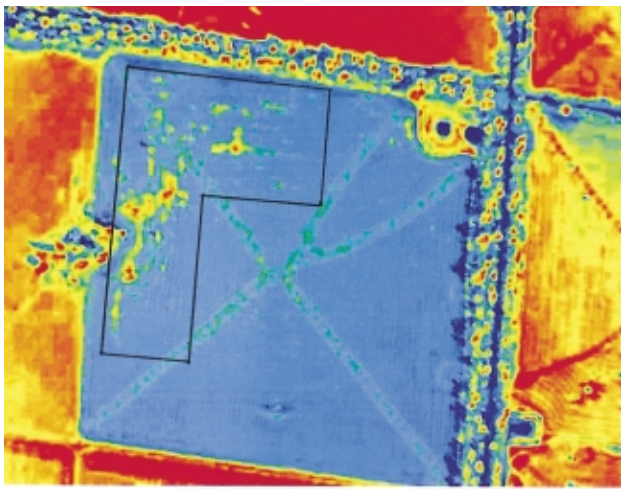
CSIRO Publishing

Australian Journal of Experimental Agriculture



VOLUME 41, 2001

. . . a journal publishing papers at the cutting edge of applied agricultural research

All enquiries and manuscripts should be directed to:

Australian Journal of Experimental Agriculture
CSIRO Publishing
PO Box 1139 (150 Oxford Street)
Collingwood, Vic. 3066, Australia

Telephone: +61 3 9662 7614
Fax: +61 3 9662 7611
Email: ajea@publish.csiro.au

Published by CSIRO Publishing
for the **Standing Committee on Agriculture and Resource Management (SCARM)**

www.publish.csiro.au/journals/ajea

Australian Journal of Experimental Agriculture, 2001, **41**, 805–813

Effects of phosphonate and salicylic acid treatments on anthracnose disease development and ripening of 'Kensington Pride' mango fruit

Zainuri[A], *D. C. Joyce*[BE], *A. H. Wearing*[C], *L. Coates*[D] *and L. Terry*[B]

[A]Agricultural Technology Programme, Department of Agronomy, Faculty of Agriculture, Mataram University, Mataram, 83125 Lombok, Indonesia.
[B]Institute for Horticultural Development, Knoxfield, Vic. 3176, Australia.
[C]School of Land and Food, The University of Queensland, Gatton College, Qld 4345, Australia.
[D]Plant Protection Unit, Queensland Department of Primary Industries, 80 Meiers Road, Indooroopilly, Qld 4068, Australia.
[E]Author for correspondence; e-mail: d.joyce@cranfield.ac.uk

Abstract. This study investigated treatment of mango (*Mangifera indica* L.) fruit with 2 host defence-promoting compounds for suppression of anthracnose disease (*Colletotrichum gloeosporioides*). Cultivar 'Kensington Pride' fruit were treated at concentrations of up to 1000 mg/L with either potassium phosphonate or salicylic acid. Applications were by various combinations of pre- and postharvest dips and vacuum infiltration. Postharvest treatments at up to 2000 mg/L salicylic acid were evaluated in a second fruiting season. Fruit were either uninoculated or inoculated with the fungal pathogen. Colour, firmness and disease-severity were assessed during shelf life at 23°C. There were no significant (P>0.05) effects of potassium phosphonate or salicylic acid on anthracnose disease severity in the first season. Moreover, phosphonate or salicylic acid treatment did not significantly affect fruit colour or firmness changes. There were significant (P<0.05) reductions in anthracnose severity in the second season, especially at the highest concentration of 2000 mg/L salicylic acid. Mango fruit skin colour and firmness changes were also slowed down significantly (P<0.05). These effects of salicylic acid were attributed to inhibition of mango fruit skin ripening (senescence).

Additional keywords: *Colletotrichum*, *Mangifera*, senescence.

Introduction

Mango is the fifth ranking fruit in the world in terms of production (Chadha 1989; Sauco 1993; Loeillet 1994). Demand for fresh mango fruit is increasing in both developing and developed countries; especially those in Europe (Loeillet 1994). There was about 120% increase in demand for mango fruit during 1985–90 (Sauco 1993). However, physiological disorders and postharvest diseases have become a major problem for the expanding Australian mango industry (Tochill *et al.* 1988).

Anthracnose caused by the fungus *Colletotrichum gloeosporioides* is one of the most important postharvest diseases of mango (Fitzell and Peak 1984). Anthracnose is usually controlled by a combination of pre- and postharvest fungicide treatments (Tuzun and Kloepper 1995). However, there is increasing public concern about health risks and environmental hazards associated with the use of pesticides (Wilson *et al.* 1994). This concern constitutes a crucial reason for seeking alternatives to conventional fungicides (Adikaram 1990). The likelihood of pathogens developing resistance to specific chemicals is another good reason for seeking alternative control measures (Agrios 1997). Accordingly, effective, safe and economic options for protecting plants are required (Tuzun and Kloepper 1995). Salicylic acid ($C_7H_6O_3$), the active ingredient of aspirin, and phosphonate [$HPO(O^-)_2$ anion], which is even less toxic to mammals, are potentially useful alternatives (Guest *et al.* 1995; Agrios 1997).

Induction of host resistance is one strategy that holds promise for control of postharvest diseases (Adikaram 1990). Host resistance can be artificially induced using chemical, physical and/or biological elicitors (Wilson *et al.* 1994). Salicylic acid has a role in the endogenous signal transduction pathway leading to expression of resistance factors (Gaffney *et al.* 1993). In tomato fruit, an increase in endogenous salicylic acid concentration is related to defence responses (Hammond-Kosack *et al.* 1992, cited by Van Kan *et al.* 1995). Exogenous salicylic acid treatment may enhance host defence responses (Gaffney *et al.* 1993; Van Kan *et al.* 1995) in fruit and vegetables, such as orange fruit and potato tubers (Gaur and Chenulu 1982), banana fruit (Ram and Vir 1986), kiwifruit fruit (Poole and McLeod 1994), and cucumber leaves (Marry *et al.* 1995; Rasmussen *et al.* 1995); but has not been tested on mango fruit.

10.1071/EA99104 0816-1089/01/060805

Salicylic acid treatment has also been shown to inhibit ethylene biosynthesis in tomato (Mattoo *et al.* 1993) and apple fruit disc tissues (Romani *et al.* 1989). An ethylene-suppression role may extend the shelf life (green life) of fruit, and thereby delay development of disease symptoms that typically develop as fruit ripen.

