *P. cinnamomi* present in the soil before inoculation. Re-isolations of the pathogen were done from at least three plants per treatment, per rootstock.

2.2.1. Glasshouse trial

One year-old clonal PRR tolerant 'Dusa™' (highly tolerant) and 'Duke 7' (tolerant) avocado plants were used. After the removal of the nurse seed, plants were replanted into 2 l containers containing a soil-perlite mix (1:1, v:v) and allowed to acclimatise for 3 months in a greenhouse at the Forestry and Agricultural Biotechnology Institute (FABI), Pretoria, South Africa (25° 45′ 19.80″ S 28° 14′ 7.59″ E), before the experiment commenced. Soil was verified to be free of *P. cinnamomi* before use. Sodium and mercury lamps supplemented natural light between 6 a.m. and 6 p.m., ensuring a 12 hour photoperiod. Plants were watered 3–4 times a week and 50 ml Hoagland's solution (Hoagland and Arnon, 1950) was used to supply nutrients once a week. Flooding was carried out by filling plastic reservoirs (45 cm × 65 cm), each containing 10–15 plants, with tap water to 1 cm above the potting mixture and was commenced

one week after inoculation with *P. cinnamomi* in order to allow establishment of infection. Inoculated plants were kept in separate reservoirs from non-inoculated plants.

2.2.2. Shadehouse trial

Responses of one year-old clonal plants of three avocado rootstocks, R0.12 (less tolerant), 'Dusa™' (highly tolerant), and R0.06 (highly tolerant), to infection and flooding were assessed. Nurse seeds were removed and plants were replanted in 10 l bags filled to 50% capacity with a bark-chip:soil mix (2:1, v:v). Soil was steam-sterilised to exclude soil microbes. Plants were allowed to acclimatise for 2 months in a 50% light exclusion shadehouse at the Hatfield Experimental Farm (University of Pretoria, 25° 47′ 7.38″ S 28° 15′ 30.44″ E) before experiments commenced. Plants were irrigated with water supplemented with Hygroponic (1 g l^{-1}, Hygrotech) and Solu-Cal (0.7 g l^{-1}, Hygrotech) twice daily for 5 min. Flooding was commenced 18 days post-infection (dpi) by filling plastic reservoirs (diameter = 30 cm) each containing a single plant with tap water to 1 cm above the potting mixture. Plants were drained after 14 days of flooding in order to assess possible recovery from stress.

2.3. Gas exchange and chlorophyll fluorescence measurements

Leaf temperature and photosynthetic photon flux density (*PPFD*) incident on the leaf surface were measured with a thermocouple in the leaf chamber and a Li-190SA quantum sensor (LI-COR), respectively. Gas exchange and chlorophyll fluorescence measurements were carried out on the third fully expanded leaf from the apex of each plant using an open-path portable photosynthesis system (LI-6400XT, LI-COR). Fluorescence measurements were performed using the 6400-40 leaf chamber pulse amplitude modulated fluorometer attached to the LI-6400XT. Steady-state fluorescence (F_s) was monitored to ensure that steady-state conditions were achieved before each measurement. Maximal fluorescence under light-adapted conditions (F'_m) was obtained by reducing all photosystem II (PSII) reaction centres by using a 1 s saturating flash. Maximal fluorescence (F_m) and minimal fluorescence (F_o) measurements were taken after dark-adapting the leaves for 30 min using aluminium foil. Minimal light-adapted fluorescence (F'_o) was determined by using far-red light (6 s) to excite PSI and force electrons to drain from PSII. Maximum quantum yield of photosystem II was defined as $F_m - F_o/F_m$ or F_v/F_m. Non-photochemical quenching (NPQ) was defined as $F_m - F_m'/F_m'$ and photochemical quenching (qP) as $(F_m' - F_s)/(F_m' - F_o')$. Actual light-harvesting efficiency ($\Phi PSII$) was calculated as $F_m' - F_s/F_m'$. The electron transport rate was defined as $(F_m' - F_s/F_m')fI\alpha_{leaf}$, where f is the fraction of absorbed quanta that is used by PSII, which is assumed to be 0.5, I is the incident radiant flux density (µmol m^{-2} s^{-1}) and α_{leaf} is leaf absorptance, calculated as a function of the fraction of blue light and kept constant for the avocado leaves at 0.8749.

2.3.1. Glasshouse trial

Average daily maximum temperature during the experimental period was 24.9 °C, with an average daily minimum temperature of 13.9 °C. Incoming solar radiation (measured by an automatic weather station) was very variable during the experimental period (varying from 6.7 to 42.8 MJ m^{-2} day^{-1}, with an average daily solar radiation of 29.08 MJ m^{-2} day^{-1}) and an average of 115 µmol m^{-2} s^{-1} and a maximum of 600 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) in the greenhouse over the course of the experiment. The low light levels were caused by overcast conditions during the experimental period; however, PAR levels still exceeded the light compensation point of container-grown avocado (63 µmol m^{-2} s^{-1}) as reported by Scholefield et al. (1980).

Gas exchange and chlorophyll fluorescence measurements were taken over a 30 day period; however, the measurements for 'Duke 7' ended at 26 dpi due to plant death. Gas exchange measurements were performed on 10 replicates (where one replicate corresponds to one tree) per treatment and fluorescence measurements on 5 replicates per treatment. Humidity within the chamber was held between 40 and 50% and leaf temperature varied between 23 and 28 °C. The reference CO_2 concentration was set at 500 µmol CO_2 mol^{-1}, the flow rate was set at 500 µmol s^{-1} and the quantum flux density within the chamber was set at 300 µmol m^{-2} s^{-1}. These conditions were set according to the prevailing conditions within the glasshouse. Measurements of leaf CO_2 assimilation (P_N), transpiration (E), stomatal conductance (g_s), and intercellular CO_2 concentration (C_i) were measured simultaneously every third day. Fluorescence measurements were measured the day after gas exchange measurements.

2.3.2. Shadehouse trial

The average daily maximum temperature in the shadehouse was 22.8 °C and the average daily minimum temperature was 5.5 °C. Average incoming solar radiation during the experimental period was 11.24 MJ m^{-2} day^{-1} and ranged from 8.37 to 13.04 MJ m^{-2} day^{-1}. PAR averaged 291.8 µmol m^{-2} s^{-1} over the experimental period with a maximum of 844.4 µmol m^{-2} s^{-1}. Gas exchange measurements were performed on 8 replicates (per treatment per rootstock) over a 46 day period, commencing three days after inoculation. Fluorescence measurements were taken from the day before flooding commenced over a period of 32 days until the trial ended. Fluorescence measurements were performed on 5 replicates per treatment per rootstock. Humidity within the sample chamber ranged from 30 to 70% over the experimental period and leaf temperature varied between 11 and 26 °C. The reference CO_2 concentration was set at 400 µmol CO_2 mol^{-1}, the flow rate was set at 500 µmol s^{-1} and the quantum flux density was adjusted according to daily conditions in the shadehouse and ranged from 500 to 800 µmol m^{-2} s^{-1}. Gas exchange parameters (P_N, E, g_s, C_i) were measured in the morning and fluorescence measurements were taken thereafter on the same day.

2.4. Plant disease rating

At the termination of each trial stem length was measured and leaf number was counted. Roots, stems and leaves were separated and dried at 70 °C for 3 days for the determination of dry mass. Relative water content (RWC) was calculated on a fresh mass basis ((fresh mass − dry mass) / fresh mass). Plant health was also visually rated at the end of the trial according to the Ciba–Geigy scale (Darvas et al., 1984). This rating was based on the assignment of a number from zero to 10 for each plant, with zero being healthy and 10 being dead (Darvas et al., 1984). Plants that were rated as healthy showed no signs of wilting or defoliation and root systems were vigorous. Dead plants showed no green tissue when the surface of the stem was scratched and leaves were either absent or dried out. These measurements were performed on a minimum of six plants per treatment, per rootstock.

2.5. Experimental design and statistical analysis

The experiments were laid out in randomised block designs, with four treatments per rootstock and 10–20 plants per treatment. Data are mean values of 6–10 plants for photosynthesis and 3–5 plants for chlorophyll fluorescence measurements. Statistical analysis was performed using JMP® 10.0.0 (SAS Institute Inc.) and GraphPad Prism 6.03 (GraphPad Software Inc.). Results are presented as means ± SD. Significance was assessed using analysis of variance (ANOVA) followed by analysis of means using Tukey's test ($P \leq 0.05$). Interaction between flooding and infection was assessed using a two-way ANOVA. Initial two-way ANOVA analysis was performed to test if there was a significant interaction between rootstocks and treatments. Significant interactions ($P = 0.0012$) were found and rootstocks were thus further analysed separately.

3. Results

3.1. Plant disease rating

P. cinnamomi was re-isolated from inoculated trees in both trials, but not from non-inoculated trees. Pathogen identity was subsequently confirmed using the species specific LPV3 primers.

