Preliminary investigations on the effect of low-pressure treatment on in vitro and in vivo growth of *Penicillium* sp. in oranges

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Abstract

***Penicillium digitatum* is the major pathogen causing postharvest decay in citrus fruit and is the organism responsible for green mould. *P. digitatum* decay is currently managed with synthetic fungicides but there is a growing consumer need to reduce the reliance on synthetic fungicides and find alternative non-synthetic treatments. Low-pressure treatments may offer a potential solution for the storage and transport of citrus, as it is a physical treatment and does not leave any chemical residues. To test the effectiveness of low pressure storage treatments on the growth of *P. digitatum*, small-scale laboratory experiments were conducted with specialist low pressure chambers. *P. digitatum* was grown on PDA agar plates and treated with low pressure (4 kPa) for 3 and 6 days at 10°C before growth assessments in air at regular (101 kPa) atmospheres. The results showed that low pressure treatments slowed the growth of *P. digitatum*. In a further experiment on oranges, *P. digitatum* infected fruit were treated with low pressure (4 kPa) at 5°C for up to 22 days. This experiment also showed a reduced growth of *P. digitatum in vivo*. These results show there is potential to control the growth of *P. digitatum* at low pressure treatments and further experimentation should be conducted.**

Keywords: citrus, storage, decay, non-chemical.

INTRODUCTION

Postharvest losses due to fungal decay are a major issue for agriculture. Several postharvest fungicides have been banned due to legislation constraints resulting in increased interest in environmentally friendly and safe alternatives (Romanazzi et al., 2016). A potential physical alternative to assist with the control of postharvest pathogens is the use of low pressure (Pristijono et al., 2017a).

Low pressure storage technology is a relatively old technology but has recently returned as a technique which can rapidly remove heat, reduce oxygen levels and rapidly remove and manage the storage atmosphere (Wang et al., 2001). Unlike other

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treatments such as heat, gamma irradiation and UV, a potential advantage of pressure treatment is the homogeneity of application during treatment (Vigneault et al., 2012). Modern low pressure systems can maintain high humidity levels >95% to lower water loss and wilting, this also lowers respiration and ethylene production to delay fruit ripening during storage (Burg, 2004). Low pressure storage at sub-atmospheric pressures has been shown to extend the storage and shelf-life of many horticultural crops such as bananas (Burg and Burg, 1966) and tomatoes (Pristijono et al., 2017b). Low pressure can also have direct effect on the growth of postharvest pathogens. Pristijono et al., (2017a) showed that low pressure treatments of 4 kPa reduced stem decay and flesh rots in green capsicums (peppers) compared to that of controls at atmospheric pressure (101 kPa).

Green mould (*P. digitatum*) is one of the most important postharvest fungi in the storage of citrus. This study examined the effectiveness of low pressure on the growth rate of *P. digitatum* on agar plates and in citrus.

MATERIALS AND METHODS

In vivo treatment

Organic Navel oranges were harvested from NSW Department of Primary Industries orchard at Somersby Research Station and washed with 50 $\mu L.L^{-1}$ chlorine and allowed to dry. A laboratory strain of *P. digitatum* spores (1 x 10^6 spores per mL) were inoculated into the wound of each orange. The inoculated fruit were kept at 25 °C for 24 hours before treatment. Inoculated fruit were either treated with low pressure (4 kPa) or remained at atmospheric pressure (101 kPa). The fruit were stored at 5 °C for 22 days before removal and assessment of growth. There were three separate chambers (independent replicates) for the low pressure treatment and atmospheric pressure. There were 10 fruit per treatment unit.

In vitro treatment

P. digitatum mycelial samples were collected from an actively growing colony (7 to 14 days old) from potato dextrose agar (PDA), sub-cultured and allowed to grow for 10 days. A 4 mm disc of the actively growing *P. digitatum* colony was then cut from the PDA plate with a circular bore cutter. The disc of agar was aseptically transferred to a fresh PDA plate and incubated for 24 hours at 25 °C before transferring to the low pressure treatment at 10 °C inside the low pressure chambers at 4 kPa. Similar treatment plates were prepared and placed atmospheric pressure (101 kPa) at 10 °C. After low pressure treatment for either 3 or 6 days, all plates were transferred to 25 °C and the growth of the fungus was measured each day. The treatment unit for each treatment was 10 agar plates which were replicated in three low pressure chambers. The control treatment plates were kept at atmosphere pressure.

