Disease Control and Storage Life Extension in Fruit

Proceedings of an International Workshop held at Chiang Mai, Thailand, 22–23 May 1997

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Cover illustration: Certain yeasts have shown potential as biological control agents for fruit rot caused by a fungus belonging to the genus *Greeneria*. The top two images show the normal appearance of *Greeneria*; the bottom two images show abnormalities in the germ tubes and mycelia of *Greeneria* following treatment with yeast isolates. For more details see paper by N. Farungsang. U. Farungsang, and S. Sangchote at page 113 in these proceedings.

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Overview of Project 9313

Tropical fruit are highly perishable commodities. Treatments which extend storage life by reducing disease and delaying ripening are essential if tropical fruit are to be shipped to distant markets. At the same time, an increasing number of international markets are demanding produce which has been treated with reduced amounts of pesticides, particularly those which are applied after harvest.

The objectives of ACIAR Project 9313 are:

- To develop non-chemical control measures for postharvest diseases of tropical fruit, and assess their impact on fruit quality, and
- To investigate the effects of key preharvest variables and postharvest treatments on fruit quality.

Papers presented during this workshop detail progress towards achieving those objectives.

The fruit crops studied in this project have reflected research priorities in each of the partner countries. In Australia the focus of research has primarily been on mango with some work on avocado, while in Thailand a wide range of tropical fruit such as mango, rambutan, mangosteen, longan and lychee have been studied.

Project 9313 has built on the results of previous Australia–Thailand ACIAR projects (8844 and 8356) and on the collaborative relationships developed in those projects.

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Production Factors Influence Fruit Quality and Response to Postharvest Treatments

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Abstract

Fruit quality attributes are largely determined before harvest, while postharvest treatments, at best, maintain quality during product storage and distribution. Therefore, the relationship between production conditions and postharvest quality needs to be well understood if maximum quality, and consistency in quality at the consumer, is to be achieved. Many production factors can affect quality at harvest and after ripening and storage, but these influences are poorly understood in subtropical and tropical fruit. Effects of production location, irrigation, soil type, rootstock, shading, nutrition etc. on visual appearance (size, colour, blemishes), eating quality (flavour, texture) storage potential (ripening, physiological disorders, disease) and tolerance to physical disinfection and disinfestation control measures (heat, cold) in fruit are discussed. It is important to use a holistic view of production and postharvest for successful horticulture management.

FRUIT quality at consumer level can be influenced by many factors. Postharvest practices can have a marked effect on quality, but most (if not all) quality parameters are also influenced by growing conditions. The primary preharvest factors are climate, nutrition and plant growth regulators (PGRs). Secondary factors such as soil quality and management, rootstock, irrigation, pruning and crop load manipulation operate at least in part through the primary factors (Monselise and Goren 1987).

A number of reviews have been written on preharvest/postharvest interactions in temperate fruit and vegetables (Winsor 1979; Ferguson 1980; Shear 1980; Sharples 1984; Beverly et al. 1993), but there is less information on these interactions in subtropical and tropical fruit. This review summarises current knowledge on the interaction between production factors, and quality and response to postharvest treatments. Special reference is given to subtropical and tropical fruit.

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The preharvest/postharvest interactions are detailed in Table 1, and summarised in the text. The reader is referred to the table for the relevant references.

Production Location and Field Temperature

There are many reports of production location affecting fruit quality. These effects are a result of the interaction of many of the factors detailed below, some of which can be manipulated easily by production practices.

Factors causing rapid or variable fruit growth can influence blemishes such as splitting and russetting. Disorders can also be affected, especially through temperatures during growth. Growing conditions that optimise fruit growth can either shorten the ripening time (persimmon) or enhance green life (banana). Temperature can also affect fruit shape, size and eating quality.

Production location can interact with the effectiveness of other postharvest treatments such as fruit waxing and heat disinfestation treatments, causing variable responses to these treatments.

Table 1. Effect of production practices on the postharvest quality of fruit. The fruit responses mentioned in the "Effect" column are those noted with increased levels of the production factor, where appropriate. CI = chilling injury, SS = soluble solids, TA = titratable acidity, TS = total solids, TSS = total soluble solids, Ca = calcium, Mg = magnesium, K = potassium, Zn = zinc, N = nitrogen, P = phosphorus, B = boron, GAs = gibberellins, GA+BA = gibberellin A_{4+7} plus benzyladenine, °Brix = concentration of sugar in solution according to the Brix scale.

Production factor	Fruit	Effect	Reference
Production location			
	Apple	Colour was affected by production location	Wagenmakers and Callesen 1995
	Banana	Conditions causing rapid fruit growth increased maturity bronzing	Campbell and Williams 1976
	Banana	Affected pulp colour and texture	Hughes and Wainwright 1994
	Avocado	Affected oil content	Lozano et al. 1989
	Mango	Affected fruit response to hot water disinfestation	Jacobi and Wong 1992
	Avocado	Affected fruit response to Ca infiltration	Wills and Sirivatanapa 1988
	Avocado	Affected days to ripen after storage	Rowell 1988
Field temperature	Tomato	Increased scarring (catface)	Wein and Turner 1994
	Mango	High temperatures increased spongy tissue	Katrodia and Sheth 1988
	Avocado	The effect of harvest date on CI was not due to fruit maturity, but preharvest temperature	Eksteen and Bester 1987
	Banana	Temperature during fruit growth affected green life	Marriott et al. 1979
	Banana	Summer and winter-harvested fruit responded differently to fruit wax treatment	Blake 1966
	Persimmon	Optimum temperatures during growth improved skin colour, and decreased shelf life and firmness due to rapid fruit growth	Sugiura et al. 1991
	Avocado	Cooler growing temperatures caused rounder fruit	Vuthapanich et al. 1998a
Cultivar			
	Citrus	Rootstock affected fruit size, and sugars and acid content	Fallahi and Mousavi 1991
	Avocado	Rootstock affected Ca and Mg uptake	Kremer-Köhne and Köhne 1992
	Avocado	Scion affected fruit Ca concentration	Witney et al. 1990 a,b
	Banana	Cultivar significantly affected green life	Marriott et al. 1979
Radiation			
Canopy position	Citrus	Affected fruit wax structure and rindstaining	El-Otmani et al. 1989
	Avocado	No effect on fruit Ca	Witney et al. 1990a
	Mango	Upper canopy fruit had higher % dry matter and ripened more quickly	Hofman et al. 1995
	Apple	Upper canopy fruit had lower fruit Ca	Ferguson et al. 1995
	Pear	Fruit from the western side of the canopy had lower Ca	Sugar et al. 1992
	Peach	Upper canopy fruit were more coloured, and had less acid, lower pH and higher sugars	Génard and Bruchou 1992
	Apple	Firmness increased with light, height, and spur length	Volz et al. 1995
	Peach	Upper canopy fruit were less firm	Génard and Bruchou 1992
	Kiwifruit	Fruit from the western side of the canopy were larger	Henzell et al. 1994

Table 1. (Continued) Effect of production practices on the postharvest quality of fruit. The fruit responses mentioned in the "Effect" column are those noted with increased levels of the production factor, where appropriate. CI = chilling injury, SS = soluble solids, TA = titratable acidity, TS = total solids, TSS = total soluble solids, Ca = calcium, Mg = magnesium, K = potassium, Zn = zinc, N = nitrogen, P = phosphorus, B = boron, GAs = gibberellins, GA+BA = gibberellin A_{4+7} plus benzyladenine, °Brix = concentration of sugar in solution according to the Brix scale.

Production factor	Fruit	Effect	Reference
Radiation (cont'd)			
Shade	Melon	Shade reduced CI	Combrink et al. 1995
	Kiwifruit	Shade increased fruit disease	Tombesi et al. 1993
	Grapefruit	Reduced sun exposure reduced chilling injury (possibly through epicuticular wax)	McDonald et al. 1993
	Kiwifruit	Shade reduced firmness and SS after storage, and increased weight loss during storage	Antognozzi et al. 1995
	Kiwifruit	Shade caused less elongated fruit	Biasi et al. 1995
	Kiwifruit	Decreased fruit mass	Tombesi et al. 1993
Bagging	Lychee	Improved visual appearance, and red skin colour	Tyas et al. 1998
	Apple	Reduced fruit cracking	Byers and Carbaugh 1995
	Banana	Increased maturity bronzing	Daniells et al. 1992
	Apple	Decreased fruit Ca and increased bitter pit	Witney et al. 1991
	Mango	Reduced red skin colour and fruit Ca, but had no effect on SS and shelf life	Hofman et al. 1997
	Banana	Increased green life, but the effects were variable	Johns and Scott 1989
Branch location			
	Apple	Central fruit on the cluster had higher sugars content	Marguery and Sangwan 1993
	Apple	Terminal fruit had higher Ca and Mg	Volz et al. 1994
	Cucumber	Fruit shape was pre-determined by axil position	Marcelis 1994
	Kiwifruit	Proportion of misshapen fruit was maximal at nodes 1 and 3	Watson and Gould 1993
Flowering			
	Apple	Late set fruit had lower fruit Ca concentration	Volz et al. 1996
	Apple	Better pollination increased fruit Ca	Volz et al. 1996
	Avocado	Late set fruit had increased disorders	Vorster et al. 1990
	Avocado	Early set fruit stored better than late set fruit	Vorster et al. 1989
	Rambutan	Early set fruit were less red, had less disease, and lower TSS and titratable acidity	Ketsa et al. 1995
Crop load			
	Apple	Flower thinning decreased fruit Ca	Deckers and Missotten 1993
	Avocado	High tree vigour reduced fruit and leaf Ca concentration	Witney et al. 1990a,b
	Avocado	CI incidence was high in a season when vegetative growth was vigorous	Donkin et al. 1994
	Apple	High tree vigour increased fruit disease	Link 1993
	Banana	Lower leaf:fruit ratio reduced maturity bronzing	Daniells et al. 1994
	Tomato	Fruit thinning increased cuticle cracking	Ehret et al. 1993
	Avocado	Fruit from less vigorous trees were more tolerant to low storage temperatures	Donkin et al. 1994

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Production factor	Fruit	Effect	Reference
Crop load (cont'd)			
	Apple	Bitter pit decreased in fruit from higher yielding trees	Baugher et al. 1996
	Avocado	Fruit from high yielding trees had reduced internal quality	Cutting and Vorster 1991
	Peach	Fruit from shoots with high leaf:fruit ratio were sweeter	Génard and Bruchou 1992
	Avocado	Fruit from high yielding trees ripened faster	Cutting and Vorster 1991
	Banana	Plants with less than six leaves produced fruit with shorter green life	Robinson et al. 1992
	Avocado	Removal of new flush during early fruit growth increased fruit (Ca+Mg)/K, and increased fruit size	Cutting and Bower 1992
Nutrition			
	Avocado	Strong correlation between fruit Ca and shelf life	Witney et al. 1990a
	Mango	B sprays during fruit growth did not increase fruit Ca	Krishnamurphy 1982
	Mango	Ca sprays increased fruit Ca and reduced spongy tissue	Gungate et al. 1979
	Papaya	Ca sprays did not increase fruit Ca	Qiu et al. 1995
	Apple	Mg sprays decreased fruit Ca and increased bitter pit	Witney et al. 1991
	Melon	High soil Ca and B decreased CI	Combrink et al. 1995
	Pear	Ca sprays decreased fruit disease	Sugar et al. 1992
	Mango	Ca sprays reduced weight loss during ripening	Singh et al. 1993
	Avocado	Zn and Ca concentrations were higher in fruit without pulp spot	Vorster and Bezuindenhout 1988
	Banana	High soil K increased TSS and ascorbic acid, and reduced acidity	Mustaffa 1988
	Pineapple	High K applications increased fruit acidity and sugars	Vis 1989
	Apple	Fruit N concentration was negatively correlated with SS	Fallahi et al. 1985
	Citrus	N and P applications improved flavour, and K increased fruit acidity	Baldry et al. 1982
	Papaya	Fruit firmness was correlated with fruit Ca concentration	Qiu et al. 1995
	Banana	High N applications reduced green life	Srikul and Turner 1995
	Mango	Ca applications increased shelf life	Singh et al. 1993
	Avocado	High N applications increased fruit weight loss and disease during ripening, and decreased shelf life	Abou-Aziz et al. 1975
Water (irrigation)			
	Mango	Fruit Ca concentrations increased with increased irrigation	Lapade 1977
	Mango	Irrigation reduced the percentage of green colour on skin	Diczbalis et al. 1995

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Production factor	Fruit	Effect	Reference
Water (irrigation) (cont'd)			
	Tomato	Fluctuating irrigation caused fruit splitting	Maroto et al. 1995
	Avocado	Irrigation did not affect fruit size, fruit mineral concentration, disease or disorders after storage	Vuthapanich et al. 1998b
	Avocado	More irrigation increased fruit disorders in storage	Arpaia and Eaks 1990
	Banana	Water stress at bunch emergence increased maturity bronzing	Daniells et al. 1987
	Peach	Effects on taste and size depended on timing of water stress	Li et al. 1989
	Apple	Excessive irrigation decreased fruit firmness through increased fruit size	Ebel et al. 1993
	Banana	Low irrigation reduced growth rate and green life	Srikul and Turner 1995
	Avocado	High and low irrigation reduced fruit Ca	Bower 1985
	Apple	Irrigation increased weight loss during storage	Velickovic 1994
	Avocado	Increased water loss during storage increased disorders and disease	Bower and Cutting 1987
	Avocado	Growing conditions can influence moisture loss after harvest, which can affect cold damage	Donkin and Cutting 1994
	Mango	Reduced irrigation during rapid fruit expansion reduced the days to ripe, lenticel spot, and fruit Ca concentration	Simmons et al. 1995
Plant growth regulators (PGRs)			
GAs	Mandarin	Increased or decreased fruit splitting, depending on fruit growth stage	Garcia-Luis et al. 1994
	Mango	Reduced TSS and TSS/acid	Khader 1991
	Apple	GA+BA decreased firmness at high concentrations	Unrath 1974
	Apple	GA+BA increased length:breadth ratio	Unrath 1974
	Banana	Increased fruit weight	Pradhan et al. 1988
Cultar®	Apple	Increased fruit Ca	Guzewski 1993
	Pepper	Decreased chilling injury	Lurie and Ronen 1993
	Avocado	Improved fruit quality	Kremer-Köhne et al. 1991
	Banana	Had no effect on flavour components	El-Otmani et al. 1992
	Mango	Increased TSS and total acidity, and reduced shelf life	Khader 1990
	Avocado	Reduced the length:breadth ratio, and increased fruit size	Wolstenholme et al. 1990
Cytokinin-active sprays	Grape	Decreased anthocyanins	Reynolds et al. 1992
	Grape	Decreased °Brix and increased TA	Reynolds et al. 1992
	Persimmon	Increased shelf life	Itai et al. 1995
	Grapes	Increased berry mass	Reynolds et al. 1992
Auxins	Citrus	Reduced fruit splitting	Almela et al. 1994
	Citrus	Increased movement of Ca from peel to flesh	Ying et al. 1995

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Production factor	Fruit	Effect	Reference
Plant growth regulators (PGRs) (cont'd)			
	Avocado	Interaction between auxin indoleacetic acid (IAA) and Ca uptake	Cutting and Bower 1989
	Pear	TIBA (auxin transport inhibitor) reduced fruit Ca concentrations and increased cork spot	Raese et al. 1995
Maturity			
	Avocado	Fruit size increased with maturity	Kaiser and Wolstenholme 1994
	Avocado	More mature fruit ripened more quickly	Eaks 1980
	Banana	Older bunches had a shorter green life	Montoya et al. 1984
	Avocado	CI increased with increased maturity in 5 out of 10 cultivars	Plumbley et al. 1989
	Avocado	CI was less in more mature fruit (later harvests)	Vorster et al. 1987
	Avocado	External CI decreased, but internal disorders increased with maturity	Donkin et al. 1994
	Avocado	Disorders decreased in more mature fruit	Vorster et al. 1989
	Mango	More mature fruit had less CI	Oosthuyse 1992
	Mango	Internal breakdown was higher in fruit with higher specific gravity (more mature)	Krishnamurphy 1982
	Plum	Disease increased with maturity	Taylor et al. 1993
	Avocado	More mature fruit lost less water during storage	Cutting and Wolstenholme 1992
	Mango	Skin was less green in more mature fruit	McKenzie 1994
	Avocado	Susceptibility to abrasion was affected by cultivar, maturity and stage of ripeness	Arpaia et al. 1987
	Apple	Over-maturity increases infiltration of Ca because of cuticle damage	Conway et al. 1994
	Avocado	Fruit Ca decreased with maturity	Cutting et al. 1992
	Avocado	More mature fruit respond more quickly to ethylene	Adato and Gazit 1974
Fruit size	Banana	Larger fruit had shorter green life	Srikul and Turner 1995
	Apple	Large fruit had lower fruit Ca	Ferguson et al. 1993
	Avocado	Freeze injury increased with fruit size	Fuchs et al. 1975
	Apricot	Gel breakdown increased with fruit size	de Klerk and von Mollendorff 1994
	Apple	Small fruit were firmer	Siddiqui and Bangerth 1995
Relative humidity (RH)			
	Melon	High RH around fruit reduced SS, decreased Ca translocation to fruit, and increased CI	Combrink et al. 1995
	Tomato	High RH around the plant increased fruit Ca	Adams and Holder 1992
	Tomato	High RH around the plant increased Ca and gold speckling, and decreased shelf life due to Ca toxicity	de Kreij et al. 1992
	Banana	High RH around the plant was associated with increased maturity bronzing	Campbell and Williams 1976

Cultivar

Scion genotype has an obvious effect on fruit quality, but rootstock genotype can also influence quality. This can be mediated through rootstock effects on mineral uptake and vegetative vigour.