Phosphonate is another chemical inductant, and is effective against oomycete (Coffey and Ouimette 1989) and non-oomycete pathogens (Heaton and Dullahide 1990). Phosphonate can act directly as an antifungal compound by inhibiting germination and sporulation processes of susceptible fungi (Coffey and Joseph 1985). However, phosphonate can also induce host resistance (Guest and Grant 1991; Griffith *et al.* 1992).

Fungicide and chemical elicitor treatments are usually applied by dipping, spraying, vacuum infiltration or trunk injection. Vacuum infiltration may give a better result, since vacuum infiltration treatment enhances uptake by fruit following extraction of gas from the tissue (Rajapakse *et al.* 1992; Yuen 1993).

The aim of this study was to investigate the potential for suppression of anthracnose disease in mango fruit using either salicylic acid or phosphonate. In view of the relatively low ratio of surface area to volume of fruit compared with leaves (Wills *et al.* 1998) and of earlier research with banana fruit (Ram and Vir 1986), relatively high concentrations of elicitor compounds were used as compared with studies using leafy material (e.g. Chen *et al.* 1993; see Discussion). The vacuum infiltration application method was tested with a view to obtaining maximum effect.

Materials and methods

Experiment 1. Pre- and postharvest dip treatment with either salicylic acid or potassium phosphonate (*0–1000 mg a.i./L*)

Mango (*Mangifera indica* cv. 'Kensington Pride') fruit from a commercial orchard near Gatton (27°34′S, 152°17′E), Queensland, were used in this experiment conducted between October 1996 and January 1997. Sample fruit on trees were marked using tags colour-coded according to the treatment. The chemical treatments were 0, 10, 100 or 1000 mg a.i./L of either salicylic acid (AR grade, Aldrich Chemical Co.) or potassium phosphonate [Fos-Ject; phosphorous (phosphonic acid) present as mono-di potassium phosphonate; 200 g a.i./L; Agrochemicals (Australia) Pty Ltd].

Chemical treatments were applied to mango fruit as a series of 3 preharvest dips and 1 postharvest dip. The chemical solutions were prepared by dissolving the required amount of either salicylic acid or potassium phosphonate in distilled water. To obtain good spreading, a wetting agent (50 μL Agral/L) was included in all treatment solutions. Preharvest application was conducted by dipping individual fruit on the tree for 30 s every second week over a 2-month period up to harvest. Fruit were harvested at the mature green stage (dry matter 16.3 ± 0.3%, n = 10) on 31 January 1997, surface-sterilised (70% v/v ethanol) and air-dried. The pedicel was then recut close to the fruit, leaving about 0.5 cm of stalk. The postharvest dip treatment (10 min) was then applied, and the fruit were left for 24 h at 23°C and 80% relative humidity (RH).

Fruit were then inoculated with *C. gloeosporioides* isolated from mango fruit [accession no. 23369 (Queensland Department of Primary Industries) from Dr Lindy Coates (plant pathologist)]. *Colletotrichum gloeosporioides* was cultured on 1/2 strength potato dextrose agar (PDA) medium and incubated under 'black light' (near ultraviolet) for 7 days. Each fruit was marked using a permanent marker pen on the skin with a 15-mm-diameter circle. Inoculation was effected by placing 25 μL of *C. gloeosporioides* spore suspension (5×10^6 spores/mL) as a drop in the centre of the marked fruit surface. Inoculated fruit were incubated on plastic trays at 25°C and 90% RH for 24 h. Thereafter, the mango fruit were transferred into mango boxes and allowed to ripen at 23°C and 80% RH for about 2 weeks.

Fruit were assessed daily during shelf-life evaluation for skin colour and firmness changes. Subjective visual skin colour ratings were: 1, 100% green; 2, 25% yellow; 3, 50% yellow; 4, 75% yellow; and 5, 100% yellow (Macnish *et al.* 1997). Hand-firmness ratings were: 1, hard; 2, firm; 3, slightly soft; 4, soft; and 5, very soft (Joyce and Shorter 1995). Anthracnose disease severity was also recorded daily as lesion diameter (mm).

Treatments in the field were arranged in a randomised complete block design (RCBD). Replication was 20-fold. Individual replicate fruit were from different trees (i.e. n = 20). During shelf life evaluation in the laboratory, the fruit were also arranged in a RCBD. Fruit of each replicate were kept in different boxes (blocks). For analysis of variance (ANOVA) as reported in the text, data were log-transformed with split-plot for time and processed using the Minitab for Windows Version 11.12 statistical package (Anon. 1996). Means and standard errors as presented in figures are for untransformed data [Mr A. Lisle (biometrician), pers. comm.].

Experiment 2. Postharvest vacuum infiltration treatment with either salicylic acid or potassium phosphonate (*0–1000 mg a.i./L*)

Mature green (dry matter 18.1 ± 0.3%, n = 10) mango cv. 'Kensington Pride' fruit were obtained from an orchard near Childers (25°14′S, 152°17′E), Queensland. They were transported by aeroplane to Brisbane on the same day as harvest (3 December 1996). The fruit were then taken to the postharvest laboratory at Gatton College (4 December 1996), where they were handled and prepared by the procedures described in experiment 1.

Salicylic acid or potassium phosphonate solutions were prepared as described above to give 0, 10, 100 or 1000 mg a.i./L. The solutions were placed into a 15-L-capacity glass vacuum desiccator connected to a vacuum pump (single stage high vacuum; Jigtool High Vacuum Pty Ltd, model SAB 50 H 5429) and gauge (Dobbie; –100 to 0 kPa range) system. Fruit to be treated were placed into the desiccator and kept submerged by a heavy plastic tray on top of them. The vacuum was drawn at –33 kPa for 5 min (Joyce and Shorter 1995). It was then released, and the fruit were allowed to absorb solution for a further 5 min. The fruit were then removed and immediately rinsed in deionised water to remove excess residual chemical. Finally, the fruit were air-dried over 24 h before inoculation.

Inoculation with pathogens, assessment and experimental design and analysis were as described in experiment 1.

Experiment 3. Postharvest dip in or vacuum infiltration with salicylic acid (*0–2000 mg/L*)

'Kensington Pride' mango fruit were harvested at commercial maturity (dry matter 14.9 ± 0.3%, n = 8) in January 1998 from the orchard used in the first experiment. For dip and for vacuum infiltration treatments as described above, fruit for each of 3 chemical (0, 1000 or 2000 mg a.i./L salicylic acid) treatments were allocated into 2 sets of 30 (10 fruit per chemical treatment). One set was left uninoculated and the other set was inoculated with *C. gloeosporioides*. Inoculation and incubation protocols are described in experiment 1. Thereafter, fruit were transferred into mango boxes and kept at 23°C and 80% RH for about 2 weeks. Fruit ripening characteristic assessments were conducted following procedures described in experiment 1.