Low-pressure treatment

A VivaFresh™ low-pressure system consisting of six aluminum chambers (0.61 L × 0.43 W × 0.58 H m total volume of 0.152m^3) all identical was used in this section of the experiment. The vacuum was produced and maintained with a two-stage rotary vacuum pump (Model 2005I, Alcatel Adixen, USA) which used a solenoid valve controlled by a proportional/integral/derivative (PID) computer control system using

VivaFresh™ software and control parameters. The low-pressure system was able to alter an air flow controller to adjust the air exchange rate to ensure that the accumulation of metabolic gasses did not occur. An air humidifier was used before entering the chamber and the internal air humidity level was monitored with wet-bulb and dry-bulb temperatures using calibrated YSI 55,000 Series GEM thermistors. The data created by the different sensors was sent to the control box and then accessed via ethernet by computers on the network.

RESULTS AND DISCUSSION

In vivo results

Effect of low pressure treatment of 4 kPa on the growth of *P. digitatum* in Navel oranges after 22 days storage at 5 °C is presented in Figure 1. The results showed that the low pressure treatment significantly reduced the growth of *P. digitatum* in the fruit. After 22 days at 5 °C, the low pressure treatment had only 4.6 mm growth compared to 16 mm of growth at atmospheric pressure (101 kPa). This difference may be due to reduced oxygen availability during low pressure storage, where the oxygen level at 4 kPa is equivalent to approximately 1% O_2 (v/v). Burg (2004) reported that low oxygen atmospheric storage of 0.1 – 0.25% O_2 have significantly inhibitory effects on pathogens and spore germination.

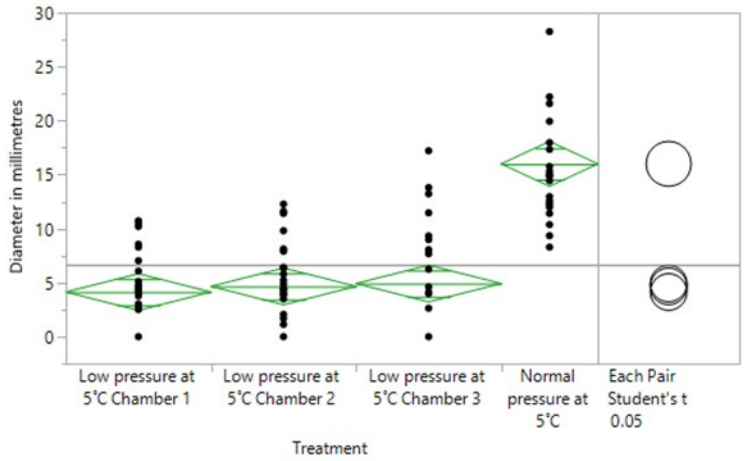

Figure 1. Effect of 3 days low pressure treatment (4 kPa) on growth of *P. digitatum* growth in Navel oranges in the three low pressure chambers after 22 days storage at 5 °C. The green diamonds represent the 95% confidence interval for the data. Overlapping marks are above and below the group mean to indicate that the chamber means are not significantly different to one another but are significantly different to normal pressure.

In vitro results

The effects of low pressure on the growth of P. digitatum on PDA petri dishes are presented in Figures 2 and 3. The low pressure treatment was applied for either three or six days at 10 °C and then returned to regular atmospheric pressure at 25 °C for measurement of fungi growth. The results show that immediately upon removal of the

plates after three days treatment, the growth of the fungus was <1 mm, while the fungus growth at atmospheric pressure was 8 mm (Figure 2). After the plates were removed from the low pressure and returned to atmospheric pressure at 25 °C, the growth rates of both treatments significantly increased. There was an increase in growth rate of the fungi which had the initial low pressure treatment (Figure 2).