3.1.1. Glasshouse trial

Classical symptoms of flooding stress were first visible in 'Duke 7' plants 10 days after flooding (17 dpi), which manifested as leaf wilting and by 15 days post-flooding (22 dpi) severe signs of wilt were visible. This wilting was more severe in plants in the combined stress treatment. Several flooded 'Duke 7' infected plants were dead at the termination of the trial, whilst the uninfected, flooded plants were all wilted. Wilting was only observed in flooded 'Dusa™' plants 16 days after the initiation of flooding (23 dpi) and approximately half of the 'Dusa™' plants experiencing the combination of stresses were wilted at the end of the trial.

Flooded treatments of both rootstocks performed poorly regardless of whether they were infected or not (Figs. 1 and 2) and leaf wilting and desiccation were apparent in the combined stress treatments in both rootstocks, but absent in plants that were inoculated but not flooded. Plants were visually rated in order to assess their health at the end of the trial (Fig. 2). The plants subjected to the combined stress had slightly higher disease ratings than uninfected flooded plants, whilst infected, non-flooded plants of both rootstocks had ratings similar to control plants (Fig. 2). In general the non-flooded plants appeared the healthiest by the end of the trial, whilst flooded treatments either displayed severe signs of stress (Fig. 1) or were dead. Root health was also assessed during disease rating and plants of both rootstocks had black and brittle roots when exposed to a combination of infection and flooding (Fig. 1). Infected plants that were not flooded had much healthier root systems than the flooded and infected plants and only a few roots exhibited the classic brown discolouration and brittleness associated with PRR. Roots from plants that were flooded in the absence of P. cinnamomi were discoloured but were not brittle.

Biomass was determined and no significant differences were found between treatments for either rootstock in terms of stem length or
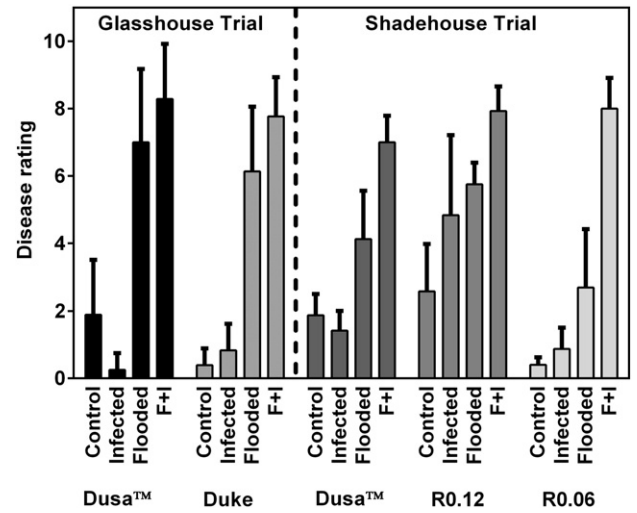

Fig. 2. Plant disease rating as determined according to the Ciba–Geigy scale. The two trials are separated by a dotted line. Rating ranges from 0 (healthy) to 10 (dead).

biomass, leaf biomass, relative water content (RWC) or total biomass (Table 1). Root biomass was significantly reduced in flooded 'Duke 7' plants when compared to control plants and reductions in infected and flooded and infected plants were also noticeable, but these differences were not significant (Table 1). The root:shoot ratio in 'Duke 7' plants was also found to be reduced in flooded, infected, and flooded and infected plants, with significant reductions seen when non-flooded, infected plants and flooded, uninfected plants were compared to control plants. No significant differences were found between treatments in 'Dusa™'.

3.1.2. Shadehouse trials

In this trial plants were flooded for two weeks, following this the water was drained from the 10 l bags and possible recovery from flooding stress was assessed. Typical symptoms of flooding stress, such as wilting, became apparent in flooded 'Dusa™' plants 13 days after flooding (31 dpi), with plants that were also infected displaying more severe wilting. Non-flooded, infected plants looked similar to
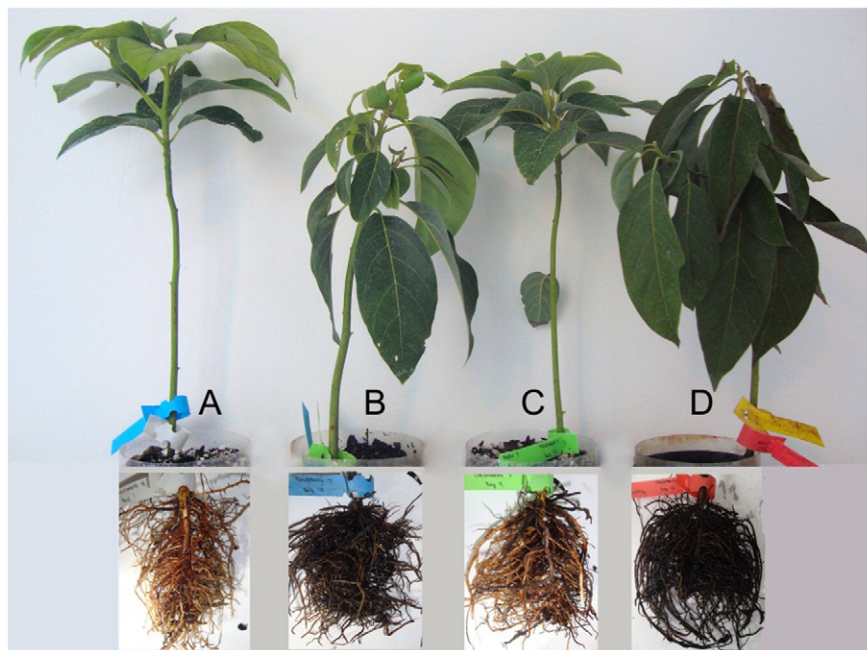

Fig. 1. Effects of Phytophthora cinnamomi and flooding on 'Duke 7' avocado plants. Healthy control plants (A), flooded plants (B), infected plants (C), and flooded and infected plants (D).

Table 1
Mean dry mass (g $\pm$ SE) of leaf, stem and root tissue, total biomass, the root/shoot dry mass ratio (R:S) and the relative water content (RWC) of clonal avocado rootstocks from the glasshouse trial.

Variable	Rootstock	Uninfected		Infected	
		Flooded	Non-flooded (control)	Flooded	Non-flooded
Leaf (g)	'Dusa™'	5.36 $\pm$ 2.77a	5.66 $\pm$ 0.61a	2.88 $\pm$ 0.53a	3.30 $\pm$ 0.39a
Stem (g)		5.35 $\pm$ 1.77a	5.52 $\pm$ 0.76a	4.07 $\pm$ 0.48a	4.49 $\pm$ 0.58a
Root (g)		2.50 $\pm$ 1.23a	2.86 $\pm$ 0.39a	2.05 $\pm$ 0.45a	3.23 $\pm$ 0.57a
R:S		0.22 $\pm$ 0.02a	0.26 $\pm$ 0.02a	0.27 $\pm$ 0.05a	0.41 $\pm$ 0.06a
RWC		0.70 $\pm$ 0.03a	0.74 $\pm$ 0.03a	0.71 $\pm$ 0.03a	0.74 $\pm$ 0.06a
Total (g)		13.20 $\pm$ 5.66a	14.04 $\pm$ 1.63a	9.00 $\pm$ 1.11a	11.02 $\pm$ 1.31a
Leaf (g)	'Duke 7'	3.18 $\pm$ 0.35a	3.68 $\pm$ 0.37a	4.38 $\pm$ 0.47a	4.29 $\pm$ 0.31a
Stem (g)		4.14 $\pm$ 0.40a	5.54 $\pm$ 0.44a	4.99 $\pm$ 0.44a	5.40 $\pm$ 0.27a
Root (g)		1.20 $\pm$ 0.15b	2.54 $\pm$ 0.27a	1.84 $\pm$ 0.26ab	1.82 $\pm$ 0.17ab
R:S		0.17 $\pm$ 0.02b	0.28 $\pm$ 0.03a	0.20 $\pm$ 0.03ab	0.19 $\pm$ 0.02b
RWC		0.70 $\pm$ 0.01a	0.74 $\pm$ 0.01a	0.70 $\pm$ 0.02a	0.71 $\pm$ 0.01a
Total (g)		8.52 $\pm$ 0.80a	11.76 $\pm$ 1.00a	11.21 $\pm$ 0.81a	11.52 $\pm$ 0.58a

Values not sharing a common letter within a row differ at $P < 0.05$. Rows without letters indicate that differences were not significant.

control plants. At this time R0.12 and R0.06 flooded plants showed similar responses, although wilting of the plants subjected to the combined stress was more severe in both rootstocks than in 'Dusa™'. Thirteen days of flooding thus resulted in wilting in all three rootstocks; however, there were uninfected, flooded plants that were not yet showing signs of wilt at this point. All infected and flooded plants in all three rootstocks were wilted at this point, with the majority displaying severe wilt.