Anthracnose disease severity determinations for *C. gloeosporioides*-inoculated fruit were likewise assessed following procedures described in experiment 1. For uninoculated fruit, however, disease severity was measured as mm^2 summed lesion area for all lesions on each individual fruit. Data for fruit that developed stem end rot lesions in the course of ripening were not included in the final analysis (i.e. were disregarded). As a result, there were 6 fruit missing, leaving 3 replicates for fruit vacuum-infiltrated with 2000 mg a.i./L and left uninoculated, 4 replicates for fruit dipped in 2000 mg a.i./L and left uninoculated, 4 replicates for fruit vacuum infiltrated with 1000 mg a.i./L and inoculated, and 3 replicates for fruit dipped in 0 mg a.i./L and inoculated with *C. gloeosporioides*. Since data were missing, analysis of variance was performed using the Genstat-5 statistical program (Payne 1989).

Results

Experiment 1. Pre- and postharvest dip treatment with either salicylic acid or potassium phosphonate (0–1000 mg a.i./L)

Fruit disease. There were no significant (P>0.05) effects, as determined by ANOVA (data not shown), of either salicylic acid or phosphonate treatments on anthracnose disease severity on cv. 'Kensington Pride' mango fruit (Fig. 1*a*, *b*). Nonetheless, trends in the data set suggest that the highest potassium phosphonate concentrations of 100 and 1000 mg/L reduced disease severity compared with the control (0 mg/L) and 10 mg/L dip treatments (Fig. 1*a*). The situation was confused for salicylic acid, where

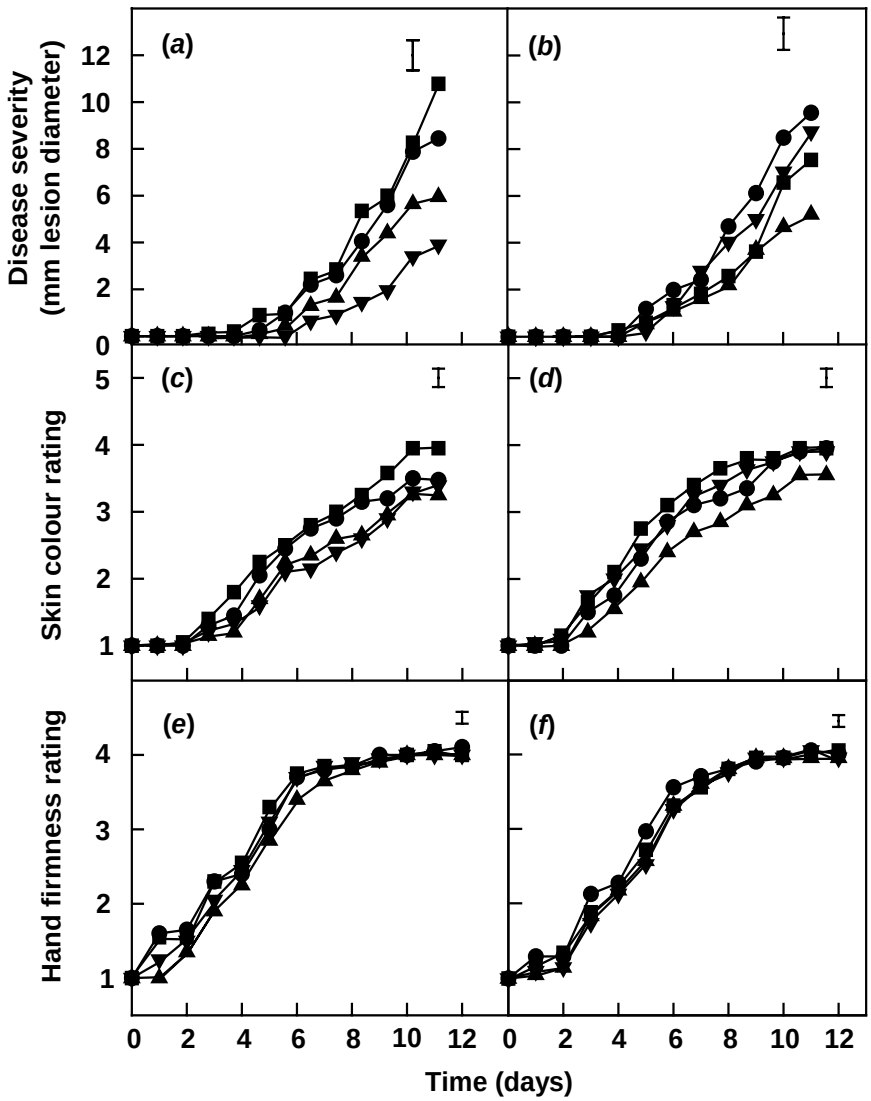


Figure 1. Effect on disease severity (*a*, *b*), skin colour (*c*, *d*) and hand-firmness (*e*, *f*) ratings of dipping mango cv. 'Kensington Pride' fruit inoculated (after chemical treatment) with *Colletotrichum gloeosporioides* in 0 (●), 10 (■), 100 (▲) or 1000 (▼) mg/L potassium phosphonate (*a*, *c*, *e*) or salicylic acid (*b*, *d*, *f*). Vertical bars show the averaged standard error (n = 1920).

100 mg/L gave the lowest disease severity level and 1000 mg/L was similar to the control (0 mg/L) and 10 mg/L dip treatments (Fig. 1*b*). Anthracnose lesions appeared on day 5 for the control and phosphonate- and salicylic acid-treated fruit (Fig. 1*a*, *b*).

Fruit skin colour and firmness. Salicylic acid and phosphonate treatments did not have significant (P>0.05, ANOVA) effects on skin colour or firmness changes in fruit inoculated with *C. gloeosporioides*. Trends in the data sets for both potassium phosphonate (100 and 1000 mg/L) and salicylic acid (100 mg/L) suggest that less colouration of the skin was correlated with reduced disease severity (compare Fig. 1*c*, *d* with *a*, *b*). Fruit skin colour (Fig. 1*c*, *d*) and firmness (Fig. 1*e*, *f*) changes generally began 3 and 1 days after inoculation, respectively.