The use of seedling rootstocks can result in differing fruit mineral concentrations and variability in fruit quality. Thus, large variability in avocado quality (ripening, maturity, disorders and disease) has been noted in fruit from different trees in the same orchard block, but on seedling rootstocks (S. Vuthapanich, unpublished results).

Radiation

Higher radiation interception by fruit and/or subtending leaves is often associated with reduced planting density, absence of artificial shade structures, more open plant canopy, upper canopy, and northern and western sides of the tree (southern hemisphere). Generally, these conditions will have similar effects on fruit quality. It is often difficult to determine whether the effects of radiation interception is through light or heat (Jackson 1980).

In general, factors that reduce radiation interception by fruit or subtending leaves result in reduced soluble solids (SS), and often higher acidity. Skin colour, especially red and often green, is also reduced. Low light can reduce fruit firmness at harvest and after storage, and increase fruit mass loss during storage, but results can be variable. This may partly be a result of light interacting with fruit development and maturity (Combrink et al. 1995). Low light may also increase disease incidence after harvest, perhaps because of reduced physical and physiological integrity of the fruit. Low light has been associated with reduced fruit size, and in some instances, less elongated fruit. Light may also affect ripening characteristics, but there is relatively little information in this area.

Disorders such as chilling injury can also be influenced by radiation during growth, possibly because of increased weight loss during storage through changes in the wax and cuticular structure (McDonald et al. 1993). The influence of fruit position in the canopy indicates similar effects of light on quality. Generally, fruit from positions receiving less light have less colour, lower SS, and higher acids, but effects on firmness can be variable. Fruit size, fruit calcium (Ca) concentration and blemishes can also be affected. In some circumstances, lower light can be beneficial under conditions of high incident radiation.

It is unclear whether the effect of light is through the fruit or the subtending leaves. Bagging of fruit has suggested a direct effect of light on fruit colour, but there is little indication of a fruit bagging effect on SS and acidity. The effect of bagging on disorders may be via Ca accumulation, since relative humidity (RH) around the fruit can affect fruit Ca accumulation. Effects on green life of banana and shelf life (days from harvest to ripe at ambient temperature) in other fruit is variable. Bagging is usually associated with less disease and improved visual appearance.

Branch Location

It is well established that central apple fruit on the spur are larger than other fruit on the same spur. Central fruit on the spur can also have higher SS and Ca concentration (apple), and fruit bearing position can also affect fruit shape (kiwifruit and cucumber). No reports concerning the effect of fruit-bearing position on quality of tropical and subtropical fruit were found, but preliminary observations suggest that mango fruit on larger panicles are often larger and more likely to remain on the tree to maturity.

Flowering and Pollination

In crops with prolonged flowering, there is a greater risk of fruit with differing maturity being harvested at the same time. Late-set avocado fruit may be larger but less mature than early-set fruit, because rapid growth of the late-set fruit occurred during the warmer part of the season. Late flowering has also been associated with lower fruit Ca concentration in apple and higher disorders during storage in avocado. Better pollination is often associated with higher fruit Ca and larger fruit, largely because of increased seed number per fruit.

Crop Load

There is generally a direct relationship between leaf:fruit ratio and fruit size. Crop load can also affect ripening patterns, possibly through an interaction with fruit maturity and mineral (especially Ca) concentrations. For example, lower leaf:fruit ratio in banana leads to a reduced green life as a result of fruit taking a longer time to attain minimum marketable size.

Sugars and SS concentrations can be increased at higher leaf:fruit ratios because of reduced competition for available carbohydrates. Firmness can be reduced, possibly through variations in fruit size and Ca. Fruit disorders and disease are generally more severe in fruit from trees with high leaf:fruit ratio or high vegetative vigour, possibly through reduced fruit Ca concentration as often found in these fruit, and their larger fruit size.

Vegetative flushing during early fruit growth has been shown to reduce avocado fruit size and Ca concentration, and increase disorders.

Nutrition

Several minerals are known to influence quality, the most notable of these being Ca. Calcium applications or high fruit Ca concentrations are often associated with increased firmness, less disease, chilling injury (CI), physiological disorders and blemishes such as skin splitting, and slower ripening. High fruit Ca also reduces weight loss during storage, which is often linked to improved storage performance.

Manipulation of fruit Ca can be difficult. Responses to soil and spray applications of Ca can be variable, and often depends on fruit type, the form of Ca used, and the concentration and frequency of application. High available soil potassium (K) and magnesium (Mg) often result in lower fruit Ca through a reduction in Ca uptake by the roots, but K and Mg sprays can also result in lower fruit Ca. High nitrogen (N) applications are often associated with low fruit Ca because of larger fruit size and a dilution of Ca in the fruit.

High N applications often have opposite effects on quality to those of Ca. Nutrition can also affect eating quality. High N can reduce firmness through increased fruit size. High K is often associated with higher acidity in citrus, but lower acidity in banana.

Water Supply

Excessive water availability can result in larger fruit, reduced flavour and firmness, and more disorders. Low water supply can also reduce fruit Ca concentration, presumably because of increased competition between leaves and fruit for available water. The timing of water stress is also important. Stress during early banana fruit growth increases maturity bronzing, and fluctuating stress increases the risk of fruit splitting.

High moisture loss from fruit during storage and ripening is often associated with higher fruit disease and disorders. Thus, production factors which increase moisture loss during storage may reduce fruit quality.

Plant Growth Regulators

Plant growth regulators (PGRs) are intimately involved in plant development, and external applications of PGRs during fruit development can have important effects. The most important effects have been obtained with gibberellins (GAs) and their inhibitors (e.g. Cultar®), and cytokinins. Generally, GAs and their inhibitors influence fruit quality through effects on competing vegetative growth.

Cultar® has often been associated with reduced CI and disorders, and less elongated fruit, with GAs having opposite effects. Cultar® has also been shown to increase fruit size, but variable effects on blemishes have been noted. Cytokinins can cause longer and larger fruit, with some effects on fruit colour and eating quality. Firmness can be reduced, presumably because of larger fruit size.

Auxin transport out of fruit has been shown to increase Ca concentrations in fruit, and Cultar® can also increase fruit Ca through reduced competition with vegetative growth during the fruit growth period. High abscisic acid (ABA) in fruit may be related to increased disorders (Cutting et al. 1988), so production factors which increase fruit stress during growth should be avoided.

Relative Humidity

Relative humidity has an important effect on Ca accumulation into fruit because of Ca translocation by mass flow in the transpiration stream. Factors that reduce evapotranspiration by plant organs will reduce Ca accumulation. Thus, higher leaf:fruit ratio, or young developing leaves in close proximity to developing fruit, will result in proportionally more water flowing to the leaves, and less to the fruit. Higher RH around fruit than around leaves will also reduce water and Ca movement into fruit. In contrast, higher RH around the whole plant will reduce leaf evapotranspiration to a greater extent than that of fruit, and result in increased fruit Ca accumulation.

Interactions

Numerous interactions exist in the relationships between production practices and quality. Interactions between fruit size, firmness, flavour and fruit Ca concentration have already been indicated. Production conditions can affect the time to reach maturity and the variability in maturity within and between trees, with subsequent effects on consistency of quality. In fruit with a long harvest period (e.g. avocado) there is an interaction between fruit maturity (usually determined by the time from flowering to harvest) and climatic conditions at harvest, and it may be difficult to determine which of these factors affect quality.

Generally, increased maturity at harvest will increase fruit size and eating quality (within limits), but decrease shelf life. Maturity also affects physiological and storage disorders, but the effects are variable. In mango, the disorders associated with uneven ripening of the flesh (e.g. spongy tissue and soft nose) are more severe in more mature fruit.

Maturity can also affect fruit response to postharvest treatments such as ethylene and heat treatment, and infiltration of chemicals.

Conclusions

This review has illustrated the many interactions that exist between production practices and fruit quality. Effective horticulture management requires an approach that recognises and capitalises on these interactions. Thus, there may be several means of addressing postharvest limitations, and these may be in both the production and postharvest areas. For example, fruit Ca concentration can be improved either by Ca soil or spray application, or by careful control of irrigation and/or leaf:fruit ratio. In fact, a combination of these factors would have the greatest impact on fruit Ca concentration, since the effect of optimum conditions in one of these parameters may be negated by sub-optimal conditions in the other. Effects on yield and other aspects of enterprise performance also need to be considered.

Research approaches which recognise these interactions, and contribute to the development of management systems that capitalise on these interactions, should be used. Ultimately, it may be possible to predict quality of fruit at harvest and after storage and distribution (e.g. Sharples 1984), which would result in improved consumer confidence because of greater consistency in quality.

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Effects of Preharvest Calcium Sprays and Fertilizers, Leaf:Fruit Ratios, and Water Stress on Mango Fruit Quality

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Abstract

Preharvest calcium chloride sprays increased fruit calcium (Ca) concentrations and the number of days to eating soft at 22°C (shelf life) by 1.3 days, but caused a 5% increase in lenticel spotting. No significant effects on skin green colour at ripe, percentage dry matter (% DM) or disease were observed. Soil fertilizer applications of gypsum did not significantly affect mango fruit shelf life or quality. Altering the leaf:fruit ratios affected fruit mass, Ca, magnesium (Mg) and potassium (K) concentrations, shelf life, % DM, disease, skin colour, and external chilling injury. Water stressing mango fruit during different stages of development and growth affected fruit Ca, Mg and K concentrations, % DM, disease, skin colour, chilling injury, fruit cuticle thickness, fruit growth rates and final fruit mass by reducing cell number or size. Pulp Ca concentrations had poor correlations with fruit shelf life in all trials. Stronger relationships were found between fruit shelf life and % DM.

WITH increasing production of mangoes from rapidly expanding growing regions, the Australian mango industry will need to increase its market access to ensure demand continue to meet supply (Hofman et al. 1995a). To facilitate access to domestic and offshore markets, mango fruit storage potential and fruit quality consistency needs to be improved. A better understanding of the interactions between preharvest cultural practices and postharvest fruit quality will yield valuable information regarding these aspects of production. Fruit quality can be thought of as a function of energy, water, and nutrient flows through the plant (Beverly et al. 1993). If there is an imbalance of flows, fruit quality will be affected.

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This study investigated the effects of preharvest cultural practices, namely, calcium (Ca) sprays and fertilizers, leaf:fruit ratios, and water stress on the postharvest quality and storage potential of 'Kensington' mango fruit, and whether increased fruit Ca concentrations were associated with improved storage potential.

Materials and Methods

Field treatments

During the 1995–96 season, 'Kensington' mango fruit growing at Ayr, northern Queensland (NQ), Australia, were treated with 0, 2, or 4% calcium chloride ($CaCl_2$) plus 0.01% Agral® at fortnightly intervals from four weeks after flowering (8 mm diameter) until fruit harvest. Individual fruit were sprayed until runoff when less than 20 mm diameter, and dipped in Ca solutions for one minute when greater than 20 mm. Control fruit were not treated. The experimental layout was a completely randomised design with four treatments replicated four times on 14 trees.

During the 1995–96 season, 'Kensington' mango fruit growing at Childers, southeastern Queensland, were treated with one of five treatments: (i) one soil application of 4 kg gypsum/tree at fruit set, (ii) one soil application of 4 kg gypsum/tree at fruit set and 0.2% Ca chelate plus 0.1% Agral® sprays weekly for six weeks after fruit set, (iii) weekly sprays of 0.2% Ca chelate plus 0.1% Agral® for six weeks after fruit set and then fortnightly until fruit harvest (iv) weekly sprays of 0.2% Ca oxide plus 0.1% Agral®for six weeks after fruit set; and (v) a control. The experimental layout was a randomised complete block with five treatments, six blocks and five trees per block.

The leaf:fruit trial was conducted during the 1995–96 season at Ayr, NQ. Eight weeks after flowering, 30 'Kensington' mango trees had branches thinned to a single fruit and girdled to isolate the fruit with 30, 60 or 120 leaves (hereafter referred to as treatment 30, 60 or 120). Branches were girdled by making a 10–15 mm wide cut through the bark to the cambium layer. Control branches were not thinned or girdled (hereafter referred to as control). The experimental layout was a randomised complete block with four treatments, three blocks and 10 trees per block. Each tree had all four treatments replicated twice on separate branches within the canopy.

The water stress trial was conducted during the 1994–95 and 1995–96 season, on a commercial 'Kensington' orchard at Ayr, NQ. The four treatments were (i) nil irrigation from the time of panicle emergence for 84 days (1994–95 season) or 56 days (1995–96 season) (hereafter referred to as Early), (ii) nil irrigation for 53 days (1994–95 season) or 56 days (1995–96 season) before harvest (hereafter referred to as Late); (iii) nil irrigation for 11 days (1994–95 season) or 14 days (1995–96 season) before harvest (hereafter referred to as Preharvest); and (iv) control. The experimental layout was a randomised complete block with four treatments replicated across four blocks. Each replication consisted of a three tree subplot.

Field assessments

Stomatal conductance (g_s) of leaves from the leaf:fruit trial and water stress trial were determined using a PP-Systems (Hitchin, Herts., U.K.) portable steady state leaf porometer. Measurements were made every one to two weeks between 0800–1100 hours on three leaves per tree which were in full sunlight adjacent to the fruit. Mature leaves adjacent to fruit were collected for starch analysis before girdling and at fruit harvest in the leaf:fruit trial.

Fruit growth was determined throughout ontogeny in the leaf:fruit trial and water stress trial. In the leaf:fruit trial, fruit length and breadth were measured using digital callipers, and length multiplied by breadth ($l \times b$) used as an estimate of fruit size. In the water stress trial, tagged fruit were harvested for growth and mineral analysis (Ca, magnesium [Mg], and potassium [K]) every fortnight. Fresh fruit weight was measured and then fruit were dried to a constant weight at 70°C, ground to 2.0 mm and assayed for mineral content using inductively coupled plasma atomic emission spectrophotometry.

To determine the effects of water stress on fruit cuticle thickness, cell size and number, 40 or 20 fruit were collected at the end of the Early water stress period (56 days after panicle emergence [DAPE]) and at fruit harvest (117 DAPE) respectively, for light microscopy analysis. Cell number was recorded by counting the number of cell layers from the epidermis to the seed. Average cell size was gauged using a measuring eyepiece. Two perpendicular measurements of 10 sequential cells midway through the fruit were made. Cuticle thickness was recorded with a measuring eyepiece at 10 random sites along each fruit section to give a mean cuticular thickness.