In general, infected 'Dusa™' plants showed similar responses to control plants, whilst plants of both flooded treatments responded similarly, which is very similar to results obtained in the glasshouse trial (Fig. 2). The less tolerant R0.12 had higher disease ratings in all three treatments when compared to controls. Flooded R0.12 plants obtained ratings similar to infected plants, whilst plants that were both infected and flooded had higher disease ratings than any other treatments. In comparison, infected R0.06 plants showed responses similar to control plants whilst flooded, uninfected plants showed a slight increase in disease rating compared to control plants (Fig. 2). However, the combination of flooding and infection resulted in higher disease ratings in these plants, comparable to observations made for 'Dusa™', R0.12, and results from the first trial (Fig. 2).

No significant differences were found in any biomass measurements for either 'Dusa™' or R0.12. The only significant differences were seen in R0.06 flooded, infected plants which showed decreases in leaf, root, and total biomass when compared to control and uninfected, flooded plants (Table 2). In 'Dusa™' and R0.12 there was a tendency for lower root biomass in flooded treatments when compared to non-flooded treatments. In R0.06, however, infected treatments had lower root biomass than uninfected treatments, with a significant reduction in flooded, infected plants. Although differences were not significant, there was a tendency for reduced root: shoot ratios in flooded treatments in all three rootstocks. This is similar to results obtained for 'Dusa™' in the glasshouse trial.

3.2. Gas exchange measurements

3.2.1. Glasshouse trial

Flooding was seen to induce changes in the gas exchange parameters of both 'Dusa™' and 'Duke 7' clonal avocado plants, whether infected with *P. cinnamomi* or not (Fig. 3). Measurements for 'Duke 7' plants ended at 26 dpi, as several plants died soon after this measurement, whilst measurements of 'Dusa™' plants continued until 30 dpi (Fig. 3).

Table 2
Mean dry mass (g $\pm$ SE) of leaf, stem, and root tissue, total biomass, the root/shoot dry ratio (R:S) and the relative water content (RWC) of clonal avocado rootstocks from the shadehouse trial.

Variable	Rootstock	Uninfected		Infected	
		Flooded	Non-flooded (control)	Flooded	Non-flooded
Leaf (g)	'Dusa™'	5.61 $\pm$ 3.92a	15.59 $\pm$ 11.32a	8.80 $\pm$ 2.77a	7.99 $\pm$ 4.01a
Stem (g)		4.47 $\pm$ 2.64a	8.35 $\pm$ 3.10a	6.55 $\pm$ 2.42a	6.45 $\pm$ 1.33a
Root (g)		1.44 $\pm$ 0.74a	4.04 $\pm$ 1.71a	1.98 $\pm$ 0.74a	4.14 $\pm$ 2.12a
R:S		0.15 $\pm$ 0.04a	0.22 $\pm$ 0.08a	0.14 $\pm$ 0.05a	0.26 $\pm$ 0.13a
RWC		0.74 $\pm$ 0.04a	0.74 $\pm$ 0.02a	0.71 $\pm$ 0.05a	0.76 $\pm$ 0.05a
Total (g)		11.51 $\pm$ 7.23a	21.03 $\pm$ 9.24a	17.33 $\pm$ 5.31a	20.02 $\pm$ 4.07a
Leaf (g)	'R0.12'	7.02 $\pm$ 1.66a	6.59 $\pm$ 3.73a	7.91 $\pm$ 6.30a	6.08 $\pm$ 4.48a
Stem (g)		4.5 $\pm$ 1.09a	7.85 $\pm$ 3.39a	6.458 $\pm$ 2.8a	7.32 $\pm$ 2.62a
Root (g)		0.50 $\pm$ 0.22a	1.89 $\pm$ 1.41a	1.44 $\pm$ 1.542a	1.60 $\pm$ 1.50a
R:S		0.04 $\pm$ 0.02a	0.12 $\pm$ 0.11a	0.07 $\pm$ 0.041a	0.10 $\pm$ 0.13a
RWC		0.61 $\pm$ 0.07a	0.72 $\pm$ 0.03a	0.66 $\pm$ 0.06a	0.73 $\pm$ 0.081a
Total (g)		12.28 $\pm$ 2.28a	15.35 $\pm$ 5.72a	14.24 $\pm$ 10.64a	14.42 $\pm$ 7.28a
Leaf (g)	'R0.06'	12.39 $\pm$ 4.46a	12.52 $\pm$ 2.29a	6.04 $\pm$ 1.87b	13.30 $\pm$ 3.54a
Stem (g)		10.19 $\pm$ 3.87a	9.43 $\pm$ 1.63a	5.30 $\pm$ 1.53a	9.22 $\pm$ 2.75a
Root (g)		4.40 $\pm$ 3.49ab	4.10 $\pm$ 1.50a	1.24 $\pm$ 0.76b	3.32 $\pm$ 0.62ab
R:S		0.18 $\pm$ 0.13a	0.19 $\pm$ 0.06a	0.14 $\pm$ 0.09a	0.15 $\pm$ 0.03a
RWC		0.58 $\pm$ 0.09a	0.74 $\pm$ 0.03a	0.69 $\pm$ 0.02a	0.69 $\pm$ 0.12a
Total (g)		30.43 $\pm$ 12.02a	26.56 $\pm$ 4.86ab	12.60 $\pm$ 2.68b	26.45 $\pm$ 6.48ab

Values not sharing a common letter within a row differ at $P < 0.05$. Rows without letters indicate that differences were not significant.
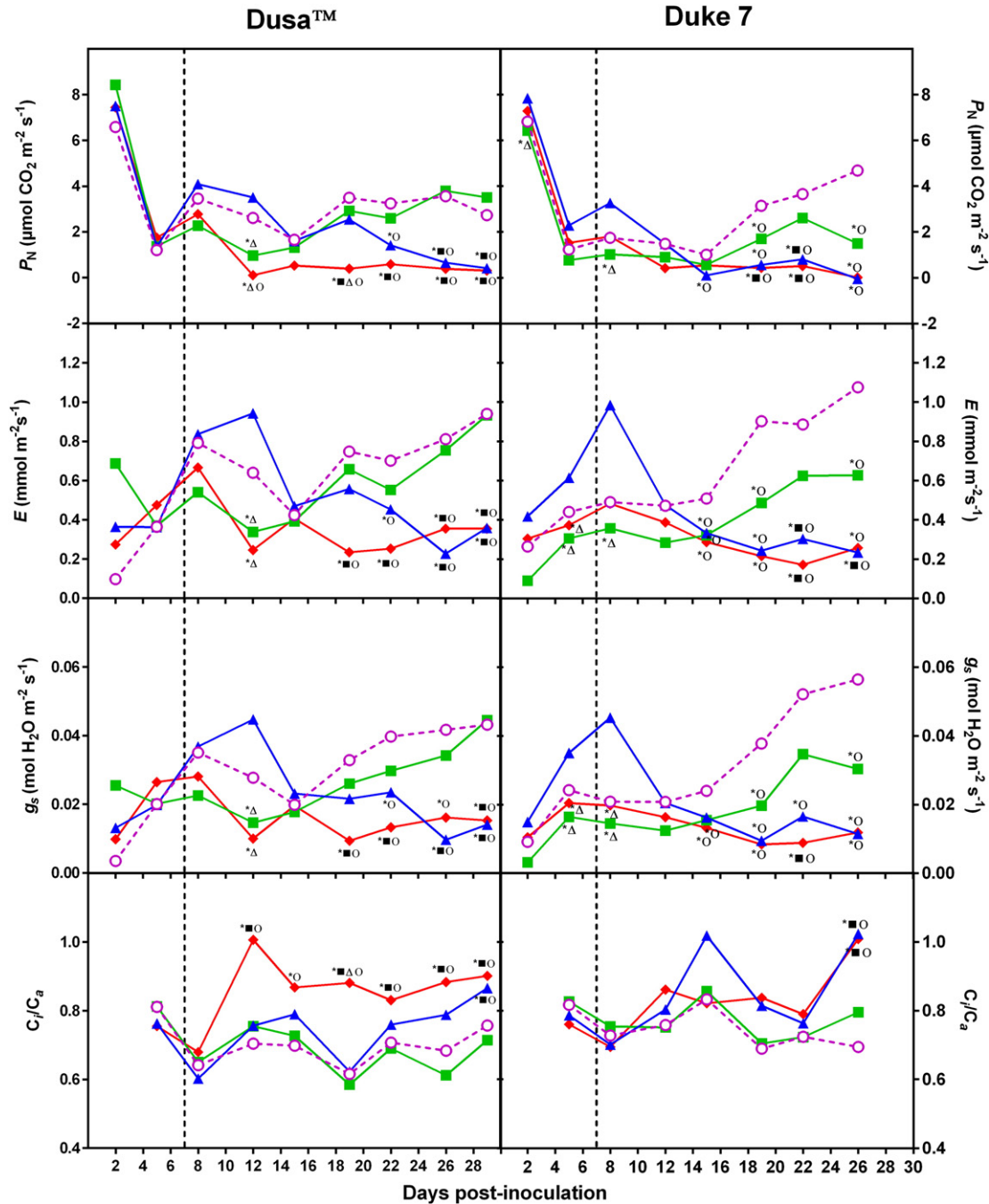
Fig. 3. Effects of *Phytophthora cinnamomi* and flooding on net photosynthesis (P_N), transpiration (E), stomatal conductance (g_s) and ratio of internal and atmospheric CO_2 concentrations (C_i/C_a) of 'Dusa™' and 'Duke 7' avocado rootstocks. Asterisks indicate significance ($P < 0.05$) with symbols to denote the treatment/s between which differences are significant; infected and flooded (diamonds), infected (squares), flooded (triangles), control (circles). Flooding began at day 7 as indicated by the dotted line.