Experiment 2. Postharvest vacuum infiltration treatment with either salicylic acid or potassium phosphonate (0 to 1000 mg a.i./L)

Fruit disease. There were no significant (P>0.05, ANOVA) reductions in anthracnose disease severity on cv. 'Kensington Pride' mango fruit as a result of treatment with either salicylic acid or phosphonate. Trends in the data suggested that higher concentrations (100 or 1000 mg/L) of

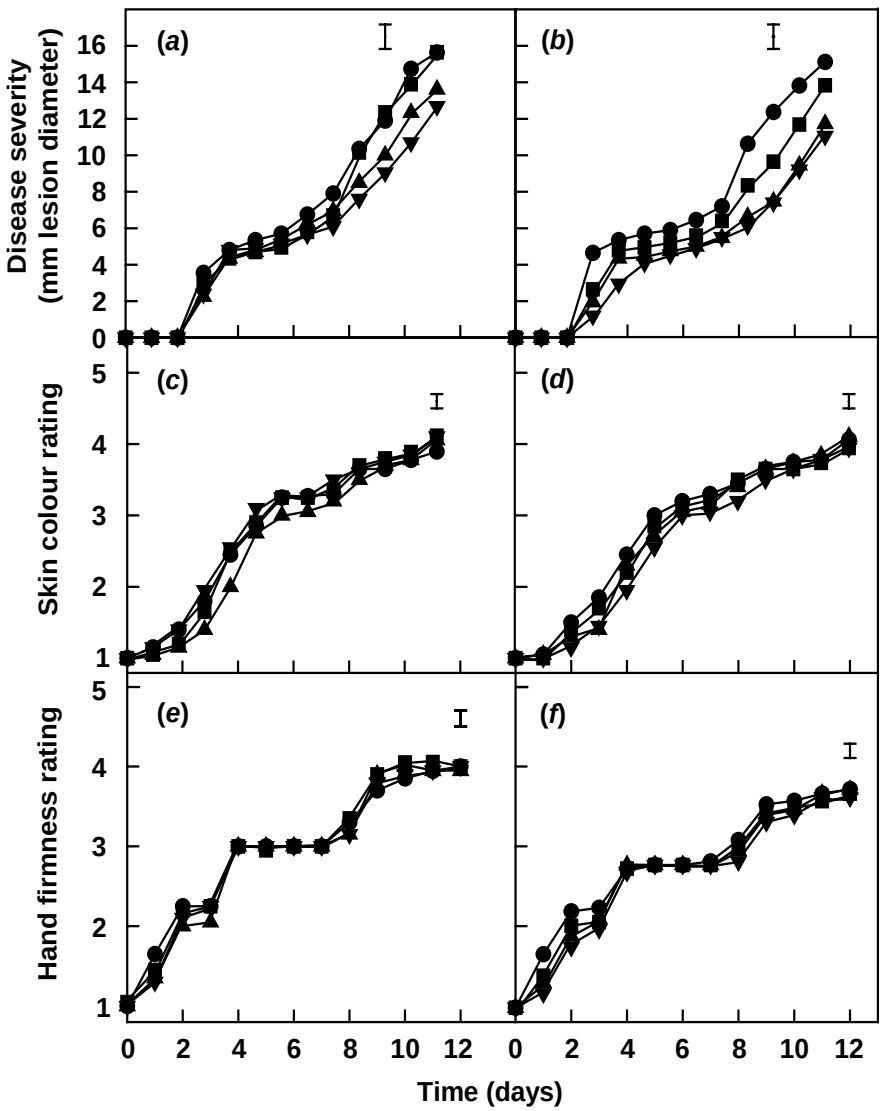


Figure 2. Effect on disease severity (*a*, *b*), skin colour (*c*, *d*) or hand-firmness (*e*, *f*) ratings of vacuum infiltration of mango cv. 'Kensington Pride' fruit inoculated (after chemical treatment) with *Colletotrichum gloeosporioides* with 0 (●), 10 (■), 100 (▲) or 1000 (▼) mg/L potassium phosphonate (*a*, *c*, *e*) or salicylic acid (*b*, *d*, *f*). Vertical bars show averaged standard errors (n = 1920).

both salicylic acid and potassium phosphonate reduced disease severity (Fig. 2*a*, *b*). Anthracnose lesions appeared on day 3 for the control and phosphonate- and salicylic acid-treated fruit (Fig. 2*a*, *b*).

Fruit skin colour and firmness. Vacuum infiltration with either salicylic acid or phosphonate did not show significant ($P>0.05$, ANOVA) effects on either colour or firmness changes in fruit inoculated with *C. gloeosporioides*. Skin colour changes were detected 2 days after inoculation, while firmness had already started to change 1 day after inoculation (Fig. 2*c–f*).

Experiment 3. *Postharvest dip in or vacuum infiltration with salicylic acid* (*0–2000 mg/L*)

Fruit disease. There were significant ($P<0.05$, ANOVA) differences in disease severity among treatments for artificially inoculated cv. 'Kensington Pride' mango fruit. Fruit treated with salicylic acid at either 1000 or 2000 mg/L had lower disease severity than the control fruit (Fig. 3). For the vacuum infiltration treatment, lesion development started on day 6 for inoculated fruit, but was delayed by further 4 days for uninoculated fruit. With vacuum treatment, both inoculated and uninoculated fruit had similar responses. With dipping, both 1000 and 2000 mg salicylic acid/L reduced disease severity, except for the 1000 mg a.i./L treatment of uninoculated fruit (Fig. 3*d*). Dipping in or vacuum infiltration with 2000 mg salicylic acid/L strongly suppressed the development of disease symptoms on artificially inoculated fruit (Fig. 3*a*, *b*). For naturally infected fruit, vacuum infiltration with 1000 mg/L salicylic acid was sufficient to suppress disease lesion development (Fig. 3*c*).