Fruit quality

Once harvested, fruit were desapped and dipped in 0.05% (v/v) Sportak® for one minute to control postharvest diseases, and air-freighted overnight to the postharvest laboratory in Brisbane. Harvested fruit were divided into two equal groups for shelf life and storage life studies.

Shelf life studies

To determine the number of days to eating soft (shelf life), fruit were ripened at 22°C in ethylene-free humidified air (93% relative humidity [RH]). Fruit were removed at eating soft (ripe) when they reached a softness of approximately 4.8 Newtons as measured by an Instron Universal Testing Machine (model 1122), fitted with an 8 mm hemispherical probe (probe penetration being 2 mm) interfaced with a computer. Fruit were then visually rated for skin greenness (percentage of the skin surface with green colour), blush area (percentage of the skin surface area with red blush), red blush intensity (0–100%, with 100% corresponding to Hunter Labscan 6000 0/45° Spectrocolorimeter L, a, b readings of 44.3, 28.5 and 15.3, respectively), lenticel spotting (1 = none, 2 = few small spots, 3 = moderate number of small spots or few large spots, 4 = large number of small

spots or moderate number of large spots and 5 = large number of large spots), disease (1 = 0%, 2 = 1–5%, 3 = 6–15%, 4 = 16–30% and 5 = 31–100% surface area affected) and pulp colour using a Roche™ yolk colour fan (1 = lightest yellow, 15 = darkest yellow, where 8–9 = typical mango pulp colour).

To determine percentage dry matter (% DM), samples of pulp were taken from the mid-cheek region of ripened fruit and dried to a constant weight at 60°C.

Storage life studies

To determine storage potential, the remaining fruit were placed at 10°C (94.5% RH) for four weeks followed by one week ripening at 22°C (59.3% RH). The quality of stored fruit was assessed similarly to non-stored fruit but chilling injury was also assessed, both external (1 = none, 2 = slight, 3 = moderate and 4 = severe, based on intensity and surface area discoloured) and internal (1 = 0%, 2 = 1–5%, 3 = 6–15%, 4 = 16–30% and 5 = 31–100% cut surface area with discoloration). Chilling injury was identified as a grey or brown discoloration of the skin and pulp.

Dried pulp from the dry matter (DM) determinations was analysed for total Ca, Mg and K concentrations by inductively coupled plasma atomic emission spectrophotometry.

Statistical analysis

Statistical analyses were conducted using Statistix® 4.0 and SAS® 6.04 data analysis software. Arcsine angular transformations were made on percentage data and back-transformed after treatment means were separated using the least significant difference (LSD) test. Non-parametric data, such as scale ratings, were analysed by a non-parametric Kruscal-Wallis test.

Results and Discussion

Preharvest calcium treatments

Mangoes treated with 4% $CaCl_2$ had higher concentrations of Ca in pulp tissue (Fig. 1), ripened 1.3 days later than controls, and had lower rates of fresh weight loss, but had a 5% increase in lenticel spotting (Table 1). No significant effects on skin green colour at ripe, % DM or disease were observed. The increased lenticel spotting may have been an expression of mild lenticel damage, which has been observed in 'Kensington' after postharvest vacuum infiltration with 8% $CaCl_2$ (Tirmazi and Wills 1981).

Soil fertilizer applications of gypsum, or different forms of preharvest Ca sprays did not significantly extend mango fruit shelf life (Fig. 2) or quality.

Table 1. Effects of preharvest calcium chloride $CaCl_2$ sprays on 'Kensington' mango fruit quality after ripening at 22°C. Values with the same letter are not significantly different at $P < 0.05$.

23	Days to eating soft	Lenticel spotting (1–5)	Fresh weight loss (g/day)
Control	14.9^{b}	3.9^{b}	1.8^{a}
0%	14.7^{b}	3.6^{c}	1.7^{a}
2%	15.0^{ab}	3.8^{b}	1.8^{a}
4%	16.2^{a}	4.1^{a}	1.5^{b}

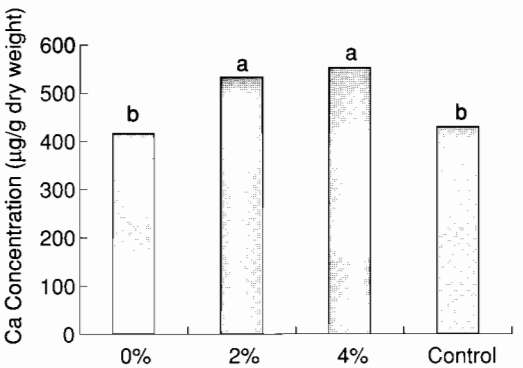

Figure 1. Effects of preharvest calcium chloride sprays on 'Kensington' mango fruit pulp calcium (Ca) concentrations after ripening at 22°C. Columns with the same letter are not significantly different at $P < 0.05$.

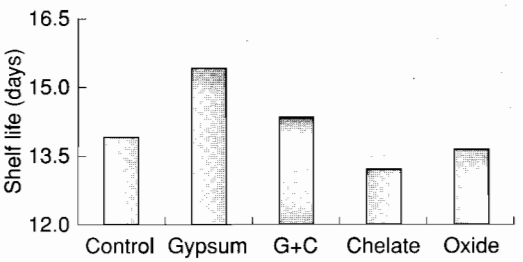

Figure 2. Effects of two different forms of preharvest calcium (Ca) sprays (Chelate and Oxide), a soil application of gypsum (Gypsum) and a combination of both (G+C) on the number of days to eating soft at 22°C (shelf life) of 'Kensington' mango fruit.

Leaf:fruit ratios

Stomatal conductance of leaves from controls and treatment 30 were up to three times higher than treatment 120 (Fig. 3). The g_s of leaves from treatment 60 was intermediate to the other treatments throughout fruit ontogeny. The starch concentrations in leaves from the control and treatment 30 were significantly lower than treatments 60 and 120, and unchanged from pre-girdling levels (Fig. 3). However, leaves from treatments 60 and 120 had significantly higher leaf starch concentrations at fruit harvest than those determined before girdling. Similar increases in starch concentrations (Schechter et al. 1994) and reduced stomatal conductance (Gucci et al. 1995) in leaves of 'thinned' branches (high leaf:fruit ratios) have been observed in apple. Higher leaf starch concentrations in treatments 60 and 120 indicated that excess photoassimilates were being accumulated during fruiting, which may have led to 'end-product inhibition' of photosynthesis and consequently reduced stomatal conductance (Schaffer et al. 1987; Layne and Flore 1995).

The rate of fruit growth increased with the number of leaves per fruit (Fig. 4). Fruit from treatment 120 grew faster and were larger than treatment 60, followed by controls and treatment 30. Differences in fruit 'size' between the control and treatment 60 only became apparent in the last four weeks before harvest as the growth rate of control fruit diminished.

Decreasing the leaf:fruit ratio to 30 resulted in smaller fruit with lower % DM, but extended the number of days to eating soft at 22°C (shelf life) by two days (Table 2). Treatment 30 fruit also had higher Ca concentrations (Table 3) and improved storage potential due to lower external chilling injury (Table 4). Increasing the leaf:fruit ratio to 60, produced larger fruit and extended the shelf life by approximately two days, but had no effect on % DM or pulp Ca concentrations. A further increase in the leaf:fruit ratio to 120 increased fruit size, % DM and the sever-

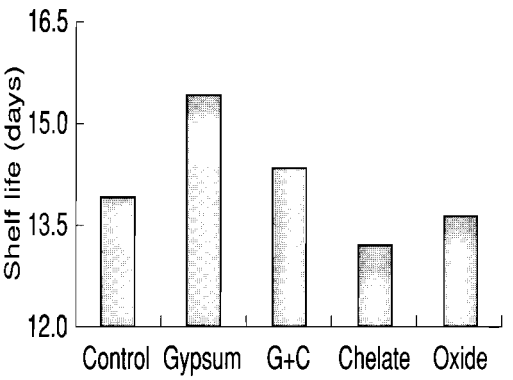

Figure 3. Effect of leaf:fruit ratios on stomatal conductance (g_s) of 'Kensington' mango. Vertical bars indicate least significant difference (LSD) at $P < 0.05$. Values beside curves correspond to leaf starch (%) concentrations at fruit harvest and before girdling. Values with the same letter are not significantly different at $P < 0.05$.

Table 3. Effects of leaf:fruit ratios on 'Kensington' mango fruit pulp calcium (Ca), magnesium (Mg) and potassium (K) concentrations after ripening at 22°C. Values with the same letter within columns are not significantly different at $P < 0.05$.

Leaf:fruit ratio	Ca	Mg	K
	(mg/100 g fresh weight)		
Control	6.6^b	6.8^a	186.1^a
30	8.4^a	4.9^c	153.1^c
60	6.5^b	5.3^c	162.6^c
120	6.0^b	5.9^b	174.9^b

Table 2. Effects of leaf:fruit ratios on 'Kensington' mango fruit ripened at 22°C. Values with the same letter within columns are not significantly different at $P < 0.05$ (FW = fresh weight, DM = dry matter).

Leaf:fruit ratio	Fruit size (g FW)	Shelf life (days)	% Skin area with		DM (%)	Disease	
			green colour	red blush		severity (1–5)	incidence (%)
Control	441.4^c	14.4^b	33.3^a	45.3^b	13.0^b	1.2^c	13.3^b
30	363.2^d	16.4^a	13.8^b	50.9^a	12.0^c	1.8^b	40.0^{ab}
60	532.5^b	16.3^a	10.6^b	55.3^a	13.7^b	2.0^b	43.3^{ab}
120	696.6^a	14.6^b	13.2^b	57.3^a	15.1^a	2.7^a	63.3^a

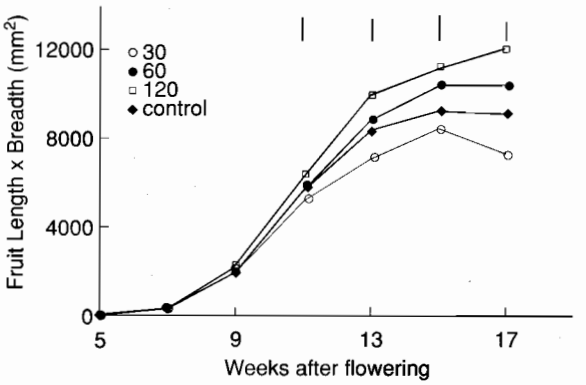

Figure 4. Effect of leaf:fruit ratios on fruit length × breadth (mm^2) of 'Kensington' mango. Vertical bars indicate least significant difference (LSD) at $P < 0.05$.

ity and incidence of disease, but had no effect on shelf life or pulp Ca concentrations. The declining Ca concentrations with increasing leaf:fruit ratio may have been due to these fruit having a faster growth rate. A faster growth rate would have resulted in a faster decline in the surface area to volume ratio (SA:V) and consequently less water (containing Ca) being lost via the transpiration stream (Kirkby and Pilbeam 1984). Perring (1979) similarly recorded a 30% decline in Ca concentrations of 'Spartan' apples due to Ca 'dilution'.

Table 4. Effects of leaf:fruit ratios on quality of 'Kensington' mango fruit after four weeks storage at 10°C and one week ripening at 22°C. Values with the same letter within columns are not significantly different at $P < 0.05$.

Leaf:fruit ratio	External chilling injury	
	severity (1–4)	incidence (%)
Control	2.0[a]	82.8[a]
30	1.4[c]	36.7[b]
60	1.7[b]	53.3[ab]
120	1.8[b]	60.7[ab]

Girdling had an independent effect on skin colour, with fruit from the girdled branches having approximately 20% less green and 5–12% more red blush on the skin at eating soft (Table 2). The increased red blush on fruit from girdled branches may have resulted from thinning large branches to a single fruit and thereby increasing fruit exposure to sunlight (Mancinelli 1985), since the higher fruit numbers on control branches often resulted in branches being weighed down and fruit hidden within the canopy foliage.

Girdling also reduced the concentrations of Mg and K in the fruit pulp tissue (Table 3). The reduced Mg and K concentrations in fruit from the girdled branches was most likely due to limited translocation of these phloem-mobile nutrients past the girdle (Tromp 1975). Fruit on branches with 120 leaves probably had higher concentrations of Mg and K owing to greater remobilisation from leaves to fruit via the phloem (Nooden 1988).

Water stress

Withholding irrigation for prolonged periods either during early or late fruit growth, significantly lowered leaf g_s (Fig. 5). Shorter periods of water stress up to two weeks, did not significantly reduce leaf g_s. The leaf g_s dropped more quickly in the Late than the Early stress period, but both treatments caused g_s to decline to approximately 20 $mmol/m^2/second$.

Mango fruit had a sigmoidal pattern of fruit growth. In the 1995–96 season, fruit showed gradual increases until 28 DAPE, followed by a rapid increase until 97 DAPE and a reduction until 116 DAPE, during seed coat hardening (Fig. 6). Water stressing during early or late fruit growth significantly reduced fruit growth rates and final fruit mass. The Preharvest stress of up to two weeks did not significantly affect growth rates or final fruit mass.

Fruit Ca concentrations during ontogeny were significantly affected by water stress in the 1994–95 season only (Fig. 7). Ca concentrations were reduced by 22% in the Early stressed fruit compared to control

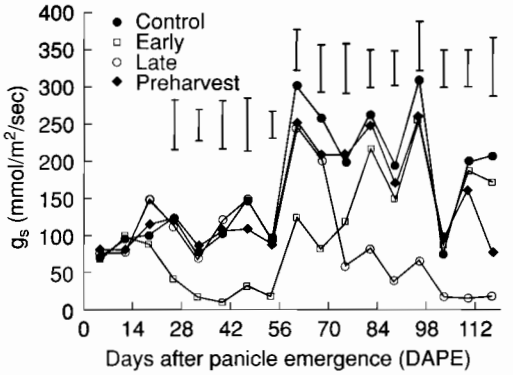

Figure 5. Effects of water stress on 'Kensington' mango leaf stomatal conductance (g_s) during the 1995–96 season. Vertical bars indicate least significant difference (LSD) at $P < 0.05$.

fruit. However, once irrigation had resumed whole fruit Ca concentrations recovered to control levels. The Late water stress also reduced fruit Ca concentrations by 19% at 147 DAPE due to a steeper decline during the period 119 to 147 DAPE. The Pre-harvest water stress did not significantly affect fruit Ca concentrations.

Early water stress significantly decreased the average fruit cell size and number by 15 and 22%, respectively, at 56 DAPE (Table 5). The cuticle layer measured in this area was also significantly increased by 29% after eight weeks water stress from flowering. At fruit maturity (117 DAPE), Early stressed fruit still had significantly reduced cell numbers (19%) but cell size had recovered and was not significantly different to control fruit. The cuticle on the Early stressed fruit had thinned by 117 DAPE, but was still significantly

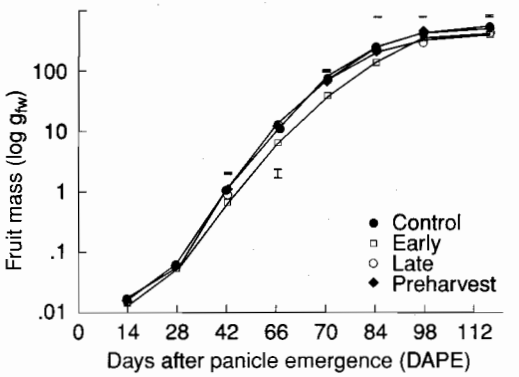

Figure 6. Effects of water stress on 'Kensington' mango fruit growth during the 1995–96 season. Vertical bars indicate least significant difference (LSD) at $P < 0.05$ (fw = fresh weight).

