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Phytochemical Changes in Mango Fruit in Response to *Alternaria alternata* Infection

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Abstract

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Changes in mango fruit quality, malondialdehyde content, and enzymatic activities in response to pathogen *Alternaria alternata* infection were studied. *A. alternata* significantly affected the appearance of mango fruit at 5 and 7 days after treatment (DAT). The quality of pathogen-infected fruit first showed a significant decrease in titratable acidity and vitamin C content and a significant increase in pH since 3 DAT. The malondialdehyde content was higher than that in the untreated controls at 3 and 7 DAT. The enzyme activities of ascorbate peroxidase and polyphenol oxidase showed significant increases since 3 DAT. Significant increases in L-phenylalanine ammonia-lyase and superoxide dismutase activities were observed at 7 DAT. These results indicate that *A. alternata* infection first significantly affects some biochemical constituents and enzyme activities in mango fruit since 3 DAT and that there was no significant effect on appearance until 5 DAT.

Keywords: *Alternaria alternata*; enzyme activity; fruit quality; mango; malondialdehyde

Mango (*Mangifera indica* L.) is a major and commercially important tropical fruit with delicious taste and high nutritive value (Evans *et al.* 2017). In 2013, China was the second greatest mango producer in the world after India (Evans *et al.* 2017). Postharvest diseases are the main factor constraining the storage and transport of mango fruit, with significant postharvest losses and quality deterioration from harvest to consumption (Tian *et al.* 2010). *Alternaria alternata* is one of the most serious fungal agents causing postharvest diseases (Prusky *et al.* 2009). *A. alternata* infects mango fruit and causes rot on the sides and stem ends, resulting in serious postharvest losses (Prusky *et al.* 2013; Diskin *et al.* 2017).

Much research has been conducted on storage technologies and nonchemical treatments to reduce the incidence of postharvest diseases and to improve and maintain fruit quality (Mulas & Schirra 2007; Perumal *et al*. 2017; Liu *et al*. 2018). Additionally, postharvest heat therapy (hot air or water treatments and short hot water rinsing and brushing) combined with other treatments could enhance the efficiency of disease management and lead to the attainment of the complete control of decay (Schirra *et al.* 2011). Various fungicides have also been evaluated as chemical methods to control a wide range of postharvest fungal diseases (Adaskaveg & Förster 2010; Alam *et al*. 2017; Perumal *et al*. 2017). In recent years, the Chinese government and Chinese consumers are placing increased emphasis on food quality and safety (Liu *et al.* 2013). Evaluating the effects of postharvest diseases on fruit quality for consumers is becoming a main objective in microbiological risk assessment for agricultural products. Previous

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reports have quantified the effects of *Candidatus liberibacter* spp. infection (citrus greening disease, Chinese: huánglóngbìng) in citrus on orange fruit and juice quality and phytochemical composition (size, colour, solids, sugars, acids, aroma volatiles, enzymes, demethylated pectin, etc.) (Bassanezi *et al.* 2009; Plotto *et al.* 2010; Baldwin *et al.* 2014). The defence enzymes of vegetative tissues, including those of mango, play an important role in response to various stresses, including pathogen infection (War *et al.* 2013; Razzaq *et al.* 2014; Farouk *et al.* 2017; He *et al.* 2017). However, there have been no relevant studies that have quantitatively assessed the effects of *A. alternata* on the quality, malondialdehyde (MDA) content, and defence enzyme activities of mango fruit.

In this study, we systematically determined the effects of *A. alternata* infection on the quality [appearance, reducing sugars (RS), titratable acidity (TA), vitamin C (Vc), total soluble solids (TTS), potential of hydrogen (pH)], MDA content, and several enzymatic activities [superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), polyphenol oxidase (PPO), L-phenylalanine ammonia-lyase (PAL) and ascorbate peroxidase (APX)] of mango fruit.

MATERIAL AND METHODS

Fruit and pathogen preparation. All mango fruit samples of the cultivar Tainong No. 1 with no disease symptoms were purchased from a Carrefour supermarket (China). Fruit of similar sizes and stages of maturity (80–85%) were selected for the experiment. All of the selected fruits were thoroughly cleaned with sterile distilled water, disinfected by immersion in a 0.8% sodium hypochlorite solution for 5 min, and then washed with sterile distilled water three times. All prepared fruit samples were placed in preservation boxes and preconditioned for 6 h before inoculation with *A. alternata* at 28°C and 90% relative humidity (Al-Haq *et al.* 2002).