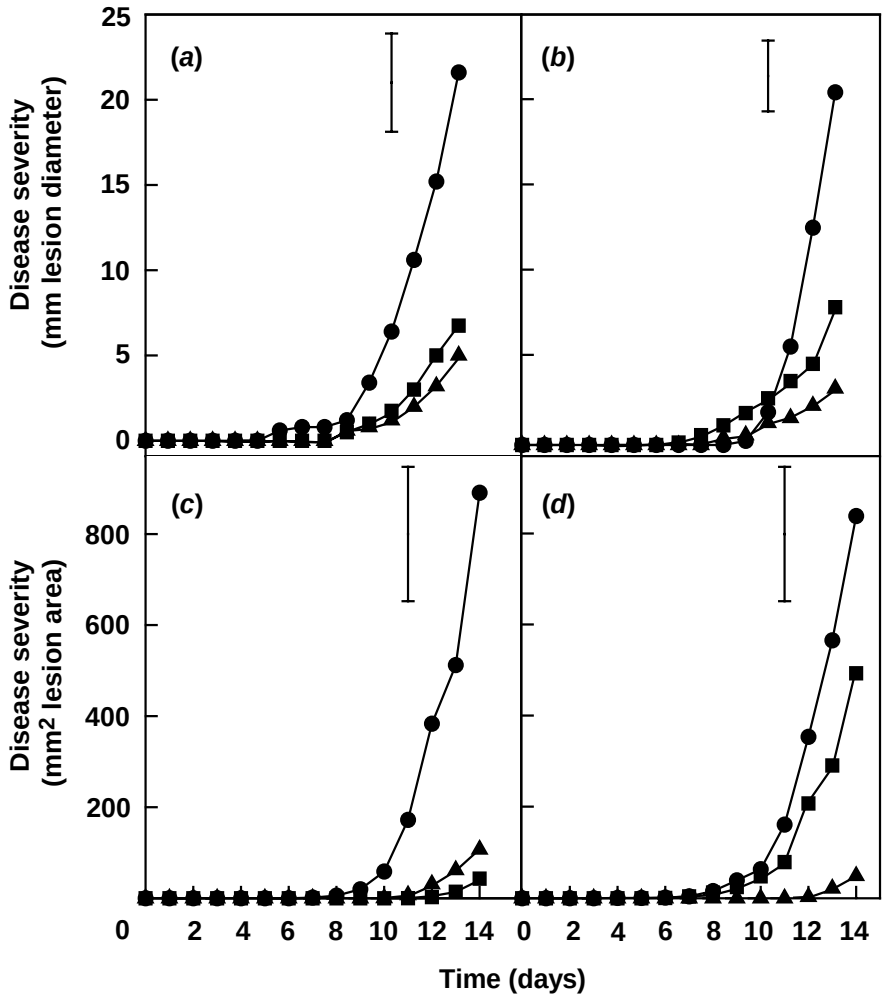


Figure 3. Effect on disease severity of vacuum infiltration (*a*, *c*) or dipping (*b*, *d*) of *Colletotrichum gloeosporioides*-inoculated (after chemical treatment) (*a*, *b*) or uninoculated (*c*, *d*) mango cv. 'Kensington Pride' fruit in 0 (●), 1000 (■) or 2000 (▲) mg salicylic acid/L. Vertical bars show l.s.d. ($P = 0.05$) for comparison of different chemical concentrations.

Fruit colour and firmness. Treatment of fruit with high concentrations (1000 or 2000 mg/L) of salicylic acid by vacuum infiltration or as a dip resulted in a significant (P<0.05) difference in skin colour ratings among the treatments. The difference became somewhat more pronounced as time progressed. Generally, fruit skin colour change commenced on day 3 and, thereafter, control fruit tended to colour earlier than those treated with salicylic acid (Fig. 4). There were clear differences in skin colour change among concentrations for inoculated fruit either vacuum- or dip-treated with salicylic acid (Fig. 4*a*, *b*). In contrast, salicylic acid treatment did not give marked differences among the concentrations for the non-inoculated fruit (Fig. 4*c*, *d*). The highest concentration (2000 mg/L) of salicylic acid resulted in the lowest skin colour rating (i.e. least yellow fruit skin). In contrast, untreated control fruit had the highest degree of yellow skin colour, indicating that they ripened earlier than the salicylic acid-treated fruit.

Similar significant treatment effects were also found for hand-firmness ratings. For fruit treated with salicylic acid either applied as vacuum infiltration or dip treatment and uninoculated or inoculated with *C. gloeosporioides*, softening generally started on day 3 with delays in the case of some salicylic acid treatments (Fig. 5). The shelf life of salicylic acid-treated mango fruit was prolonged by 2.1 and 2.2 days for the 1000 and 2000 mg salicylic acid/L treatments, respectively (Table 1).

Discussion

In the first experiments reported herein, neither potassium phosphonate nor salicylic acid treatment at concentrations of 10, 100 or 1000 mg a.i./L significantly reduced anthracnose

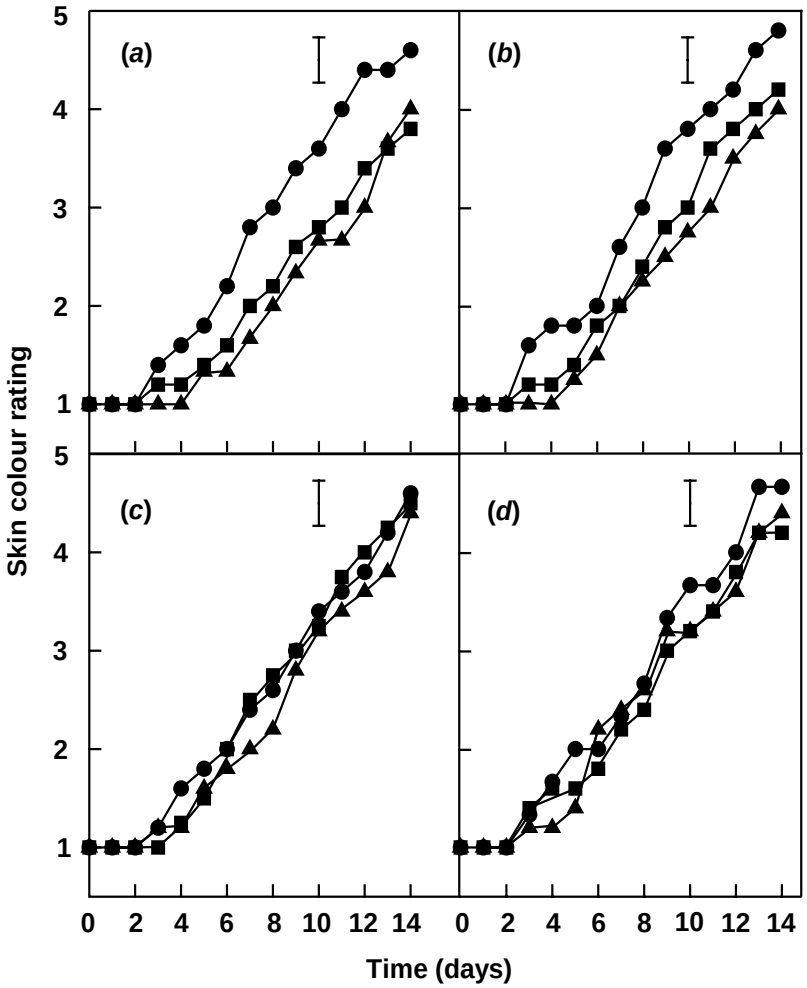


Figure 4. Effect on skin colour rating of vacuum infiltration (*a*, *c*) or dipping (*b*, *d*) of *Colletotrichum gloeosporioides*-inoculated (after chemical treatment) (*a*, *b*) or uninoculated (*c*, *d*) mango cv. 'Kensington Pride' fruit in 0 (●), 1000 (■) or 2000 (▲) mg salicylic acid/L. Vertical bars show l.s.d. (P = 0.05) for comparison of different chemical concentrations.