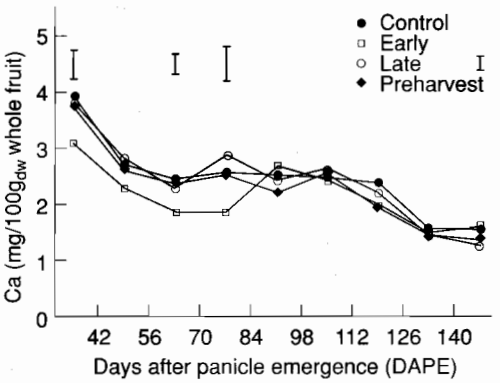

Figure 7. Effects of water stress on 'Kensington' mango fruit calcium (Ca) concentrations during ontogeny in the 1994–95 season. Vertical bars indicate least significant difference (LSD) at $P < 0.05$ (dw = dry weight).

Table 5. Effects of water stress on 'Kensington' mango fruit cell number, size and cuticle thickness during ontogeny in the 1995–96 season. Values with the same letter are not significantly different at $P < 0.05$ (DAPE = days after panicle emergence).

Treatment	56 DAPE			117 DAPE		
	Cell number	Cell size (µm)	Cuticle thickness (µm)	Cell number	Cell size (µm)	Cuticle thickness (µm)
Control	247.7^a	27.1^a	13.7^b	345.9^a	97.5^a	12.9^c
Early	194.2^b	23.2^b	16.7^a	280.1^b	96.3^a	14.3^b
Late				349.6^a	68.0^b	17.5^a
Preharvest				319.8^a	95.7^a	13.5^{bc}

Table 6. Effects of water stress on 'Kensington' mango fruit quality after ripening at 22°C. Values with the same letters are not significantly different at $P < 0.05$ (DM = dry matter, FW = fresh weight).

Treatment	Fruit mass (g FW)	Shelf life (days)	Disease (1–5)	DM (%)	% Skin area with		Blush intensity (%)	Pulp colour (1–15)	Lenticel spotting (1–5)
					green colour	red blush			
Control	513.0[a]	13.6[b]	2.7[c]	13.9[bc]	6.7[a]	24.4[b]	20.8[bc]	9.6[c]	3.7[a]
Early	343.5[b]	15.2[a]	3.2[a]	12.7[c]	11.1[a]1	20.9[b]	16.3[c]	8.7[d]	2.6[c]
Late	350.2[b]	11.7[c]	2.4[c]	17.4[a]	5.3[a]	35.2[a]	46.1[a]	10.9[a]	2.9[b]
Preharvest	478.8[a]	13.4[b]	2.7[b]	14.4[b]	7.3[a]	35.2[a]	29.1[b]	9.8[b]	3.6[a]

thicker than control fruit. The Late water stress reduced the average cell size by 30% and increased the cuticle thickness by 26%. The Preharvest water stress treatment did not significantly affect fruit cell number, size or cuticle thickness.

Early water stressed fruit took significantly longer to ripen at 22°C and had less lenticel spotting, but were significantly smaller in size (33%), had lower % DM, lighter pulp colour, more disease, and appeared to be slightly greener than the control (Table 6). Late water stressed fruit ripened significantly earlier, had less lenticel spotting, higher % DM, more blush, less disease and darker pulp, but were smaller in size (32%) and appeared slightly greener than the control. Preharvest water stress did not significantly affect fruit quality at 22°C.

After cold storage the Late water stressed fruit had significantly more internal and external chilling injury, more disease and retained more green skin colour (Table 7). Early water stress did not significantly affect chilling injury susceptibility but fruit retained more green skin colour.

Table 7. Effects of water stress on 'Kensington' mango fruit quality after four weeks storage at 10°C and one week ripening at 22°C.

Treatment	% Chilling injury		Disease (1–5)	Green skin (%)
	internal	external		
Control	7.2[b]	5.2[b]	3.9[c]	2.7[b]
Early	0.0[b]	2.5[b]	4.0[c]	9.8[a]
Late	37.7[a]	61.7[a]	4.5[a]	12.2[a]
Preharvest	5.0[b]	12.5[b]	4.3[b]	3.2[b]

Early water stress did not affect fruit pulp Ca or K concentrations, but decreased Mg concentrations following cold storage (Table 8). Late water stress increased fruit pulp Ca and Mg concentrations, but did not affect K concentrations. Preharvest water stress did not affect Ca or Mg pulp concentrations but increased K concentrations.

Table 8. Effects of water stress on 'Kensington' mango fruit pulp calcium (Ca), magnesium (Mg) and potassium (K) concentrations after four weeks storage at 10°C and one week ripening at 22°C. Values with the same letter are not significantly different at $P < 0.05$.

Treatment	Ca	Mg	K
	(mg/100 g fresh weight)		
Control	6.4[b]	7.8[b]	189.5[b]
Early	6.8[b]	6.5[c]	190.8[b]
Late	9.0[a]	10.0[a]	208.3[ab]
Preharvest	6.4[b]	7.3[bc]	220.8[a]

Poor correlations were found between fruit pulp Ca concentrations and fruit shelf life in all trials (Table 9). Correlations were not significantly improved when ratios of Ca, Mg and K were used. While strong positive relationships between fruit shelf life and Ca concentrations for other subtropical fruit such as avocado (Cutting et al. 1992) have been reported, only weak relationships for mango fruit (Hofman et al. 1995b) have been found, indicating Ca nutrition was not a major determinant in mango fruit ripening. Stronger relationships were found between % DM and shelf life. The negative correlations of fruit shelf life and % DM confirms previous reports of reduced mango shelf

life with increasing fruit maturity (Hofman et al. 1995b).

Table 9. Correlation coefficients (r^2) between the number of days to eating soft (shelf life) or percentage dry matter (% DM) and fruit pulp calcium (Ca) concentrations, for the preharvest Ca spray trial, leaf:fruit ratio trial and water stress trial fruit (n.s., *, **, *** indicate no significance, significant at $P < 0.05$, < 0.01 or < 0.001, respectively).

Trial	Shelf life and Ca	Shelf life and % DM
Preharvest Ca sprays	$0.10^{n.s.}$	-0.59***
Leaf:fruit ratios	0.26**	-0.24**
Water stress	$0.23^{n.s}$	-0.51***

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Effects of Fruit Position and Preharvest Calcium Dips on 'Nam Doc Mai' Mango Fruit Quality

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Abstract

To investigate the effects of fruit position and preharvest calcium dips on fruit quality in 'Nam Doc Mai' mango, two experiments were conducted. In the first experiment, fruit were harvested from labelled flowers on the eastern and western faces of trees and ripened at 25°C for assessment of fruit quality. No marked differences were observed in either disease incidence or ripening behaviour of fruit harvested from either side of the tree, although fruit from the eastern face matured one week earlier than that from the western face. In the second experiment, 'Nam Doc Mai' mango fruits at "70% mature green stage" (88 days after full bloom) were treated with four different formulations of calcium: calcium chloride ($CaCl_2$), calcium sulfate, calcium nitrate and calcium lactate. Each formulation was applied at a concentration of either 2% or 4% by dipping attached fruit in the appropriate solution three times at 7-day intervals. One week after the third dipping date, fruits were harvested when mature (115–117 days after full bloom) and ripened at 25°C. None of the treatments applied resulted in any significant effect on fruit quality, however calcium chloride was found to affect fruit quality in a subsequent trial when applied at an earlier developmental stage (60, 67 and 74 days after full bloom), with a higher proportion of mature green fruit being produced.

POOR fruit quality and short shelf life are serious problems of perishable crops including mango fruit. Mango is one of the most popular fruits in Thailand as it grows very well in almost all regions of the country. Growing conditions and preharvest factors show marked effects on fruit quality and shelf life as well as disease development (Monselise and Goren 1987).

'Well begun is half done' is a very apt phrase in relation to optimising/modifying conditions during fruit development in order to maximise the quality of fruit at harvest and in the postharvest period. A number of studies have been reported on preharvest and postharvest interactions in temperate fruits and vegetables (Windsor 1979; Shear 1980; Sharples 1984) which may also be relevant to tropical fruits. For Thai mangoes, there is little information on the effect of fruit position on the tree canopy and preharvest treatment on fruit quality. Calcium is the most important nutrient affecting the postharvest quality of many crops such as apples and tomatoes (Conway et al. 1992), and mangoes (Singh et al. 1993). Calcium plays an important role in many aspects of plant growth and senescence (Ferguson and Drobak 1988; Poovaiah 1988). Application of calcium onto attached fruit is one way to promote fruit quality.

In this paper we present the results of a study into the effects of fruit position and preharvest calcium dips on fruit quality in Thai mango cultivar 'Nam Doc Mai'.

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Materials and Methods

Ten-year-old 'Nam Doc Mai' mango trees of uniform size and vigour were selected. Trees were located in

two orchards: Prapat and Sons' orchard, Amphur Sansai, Chiang Mai (orchard 1), and Chiang Mai Agricultural and Technology College's orchard, Amphur Sanpatong, Chiang Mai (orchard 2).

Experiment 1

Fourteen flower clusters from each of the eastern and western faces of ten uniform trees were labelled at the flower bud stage (7 days before blooming). Percentage of fruit set per cluster was checked at 7-day intervals for 2 months. The fruit were collected when they reached the normal harvesting date (115–117 days after full bloom). Fruit weight, size and development of decay were assessed.

Experiment 2

Ten uniform trees were used for the study on the effect of preharvest calcium dips on mango fruit quality. Labelled fruit at 4 weeks before harvesting date were treated with four different formulations of calcium: calcium chloride ($CaCl_2$), calcium sulfate, calcium nitrate and calcium lactate, at a concentration of either 2 or 4% w/v plus Triton X-100 at 0.1 v/v. Fruit were dipped at 7-day intervals, three times in all. Fruit were collected at normal harvesting date (115–117 days after full bloom) and kept at 25°C for ripening. Fruit weight and percentage of decay were measured. In the following season the trial was conducted using only $CaCl_2$, at concentrations of 2 and 4% w/v. The $CaCl_2$ treatments were applied at 60, 67 and 74 days after full bloom. Treated and non-treated (water dipping as a control) fruit were harvested at 115–117 days after full bloom and assessed for fruit maturity stage and percentage of decay after ripening at 25°C for 10 days.

Results and Discussion

Fruit position

There was a marked difference in percentage of fruit set per cluster between the eastern and western sides of the tree canopy (Table 1). The number of fruit set per cluster from the eastern side (62.9%) was much greater than that of the western side (38.6%). This data was collected from orchard 1 only. Measurement of fruit weight from both orchards 1 and 2 showed that the average weight of fruit was greater in fruit obtained from the eastern side of the tree canopy than from the western side (Table 2). There were no significant differences in the size and shape of fruit with respect to fruit width, fruit length and fruit width:fruit length ratio (Table 3).

Table 1. Percentage of fruit set per cluster on the eastern and western sides of the tree canopy in mango cv.'Nam Doc Mai' (orchard 1).

Position on tree canopy	Percentage of fruit set per cluster[a]	
	Fruit set (%)	Non-fruit set (%)
East	62.9a	37.1x
West	38.6b	61.4y
Average	50.8	49.3

[a] Means are from 10 replications (140 clusters). Means in columns followed by different letters are significantly different at the 0.05% level (least significant difference test).

Table 2. Fruit weight of 'Nam Doc Mai' mango fruit at time of normal harvest

Position on tree canopy	Fruit weight (g)[a]		Average
	Orchard 1	Orchard 2	
East	285.2a	196.3x	240.8
West	268.8b	160.1y	214.5
Average	277.0	178.2	227.7

[a] Means are from 10 replications (100 fruits). Means in columns followed by different letters are significantly different at the 0.05% level (least significant difference test).

Table 3. Fruit width, fruit length and fruit width:fruit length ratio of 'Nam Doc Mai' mango

Position of tree canopy	Fruit width[a] (cm)	Fruit length[a] (cm)	width:length ratio
East			
Orchard 1	6.7	13.6	0.49
Orchard 2	5.9	11.5	0.51
West			
Orchard 1	6.5	13.8	0.47
Orchard 2	5.4	11.3	0.47

[a] Means are from 10 replications (100 fruits) Means in columns are not significantly different at 0.05% level (least significant difference test).

Fruit from the eastern side of the tree tended to mature earlier than fruit from the western side by about 5–7 days. There was no significant difference in postharvest disease incidence between the fruit from the eastern and western faces of tree canopy. Our results support previous reports of cultural practice and fruit position affecting fruit size (Monselise and Goren 1987).

Preharvest calcium dipping

The results of using four formulations of calcium on fruit weight at harvest and fruit decay after storage at 25°C for 10 days, are presented in Table 4.

Table 4. Effects of calcium formulations on fruit weight at harvest and percentage of fruit decay after ripening at 25°C for 10 days.

Treatment	Fruit weight (g)[a] at harvest	% fruit decay[a] after storage
Control	292.8a	100a
Calcium nitrate $Ca(NO_3)_2 4H_2O$	285.3a	95a
Calcium chloride $CaCl_2.2H_2O$	291.7a	86b
Calcium sulfate $CaSO_4.2H_2O$	287.4a	100a
Calcium lactate $(CaO_6C_6H_{10}.4H_2O)$	294.9a	92a

[a] Means are from 10 replications (100 fruits). Means in columns followed by different letters are significantly different at 0.05% level (least significant difference test).

There were no significant differences in fruit weight at harvest between any of the four treatments and the control, however the percentage of fruit decay in the $CaCl_2$ treatment was significantly lower than in the other treatments. A second trial was subsequently conducted using only $CaCl_2$, at concentrations of 2 and 4%. Moreover, the application date was changed to an earlier stage than the previous experiment. This $CaCl_2$ treatment resulted in a higher percentage of mature green fruit than the control (Table 5).

Calcium treatment tended to delay fruit ripening as represented by the percentage of fruit at different stages (Table 5). This supports the report by Singh et al. (1993). There were no significant differences in percentage of fruit decay between each treatment (Table 6). Decay symptoms were mostly anthracnose and reached nearly 100% within 10 days. Preharvest calcium dipping did not affect postharvest decay but was able to delay ripening.

Table 5. Fruit stage at time of normal harvest after treatment with calcium chloride ($CaCl_2$). Mature green (M) = 100% green colour fruit, firmness > 17kg/cm^2; Breaker (B) = 0–20% of total area yellowish, green firmness 15–17 kg/cm^2; Ripe (R) = >20% of total area yellow, firmness < 15 kg/cm^2.

Treatment	Fruit stage at harvest[a]		
	M	B	R
Control	67.0a	22.0d	11.0z
2% $CaCl_2$	72.7b	24.7de	2.6x
4% $CaCl_2$	75.0b	18.7c	6.3y

[a] Means are from 10 replications. Means in columns followed by different letters are significantly different at the 0.05% level (least significant difference test).

Table 6. Percentage of fruit decay at time of harvest, and after ripening at 25°C for 10 days, following field treatment with with $CaCl_2$

Treatment	Percentage of fruit decay[a]	
	at harvest	after storage
Control	2.7	100.0
2% $CaCl_2$	4.0	94.7
4% $CaCl_2$	3.8	95.0

[a] Means are from 10 replications (100 fruits). Means in columns are not significantly different at the 0.05% level (least significant difference test).

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Mango Fruit Quality Is Affected by Production Conditions

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Abstract

To investigate the effects of preharvest factors on variability in postharvest quality of 'Kensington' mango, fruit were obtained from several production districts over three seasons, and from 12 orchards in the Burdekin district, Queensland, at a single harvest. The effect of time of flowering and location of the fruit in the canopy on quality was also investigated. Fruit quality was assessed at ripe, without storage or after storage at 10°C for four weeks.

Harvest date increased the percentage dry matter (% DM) and eating quality, and reduced the days from harvest to eating soft (DTES). Production location and/or practices affected several aspects of quality, including disease and chilling injury severity. Quality was generally higher in fruit from the tropical areas of Northern Territory and the Atherton Tablelands (Queensland), but not consistently in all seasons tested, and not for all quality parameters. Fruit harvested from farms in the Burdekin district showed significant differences in % DM, DTES, the percentage of green skin at eating soft (% GS), and lenticel spotting. Fruit from late flowering panicles, from low canopy position, and from the eastern and northern sides of the tree ripened more slowly, and fruit from later flowering panicles, and from the lower parts of the canopy had lower total soluble solids. The % GS was highest on fruit from the northern aspect of the tree. However, these attributes only accounted for 5–24% of the variation noted in these fruit.