The pathogenic fungus *A. alternata* was obtained for the experiment from the China Center of Industrial Culture Collection (China). *A. alternata* was grown on potato dextrose agar (PDA) (Beijing Land Bridge Technology Co. Ltd., China) at 28°C for 5 days under aerobic conditions. A spore suspension of 1×10^8 spores/l was prepared from a 5-day old culture of *A. alternata* using sterile distilled water (Ellaiah *et al.* 2004).

Experimental design. The mango fruit samples were artificially inoculated with *A. alternata* as described by Al-Haq *et al.* (2002). Sterile distilled water without *A. alternata* was set as the control. Both treatments were performed with four replicates. Each treatment included 40 fruits in a single replicate. All fruits (320) were held in incubators at 28°C and 90% relative humidity to stimulate ripening and rot development. In each treatment, 10 mango fruits per replicate were randomly selected for the evaluation of appearance at regular intervals of 1, 3, 5, and 7 days after the treatment (DAT). And then, the selected fruits per replicate were peeled and the stones were removed, and the remaining flesh was mixed by shaking with a bag mixer (BagMixer®400; Interscience International, France). The mixed samples were used for the fruit quality assays (RS, TA, Vc, TSS, and pH), MDA content and enzyme activity measurement (SOD, POD, CAT, PPO, PAL, and APX enzymes).

Quality evaluation. During each evaluation, the appearance of 10 mango fruits infected by *A. alternata* and 10 control fruits per replicate were evaluated using a 0–3 scale; where: 0 – fresh yellow-green colour and healthy with no disease, 1 – fresh yellow colour with 1–10% fruit rotted, 2 – bright yellow colour with 11–30% fruit rotted, 3 – dark yellow colour with 31–100% fruit rotted.

The titratable acidity (TA) estimation was done by titration with NaOH and expressed as a percentage. The Vc content was determined by the 2,6-dichloro-indophenol titration method. The potential of hydrogen (pH) was measured with a pH meter (Mettler-Toledo GmbH, Switzerland). The TSS measurement was realized using the PAL-1 digital refractometer (Atago, Japan). The RS analysis was assayed by the DNS method (Pal & Chakraborty 2013).

MDA content and enzyme activity determination. The MDA content and the enzyme activity of SOD, POD, CAT, PPO, PAL, and APX were determined using the appropriate test kits (Suzhou Keming Biotechnology Co. Ltd., China). The MDA content was expressed as mol/kg. The enzyme activities of SOD, POD, CAT, PPO, PAL, and APX were expressed as U/kg based on protein content.

Statistical analysis. The laboratory studies were performed with four replicates. The data were expressed as a mean ± SD. The data were analysed by the analysis of variance (ANOVA method) using the SPSS (v22.0 for Windows; IBM). Significant differ-

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ences between the means were tested using Duncan's multiple range test at $P < 0.05$.

RESULTS AND DISCUSSION

Fruit quality. The appearance scores of *A. alternata*-infected mango fruit and untreated controls over the entire experimental period are shown in Figure 1. The appearance scores for fruit infected by *A. alternata* progressively increased and showed a significant increase ($P < 0.05$) at 5 and 7 DAT.

In addition, *A. alternata* infection affected biochemical constituents such as RS, TA, Vc, TSS, and pH (Figure 2). Compared to the untreated controls, the RS content of infected mango fruit also remained stable until 7 DAT, at which point a significant reduction occurred. The TA content of *A. alternata*-infected mango fruit showed a sharper reduction, which was significantly lower than that observed for the untreated controls at 3, 5, and 7 DAT. Compared to the untreated controls, the Vc content of *A. alternata*-infected mango fruit showed a significant and rapid reduction at 3 and 5 DAT and then remained stable at a low level. There was a significant increase in the TSS content of *A. alternata*-infected mango fruit at 3 DAT and then a decrease to the original level at the end of the experiment. The pH of *A. alternata*-infected mango fruit showed an increasing trend and was significantly higher than that of the untreated control fruit during the entire experimental period.

