South African Journal of Botany 95 (2014) 40–53

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

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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ARTICLE INFO

Article history:
Received 20 March 2013
Received in revised form 12 August 2014
Accepted 12 August 2014
Available online 7 September 2014

Edited by AK Cowan

Keywords:
Phytophthora
Avocado
Fluorescence
Gas exchange
Flooding

ABSTRACT

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.

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

Abbreviations: ΦPSII, photosystem II quantum efficiency; C_i, internal CO_2 concentration; C_a, atmospheric CO_2 concentrations; *E*, transpiration; ETR, electron transport rate; F_m, maximal fluorescence; F'_m, maximal light adapted fluorescence; F_o, minimal fluorescence; F'_o, minimal light-adapted fluorescence; F_s, steady-state fluorescence; g_s, stomatal conductance; NPQ, non-photochemical quenching; qP, photochemical quenching; P_N, net assimilation rate; PRR, Phytophthora root rot.

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http://dx.doi.org/10.1016/j.sajb.2014.08.004
0254-6299/

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