There were good correlations between % DM and eating quality, but this varied with production district. There were no significant relationships between fruit quality and fruit mineral concentrations, except for significant but weak correlations between calcium and DTES, nitrogen and % GS, and potassium and % GS.

These results indicate that preharvest factors can have important effects on mango fruit quality, but the factors measured only accounted for a small portion of the variation in fruit quality. Research is in progress to identify the influence of production factors on quality to a greater extent.

CONSISTENT quality of horticultural products is becoming increasingly important in industry profitability. Considerable emphasis has been placed on maintaining quality after harvest. However, research in temperate fruits indicates that production factors have a significant impact on quality at harvest, and on the capacity of fruit to withstand the stresses of postharvest treatments (Ferguson 1980; Beverly et al. 1993). There is growing evidence of similar influences in subtropical and tropical fruit (Monselise and Goren 1987; Arpaia 1994; Hofman and Smith 1994).

Horticulture industries in Australia are placing increasing emphasis on fruit quality at all levels, and aspects such as skin colour, internal disorders, and differences in ripening rate are important commercial factors. Production practices can influence these characteristics. For example, high nitrogen (N) can increase green colour of the skin, (Smith 1989; McKenzie 1994), and high fruit calcium (Ca) can retard green colour loss during ripening (Wills et al. 1988). Flavour can be affected by time of harvest and use of Cultar® sprays during fruit growth (Khader 1990). Shelf life (the time from harvest to eating ripe

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at room temperature) can be affected by harvest maturity and Ca (Singh et al. 1987; Wills et al. 1988). Internal disorders of mango can be affected by temperatures during fruit growth, fruit nutrition, and maturity (Hofman and Smith 1994).

This paper summarises some of the results obtained over the last four years on factors contributing to the variability in fruit quality of 'Kensington' mangoes. This is part of an ongoing program to identify how to increase the consistency and predictability of subtropical and tropical fruit quality to the consumer.

Materials and Methods

Production location and maturity

1993–94

Mango fruit (*Mangifera indica*, 'Kensington Pride') were obtained from three sites. A commercial orchard on the Atherton Tablelands (tropical, north Queensland), on a shallow nodular yellow podzolic soil, provided two adjacent sites; site 1 with gravel causing poor water and nutrient retention, and site 2 without the gravel. The third site (site 3) was on a commercial orchard near Nambour (subtropical, southeastern Queensland) on a gleyed podzolic soil (or yellow ferrosol). Fruit were sampled from five trees from each of sites 1 and 2, and 10 trees from site 3. Panicles on the northern side of the tree and at mid-canopy height were tagged at the same stage of flowering to minimise the effects of flowering date on maturity at harvest.

Harvesting started at earliest commercial maturity. Six fruit per tree for sites 1 and 2, and four fruit per tree for site 3, were harvested at weekly intervals for 4 weeks. All fruit were de-sapped at the laboratory, weighed and dipped in prochloraz (0.05% v/v Sportak®) for 30 seconds. Three fruit from each tree (two for site 3) were ripened at 22°C in ethylene-free air (passed through Purafil®). Fruit were assessed at eating soft for quality. The remaining three fruit per tree (two for Site 3) were placed in standard cartons and held at 10°C for 4 weeks and ripened at 22°C for a further 10 days. Fruit quality was assessed during storage and ripening.

1994–95 and 1995–96

Six fruit were harvested from each of six trees in each of four orchards in the production districts of the Northern Territory (NT; tropical), Atherton Tablelands (Queensland; tropical), the Burdekin district (Queensland; dry tropical), and the Sunshine Coast, Queensland; subtropical). Three fruit per tree were ripened at 22°C as above, and the remaining three stored at 10°C for 4 weeks, then ripened as above. Fruit were obtained from 12 growers in the Burdekin area at peak commercial harvest (7 and 8 December) in 1994. Three mature fruit from each of five trees were picked at random around the tree from each farm. The fruit were airfreighted to Brisbane, ripened at 22°C and assessed for quality.

Canopy position and date of flowering

About 250 panicles on 10 trees on a commercial orchard at the Sunshine Coast (SC) were tagged when the trees had reached peak flowering, and characterised for the stage of flowering (early flowering = the first flowers on the panicle had just opened; mid-flowering = 50% of the flowers opened, and late = all flowers were open). This corresponded to a spread of flowering from early to late of about 4 weeks. Panicles were also described for the number of fruit per panicle at harvest, and position on the tree (north, east, etc., and upper, middle or lower part of the canopy). Fruit from all categories were harvested at the same time, ripened at 22°C, and assessed for quality.

Quality assessment

The number of days from harvest to eating soft (DTES, judged by hand pressure) was recorded for every fruit. Eating soft corresponded to a firmness of 5 Newtons, as measured on an Instron Universal Testing Machine (Model 1122), fitted with an 8 mm hemispherical probe (probe penetration of 2 mm). Skin colour was visually rated as the percentage of skin area containing green colour (full green = 100%; full yellow = 0%). The severity of body rots (caused mainly by *Colletotrichum* spp.) on the side of the fruit, and stem-end rots (SERs; caused mainly by *Dothiorella* spp.) at the stem end of the fruit, were rated as either the percentage of the fruit surface area affected, or as 1 = no disease, 2 = 1–5%, 3 = 6–15%, 4 = 16–30% and 5 = 31–100% of the surface area affected. Chilling injury (CI) following storage and ripening was rated on the 1 to 5 scale above. Lenticel spotting severity was assessed on a 1–5 scale of 1 = none, 2 = few small spots, 3 = moderate number of small spots or few large spots, 4 = large number of small spots or moderate number of large spots, and 5 = large number of large spots.

Measurement of total soluble solids (TSS) was made with an Atago bench refractometer corrected to 20°C. Eating quality (1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely) was determined at eating soft by a panel of 12 trained tasters. Percentage dry matter (% DM) was determined by drying representative portions of the flesh at 65°C to constant weight in a vacuum oven.

Dried flesh samples from the ripe fruit were frozen in liquid nitrogen, ground with a mortar and pestle, further dried at 70°C for 2 days, then finely ground in a shatter box. A 0.3–0.5 g subsample was digested in 15 mL nitric/perchloric acid and analysed for mineral concentrations by inductively coupled plasma atomic emission spectrophotometry or atomic absorption spectroscopy. Nitrogen was determined using Kjeldahl digestion.

Results

Production location and maturity

1993–94

The DTES decreased with later harvests in fruit from all sites (Table 1). Fruit from site 3 at harvests 2 and 3 ripened more quickly than those from site 1. The severity of SERs decreased with harvest. Site 3 fruit had more SERs than site 2 fruit at harvest 1. No SERs were recorded for site 1, and two fruit in harvests 2, 3 and 4, whereas in site 3, SERs were present in those harvests. There was low body rots severity on fruit from all sites at harvest 1, but no body rots recorded for subsequent harvests from sites 1 and 2. Body rots were present on site 3 fruit in harvests 3 and 4.

Table 1. The effect of harvest time (weekly intervals, 1993–94) and production location on the days from harvest to eating soft at 22°C, the severity (% of the fruit surface area affected) of stem-end rots and body rots at eating soft (no storage), and chilling injury (% of the fruit surface area affected) following storage at 10°C for 4 weeks, and then at 22°C for 10 days, of 'Kensington Pride' mango.

Quality parameter	Week of harvest	Site		
		North Qld		Southeastern Qld
		Site 1	Site 2	Site 3
Days to eating soft	1	22.80^{a}	22.80^{a}	21.65^{ab}
	2	24.00^{a}	20.20^{b}	17.30^{c}
	3	17.27^{c}	15.20^{cd}	13.95^{de}
	4	13.73^{de}	11.80^{e}	13.85^{de}
Stem-end rots	1	5.0^{ab}	0.6^{a}	$15.4^{b;x}$
	2	0.0	0.0	7.5^{xy}
	3	0.0	0.0	3.0^{y}
	4	0.0	0.0	5.3^{y}
Body rots	1	0.04^{a}	0.49^{a}	$1.0^{a;x}$
	2	0.0	0.0	2.2^{xy}
	3	0.0	0.0	-
	4	0.0	0.0	7.3^{y}
Chilling injury	1	0.0^{c}	0.0^{c}	5.3^{ab}
	2	0.0^{c}	0.0^{c}	10.2^{ab}
	3	2.2^{bc}	1.7^{bc}	12.2^{a}
	4	2.2^{bc}	0.08^{c}	10.2^{ab}

Note: The disease and chilling injury data are angular transformed. Numbers with the same letters for each quality parameter are not significantly different when separated by least significant difference (LSD) at P = 0.05.

Fruit with higher dry matter had higher eating quality ($P<0.01$), with no difference in the relationship between sites 1 and 2 (eating quality = 0.25 (% DM) + 1.82). Fruit from site 3 had the same slope as for sites 1 and 2, but an intercept of 3.48 (i.e. higher eating quality at the same % DM than fruit from sites 1+2). For sites 1 and 2, DTES = –1.55 (% DM) + 43.4 (R^2 = 0.59, P = 0.01), and for site 3, DTES = –0.62 (% DM) + 24.3 (R^2 = 0.14, P = 0.05).

There were significant negative correlations between % DM and percentage green skin (% GS) at eating soft (angular transformed) for all sites (R^2 = 0.19, P = 0.01), indicating that fruit with higher % DM has less green on the skin at eating soft. There was also a significant positive correlation between DTES and % GS at eating soft for site 1 (R^2 = 0.22, P = 0.01) and site 3 (R^2 = 0.19, P = 0.01) indicating that fruit which ripened more quickly had less % GS at eating soft.

1994–95 and 1995–96

Days to eating soft decreased with harvest in both years (Fig. 1). For harvests with similar % DM, Burdekin fruit had the shortest DTES in both seasons. In most of the production locations there was a trend to decreasing % GS with harvest but there was no difference between locations at similar % DM. Stem-end rots increased with later harvests in 1994–95 in the Atherton and Burdekin areas only, but there was no harvest effect in 1995–96. At similar % DM, NT fruit had lower SERs in 1995–96, but there was no locality effect in 1994–95. Body rots severity was not affected by harvest in either season except for higher severity in later harvested Atherton fruit in 1995–96. At a similar % DM, fruit from the SC had higher severity in 1995–96 than those from the other locations.

Eating quality was acceptable at all harvests and locations in both seasons, even when the % DM was below 14% (Table 2). In 1994–95, eating quality was lowest in SC fruit, but there was no locality effect in 1995–96 for fruit at similar % DM.

After storage and ripening, later harvested fruit had higher incidence of SERs in 1994–95, but not in 1995–96, and not in regard to body rots (Table 3). Severity of SERs was lowest in Atherton fruit in 1994–95 only, and in NT fruit in 1995–96. The severity in fruit from other locations was generally similar. There was little harvest date, locality or season effect on body rots severity.

There was no locality influence on the concentrations of Ca, magnesium (Mg), potassium (K) and boron (B) in fruit, except for higher Mg and lower B in NT

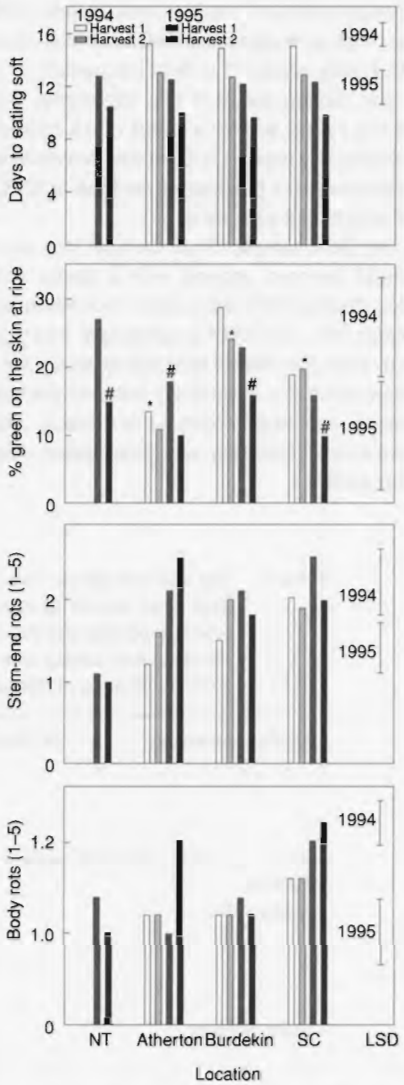

Figure 1. Effect of production location (NT= Northern Territory, Atherton = Atherton Tablelands, SC= Sunshine Coast), harvest time (two weeks apart) and season (1994–95 and 1995–96) on the days to eating soft, the percentage of the skin with green colour at ripe, and the severity of stem-end rots and body rots (1= 0, 2 = 1–5%, 3 = 6–15%, 4 = 16–30% and 5 = 31–100% of the surface area affected by disease) of 'Kensington Pride' mango ripened at 22°C directly after harvest. Bars indicate the average least significant difference (P=0.05) for comparison between season, harvest and location. "*" and "#" indicate those harvests from each of the production locations with similar percentage dry matter (% DM) for 1994–95 and 1995–96 , respectively.

Table 2. Effect of production location (NT = Northern Territory, Atherton = Atherton Tablelands, SC = Sunshine Coast) and harvest date (two weeks apart) on the percentage dry matter (% DM) and eating quality (EQ) of ripe, unstored 'Kensington Pride' mango.

		1994		1995	
Location	Harvest	% DM	EQ	% DM	EQ
NT	1	-	-	13.9^{ce}	6.9^{bd}
	2	-	-	15.3^{ad}	7.3^{ac}
Atherton	1	15.0^{bc}	6.82^{ab}	$15.1b^{def}$	7.3^{ab}
	2	16.7^{a}	7.04^{a}	16.0^{ac}	7.3^{ab}
Burdekin	1	13.7^{cde}	7.04^{a}	13.1^{ef}	7.0^{bcd}
	2	15.3^{ab}	7.04^{a}	16.5^{ab}	7.7^{a}
SC	1	12.8^{d}	6.35^{c}	13.5^{cdf}	7.1^{ad}
	2	14.5^{bc}	6.62^{bc}	15.0^{ae}	6.7^{bc}
av LSD		1.3	0.32	2.0	0.6

Note: Eating quality was rated as general acceptability on a scale of 1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely). Numbers with the same letter are not significantly different when separated by least significant difference (LSD) at P = 0.05. Different LSDs were used for the comparisons between districts and harvests, but the average LSD is presented.

Table 3. Effect of production location (NT = Northern Territory, Atherton = Atherton Tablelands, SC = Sunshine Coast) and harvest date (two weeks apart) on the severity of stem-end rots (SERs), body rots and chilling injury (CI) following storage at 10°C for 4 weeks, and then at 22°C for 10 days, of 'Kensington Pride' mango.

		1994–95			1995–96		
Location	Harvest	SERs	Body rots	CI	SERs	Body rots	CI
NT	1				2.3^{c}	1.2^{c}	1.3^{a}
	2				2.6^{c}	1.8^{ab}	1.3^{a}
Atherton	1	1.96^{d}	1.17^{a}	1.46^{b}	4.7^{ab}	1.7^{abc}	1.2^{a}
	2	3.05^{c}	1.34^{a}	1.85^{a}	4.8^{ab}	2.0^{abc}	1.5^{a}
Burdekin	1	3.78^{bc}	1.4^{a}	1.31^{b}	3.9^{b}	1.3^{bc}	1.6^{a}
	2	4.37^{a}	1.37^{a}	0.97^{b}	4.6^{a}	1.7^{abc}	1.5^{a}
SC	1	3.26^{c}	1.43^{a}	1.13^{b}	4.5^{ab}	1.8^{abc}	1.2^{a}
	2	3.92^{ab}	1.65^{a}	1.29^{b}	4.7^{ab}	2.2^{a}	1.1^{a}
av LSD		0.767	0.442	0.456	1.09	0.96	0.59

Note: Ratings used the scale of 1= 0, 2 = 1–5%, 3 = 6–15%, 4 = 16–30% and 5 = 31–100% of the surface area affected by disease or chilling injury. The average least significant difference (LSD) (P = 0.05) is for comparison of site by harvest means. Numbers with the same letter are not significantly different when separated by LSD at P = 0.05. Different LSDs were used in the comparisons, but the average LSD is presented.

fruit in 1995–96 (data not presented). Calcium was correlated to DTES (r = 0.42, P =0.001) and internal flesh colour (r = –0.55, P = 0.001), and N (r = 0.40, P = 0.001) and K (r = 0.41, P = 0.001) correlated to the % GS in the ripe fruit (no storage; data not presented).