Net CO_2 assimilation (P_N) was seen to be reduced by flooding in both root-stocks. In 'Dusa™' these reductions occurred earlier in plants subjected to the combined stress and P_N was almost zero (0.1 µmol CO_2 m^{-2} s^{-1}) 12 dpi (5 days after commencement of flooding) in these plants. Although infected, non-flooded plants also showed reductions in P_N at this point, plants were able to recover and P_N returned to levels seen in control plants by the end of the trial. Flooded plants that were not inoculated with *P. cinnamomi* exhibited increased values for P_N up until 15 dpi, after which they decreased to levels seen in the combined stress treatment. These increased initial values were also seen in 'Duke 7' flooded plants, which had the highest values for P_N up until 12 dpi (Fig. 3). Plants from the combined stress treatment did not show these increases in either rootstock. Low light levels

(18 MJ m^{-2} day^{-1}) 15 dpi, caused by cloudy conditions, are the most likely cause of observed reductions in P_N across all treatments in both rootstocks. Whilst P_N returned to similar levels measured prior to the cloudy day in most of the 'Dusa™' treatments, a similar recovery in P_N was not observed in combined stress plants and these plants continued to exhibit low P_N values for the rest of the trial period, which were significantly lower than P_N in non-flooded plants (Fig. 3). Both flooded 'Dusa™' treatments exhibited reductions in P_N that were significantly reduced compared to control plants 22 dpi and from 26 dpi, until the trial was terminated, P_N in these plants was significantly reduced compared to control and non-flooded, infected plants.

'Duke 7' plants that were flooded (with or without infection) could not recover from the low light conditions experienced at 15 dpi and

rates for P_N remained low, with significant reductions observed for the remainder of the experiment when compared to the control. Infected, non-flooded 'Duke 7' plants showed increases in P_N after 15 dpi, however, the rate of P_N remained significantly (22 dpi, 26 dpi) lower than control plants. As seen in 'Dusa™', non-flooded 'Duke 7' plants exhibited higher overall levels of P_N when compared to the flooded treatments. However, differences were evident between the responses of the two rootstocks to infection alone. Infected 'Dusa™' plants were capable of maintaining P_N levels similar to control plants, whilst infected 'Duke 7' plants showed reductions in P_N throughout the trial when compared to control plants. Infected 'Duke 7' plants were also found to have lower ($P = 0.02$) rates of P_N at 26 dpi when compared to infected 'Dusa' plants.

Lower values for both g_s and E were apparent 12 dpi in 'Dusa™' infected plants (both flooded and non-flooded) when compared to uninfected treatments. The highest values for g_s and E at this time point were observed in flooded, uninfected 'Dusa™', as noted for P_N. Similar results were observed in 'Duke 7' plants; however, the peak in g_s and E in flooded, uninfected plants was observed earlier (8 dpi) than in 'Dusa ™' plants and by 12 dpi had declined again. This is consistent with the peak in P_N at 8 dpi in 'Duke 7' plants (Fig. 3). By 19 dpi plants of both rootstocks exposed to the combined stress had significantly lower values of g_s and E than control plants. 'Duke 7' uninfected, flooded plants and infected, non-flooded plants also showed reduced values at this point. 'Dusa™' uninfected, flooded plants only showed reductions in g_s and E at 22 dpi. At this point flooded treatments of both rootstocks were showing significant reductions in g_s and E relative to non-flooded treatments. Following an initial decline in g_s and E in response to infection (no flooding) both 'Dusa™' and 'Duke 7' plants displayed some recovery from 19 dpi relative to the combined stress treatment. By the end of the trial g_s and E in infected 'Dusa™' plants had completely recovered relative to the control. However, as observed with P_N, g_s and E did not return to control levels in infected 'Duke 7' plants (Fig. 3).

The C_i/C_a ratio of 'Dusa™' plants subjected to the combined stress exhibited higher values than any other treatment from 12 dpi until the end of the trial. Flooded plants displayed increases from 22 dpi, whilst non-flooded infected plants and control plants had similar values throughout the trial. 'Duke 7' plants exhibited a different trend from 'Dusa™' plants, as 15 dpi flooded and uninfected 'Duke 7' plants had the highest C_i/C_a values and 26 dpi both flooded treatments exhibited significantly higher values than non-flooded treatments. Interaction between treatment (flooding and infection and combined stress) was assessed (Table 3). The major cause of variation, in both 'Dusa™' and 'Duke 7', found between values in treatments was due to flooding. Infection was less dominant in the stress response.

3.2.2. Shadehouse trials

Plants in the shadehouse were inoculated with *P. cinnamomi* and 18 days later they were flooded for two weeks after which plants were drained and recovery was monitored. A trend similar to that seen for P_N, E, and g_s in 'Dusa™' for the glasshouse trial was also seen for 'Dusa™' in the shadehouse trial, with 'Dusa™' plants under the combined stress treatment once again showing lower values for these parameters when compared to other treatments. Reductions in P_N were first apparent in infected and flooded 'Dusa™' plants at 21 dpi, but increased back to control levels at 23 dpi (Fig. 4). By 27 dpi values had dropped again and were significantly different from both non-flooded treatments. Low levels were maintained throughout the rest of the trial and plants did not show recovery in P_N after drainage at 32 dpi. P_N was also reduced in the flooded, uninfected treatment, although these differences were only significant 27 dpi and 41 dpi. After drainage P_N in flooded plants was able to recover to levels that were not significantly different from control levels by 49 dpi. Infected plants did not differ from control plants and maintained similar rate of P_N throughout the trial. Flooded R0.12 plants, a rootstock less tolerant to PRR, showed significantly reduced values for P_N compared to the control towards the end of the flooding period (27 dpi). These reductions were maintained in both flooded treatments until the end of the trial and plants did not recover after drainage. Unlike 'Dusa™', R0.12 infected plants that were not flooded started showing significant reductions in P_N when compared to control plants from 33 dpi, which continued until the trial was terminated.

In general, the more tolerant R0.06 only exhibited significant reductions in P_N, E, and g_s in the combined stress treatment (Fig. 4). Significant reductions in P_N in these plants relative to the control were first evident at 25 dpi and by 27 dpi P_N levels were significantly reduced compared to all other treatments. These low levels were maintained until termination of the trial and plants did not recover after drainage. Flooded plants that were uninfected did not show significant differences from non-flooded, infected plants. There were no significant differences between plants that were infected in the absence of flooding and control plants (Fig. 4).

Transpiration and g_s, showed similar trends to P_N for each rootstock (Fig. 4). Infected, flooded 'Dusa™' plants had the lowest rates of both E and g_s. Decreases were seen in E in both flooded treatments as early as the first day after the start of flooding (19 dpi). Flooded, infected plants maintained these lower values until the termination of the trial. During the flooding period (27 dpi and 30 dpi) flooded, uninfected plants also showed reductions in E when compared to control plants (Fig. 4). By 31 dpi these plants had recovered to control levels whereas the flooded, infected plants maintained significantly reduced levels as compared to controls, even after drainage. Similar results were obtained for g_s, with flooded treatments both showing significant reductions as compared to control plants from 27 dpi until 31 dpi when there was an increase in g_s for all treatments. After drainage flooded plants that were not infected recovered to control values, whilst infected, flooded plants could not recover and maintained lower levels of g_s relative to other treatments, although these differences were not always significant.