With the advancement of ripening in mango fruit, TSS content and pH increased, and a reverse trend was observed for TA and Vc contents (Razzaq *et al.* 2014). Significant differences in appearance were observed at 5 and 7 DAT (Figure 1), with approxi-

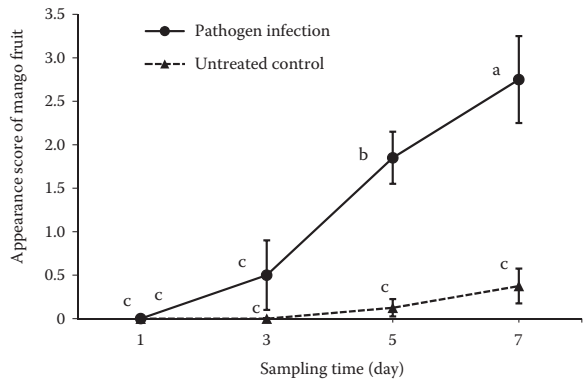

Figure 1. Appearance scores of mango fruit with *A. alternata* infection ($P < 0.05$)

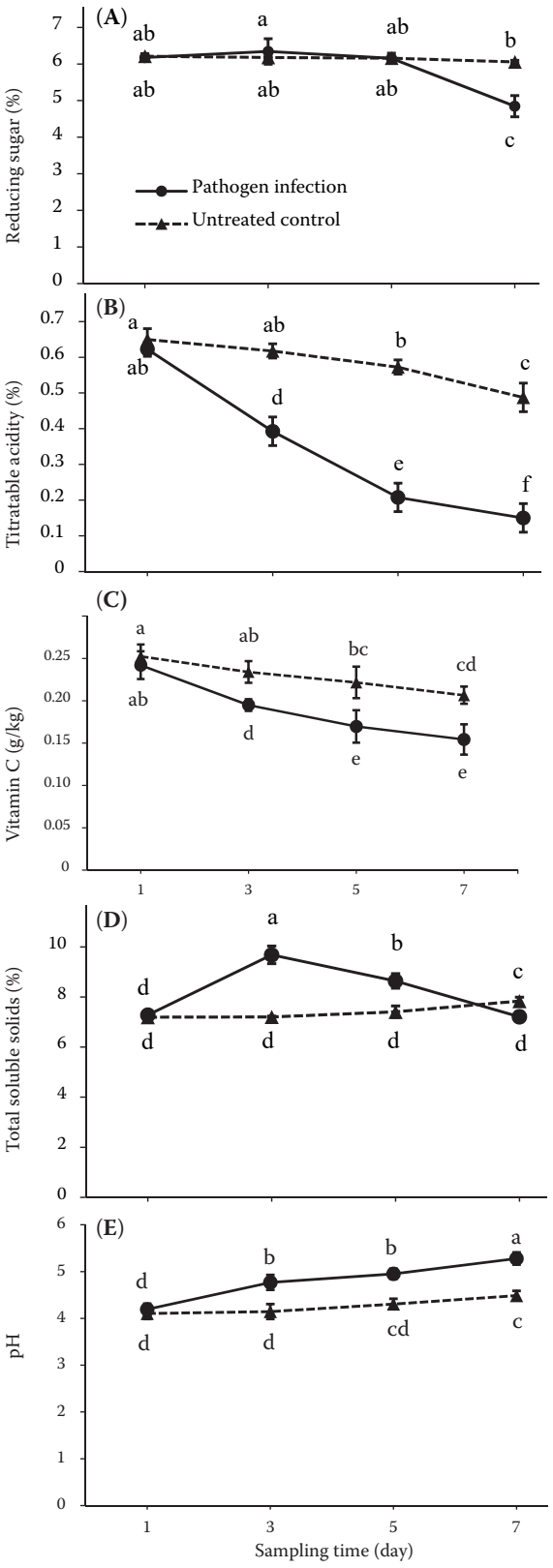

Figure 2. Chemical content changes in mango fruit with *A. alternata* infection ($P < 0.05$): (**A**) reducing sugar, (**B**) titratable acidity, (**C**) vitamin C content, (**D**) total soluble solids, and (**E**) pH