disease. However, a slight tendency for disease reduction with increasing concentration (100 or 1000 mg/L) was discerned (Figs 1*a* and 2*a*, *b*). Significant disease reduction was recorded in the subsequent experiment with salicylic acid applied at 1000 and 2000 mg/L (Fig. 3). Thus, salicylic acid concentrations of 100 or 1000 mg a.i./L might be regarded as marginally effective. Compared with exogenous concentrations shown to be effective in eliciting host defence responses in other systems [e.g. 0.5 mmol/L (69 mg/L) injected into tobacco leaves; Chen *et al.* 1993], the upper concentrations are relatively high. However, Ram and Vir (1986) reported that disease development in banana was inhibited by application of 2000 mg/L salicylic acid. Direct antifungal effects at such high concentrations cannot be discounted. For example, in an accompanying *in vitro* study with the fungal bioassay organism *Cladosporium* sp. (see below) on 1/2 potato dextrose agar, colony growth rates at 0, 50, 100, 500, 1000 and 2000 mg salicylic acid/L of agar were 3.3 ± 0.1, 2.7 ± 0.2, 2.5 ± 0.1, 0, 0, 0 mm/day, respectively ($n = 4$).

Salicylic acid may act, at least in part, to delay development of disease symptom through retarding fruit ripening. Exogenous application of salicylic acid has been shown to inhibit ethylene synthesis by plant tissue (Leslie and Romani 1986; Romani *et al.* 1989). Reduced ethylene synthesis was attributable to inhibition of ACC oxidase activity (Mattoo *et al.* 1993). Mango fruit are climacteric and ethylene is, thus, involved in co-ordination of ripening (Lizada 1993).

When mango fruit were dip-treated with up to 1000 mg/L salicylic acid in the first experiment (1996–97 season; Fig. 1)

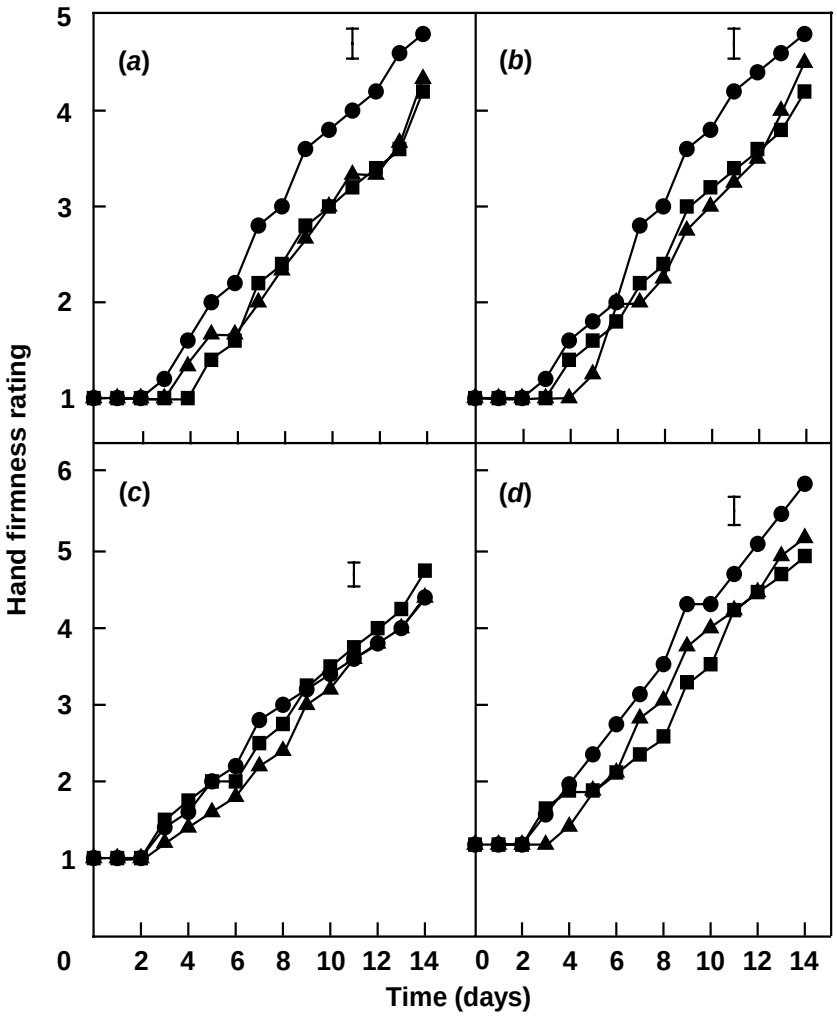


Figure 5. Effect on hand-firmness rating of vacuum infiltration (*a*, *c*) or dipping (*b*, *d*) of *Colletotrichum gloeosporioides*-inoculated (after chemical treatment) (*a*, *b*) or uninoculated (*c*, *d*) mango cv. 'Kensington Pride' fruit in 0 (●), 1000 (■) or 2000 (▲) mg salicylic acid/L. Vertical bars show l.s.d. ($P = 0.05$) for comparison of different chemical concentrations.