Trends for E and g_s in R0.12 also closely resembled the trend seen for P_N (Fig. 4). E and g_s were reduced in flooded treatments, although significant reductions in flooded, uninfected plants were seen later (27 dpi) than reductions in the combined stress treatment (19 dpi). By 35 dpi both flooded treatments and non-flooded, infected plants exhibited significant reductions in E as compared to control plants. However, by the end of the trial E in flooded, uninfected plants was able to recover to levels similar to the control after drainage, whilst both infected

Table 3

Summary of a two-way ANOVA to determine variation caused by individual stresses and the interaction between the stresses of clonal avocado rootstocks from the glasshouse trial.

| Rootstock	Variable	% variation flooding	P-value	% variation infection	P-value	% variation interaction (F × I)	P-value
'Dusa™'	P_N	56.13	<0.0001****	0.343	0.592	1.401	0.288
	g_s	33.03	0.0001***	0.509	0.599	1.380	0.388
	E	40.65	<0.0001****	0.205	0.733	1.418	0.371
	C_i/C_a	25.22	0.0007***	0.513	0.601	7.786	0.047*
Duke 7	P_N	46.94	<0.0001****	7.659	0.0096**	8.418	0.007**
	g_s	42.98	<0.0001****	4.537	0.08	4.877	0.07
	E	46.71	<0.0001****	3.515	0.111	4.504	0.0725
	C_i/C_a	50.45	<0.0001****	0.9393	0.414	1.796	0.261

Significance is indicated by an asterisk.
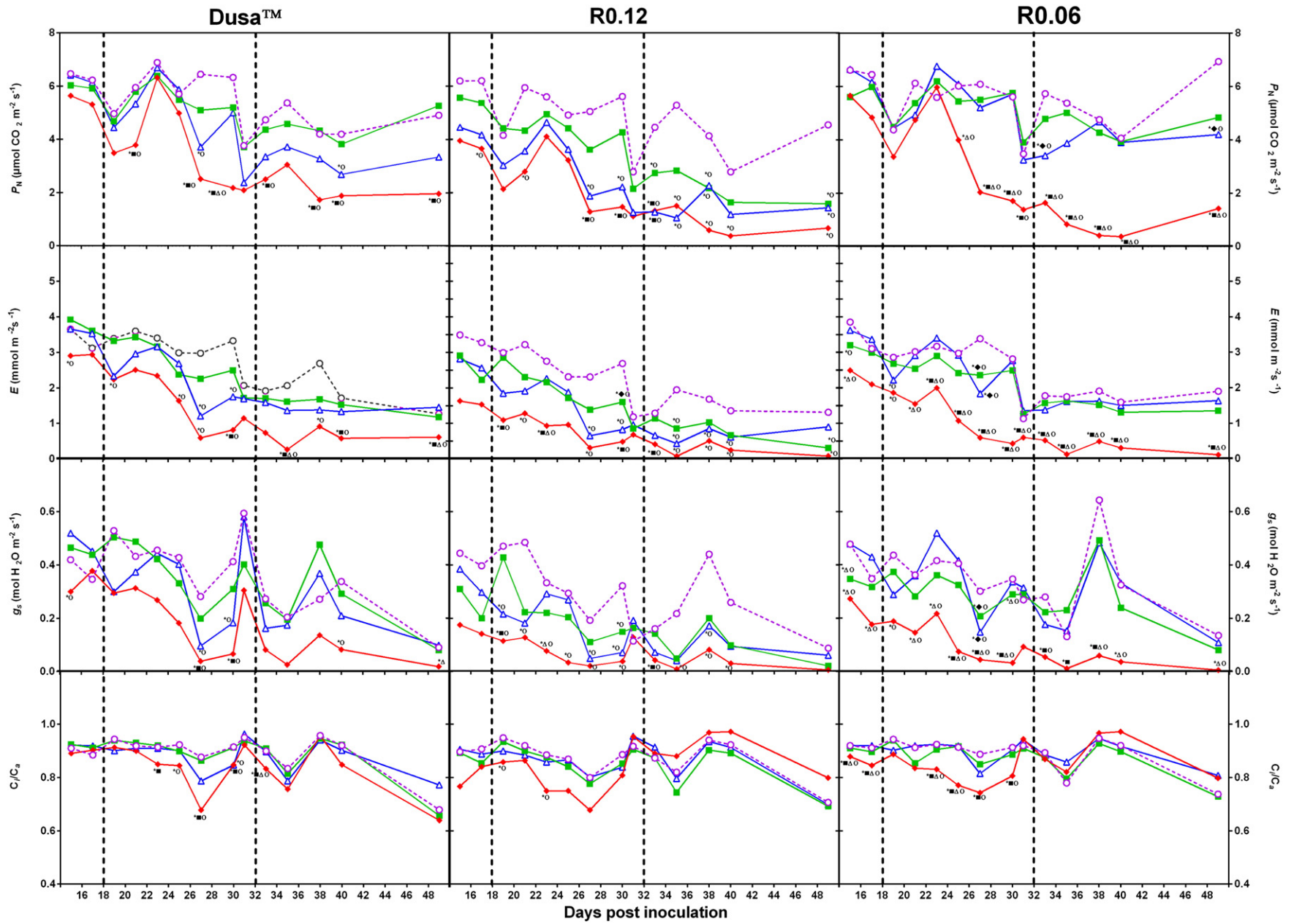
Fig. 4. Effects of *Phytophthora cinnamomi* and flooding on net photosynthesis (P_N), transpiration (E), stomatal conductance (g_s) and ratio of internal and atmospheric CO_2 concentrations (C_i/C_a) of 'Dusa™', R0.12 and R0.06 avocado rootstocks. Asterisks indicate significance ($P < 0.05$) with symbols to denote the treatment/s between which differences are significant; infected and flooded (diamonds), infected (squares), flooded (triangles), control (circles). Flooding began at day 18 as indicated by the dotted line and plants were drained at day 32 as indicated by the second dotted line.

treatments exhibited values significantly lower than control levels. Lower values of g_s were also seen in flooded, infected plants and were maintained until the termination of the trial. At 38 dpi both flooded treatments exhibited reduced values relative to controls, however, uninfected plants eventually returned to levels similar to infected, non-flooded plants. Differences between treatments were no longer significant by 49 dpi (Fig. 4).

Flooded, infected plants of R0.06 consistently showed lower E and g_s when compared to all other treatments and did not recover after drainage, but remained low until the trial was terminated (Fig. 4). Uninfected, flooded plants and infected, non-flooded plants showed significant reductions relative to control levels for both E and g_s at 27 dpi; however, values were still higher than those seen in flooded, infected plants. Hereafter values in infected plants and flooded, uninfected plants returned to control levels.

Significant reductions were observed in the C_i/C_a ratio of flooded and infected 'Dusa™' plants from 23 dpi until 33 dpi compared to non-flooded plants, which corresponded to a rapid decline in g_s at this time (Fig. 4). However, at 36 dpi following drainage no significant differences in the C_i/C_a ratio were observed between treatments. Similar results were obtained in flooded, infected R0.12 plants, with decreases seen at 19 dpi and 27 dpi but returning to control levels thereafter. R0.06 exhibited lower C_i/C_a ratios in infected, flooded plants until 30 dpi after which they returned to levels similar to other treatments. No other treatments showed significant changes in C_i/C_a (Fig. 4). In general the flooding component contributed the greatest variation in values in all three rootstocks (Table 4). Interestingly the variation in values caused by the combined treatment was most noticeable in R0.06, which reflects the results obtained for the photosynthetic parameters.

3.3. Fluorescence measurements

3.3.1. Glasshouse trials

No significant differences in maximum quantum efficiency (F_v/F_m) between treatments were seen for 'Dusa™'. Differences were only observed in 'Duke 7' at the end of the trial (23 dpi) when F_v/F_m of plants exposed to a combination of stresses dropped to levels significantly lower than both non-flooded, infected plants and control plants (data not shown). Although reductions in PSII efficiency ($\Phi PSII$) relative to the control for infected, flooded and flooded, uninfected 'Dusa™' plants were observed at 23 dpi, the difference only became significant at 30 dpi (Fig. 5). 'Duke 7' plants displayed a similar trend with differences in $\Phi PSII$ between flooded and non-flooded treatments occurring slightly earlier at 16 dpi, but only becoming significant at 23 dpi. Flooded 'Duke 7' plants that were also infected had lower values than plants that were only flooded and $\Phi PSII$ values in these plants were significantly different from infected, non-flooded plants, in addition to control plants. These infected, non-flooded plants also showed reductions in $\Phi PSII$ relative to the control plants, but differences were not significant.

A similar trend to that observed in $\Phi PSII$ values was noted in values for photochemical quenching (qP) in both rootstocks (Fig. 5). Flooding was observed to reduce qP with significant differences observed between flooded 'Dusa™' treatments and non-flooded treatments at 30 dpi. 'Duke 7' plants exhibited differences between flooded and control treatments from as early as 16 dpi, but differences only became significant at 20 dpi, where infected, flooded plants showed reductions relative to control plants. By 23 dpi both flooded treatments showed significantly lower values than non-flooded plants. No significant differences were seen in NPQ between treatments in 'Dusa™'. 'Duke 7' plants that were both flooded and infected only showed differences in NPQ at 23 dpi where these plants were significantly lower than non-flooded, infected plants. However, these plants showed values similar to control plants and thus it is the infected, non-flooded plants that had increased values for NPQ. It is interesting to note that 'Dusa™' infected, flooded plants had the highest NPQ at 30 dpi whilst in 'Duke 7' the same treatment had the lowest NPQ. The electron transport rate (ETR) of both rootstocks exhibited similar patterns to g_s, E and $\Phi PSII$ for the respective rootstocks (data not shown), with significantly lower values observed in flooded 'Dusa™' and 'Duke 7' plants, both infected and uninfected, than in the non-flooded treatments at the end of the trial period. In general, flooding reduced values for $\Phi PSII$, qP, and ETR, as compared to non-flooded treatments. When significance was assessed to see if there were any differences within the same treatments between rootstocks, no significant differences were found in $\Phi PSII$, qP, NPQ, F_v/F_m, or ETR.