The % DM, DTES, % GS, lenticel spotting and incidence of SERs was significantly influenced by orchard location and/or production practices in the Burdekin district (Table 4). Fruit from farms which had a high % GS also had large variability in skin colour between individual fruit. For example, the % GS from orchard 8 ranged from 0 to 85% between fruit, while those from Orchard 5 ranged from 0 to 20%. Fruit from orchards in the Horseshoe Lagoon (north) area generally had lower % DM, ripened more slowly, and had higher % GS than those from Horseshoe Lagoon (south).

There was no obvious relationship between % DM and DTES, or % DM and % GS across all farms. Also, the DTES did not correlate to % GS.

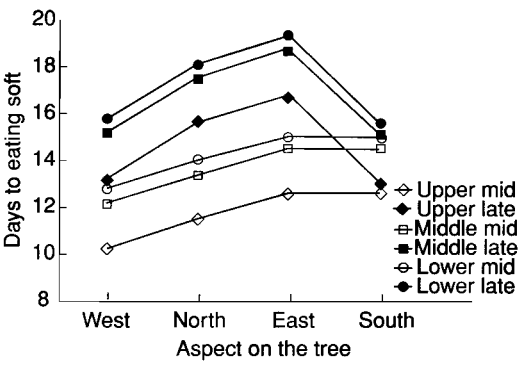

Figure 2. Effect of aspect on the tree (west, north, east or south), canopy height (upper, middle or lower canopy height) and flowering time (mid or late flowering) on the days from harvest to eating soft of 'Kensington Pride' mango ripened at 22°C immediately after harvest.

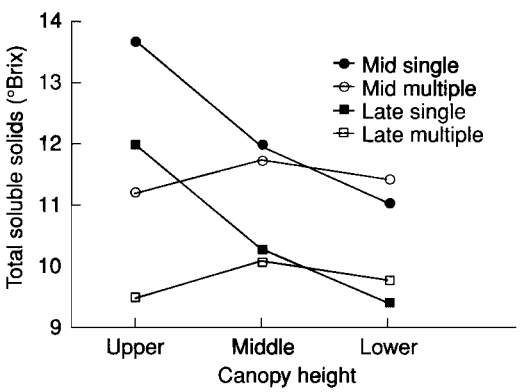

Figure 3. Effect of canopy height (upper, middle or lower canopy), flowering time (mid or late flowering) and the number of fruit on the panicle (one fruit per panicle or multiple fruit per panicle) on the total soluble solids of ripe 'Kensington Pride' mango ripened at 22°C immediately after harvest.

Canopy position and date of flowering

Fruit from the eastern side of the tree, from the lower parts of the canopy, and from the late flowering period, generally ripened more slowly than those from the western part of the tree, or from the middle flowering period (Fig. 2). The quantity of TSS was higher in fruit from the upper parts of the canopy, in fruit from the mid flowering period, and in fruit from single fruit panicles in the upper part of the canopy (Fig. 3). In addition, fruit from the northern side of the canopy were generally more green at eating soft than those from the other aspects (Fig. 4).

The measured characteristics of canopy height, aspect, and time of flowering accounted for only 23.4% of the variation in DTES. Fruit height, flowering time, and fruit per panicle accounted for 22.7% of the variation in TSS, and fruit aspect accounted for only 5.8% of the variation in % green on the skin at eating soft.

Fruit with higher % DM generally ripened more quickly. Also, fruit that ripened more slowly had a higher % GS (r = 0.39, P = 0.05). The % DM did not show any relationship to % GS at ripe.

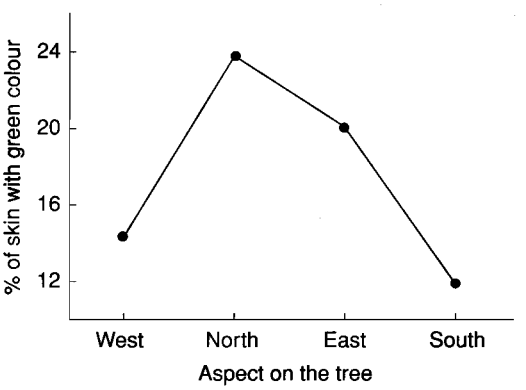

Figure 4. Effect of aspect on the tree (west, north, east or south) on the percentage of the skin with green colour in ripe 'Kensington Pride' mango ripened at 22°C immediately after harvest.

Table 4. 'Kensington Pride' mango fruit quality characteristics from 12 different orchards in the Burdekin district of northern Queensland, harvested over two consecutive days during the normal commercial harvesting period (DTES = days from harvest to eating soft, % GS = the percentage of the skin surface area with green colour at eating soft).

Orchard	Growing area	% Dry matter	Fruit mass (g)	DTES	% GS	Lenticel spotting	Body rots	Stem-end rots
1	Horseshoe Lagoon (north)	14.4	399^{a}	12.9^{abc}	31^{b}	1.9^{c}	1.0	1.1^{b}
2	Horseshoe Lagoon (north)	15.1	393^{ab}	12.7^{bc}	8^{cde}	1.9^{c}	1.1	1.0^{b}
3	Horseshoe Lagoon (north)	15.3	401^{a}	12.5^{bc}	14^{cd}	2.1^{bc}	1.0	1.5^{ab}
4	Horseshoe Lagoon (north)	15.0	400^{a}	13.9^{ab}	13^{cd}	2.5^{a}	1.1	2.0^{a}
5	Horseshoe Lagoon (south)	17.5	396^{ab}	9.8^{d}	1^{f}	2.5^{a}	1.0	1.0^{b}
6	Horseshoe Lagoon (south)	14.4	336^{cd}	11.6^{cd}	5^{def}	2.1^{bc}	1.0	2.0^{a}
7	Horseshoe Lagoon (south)	17.8	402^{a}	12.5^{bc}	2^{ef}	2.4^{ab}	1.0	1.4^{ab}
8	Mona Park	16.0	415^{a}	10.3^{d}	48^{a}	2.1^{abc}	1.1	1.2^{b}
9	Giru	16.3	364^{bc}	14.6^{a}	19^{bc}	2.2^{abc}	1.0	1.6^{ab}
10	Laughton Park	15.9	387^{ab}	11.5^{cd}	10^{cd}	2.5^{a}	1.0	1.1^{b}
11	Mt Kelly	17.1	326^{d}	11.7^{cd}	8^{cde}	2.5^{a}	1.0	1.1^{b}
12	Gumlu	16.9	345^{cd}	10.3^{d}	5^{def}	2.1^{abc}	1.0	1.3^{b}
	LSD (0.05)	-	34	1.9		0.4	n.s.	0.55

Note: Fruit were ripened at 22°C. Horseshoe Lagoon (north) and (south) are orchards on the north and south of the Bruce Highway, respectively. Numbers with the same letter for each quality parameter are not significantly different when separated by least significant difference (LSD) at P = 0.05.

Discussion

Our results indicate that a number of production factors can influence postharvest characteristics. Similar results have been obtained in other fruit. Production location can affect quality in apples (Luton and Holland 1986), fruit firmness can be affected by growing medium (Cronin and Houlihan 1985), nutrition (Richardson 1986) and pruning (Miller 1982), physiological disorders and disease by irrigation (Brooks 1916) and nutrition (Ferguson 1980), and skin colour by nutrition (Fallahi et al. 1985) and position in the canopy (Marini 1985).

The 1993–94 results indicated similar fruit quality (DTES, disease and CI) from the adjacent sites 1 and 2, but more disease and CI in fruit from site 3. This difference may be related to the higher rainfall received at site 3 during fruit maturation and harvesting. Varying rainfall patterns may also explain the lower disease severity in NT fruit in 1994–95 and the higher disease severity in SC fruit in 1995–96. Fruit from NT are generally harvested in October and November before the start of the wet season, but SC fruit are harvested in January during the wet season. However, it is likely that other production factors may also have influenced disease severity.

The results also indicated a large variation in fruit quality between orchards in the same production district. In the Burdekin experiment, differences in climate between the Horseshoe Lagoon sites would have been small, suggesting that either cultural practices or soil or water quality factors may be important influences.

Some of the production factors may have affected fruit quality through maturity (as measured by % DM). The effect of flowering date would be through this mechanism, and the characteristics noted in fruit from the later flowering panicles are typical of less mature fruit (e.g. longer DTES and lower TSS). Likewise, fruit on the eastern side of the tree and from the lower canopy had similar characteristics. Similar results of canopy position were obtained by Génard and Bruchou (1992) in peaches, and suggests that the quantity and quality of light received by the fruit and/or the subtending leaves is important in fruit quality.

The % DM did not have an over-riding influence on fruit quality, since the relationship of % DM to some aspects of quality was weak. Also, the percentage of variation in DTES, TSS and % GS accounted for by flowering time, canopy position, aspect, and the number of fruit per panicle was only small (5–24%), suggesting that other factors, or a greater interaction of the factors measured in this experiment (e.g. interaction between light and aspect) may be involved in fruit quality. However, we have noted that fruit of the same size from the same panicle can have DTES differing by up to 10 days, indicating that small differences in flowering date, or other physiological factors, are very important. More precise measures of flower anthesis (rather than panicle flowering date) and fruit light exposure (rather than canopy position as measured here; e.g. Génard and Bruchou 1992) may be helpful in assessing the factors influencing this variation. Other factors which may contribute to quality are nutrition (especially mineral distribution within the tree), irrigation, and leaf to fruit ratio (Monselise and Goren 1987). These factors can all alter the relative balance of water, carbohydrates and mineral flows into the fruit, and affect fruit quality (Beverly et al. 1993).

Acknowledgments

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Postharvest Water Loss, Postharvest Calcium Treatment and 'Kensington' Mango Fruit Quality

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Abstract

Postharvest diseases of mangoes tend to remain quiescent until the fruit ripen. Accordingly, in the context of optimum postharvest handling, it may be desirable to delay the onset of ripening until fruit are in the hands of the retailer or consumer. Reduced rates of postharvest water loss and elevated fruit calcium levels have been associated with shelf life extension of fruit. The effects of varying rates of postharvest water loss and of postharvest calcium infiltration on ripening 'Kensington' mango fruit are reported in this paper. Fruit ripening was generally delayed in both 'flow-through' and 'static' relative humidity control systems by reduction in the rate of postharvest water loss. Partial pressure infiltration of calcium also resulted in slightly delayed ripening. However, increased levels of lenticel spotting and uneven skin colour development were associated with calcium infiltration, and were considered unacceptable from a visual quality perspective. The lenticel blackening effect of calcium treatment appeared to be due to 'salt burn' in the vicinity of lenticels. Thus, while reduced postharvest water loss is likely to confer an extended 'green life' advantage for 'Kensington' mangoes, calcium infiltration treatments are impractical because of the risk of injury.

MANY postharvest pathogens of harvested horticultural produce are 'weak' pathogens insofar as, although they may have established an infection, they remain in a latent or quiescent state. Once a harvested product starts to ripen or senesce, such pathogens are released from their 'dormant' or 'arrested' state and invade the host tissue, eventually causing visible disease symptoms (Sommer 1985a). This behaviour is common to all manner of pathogens, including *Dothiorella dominicana* and *Colletotrichum gloeosporioides*—postharvest pathogens which cause stem-end rot and anthracnose diseases of mango and which are considered to be endophytic and exophytic, respectively (Sommer 1985b; Coates and Gowanlock 1994; Johnson and Sanghote 1994). Release of pathogens from their quiescent state is most probably caused by both a progressive increase in substrate supply for pathogen growth, and a decrease in the capacity of the host tissue to defend itself. In the first case, metabolic substrates (e.g. sugars) may leak out of cells in association with increasing membrane dysfunction during ripening and senescence. In the second case, levels of endogenous antifungals may decline as normal growth metabolism gives way to ripening and senescence metabolism. Increases both in attractiveness for consumption by animals and in susceptibility to decay during ripening and senescence are desirable phenomena in a 'natural systems' sense, since they facilitate seed dispersal (i.e. reproduction) and nutrient recycling.

In the context of commercial handling of harvested fruit, vegetables and ornamentals, one aim of postharvest physiologists and technologists is to regulate ripening and senescence (Wills et al. 1981). Where climacteric fruit (e.g. banana) are concerned , the specific objective is to have the fruit ripen when it is about

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to be eaten. In the cases of non-climacteric fruit (e.g. oranges) and vegetative and floral tissues (e.g. coriander, broccoli), the objective is to delay senescence in order that the tissue remains fit for consumption.

Water loss and tissue calcium content are two factors known to influence the physicochemical status of harvested horticultural crops (Joyce and Patterson 1994; Yuen 1994). Low rates of postharvest water loss can delay ripening and senescence, in addition to minimising wilting and/or shrivelling, preventing loss in saleable weight and maintaining nutritional value (e.g. vitamin C content). Similarly, in association with promoting the maintenance of cell wall and membrane stability, high tissue calcium concentrations have been correlated with delayed ripening and senescence, reduced incidence of physiological disorders (e.g. bitter pit) and increased resistance to decay, particularly that caused by wound invading microorganisms.

Mango is a commercially important crop in tropical regions of the world (Tjiptono et al. 1984). However, mango fruit have relatively short postharvest longevity, and hence limited commercial storage, transport and marketing life. This is because, being chilling sensitive, they cannot be kept for sustained periods of time at temperatures of 10–15°C (Lizada et al. 1984). In Australia, among a broad range of mango varieties, 'Kensington' is most important in fiscal terms.

This paper reports on a series of separate but related studies aiming to increase the postharvest life of harvested 'Kensington' mango fruit either by limiting postharvest water loss or by infiltrating with calcium chloride solution.

Materials and Methods

'Kensington' mango (*Mangifera indica*) fruit were harvested from farms at Alligator Creek in northern Queensland (NQ) and from Childers and Nambour in southeastern Queensland (SEQ). Fruit were harvested at commercial (mature green) maturity, except in the case of one experiment with fruit from Nambour. In this experiment, early (3 weeks early) and late (3 weeks late) maturity fruit were harvested for comparison with middle (commercial harvest time) maturity fruit. Harvested fruit were transported as quickly as possible by air (NQ) or road (SEQ) to postharvest laboratories in Brisbane, Queensland. Experiments were conducted in controlled temperature (20–23°C) facilities at St Lucia and Hamilton. In all experiments, except that on fruit from Alligator Creek, mangoes were dipped in Sportak®(0.55 mL/L; a.i. 0.45% prochloraz) fungicide for 2 minutes.

Water loss

The effect of water loss on postharvest characteristics of mango fruit was investigated using both flow-through and static relative humidity (RH) control systems. In the flow-through system, the RH of air flows passing through storage drums was regulated by mixing dry compressed air with that which had been bubbled through distilled water, in varying proportions. In the static system, RH in the headspace around fruit was regulated by varying the hole size in otherwise sealed, moisture and vapour impermeable, plastic containers.

Calcium infiltration

Reduced pressure infiltration of 4% w/v calcium chloride (commercial grade) solution into mango fruit was achieved using a large glass vacuum desiccator filled with solution, a vacuum gauge to monitor partial pressure levels and a vacuum pump to reduce the pressure in the headspace over the solution in the desiccator. Fruit were first held submerged in the solution for 0.5 minutes, then a partial pressure was drawn and maintained for 4.5 minutes, after which time atmospheric pressure was restored and the fruit kept submerged for a further 5 minutes. Infiltrated fruit were then rinsed in running deionised water.

Dye infiltration

In order to visualise the pathway of partial pressure-induced solution infiltration into fruit, mangoes were also infiltrated with Brilliant Blue food dye (50 g/L).