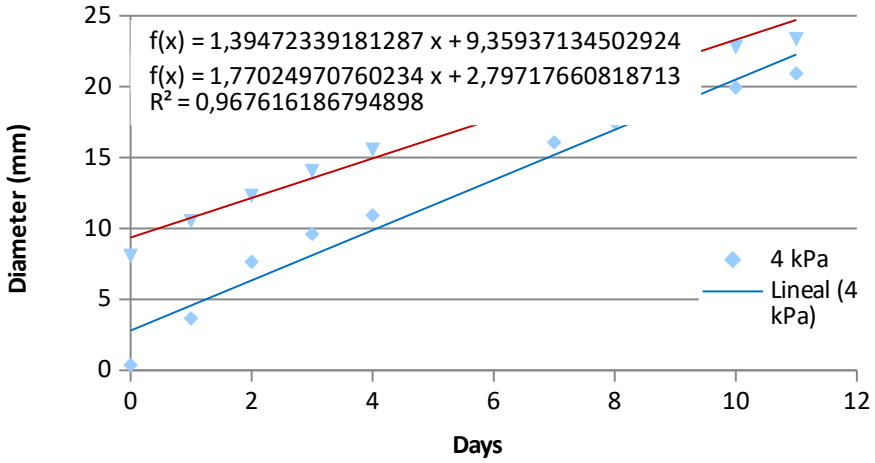

Figure 2. The diameter (mm) of *P. digitatum* colony at 25 °C on PDA petri plates following treatment at 4 kPa (or101 kPa) at 10°C for 3 days.

The growth of the *P. digitatum* colony after six days low pressure treatment was 2.5 mm, while the growth at atmospheric pressure was 14 mm in diameter (Figure 3). After the plates were removed from the low pressure treatment, the growth rate significantly increased where with the growth of the fungi more than doubled on the first day at atmospheric treatment after removal.

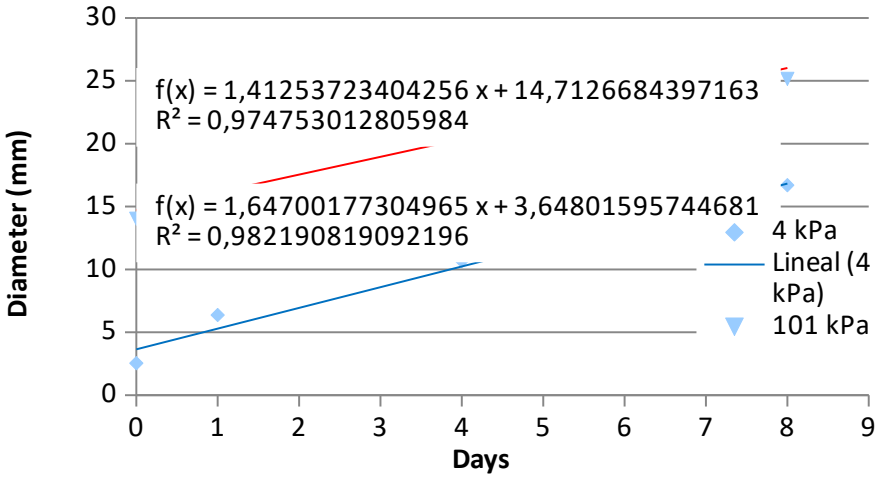

Figure 3. The diameter (mm) of *P. digitatum* colony at 25 °C on PDA petri plates following treatment at 4 kPa (or101 kPa) at 10°C for 6 days.

CONCLUSIONS

While *P. digitatum* growth at these low treatment temperatures (5 and 10 °C) is relatively slow, there were significant difference in the growth of *P. digitatum* between the low treatments and the control treatments at atmospheric pressure. Both in vitro and in vivo, *P. digitatum* growth was lower when under the low pressure treatment. This difference in growth requires further study but it may be due to low oxygen levels as the partial pressure of oxygen is reduced in the low pressure treatment reducing the metabolic rates of the fungi at the low pressure.

ACKNOWLEDGEMENTS

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