3.3.2. Shadehouse trial

Maximum quantum yield of PSII (F_v/F_m) was stable for the majority of the trial in 'Dusa™', with slight increases in infected treatments seen at 33 dpi and decreases seen in uninfected, flooded plants at the end of the trial; however no significant differences in F_v/F_m were noted in any of the rootstocks (data not shown). A trend similar to that seen in the glasshouse trial was observed in the shadehouse trial for $\Phi PSII$ values in 'Dusa™'. Non-flooded treatments had higher values for $\Phi PSII$ than flooded treatments (Fig. 6). At 33 dpi (1 day after drainage) 'Dusa™' plants in the combined stress treatment started showing significant reductions in $\Phi PSII$ relative to the control. From 38 dpi $\Phi PSII$ in these plants was also significantly lower than both non-flooded treatments, which was maintained until the end of the trial. Flooded, uninfected plants also showed significant reductions in $\Phi PSII$ relative to the control from 40 to 45 dpi. Infected, non-flooded plants showed similar levels to control plants for most days (Fig. 6). The less tolerant rootstock R0.12 showed clear reductions in $\Phi PSII$ for all treatments relative to control plants (Fig. 6). By 27 dpi flooded, infected plants showed significant reductions relative to control plants and by 31 dpi all treatments showed significant reductions relative to the control. $\Phi PSII$ values remained low in these plants until 45 dpi when only flooded, infected plants still showed significantly reduced values relative to the control plants, but at 49 dpi these levels were no longer significantly lower than control plants. In the more tolerant R0.06 rootstock, only the flooded and

Table 4
Summary of a two-way ANOVA to determine variation caused by individual stresses and the interaction between the stresses of clonal avocado rootstocks from the shadehouse trial.

Rootstock	Variable	% variation flooding	P-value	% variation infection	P-value	% variation interaction (F × I)	P-value
'Dusa™'	P_N	36.40	<0.0001****	30.04	<0.0001****	5.485	<0.0459*
	g_s	56.37	<0.0001****	12.04	0.0083**	0.052	0.8516
	E	54.69	<0.0001****	16.10	0.0021**	0.059	0.8362
	C_i/C_a	42.03	0.0006***	0.010	0.9505	0.013	0.9453
R0.12	P_N	56.09	<0.0001****	6.363	0.0538	0.521	0.5660
	g_s	37.38	0.0002***	12.16	0.0185*	5.553	0.0995
	E	48.05	<0.0001****	11.06	0.0138*	2.999	0.1773
	C_i/C_a	14.76	0.0515	6.993	0.1703	0.011	0.9547
R0.06	P_N	17.39	0.0019**	15.87	0.0028**	18.35	0.0015**
	g_s	18.97	0.0005***	34.33	<0.0001****	15.96	0.0012**
	E	22.22	<0.0001****	35.39	<0.0001****	20.16	<0.0001****
	C_i/C_a	9.913	0.0543	20.47	0.0077**	6.666	0.11

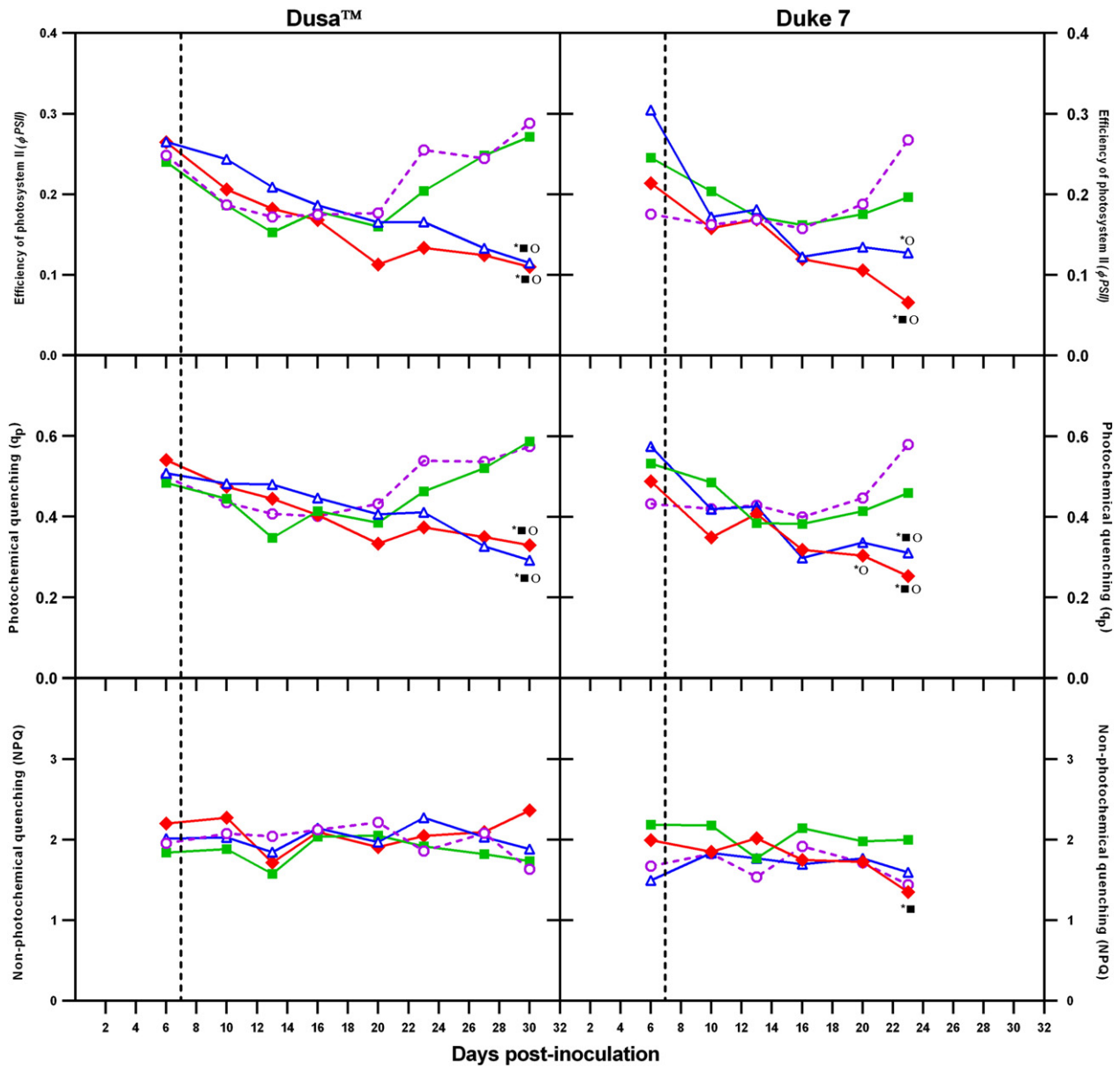Significance is indicated by an asterisk.

Fig. 5. Effects of *Phytophthora cinnamomi* and flooding on PSII efficiency (*ΦPSII*), photochemical quenching (qP) and non-photochemical quenching (NPQ) of 'Dusa™' and 'Duke 7' avocado rootstocks. Asterisks indicate significance with symbols to denote the treatment/s between which differences are significant; infected and flooded (diamonds), infected (squares), flooded (triangles), control (circles). Flooding began at day 7 as indicated by the dotted line.

infected treatment exhibited significant lower *ΦPSII* relative to the control, which occurred from 31 dpi until the end of the trial (Fig. 6). No significant differences were noted in photochemical quenching 14 days after flooding or during recovery in 'Dusa™' or R0.12 (Fig. 6). R0.06 plants that were both flooded and infected showed significant reductions from control values from 27 dpi (9 days after commencement of flooding) to 40 dpi. From this point until the end of the trial no significant differences were observed between treatments.

'Dusa™' plants that were both flooded and infected exhibited increased NPQ relative to the non-flooded treatments from 31 dpi to 45 dpi (Fig. 6). Flooded, uninfected plants also showed lower values for NPQ than infected, flooded plants at 31 dpi and 33 dpi. There were no significant differences between treatments by the end of the trial. No significant differences in NPQ were seen between treatments of R0.12, although control plants maintained the lowest values throughout the trial. As seen in Dusa, R0.06 flooded, infected plants showed significantly higher values for NPQ (31 dpi) than infected, non-flooded plants (Fig. 6). These differences were maintained until 35 dpi when values

were significantly different from all other treatments, including control plants. By 38 dpi NPQ values for flooded, infected plants were no longer significantly different from the control. The ETR exhibited similar patterns to g_s, E and *ΦPSII* for each rootstock, with the lowest values occurring in the combined stress treatments for all three rootstocks (data not shown).