Assessments

Temperature and RH were monitored with Vaisala temperature and RH probes and meters. Fruit softening was monitored subjectively by hand using the following scale: 1 = hard, 2 = springy, 3 = firm (slightly soft), 4 = soft (edible) and 5 = oversoft. In some experiments, firmness was also measured objectively, using either a Chatillion force gauge or a modified McGlasson Tomato Firmness Tester (see Table captions). Postharvest weight change (water loss) was determined gravimetrically using analytical balances.

Calcium was analysed by atomic absorption spectroscopy on oven dried and dry ashed tissue samples dissolved in 1M hydrogen chloride (HCl). Light

microscopy was performed on tissue sections fixed in 3% glutaraldehyde, dehydrated in an ethanol series (30, 50, 70, 90, 100, 100% 1 hour/step except overnight in 70%), infiltrated overnight and embedded in Historesin overnight (until set), sectioned with glass knives, and stained with 0.05% toluidine blue O. Scanning electron microscopy was used to study skin tissue samples which had been snap frozen in liquid nitrogen, freeze dried and gold sputtered. The microscopes used were a Olympus BH-2 and a Jeol WINSEM respectively.

Experimental

Mango fruit were sorted and fruit with defects were discarded. Fruit were then randomly assigned into treatment units. Details of experimental design, replication and analysis are provided in table captions (see Results and Discussion).

Results and Discussion

Water loss in the flow-through system

Water (weight) loss rates from 'Kensington' fruit kept at between 5 and >95% RH inclusive ranged from 1.2 to 0.2 %/day (Table 1). Reducing the rate of water loss significantly delayed both fruit softening and the time taken to reach the respiratory climacteric peak. Holding fruit at >95% RH compared to 5% RH increased the time to peak respiration by a factor of 1.6.

Similarly, in a separate study where 'Kensington' fruit were held between 20 and 98% RH, fruit held at lower RH showed that RH significantly increased water loss rates and led to a higher degree of fruit softening as judged by deformation under an applied load (Table 2). In contrast, softening as judged by hand firmness determinations was not promoted at lower RH.

Water loss in the static system

Fruit ripening responses similar to those obtained in the flow-through RH experiments were obtained when RH was controlled in a static RH control system. In two sequential experiments, RH was controlled at between 57 and 93%, which gave an overall range of water loss rates from 1 to 0.2 %/day (Table 3). In both experiments, there was a general trend for delayed ripening in association with reduced water loss. Significant differences in greenlife between RH treatments, as assessed by fruit firmness, were evident in the two experiments.

Table 1. 'Kensington' mango fruit weight loss and shelf life parameters (hand firmness score on day 16; time [days] to the respiratory climacteric peak) in a flow-through humidity control system at 20°C. Within columns, means followed by different letters are significantly different at P=0.05 (Duncan's multiple range test [DMRT]). (Experimental: Fruit from Nambour, completely randomised design, number of fruit in trial [n] = 16, analysis of variance [ANOVA].)

Relative humidity (%)	Weight loss (%/day)	Shelf life parameters	
		Hand firmness on day 16 (score)	Time to climacteric peak (days)
5	1.23 [a]	3.9 [a]	8.82 [b]
60	0.72 [b]	3.0 [b]	9.28 [b]
>95	0.19 [c]	2.2 [c]	13.7 [a]

Table 2. 'Kensington' mango fruit weight loss and shelf life parameters recorded on day 8 (hand firmness score; flesh deformation [mm] under 500 g applied for 30 sec measured with the modified McGlasson Tomato Firmness Tester) in a flow-through humidity control system at 23°C. Within columns, means followed by different letters are significantly different at P=0.05 (least significant difference [LSD]). (Experimental: Fruit from Alligator Creek, completely randomised design, n = 50, analysis of variance.)

Relative humidity (%)	Weight loss (%/day)	Shelf life parameters	
		Hand firmness on day 8 (score)	Deformation on day 8 (mm)
20	1.39 [a]	3.7 [a]	1.27 [a]
60	0.73 [b]	4.2 [a]	1.02 [b]
98	0.28 [c]	3.8 [a]	0.91 [c]

Experiments with the flow-through system were extended to the static system because the latter may be implemented in commercial handling systems. For example, after determining optimum ratios of commodity mass to hole area, cartons with moisture vapour impermeable walls might be made with suitably located and sized holes that could control water

loss while still providing sufficient ventilation to avoid the risk of undesirable 'physiological gas' levels (i.e. low oxygen, high carbon dioxide and ethylene).

Calcium concentrations

Postharvest infiltration of mangoes with 4% w/v calcium chloride at –33 kPa resulted in a consistent increase in the skin calcium concentration by a factor of approximately 1.3 across early, middle and late maturity fruit (Table 4). Unexpectedly, calcium infiltration actually shortened the shelf life of commercially mature fruit by 1.6 days (12%), but was without significant effect on the shelf lives of early and late maturity fruit. In the case of the early harvested fruit, partial pressure infiltration with calcium chloride solution caused lenticel darkening (blackening).

In associated experiments, 'Kensington' mango fruit were infiltrated with calcium chloride solution with and without Brilliant Blue dye at partial pressures of 0, –33, –66 and –99 kPa. Co-infiltration with dye revealed that some but not all lenticels were penetrated by solution (Fig. 1a). This visual observation was supported by analytical data showing that the calcium concentration of co-infiltrated coloured lenticels was approximately two fold higher than that of uncoloured lenticels sampled from the same fruit (Table 5). Despite calcium concentration data showing a considerable difference between uncoloured and coloured lenticels in terms of calcium penetration, no differences in lenticel morphology were evident upon

Table 3. 'Kensington' mango fruit weight loss and greenlife (shelf life) parameters (time [days] to reach a hand firmness rating of slightly soft; Chatillion force gauge [time {days} to achieve depression of the flesh by 1 mm under 7 Newtons; experiment 1, fruit from Childers]; modified McGlasson Tomato Firmness Tester [time {days} to achieve depression of the flesh by 0.55 mm under 500 g applied for 30 sec.; experiment 2, fruit from Nambour]) in a static humidity control system at 20°C. Within columns, means followed by different letters are significantly different at P=0.05 (least significant difference). (Experimental: completely randomised design, n = 10, analysis of variance.)

RH (%)	Weight loss (%/day)	Greenlife	
		By hand firmness (days)	By machine firmness (days)
Childers fruit			
57	0.95 [a]	9.1 [a]	10.8 [a]
59	0.87 [b]	8.4 [a]	10.2 [a]
60	0.79 [c]	9.9 [b]	14.1 [b]
61	0.74 [c]	9.4 [a]	12.9 [b]
71	0.64 [d]	10.7 [b]	14.1 [b]
74	0.57 [e]	10.0 [b]	12.9 [b]
83	0.41 [f]	10.5 [b]	14.3 [b]
Nambour fruit			
60	0.91 [a]		2.4 [a]
85	0.56 [b]		6.3 [b]
88	0.23 [c]		10.6 [c]
91	0.17 [d]		11.2 [c]
93	0.17 [d]		11.6 [c]

Table 4. Fruit skin calcium concentration and shelf life (time [days] to eating soft) at 22°C for 'Kensington' mangoes harvested 3 weeks prior to (early), at (middle) or 3 weeks later (late) than normal commercial maturity and either left untreated (control) or subjected to partial pressure infiltration at –33 kPa of 4% w/v calcium chloride solution. Within columns, means followed by different letters are significantly different at P=0.05 (least significant difference) (Experimental: Fruit from Nambour, completely randomised design, n = 10, analysis of variance.)

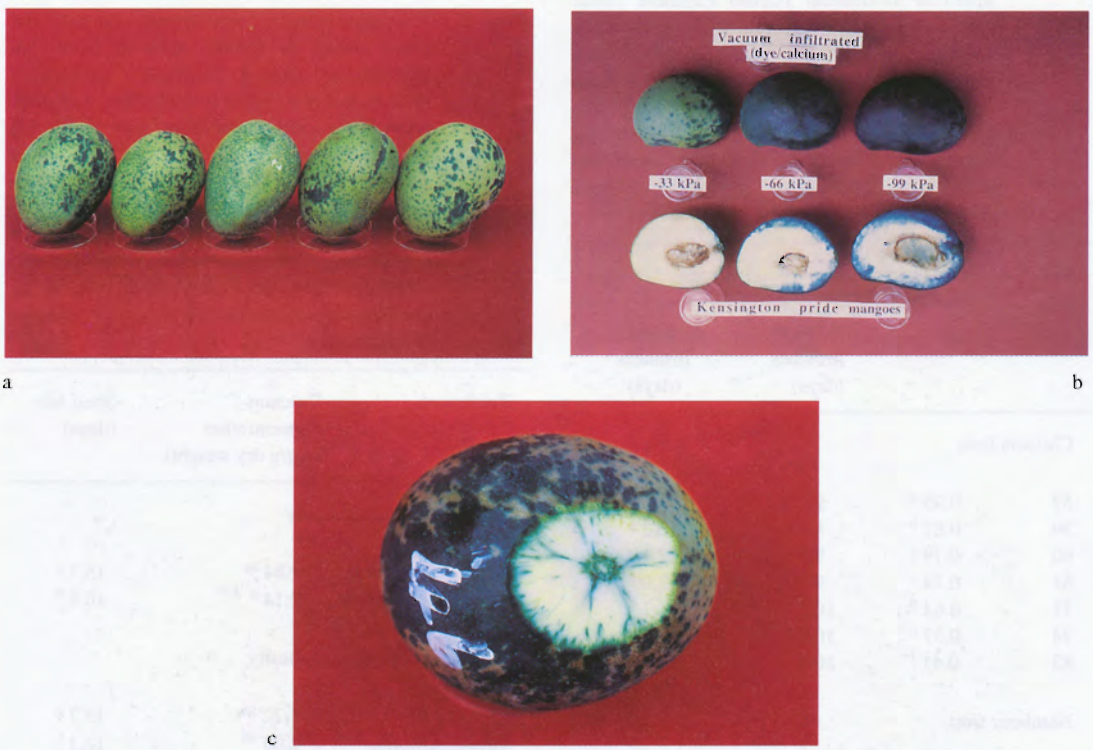
Treatment	Calcium concentration (mg/g dry weight)	Shelf life (days)
Early (–3 weeks) maturity		
control	1.64 [bc]	15.7 [a]
calcium chloride	2.14 [a]	15.5 [a]
Middle (commercial) maturity		
control	1.72 [abc]	13.7 [b]
calcium chloride	2.08 [ab]	12.1 [c]
Late (+ 3 weeks) maturity		
control	1.34 [c]	6.6 [d]
calcium chloride	1.95 [ab]	6.4 [d]

Table 5. 'Kensington' mango fruit lenticel (bulked 2mm diameter by 1 mm deep surface [epidermal] tissue cores centred on lenticels) calcium concentrations for uncoloured and coloured lenticels sampled from fruit co-infiltrated at –33 kPa with 4% w/v calcium chloride plus Brilliant Blue dye solution. Means ± standard error are presented. (Experimental: completely randomised design, n = 9.)

Lenticel sample	Calcium concentration (mg/g dry weight)
uncoloured	0.83 ± 0.07
coloured	1.86 ± 0.09

Table 6. Mango fruit skin calcium concentration and shelf life (time [days] to eating soft) at 22°C for 'Kensington' mangoes either left untreated (control) or subjected to partial pressure infiltration of 4% w/v calcium chloride solution at –33, –66 or –99 kPa. Within columns, means followed by different letters are significantly different at P=0.05 (least significant difference). (Experimental: Fruit from Nambour, completely randomised design, n = 10, analysis of variance.)

Treatment	Calcium concentration (mg/g dry weight)	Shelf life (days)
control	1.51 ^{cde}	14.1 ^{b}
–33 kPa	1.93 ^{cd}	14.1 ^{b}
–66 kPa	3.42 ^{b}	14.2 ^{ab}
–99 kPa	5.01 ^{a}	15.0 ^{a}



a b

c

Figure 1. Photographs of 'Kensington' mango fruit from Nambour (southeastern Queensland) co-infiltrated with 4% w/v calcium chloride plus Brilliant Blue dye solution showing (a) variation in dye penetration via lenticels into 5 replicate fruit after infiltration at –33 kPa, (b) lateral movement of dye into fruit infiltrated at –33, –66 and –99 kPa, and (c) movement of dye via vascular tissue into fruit infiltrated at –66 kPa.

examination by either light or electron microscopy (Figs. 2 and 3, respectively).

Decreasing the partial pressure of infiltration enhanced penetration of 'Kensington' mango fruit by dye (Fig. 1b) and calcium chloride solution, as shown by dramatically increasing skin calcium concentrations with decreasing partial pressure (Table 6). Dye was observed to penetrate through vascular tissue (Fig. 1c), as well as via lenticels. Treatment at –99 kPa caused a small but significant increase in the shelf life of 'Kensington' fruit. However, fruit quality was reduced by lenticel blackening and uneven skin coloration following calcium infiltration at the lower partial pressures of –66 and –99 kPa (Fig. 4). Calcium treatment at these lower partial pressures resulted in unacceptable levels of lenticel darkening within 3 days of treatment.

Overall, only a small, if any, gain in shelf life was achieved by infiltration of 'Kensington' fruit with calcium chloride (Tables 4 and 6). Furthermore, associated potential problems of uneven skin coloration and lenticel blackening due to 'salt burn' probably represent unacceptable risk from a commercial perspective (Fig. 4). Thus, partial pressure calcium infiltration of 'Kensington' mango fruit appears impractical.

Conclusion

From the perspective of delaying ripening of harvested mango fruit, and thus the onset of disease development, the reduction of water loss throughout the postharvest handling chain clearly merits further investigation with a view to commercialisation of appropriate packaging technology. In contrast, despite allusions in the literature to the contrary, postharvest partial pressure infiltration of mango fruit with calcium is unlikely to be commercially feasible because shelf life gains can be only small or nonexistent, and because the risk of inducing disorders (such as lenticel darkening and uneven skin coloration) is high.

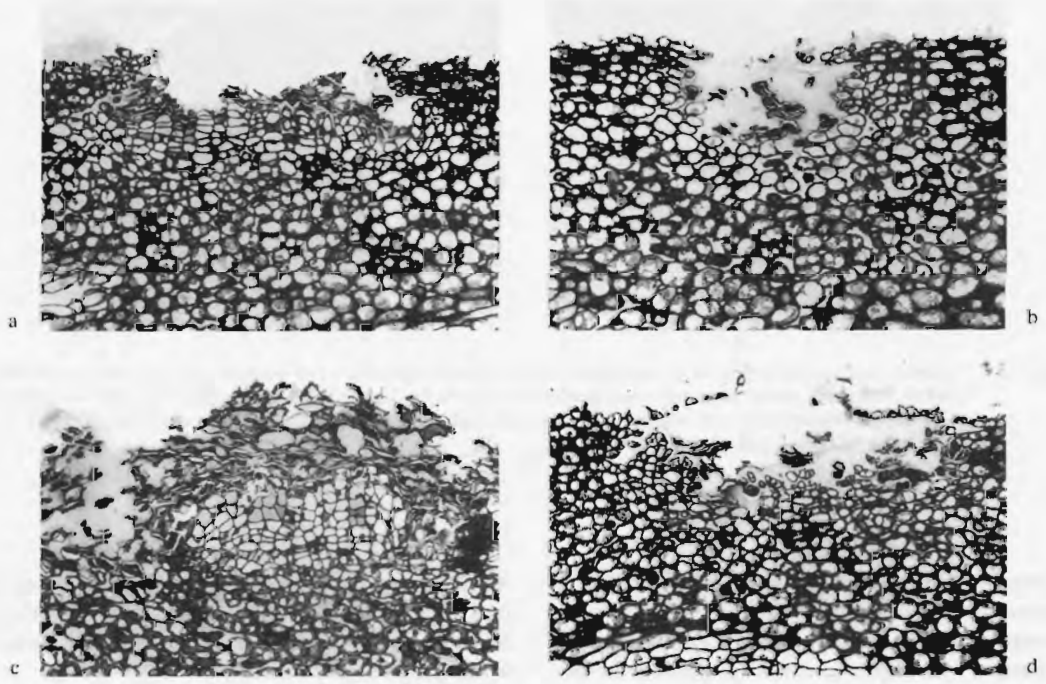


Figure 2. Light micrographs of transverse sections through uncoloured (a, b; two replicates) and coloured (c, d; two replicates) lenticels following partial pressure co-infiltration at –33 kPa of 'Kensington' mango fruit with 4% w/v calcium chloride plus Brilliant Blue dye solution. (The 4 µm thick tissue sections were photographed at a magnification of ×200.)