Table 1. Concentration main-factor mean ± s.e.m. of shelf life [time to reach eating-soft stage (hand-firmness rating 4)] of *Colletotrichum gloeosporioides*-inoculated (after chemical treatment) or uninoculated cv. 'Kensington Pride' mango fruit treated with 0, 1000 or 2000 mg salicylic acid/L applied by either vacuum infiltration or dip treatment

Salicylic acid conc. (mg/L)	*n*	Shelf life (days)
0	18	9.6 ± 0.68
1000	19	11.7 ± 0.35
2000	17	11.8 ± 0.35

there was no significant effect of delaying their ripening. Moreover, application of salicylic acid by the vacuum infiltration method, which is proposed to enhance chemical uptake into fruit (Yuen 1993), had no effect on mango fruit ripening (Fig. 2). However, when a wider salicylic acid concentration range (0, 1000 or 2000 mg/L) was tested in the second season, a clear effect of slowing fruit ripening was determined (Figs 4 and 5). Shelf life, characterised by fruit firmness change, was extended by about 2 days with salicylic acid treatment (Table 1). This observation agrees with the finding that salicylic acid application (32.5 mg/L) inhibited ethylene biosynthesis in pear and tomato fruit and in apple fruit disc tissues (Romani *et al.* 1989).

In an associated experiment with mango cv. 'Haden' fruit (14 ± 0.3% dry matter; 10 January 1998 harvest at Childers), enhanced disease resistance and delayed ripening were similarly recorded for fruit treated at 2000 mg/L salicylic acid. The positive effect of enhanced disease resistance was evident as significant inhibition of disease lesion expansion (data not shown). Shelf lives (cf. Table 1; time to eating soft; $n = 20$) for 0 and 2000 mg salicylic acid/L treatments were 8.3 ± 0.5 and 9.8 ± 0.4 days, respectively.

That development of disease was positively associated with fruit ripening, namely colouring and softening, was further evidenced by highly significant ($P<0.01$) linear correlation coefficients between colour or firmness and disease severity parameters of $r = 0.32$ and 0.39 ($n_{x,y} = 840$) or 0.35 and 0.34 ($n_{x,y} = 560$) for cvv. 'Kensington Pride' and 'Haden', respectively.

Salicylic acid applied exogenously can induce or enhance plant disease resistance (Malamy and Klessig 1992; Raskin 1992; Walters *et al.* 1993; Stermer 1995). Moreover, salicylic acid also has an endogenous role in mediating natural disease resistance (Meuwly *et al.* 1994).

In a follow up experiment with 'Kensington Pride' mango (dry matter 14.2 ± 0.9%, $n = 5$; 29 January 2000 harvest at Gatton), the possibility of salicylic acid enhancement of natural disease resistance compounds in the fruit skin was investigated. A thin layer chromatography (TLC) bioassay technique (Adikaram and Ratnayake Bhandara 1998) was used for skin tissue extracts (0.2 mL extract/g fresh weight; Droby *et al.* 1986). For 3 TLC running-solvent systems of increasing polarity, viz. hexane:ethyl acetate:methanol; 60:40:10, 60:40:20 and 60:40:30 (v/v/v), similar inhibition patterns of *Cladosporium* sp. bioassay organism growth were observed for untreated control fruit and those either dipped or vacuum-infiltrated with 2000 mg/L salicylic acid (data not shown). Control treatments (50 µL) of cycloheximide (500 mg/L) and salicylic acid (2000 mg/L) both gave inhibition zones (Rf value about 0.2 in 60:40:10), whereas the final extractant, ethanol [99% (v/v)], did not. Similarly, there were no differences between treatments when *C. gloeosporioides* was used as the bioassay organism with the hexane:ethyl acetate:methanol (60:40:10) running solvent. Thus, no evidence was obtained for enhancement of endogenous antifungal activity in the skin of 'Kensington Pride' mango fruit by salicylic acid.

Conclusion

Treatment of 'Kensington Pride' mango fruit with 2000 mg/L salicylic acid suppressed anthracnose disease. The data suggest that this beneficial effect may be attributable to a delay in fruit ripening that is possibly mediated by an anti-ethylene effect. Delaying expression of disease symptoms and fruit ripening for 2 days might constitute an advantage for farmers and retailers. Future work could extend the work started with phosphonate and should examine treatment with salicylic acid at different stages of maturity and for various mango cultivars. In addition, human health implications of salicylic acid treatment and potential enhancement of endogenous disease resistance factors may warrant detailed investigation.

Acknowledgments

This work was supported in part by AusAid, the Australian Centre for International Agricultural Research, and the Horticultural Research and Development Corporation. We thank Professor Dov Prusky (plant pathologist) and Mr Alan Lisle (biometrician) for advice, and Ms Christine Crossley, Mr Tony Cooke, Ms Catherine Raymont, Mr Victor Robertson, Ms Angela van der Kruyssen, and Ms Suzy Brangwin for technical assistance.

References

Adikaram NKB (1990) Possibility of control of postharvest fungal diseases by manipulating of host defence system. In 'Proceedings of the 3rd conference on plant protection in the tropics'. Vol. V. Genting Highlands, Pahang. pp. 31–36.

Adikaram NKB, Ratnayake Bhandara BM (1998) Methodology for studying defence mechanisms against fungal pathogens — an overview. *ACIAR Proceedings* **80**, 177–185.

Agrios GN (1997). 'Plant pathology (4th edn).' (Academic Press: San Diego)

Anonymous (1996) 'Minitab reference manual, release 11 for Windows.' (Minitab Inc.: State College, Pl, USA)

Chadha KL (1989) World mango industry. *Acta Horticulturae* **231**, 3–11.

Chen Z, Silva H, Klessig DF (1993) Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science* **262**, 1883–1886.

Coffey MD, Joseph MC (1985). Effect of phosphorous acid and Fosethyl AL on the life cycle of *Phytophthora cinnamomi* and *P. citricola*. *Phytopathology* **75**, 1042–1046.

Coffey MD, Ouimette DG (1989) Phosphonate: antifungal compounds against oomycetes. In 'Nitrogen, phosphorus, and sulphur utilization by fungi'. (Eds L Boddy, R Marchant, DJ Read) pp. 108–129. (Cambridge University Press: Cambridge)

Droby S, Prusky D, Jacoby B, Goldman A (1986) Presence of antifungal compounds in the peel of mango fruits and their relation to latent infections of *Alternaria alternata*. *Physiological and Molecular Plant Pathology* **29**, 173–183.

Fitzell RD, Peak CM (1984) The epidemiology of anthracnose disease of mango: inoculum sources, spore production and dispersal. *Annals of Applied Biology* **104**, 53–59.

Gaffney T, Friedrich L, Vernooji B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H, Ryal J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **261**, 754–756.

Gaur A, Chenulu W (1982) Chemical control of postharvest diseases of *Citrus reticulata* and *Solanum tuberosum*. *Indian Phytopathology* **35**, 628–632.