4. Discussion

'Duke 7' has been the industry standard rootstock for several years, and has recently been replaced by the more tolerant 'Dusa™' rootstock in commercial nurseries in South Africa and California (Van Rooyen, 2011). The response of 'Dusa™' to flooding and *P. cinnamomi* infection was assessed in this study to determine possible physiological markers for PRR tolerance in rootstocks and to determine if tolerance to PRR is associated with higher tolerance to flooding. In order to achieve this 'Dusa™' was first compared to the previous industry standard 'Duke 7' rootstock in a glasshouse trial. Following this 'Dusa™' was compared
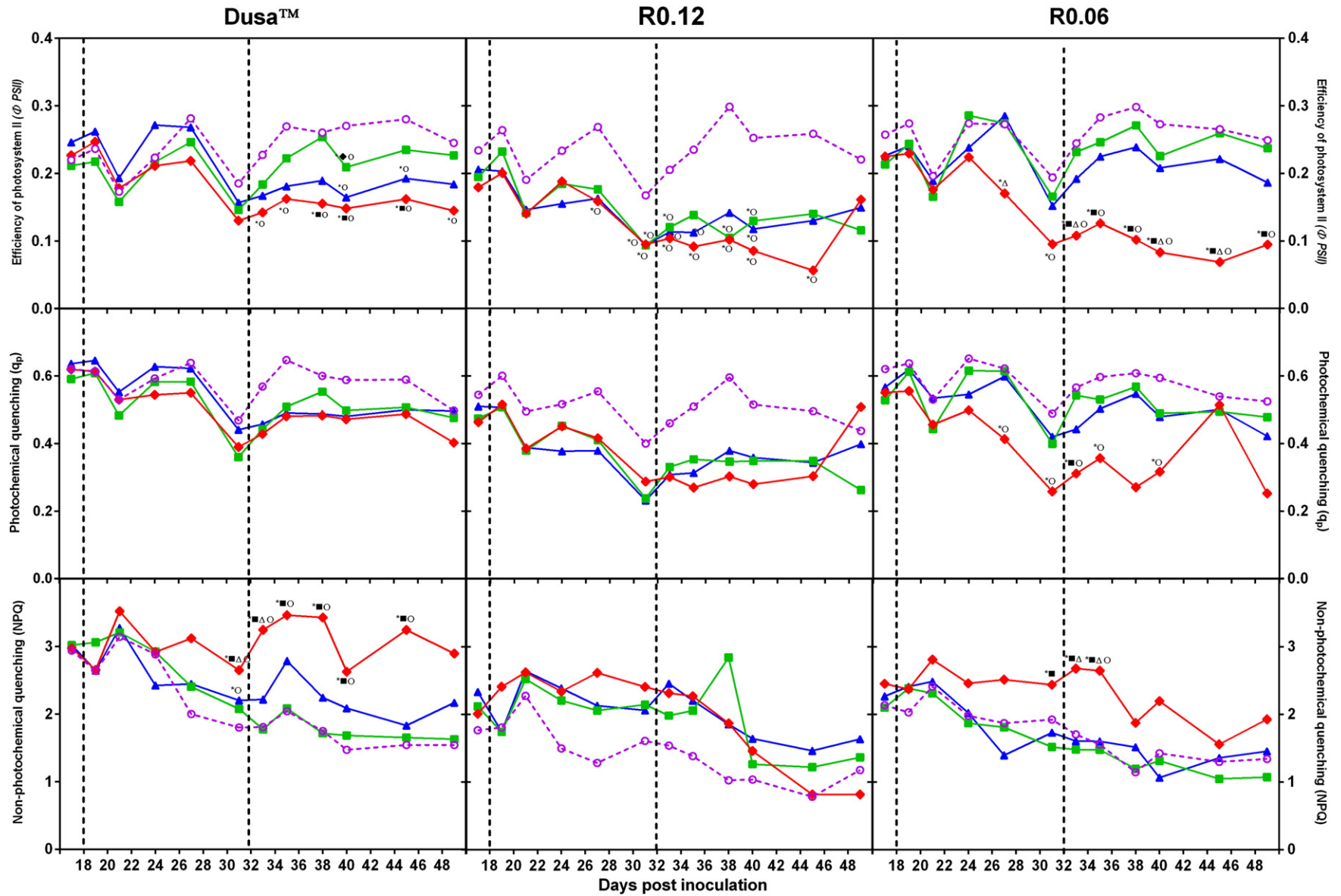
Fig. 6. Effects of *Phytophthora cinnamomi* and flooding on PSII efficiency (*ΦPSII*), photochemical quenching (qP) and non-photochemical quenching (NPQ) on 'Dusa™', R0.12 and R0.06 avocado rootstocks. Asterisks indicate significance with symbols to denote the treatment/s between which differences are significant; infected and flooded (diamonds), infected (squares), flooded (triangles), control (circles). Flooding began at day 18 as indicated by the dotted line and plants were drained at day 32 as indicated by the second dotted line.

to two other rootstocks initially selected for tolerance to PRR. R0.12 was selected for tolerance to PRR but screening showed it to be inferior. R0.06 is a new selection that has not yet been tested on a commercial scale and is thought to be superior to 'Dusa™'. In addition to comparing rootstocks, reductions in P_N, associated with flooding and infection, were investigated to determine whether these reductions were due to stomatal or non-stomatal limitations.

In both trials flooding was seen to have an immediate impact on root health, which was greater than that observed when plants were only infected with *P. cinnamomi*. However, the combination of flooding and *P. cinnamomi* had the most devastating impact on plant health and caused extensive root necrosis and wilting (Fig. 1). Although uninfected plants that were flooded also showed signs of wilting, this was only apparent at a later date. Visible symptoms of stress were generally more severe in 'Duke 7' plants as compared to 'Dusa™', which was also evident in photosynthetic parameters. These symptoms of flooding stress were first observed in 'Duke 7' plants 10 days after flooding was commenced and for 'Dusa™' 16 days after flooding was commenced. These visible symptoms were, however, preceded by differences in P_N, g_s and E (Fig. 3), as has previously been described in avocado (Ploetz and Schaffer, 1989). Differences in P_N and g_s between flooded and control plants were evident as early as 5 days after flooding commenced (12 dpi, Fig. 3) in the glasshouse trial and changes in P_N and g_s were seen within 3 days of the commencement of flooding in the shadehouse trial. The rapidity with which these symptoms appeared and the poor health of the plants by the end of the trial (Fig. 2) was not unexpected, as avocado is considered a flood-sensitive species (Schaffer et al., 1992).

Leaf wilting and decline in general plant health of flooded plants were observed in all rootstocks when compared to non-flooded controls. In general, no significant reductions in biomass were apparent for any rootstock in both the glasshouse trial and shadehouse trial. Flooded 'Duke 7' plants showed reductions in root biomass relative to control plants in the glasshouse trial (Table 1) and the only significant changes seen in biomass in the shadehouse trial (Table 2) were reductions in leaf and root biomass in R0.06 plants that were flooded in combination with infection. This may suggest that changes in carbon allocation are a long-term response to flooding in avocado and that reductions in root biomass are caused directly by the root rot.

'Dusa™' is considered a more PRR-tolerant rootstock than 'Duke 7' (Smith et al., 2011; Whiley et al., 2002), and this is supported by the physiological response of the plants to infection by *P. cinnamomi*. Infected 'Dusa™' plants showed an initial decline in P_N, g_s, and E but by the end of the trial there were no differences between control and infected plants (Fig. 3). The reopening of stomata has previously been related to flood tolerance (Zentmeyer, 1980) and thus the recovery of g_s and P_N to control levels in infected 'Dusa™' plants could indicate recovery from stress, in this case infection. This could possibly be due to regeneration of feeder roots in these plants. P_N, g_s and E were significantly reduced in infected 'Duke 7' plants as compared to control plants and could not recover to control levels, suggesting lower relative PRR tolerance in 'Duke 7' plants. This appears to be a general response to infection by *P. cinnamomi* as similar reductions in P_N and g_s have been noted in other species (Corcobado et al., 2013; Dinis et al., 2011). Infected 'Dusa™' and R0.06 plants in the shadehouse trial also showed recovery of P_N, E, and g_s to control levels after an initial decline (Fig. 4). However this was not true for the less tolerant R0.12, where infected plants showed lower levels when compared to control plants throughout the trial. Treatments that were flooded in the absence of *P. cinnamomi* showed initial increases in P_N, g_s, and E in both 'Dusa™' and 'Duke 7' rootstocks, whilst plants exposed to both stresses did not show this increase and had lower values for these parameters (Fig. 3). However, by the end of the trial all flooded treatments, across both rootstocks, had significantly reduced values for P_N, g_s and E when compared to non-flooded treatments. These reductions were also seen in 'Dusa™' in the shadehouse trial. Interestingly the less tolerant rootstock R0.12 showed significant reductions in P_N, g_s and E when plants were flooded in the

absence of *P. cinnamomi*. Reductions in P_N, g_s and E in 'Dusa™' were not as severe and were not apparent in the more tolerant rootstock R0.06, where both non-flooded, infected plants and uninfected, flooded plants generally showed levels similar to control plants for these parameters (Fig. 4). Reductions in P_N, g_s, and E have been observed in a number of plants when flooded (da Silva et al., 2011; Fleischmann et al., 2002; Schaffer and Ploetz, 1989) and are characteristic of flood-sensitive plants such as avocado (Kozlowski, 1997; Schaffer et al., 1992). This may suggest that the more tolerant R0.06 is also more tolerant to flooding in the absence of *P. cinnamomi* than the other rootstocks evaluated, but that this tolerance is compromised when plants are flooded in the presence of *P. cinnamomi*.