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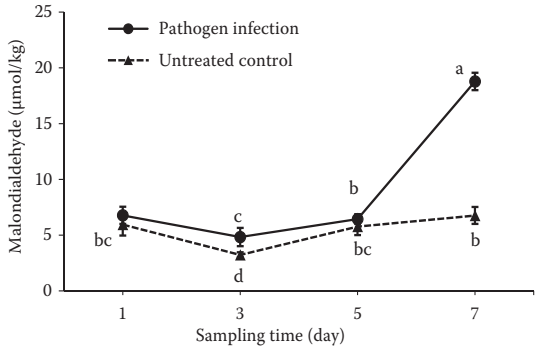

Figure 3. The malondialdehyde content changes in mango fruit with *A. alternata* infection ($P < 0.05$)

mately 2 to 3 cm diameter lesions. Earlier than the decline in appearance, the quality of the mango fruit decreased gradually with a significant loss of RS, TA, and Vc contents and an obvious increase in pH after 3 DAT (Figure 2). Infection by and development of the pathogen *A. alternata* led to enhanced respiration and rapid consumption of nutrients in the mango fruit followed by a decrease in RA, TA, and Vc contents and then an increase in pH.

MDA content. *A. alternata* infection affected the MDA content of mango fruits (Figure 3). A significantly higher MDA content was observed in *A. alternata*-infected mango fruit compared with the untreated control fruit (Figure 3), which could be attributed to the important lipid oxidation of MDA in response to *A. alternata* infection (Huang *et al.* 2007; War *et al.* 2013).

Enzyme activity. The enzymes APX, PAL, PPO, POD, SOD, and CAT play an important role in plant defence against various stresses, including pathogen infection (War *et al.* 2013; Razzaq *et al.* 2014; Farouk *et al.* 2017; He *et al.* 2017). SOD as the first line of defence can convert oxygen radicals (O_2^-) into H_2O_2 to scavenge the toxic free radicals, which could be used to explain the significant increase of SOD in *A. alternata*-treated fruit at 7 DAT compared with the untreated control (Figure 4), which was similar