Griffith JM, Davis AJ, Grant BR (1992) Target sites of fungicides to control oomycetes. In 'Target site of fungicide action'. (Ed. CU Koller) pp. 69–100. (Chemical and Rubber Co. Press: Boca Raton, FL, USA)

Guest DI, Grant BR (1991) The complex action of phosphonates as antifungal agents. *Biological Reviews* **66**, 159–187.

Guest DI, Pegg KG, Whiley, AW (1995) Control of *Phytophthora* diseases of tree crops using trunk-injected phosphonates. *Horticultural Reviews* **17**, 299–330.

Hammond-Kosack KE, Oliver R, Harrison K, Ashfield T, Jones J (1992) Studies on the mechanism by which tomato *cf* (*Cladosporium fulvum*) resistance genes activate plant defense. In 'Advances in molecular genetics of plant–microbe interactions'. Vol. 2. (Eds EW Nester, DPS Verma) pp. 457–461. (Kluwer Academic Publishers: London)

Heaton JB, Dullahide SR (1990) Efficacy of phosphonic acid in other host plant pathogen systems. *Australasian Plant Pathology* **19**, 133–134.

Joyce DC, Shorter, AJ (1995) Partial pressure calcium infiltration of mangoes did not delay ripening. In 'Proceedings of the Australasian postharvest conference'. 18–22 September 1995, Melbourne, Australia. pp. 261–265.

Leslie CA, Romani RJ (1986) Salicylic acid: a new inhibitor of ethylene biosynthesis. *Plant Cell Reports* **5**, 144–146.

Lizada C (1993) Mango. In 'Biochemistry of fruit ripening'. (Eds GB Seymour, JE Taylor, GA Tucker) pp. 255–271. (Chapman and Hall: London)

Loeillet D (1994) The European mango market: a promising tropical fruit. In 'Symposium on tropical orchards'. Montpellier, France, 30 Aug.–5 Sept. 1993, pp. 434–435.

Macnish AJ, Joyce DC, Hetherington SE (1997) Packaging to reduce water loss can delay ripening of mango (*Mangifera indica* L. cv. 'Kensington Pride') fruit. *Australian Journal of Experimental Agriculture* **37**, 463–467.

Malamy J, Klessig DE (1992) Salicylic acid and plant disease resistance. *Plant Journal* **2**, 643–654.

Marry AJ, Taylor PN, Friend J (1995) Systemic acquired resistance in cucumber leaves is correlated with resistance of the leaves to maceration by cell wall degrading enzymes. *Aspects of Applied Biology* **42**, 349–353.

Mattoo AK, Li N, Liv D, Pech JC, Latche A, Balague C (1993) Tomato ACC synthase regulation of gene expression and importance of the c-terminal region in enzyme activity. In 'Cellular and molecular aspects of the plant hormone ethylene. Proceedings of the international symposium on cellular and molecular aspects of biosynthesis and action of the plant hormone ethylene'. France, 31 Aug.–4 Sept. 1992. (Eds JC Pech, A Latche) pp. 223–231. (Kluwer Academic Publishers: London)

Meuwly P, Molders W, Summersmatter K, Sticher L, Metraux JP (1994) Salicylic acid and chitinase in infected cucumber plants. *Acta Horticulturae* **381**, 371–374.

Payne RW (1989) 'Genstat 5 reference manual.' (Oxford University Press: Oxford)

Poole PR, McLeod LC (1994) Development of resistance to picking wound entry by *Botrytis cinerea* storage rots in kiwifruit. *New Zealand Journal of Crop and Horticultural Science* **22**, 387–392

Ram V, Vir D (1986) Efficacy of fungicides XXXVI. Relative evaluation of various postharvest chemical treatments against spoilage of banana fruits caused by *Culvularia lunata*. *Indian Phytopathology* **39**, 594–595.

Rajapakse NC, Hewett EW, Banks NH, Cleland DJ (1992) Vacuum infiltration with calcium chloride influences oxygen distribution in apple fruit flesh. *Postharvest Biology and Technology* **1**, 221–229.

Raskin I (1992) Role of salicylic acid in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **43**,439–463.

Rasmussen JB, Smith JA, Williams S, Burkhart W, Ward E, Somerville SC, Ryals J, Hammerschmidt R (1995) cDNA cloning and systemic expression of acidic peroxidase associated with systemic acquired resistance to disease in cucumber. *Physiological and Molecular Plant Pathology* **46**, 389–400.

Romani RJ, Hess BM, Leslie CA (1989) Salicylic acid inhibition of ethylene production by apple discs and other plant tissues. *Plant Growth Regulation* **8**, 63–69.

Sauco VG (1993) The situation of mango culture in the world. *Acta Horticulturae* **341**, 31–41.

Stermer BA (1995) Molecular regulation of systemic induced resistance. In 'Induced resistance to disease in plants'. (Eds R Hammerschmidt, J Kuc) pp. 111–140. (Kluwer Academic Publishers: London)

Tochill BL, Wright RM, Baker I (1988) Australian mango industry. *Acta Horticulturae* **231**, 17–38.

Tuzun S, Kloepper J (1995) Practical application and implementation of induced resistance. In 'Induced resistance to disease in plants'. (Eds R Hammerschmidt, J Kuc) pp. 152–168. (Kluwer Academic Publisher: The Netherlands)

Van Kan JAL, Cozijnsen T, Danhash N, De Wit PJGM (1995) Induction of tomato stress protein mRNAs by ethephon, 2,6-dichloroisonicotinic acid and salicylate. *Plant Molecular Biology* **27**, 1205–1213.

Walters DR, Mitchell AF, Hampson J, McPherson A (1993) The induction of systemic resistance in barley to powdery mildew infection using salicylates and various phenolic acids. *Annals of Applied Biology* **122**, 451–456.

Wills R, McGlasson B, Graham D, Joyce, D (1998) 'Postharvest — an introduction to the physiology and handling of fruit, vegetables and ornamentals.' (University of NSW Press: Sydney)

Wilson CL, El-Ghaouth A, Chalutz E, Droby S, Steven C, Lu JY, Khan V, Arul, J (1994) Potential of induced resistance to control postharvest diseases of fruit and vegetables. *Plant Diseases* **78**, 837–844.

Yuen CMC (1993) Calcium and fruit storage potential. In 'International conference for postharvest handling of tropical fruit'. (Eds BR Champ, E Highley, GI Johnson) *ACIAR Proceedings* **50**, 218–227.

Received 8 July 1999, accepted 25 April 2001