Reductions in P_N, g_s and E may be as a result of damage to the roots caused by PRR or by the lack of O_2 in the growing medium resulting from flooded conditions, as plants try to compensate for reduced water uptake by the roots and to maintain leaf water status (Davies and Flore, 1986). This reduced water uptake by the roots has been attributed to changes in root permeability and conductivity (Else et al., 1995; Jackson et al., 1996; Nicolas et al., 2005; Pezeshki, 2001). The decrease in P_N is suggested to be a consequence of stomatal closure, which reduces CO_2 diffusion into the leaf (Kozlowski, 1997; Schaffer et al., 1992). Whilst this was the likely cause of reduced P_N in 'Duke 7' plants infected with *P. cinnamomi*, stomatal limitations were unlikely to be the sole cause of reduced P_N in flooded and flooded and infected plants. This is in agreement with Schaffer et al. (1992) who attributed changes in P_N in flooded avocado plants to non-stomatal limitations that are associated with the biochemistry of the photosynthetic reactions. Support for non-stomatal limitations to P_N in flooded plants was found in the C_i values from this study. If stomatal closure was limiting CO_2 diffusion into the leaf and therefore P_N, it would be expected that there would be a parallel decrease in C_i (Farquhar and Sharkey, 1982); however, in flooded and infected 'Dusa™' plants there is an increase in the C_i/C_a ratio from 12 dpi (Fig. 3), indicating a reduced mesophyll capacity for assimilation (Farquhar and Sharkey, 1982). An increase in C_i/C_a was also seen in 'Dusa™' plants that were flooded without infection, although only towards the end of the trial. This reduced mesophyll capacity has been attributed to changes in chlorophyll content, altered water and nutrient uptake, changes in enzyme efficiencies, and damage to the photosystem (Else et al., 2009; Pezeshki, 2001). Similar non-stomatal limitations to P_N were found in 'Verna' lemon trees in response to flooding (Gimeno et al., 2012). Whilst the C_i/C_a ratio was consistently higher in 'Dusa™' flooded and infected plants from 12 dpi as compared to non-flooded treatments, a similar increase in the C_i/C_a ratio was only observed in flooded and flooded and infected 'Duke 7' plants at the end of the trial, suggesting slightly different physiological responses of the two rootstocks to flooding (Fig. 3).

In flooded 'Dusa™' and flooded and infected 'Duke 7' plants it would appear that the photosystems are only damaged once the plants have begun to senesce and it is therefore a long term response to flooding, as has been previously reported (Davies and Flore, 1986; Kozlowski, 1997). The reduced mesophyll capacity in this study in 'Dusa™' flooded and infected plants is therefore most likely a result of changes in enzyme efficiencies (Kozlowski, 1997). However, in contrast to the glasshouse study, C_i/C_a ratios in 'Dusa™' plants within the shadehouse trial decreased in flooded, infected plants (Fig. 4). This indicates that the decreases in P_N could indeed be due to stomatal limitation in these plants. These reductions were also seen in the less tolerant R0.12 and the more tolerant R0.06 rootstocks, suggesting that this is a conserved response between rootstocks. The difference between the two trials could be due to a variation in temperature or light levels, which in combination with the stress imposed by flooding and infection may have limited P_N and thus resulted in increased C_i/C_a in plants in the glasshouse.

When the interaction between flooding and infection was assessed it was found that in both 'Dusa™' and 'Duke 7' flooding seemed to have a greater impact on photosynthetic parameters than infection

(Table 3). This was confirmed in the shadehouse trials for all three root-stocks assessed, including 'Dusa™', although in this trial infection did seem to play a more significant role in the change in photosynthetic parameters (Table 4). Interestingly the impact of the combination of treatments was much higher in R0.06 than in the other rootstocks, which reflected the drastic response of the rootstock under these conditions.

Fluorescence measurements were used to further evaluate photosynthetic performance in flooded and infected plants. The F_v/F_m ratio, used as an indication of stress (Ball et al., 1994; Maxwell and Johnson, 2000) or photoinhibition (Dias and Marenco, 2006), was generally unchanged in both trials and could not be used as a useful indicator of early responses to flooding or infection in avocado. Differences in $\Phi PSII$ were noticeable at the onset of visible symptoms between flooded and non-flooded treatments of both 'Dusa™' and 'Duke 7' plants, with flooded plants exhibiting lower values for $\Phi PSII$ than non-flooded plants. Similar differences were seen in 'Dusa™' plants from the shadehouse trial. The less tolerant R0.12 rootstock exhibited reductions in $\Phi PSII$ for all treatments relative to control plants, illustrating the greater effect of infection on a less tolerant rootstock which greatly compromises P_N. The more tolerant R0.06 only exhibited reductions in $\Phi PSII$ for plants that were exposed to the combination of flooding and infection, with plants that were uninfected but flooded performing at similar levels to control plants. This highlights the importance of the combination of stress on avocado, with R0.06 tolerating either stress well but being very susceptible to the combination of the two stresses.

This reduction in $\Phi PSII$ indicates a decrease in the proportion of radiation absorbed by chlorophyll associated with PSII that is used in photochemistry (Maxwell and Johnson, 2000) and was accompanied by a decrease in qP and an increase in NPQ in some rootstocks (Fig. 6), which in turn suggests increased protection through xanthophyll cycling (Müller et al., 2001). Decreases in $\Phi PSII$ of 'Dusa™' and 'Duke 7' grown in the glasshouse were not accompanied by an increase in NPQ (Fig. 5), possibly suggesting a reduced ability of these plants to dissipate excess energy resulting from a decline in photochemistry. Lower light levels in the glasshouse as compared to the shadehouse could also account for the differences in NPQ, as lower light levels translate to less energy that needs to be dissipated.

5. Conclusions

From this study it is evident that in the avocado rootstocks evaluated, the initial physiological response to flooding is different to that of infection. Whilst infected plants showed an initial decline in P_N and g_s, flooded plants showed an initial increase in these parameters. However, the combination of the two stresses induced a severe decline in both P_N and g_s. This suggests that the combination of the two stresses brings about an additional response that is more than just additive, as suggested by Mittler (2006). Avoiding waterlogged conditions in avocado orchards is therefore critical to maintaining healthy, productive trees. In addition, P_N and g_s for flooded, uninfected plants decreased to very low levels in all rootstocks except R0.06 by the termination of the trials. Tolerance to *P. cinnamomi* is therefore not related to an increased flooding tolerance in either 'Dusa™' 'Duke 7', or R0.12 rootstocks, although R0.06 seems to exhibit increased tolerance to both flooding and infection but not to the combination of the two stresses. Tolerance to *P. cinnamomi* infection does, however, appear to be related to the ability of plants to reopen stomata and allow P_N to recover to pre-infection levels, which could be related to root repair or regeneration following infection and could be used as a physiological marker for tolerance. This was made evident in this study by the greater ability of infected 'Dusa™' and R0.06 plants to restore both P_N and g_s to levels observed in the control, when compared to 'Duke 7' and R0.12 plants.

The early decline in both P_N and g_s in flooded and infected plants was not matched by changes in fluorescence parameters and these only began to change once visible symptoms were apparent in flooded and flooded and infected plants. Long term flooding did, however, indicate possible photosystem damage in both 'Dusa™' and 'Duke 7' plants as indicated by a reduced F_v/F_m ratio relative to the control at the end of the trial. This decrease was, however, not seen in shadehouse trials. Whilst this suggests that the initial decline in P_N is as a result of stomatal limitations, this appears only to be the case for infected plants. Due to a high C_i/C_a ratio in 'Dusa™' flooded and infected plants non-stomatal limitations appear to be responsible for the decline in P_N in these plants, which could possibly be attributed to reduced enzymatic efficiencies. Plants from the shadehouse trial did not show a similar response, with C_i/C_a levels dropping in flooded, infected plants, which suggests that the primary limitation to photosynthesis in these plants was a stomatal limitation. Increased NPQ in these plants may have prevented damage to the photosystem and limited the contribution of non-stomatal limitations to P_N in these plants. Assessing photosynthetic parameters may therefore be useful in rootstock selection for both improved PRR tolerance and flooding tolerance. In this respect, tolerance would be associated with a recovery in photosynthetic parameters following imposition of stress, which could be related to an increased ability to dissipate excess energy through xanthophyll cycling thereby preserving the integrity of the photosynthetic apparatus.

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