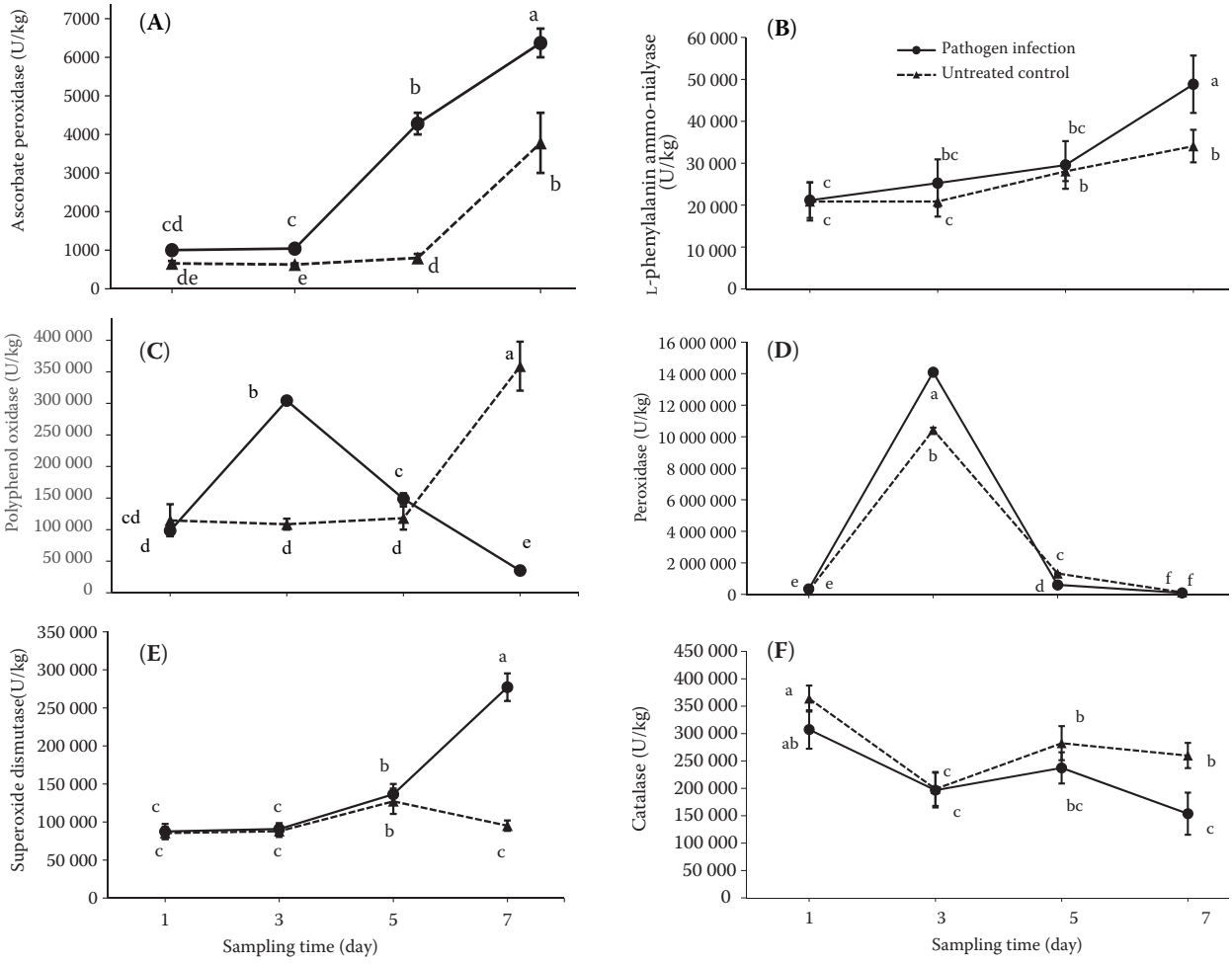

Figure 4. Enzyme activity changes in mango fruit with *A. alternata* infection ($P < 0.05$): (**A**) ascorbate peroxidase, (**B**) L-phenylalanin ammo-nialyase, (**C**) polyphenol oxidase, (**D**) peroxidase, (**E**) superoxide dismutase, and (**F**) catalase

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as the changes in MDA content (Figure 3). APX can reduce H_2O_2 to water and utilize AsA as an electron donor (Khan *et al.* 2011; Joshi & Chinnusamy 2014), which could inhibit pathogen infection (Barbehenn *et al.* 2005; War *et al.* 2013). There was a similar but more sensitive trend of change in APX activity (since 3 DAT) like in the appearance score (since 5 DAT), which means that APX activity significantly increased with the aggravation of *A. alternata* infection (Figures 3 and 4). There was no significant difference in the PAL activity between the *A. alternata*-infected fruit and the untreated controls during the experiment until 7 DAT, at which point a significant increase occurred in the infected fruit. CAT and POD can dismutate or decompose H_2O_2. The activity of CAT in the untreated control fruit rapidly decreased at 3 DAT and then experienced a fast increase at 5 DAT and finally remained stable to the end of the experiment, but that in *A. alternata*-infected fruit experienced a decrease, an increase, and then a decrease again, and the final level was significantly lower than that in the untreated control fruit. Associated significant changes in enzyme activity, such as those of APX and PPO, were also first observed since 3 DAT (Figure 4).

The balance in reactive oxygen species (ROS) metabolism was destroyed by *A. alternata* infection, followed by the stimulation of lipid membrane peroxidation with an increase in MDA content and APX, PPO, and POD enzyme activities (Figures 3 and 4). The stable increase in APX, PAL, and SOD in *A. alternata*-infected mango fruit compared to the untreated controls demonstrated that *A. alternata* infection significantly affected the enzyme systems of mango fruit.

Evaluating the risk of *A. alternata* to mango fruit is helpful in providing a more scientific basis for the suggestions given to consumers. There was no significant effect on appearance until 5 DAT, whereas significant losses in some biochemical constituents, such as TA, Vc, and pH, was first observed at 3 DAT. Similarly, the significant decrease in some enzyme activities, such as those of APX and PPO, was also first observed at 3 DAT. These results indicated that if the appearance of the pathogen-infected mango fruit is not very good, the loss of fruit quality and relevant enzyme activities could be worse.

CONCLUSIONS

A. alternata infection first significantly affected some biochemical constituents and enzyme activities of mango fruit after 3 DAT, and there was no significant effect on appearance until 5 DAT. Apart from the infected areas, evaluating the risk of the remaining mango flesh at different distances from the pathogen damage is more meaningful in providing scientifically based recommendations to consumers. In addition, the possible risk of biotoxins produced by *A. alternata* should also be considered in future research.

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