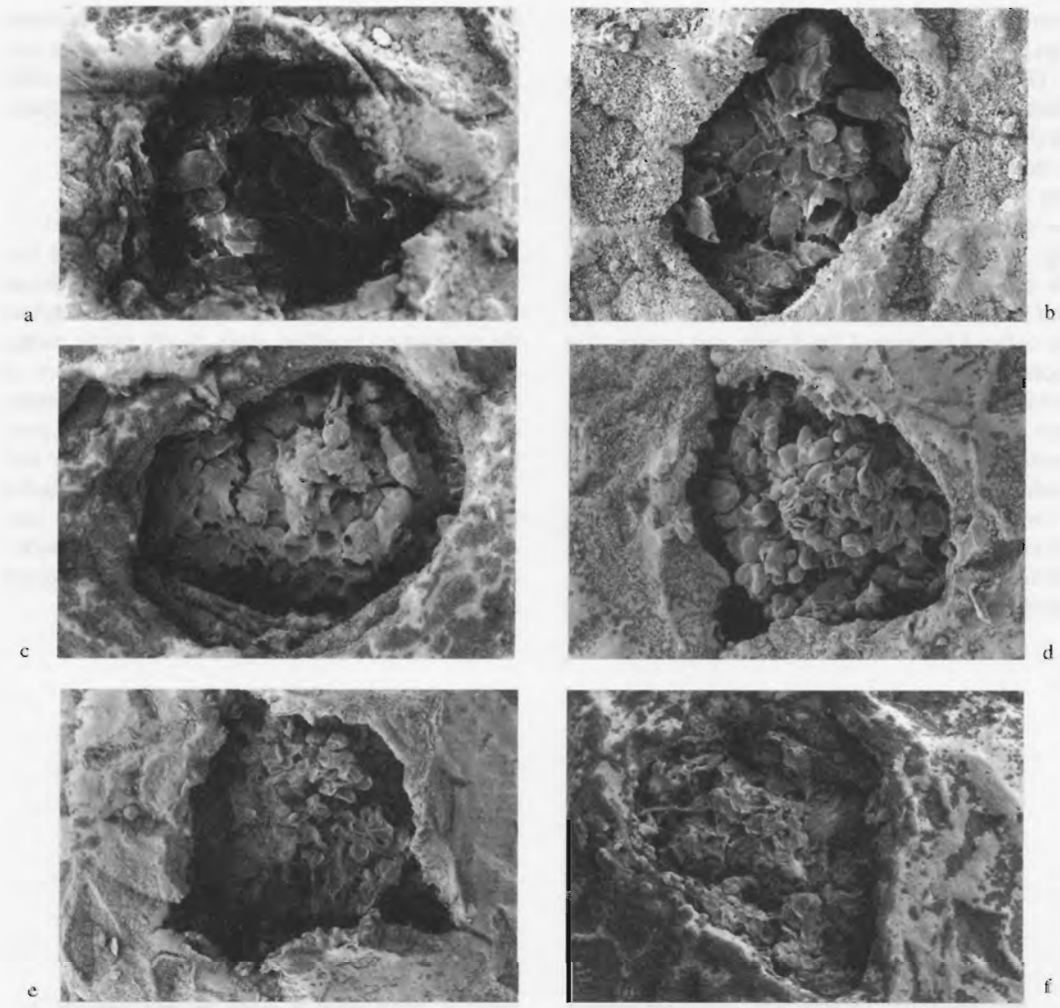


Figure 3. Scanning electron micrographs of uncoloured (a, b, c; three replicates) and coloured (d, e, f; three replicates) lenticels following partial pressure co-infiltration at –33 kPa of 'Kensington' mango fruit with 4% w/v calcium chloride plus Brilliant Blue dye solution. (Lenticels were photographed at magnifications of × 850 [a], × 550 [b], × 500 [c], ×370 [d], × 230 [e], and × 330 [f].)

Despite obvious problems caused by infiltration of calcium chloride solution into mango fruit, the partial pressure infiltration technique itself may have another application with respect to control of postharvest disease. It is tempting to speculate, on the basis of observed patterns of dye infiltration, that partial pressure infiltration of water soluble fungicides may better 'target' organisms located beneath the waxy cuticle or in the vascular system. Furthermore, better targeting may allow reduction in the concentrations at which fungicides are applied. These propositions warrant investigation in future research.

Fuller accounts of some of the studies summarised in the present report may be found elsewhere (Joyce and Shorter 1996; Macnish 1995; Akkaravessapong 1996; Macnish et al. 1997).

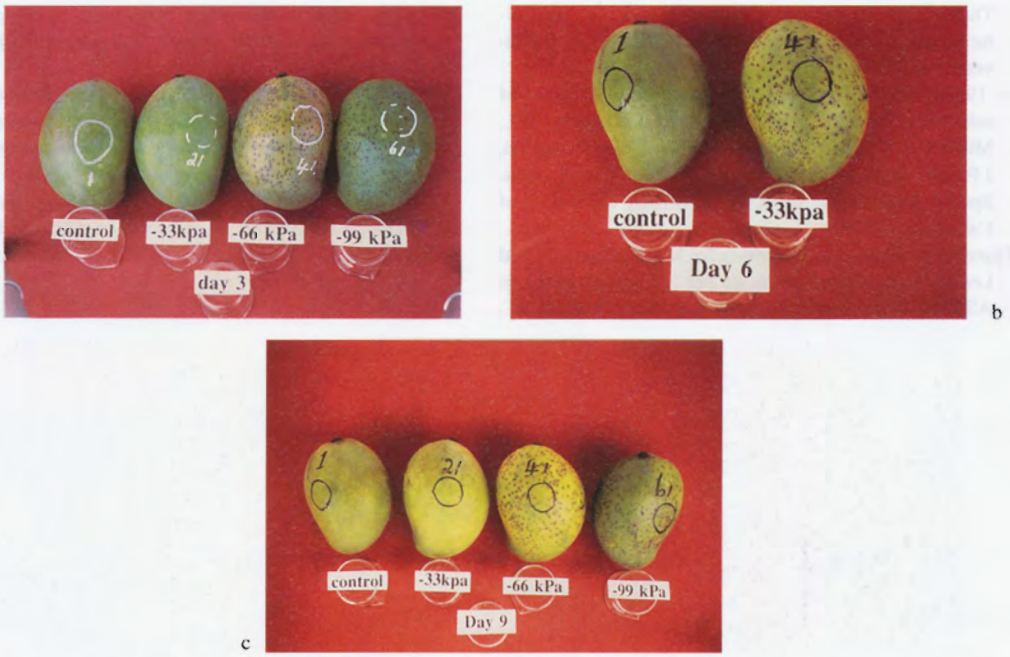


Figure 4. Photographs taken (a) 3, (b) 6 and (c) 9 days after infiltration of 'Kensington' mango fruit with 4% w/v calcium chloride solution at 0 (control), –33, –66 and –99 kPa.

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Effects of Carbon Dioxide Pulsing During the Postharvest Period on 'Nam Doc Mai' Mango Fruit

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Abstract

Harvested 'Nam Doc Mai' mango fruit were treated with 20, 50 or 100% carbon dioxide (CO_2) or normal air (control) for 24 and 48 hours, then inoculated with 10^6 spores/mL *Colletotrichum gloeosporioides* and kept at 25°C. Decay symptoms in fruit exposed to CO_2 were lower than in unexposed fruit. The treatment of 50 and 100% CO_2 for 48 hours showed better results in delaying the expansion of lesions than that of 24 hours.

To study this further, mycelial growth, spore germination, and germ tube growth of *Colletotrichum gloeosporioides* in each treatment were investigated. The growth of mycelia decreased markedly with an increase in CO_2 concentration. Germ tube growth in 20 and 50% CO_2 was slower than in normal air. In an atmosphere of 20 or 50% CO_2, spore germination was slowed, but not totally inhibited. With exposure to 100% CO_2, spore germination did not occur throughout the trial period. Application of 20 or 50% carbon dioxide stimulated appressorium formation. Levels of antifungal compounds in crude extracts of mango peel were determined. The best active band was clearly observed at Rf 0.21. The extract containing the highest level of the antifungal compound(s) came from peels in the 24-hour, 20% CO_2 treatment.

MANGO (*Mangifera indica* Linn.) is an important fruit crop in Thailand as well as in the other Asian countries. The marketing of mangoes is restricted by improper handling which results in short shelf life. Use of fungicides to control postharvest diseases is becoming more restricted because of the harmful effects of their residues. Non-fungicide treatments such as exposure to high carbon dioxide (CO_2) levels have been shown to affect fruit respiration and fungal growth during the postharvest period (Wells and Uota 1970; El-Gorrani and Sommer 1979; Prusky et al. 1991). In Thai mangoes, anthracnose is caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc., and becomes apparent in the ripe fruit. Droby et al. (1986) reported that the presence of natural antifungal compounds in the peel of unripe mango fruit was much higher than that of ripe mango fruit and showed a relationship to disease symptom development.

In this study the relationship between postharvest disease development caused by *C. gloeosporioides*, the effect of exposure of the fruit to high CO_2 concentrations, and level of antifungal compounds in 'Nam Doc Mai' mango fruit, was investigated.

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Materials and Methods

Fungus

Colletotrichum gloeosporioides was isolated from an anthracnose lesion of a naturally infected, ripe 'Nam Doc Mai' mango fruit. The fungus was maintained on potato dextrose agar (PDA) at 25°C. Portions of the isolate were prepared for studies on (i) mycelial growth, and (ii) spore germination and appressorium formation, under various conditions.

Fruit

'Nam Doc Mai' mangoes were harvested at the 'mature green' stage and selected for healthy fruit without any wounds or insect damage. Uniform fruit (300–350 g per fruit) were used for each experiment.

Effect of carbon dioxide pulsing on anthracnose disease symptoms

Mature green 'Nam Doc Mai' mangoes were exposed to 20, 50 or 100% CO_2 for 24 or 48 hours before being inoculated with 10^6 spores/mL of a *C. gloeosporioides* spore suspension. The possibility of natural infection was reduced by using only unwounded fruit. The inoculated fruit were incubated at 25°C in a moist chamber (95–99% relative humidity [RH]) for 2 days, then the diameter of anthracnose lesion was measured. Treated fruit were removed to normal air at 25°C for further investigation. The peels of mangoes were collected for analysis of extracted compounds.

Effect of carbon dioxide pulsing on mycelial and germ tube growth, spore germination and appressorium formation of *C. gloeosporioides*

For mycelial growth, mycelium was taken from the *C. gloeosporioides* isolate on PDA medium using a cork borer of 0.5 cm diameter, transferred to fresh PDA medium and kept at 25°C for 48 hours, after which time the colony diameter was about 1.5 cm. The plates were then randomly placed in 8-litre gas-light chambers. Carbon dioxide was supplied to the chambers via pressure reduction valves. The CO_2 concentrations were adjusted to 20 or 50% CO_2 by mixing with air through a manifold. The levels of CO_2 were measured by gas chromatography (GC-9A Shimadzu). The results in terms of colony diameter were compared with 100% CO_2 and air (as a control) after incubation at 25°C for 24 hours.

For examination of spore germination and appressorium formation, a spore suspension 5×10^5 spores/mL was prepared and its concentration rechecked before use. 0.02 mL of the spore suspension was put on a millipore filter and exposed to 20, 50 or 100% CO_2 and compared to a normal air control for 4, 6 ,8 10, 12, 14 or 16 hours at 25°C, then lactophenol cotton blue was immediately added to stop spore growth. The results were observed, photographed and measured under a light microscope.

Extraction of compounds from the peel

Peel tissue, 1–2 mm thick, from the CO_2-exposed fruit was homogenised for 5 min in cold 95% ethanol (150 mL/50g fresh weight tissue), using a blender. After filtration the extract was concentrated at 40°C under reduced pressure to about one-third of the original volume. The concentrated extract was partitioned twice with equal volumes of dichloromethane. The organic layers were pooled, dried with anhydrous magnesium sulphate ($MgSO_4$) and concentrated to dryness under reduced pressure (Droby et al. 1986).

Separation by thin layer chromatography and subsequent bioassay of the compounds extracted from the peel

The crude extracts from each treatment were chromatographed on plates coated with silica gel 60 GF254 (Merck), 1 mm thick, using developing solvent consisting of a mixture of n-hexane:ethyl acetate:methanol (60:40:1). Plates were developed and left for one day before spraying with spore suspensions of *Cladoporium cladosporioides* and *C. gloeosporioides* (10^6 spores/mL in potato dextrose broth) (Droby et al. 1986). After incubation in a moist chamber for 2 days, clear zones were seen. These bands contained naturally occurring antifungal compounds.

Results and Discussion

Carbon dioxide treatments of 'Nam Doc Mai' mangoes at 20, 50 or 100% for 24 or 48 hours were variably able to delay the expansion of anthracnose lesion diameter to a greater degree than the control (data for the 24-hour treatment, where the 20% concentration of CO_2 had the greatest effect, are shown in Figure 1). Treatment with CO_2 for 48 hours rather than 24 hours resulted in even less lesion development (data not shown). After inoculation and storage for 14 days, expansion of the lesions in fruit treated with CO_2 for 48 hours was less than the other treatments, as shown in Figure 2. These results, showing CO_2 pulsing before storage leads to a reduction in decay caused by *C. gloeosporioides*, are similar to studies using other crops (Stewart 1978; Kader 1986; Sitton and Patterson 1992). The effects of short periods of CO_2 pulsing on postharvest fruit condition depend upon the maturity of the fruit, temperature, and CO_2 concentration (Wang 1979). In a preliminary observation, CO_2 injury was detected in mango fruit kept at 25°C under 100% CO_2 for 72 hours (data not shown). The higher level of CO_2 and longer exposure period may delay the ripening process in the treated fruit.

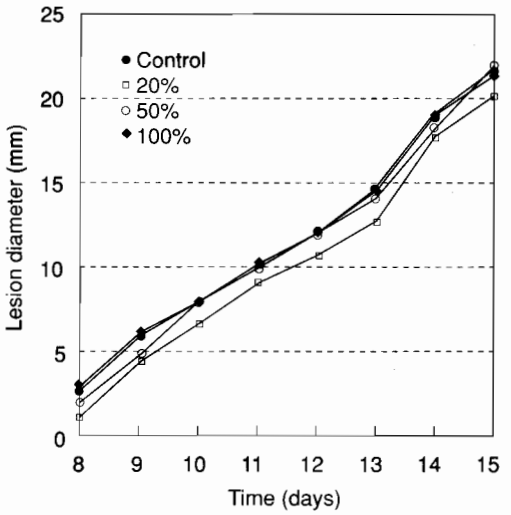

Figure 1. Diameter of anthracnose disease lesions on 'Nam Doc Mai' mango skin after inoculation with *Colletotrichum gloeosporioides* spores and subsequent treatment with 20, 50 or 100% carbon dioxide (CO_2) for 24 hours compared with the control (normal air) and storage at 25°C.

Mycelial growth and spore germination of *C. gloeosporioides* were measured after treatment with CO_2. Carbon dioxide treatment at 100% for 24 hours slowed mycelial growth (as represented by colony diameter) to a greater extent than the other treatments (Table 1). Mycelial growth was retarded by all treatments, but not totally inhibited. Similar results were observed in relation to *C. gloeosporioides* spore germination, as presented in terms of germ tube length and germination percentage (Tables 2 and 3, respectively).

Under conditions of 50% CO_2 *C. gleoesporioides* had the highest percentage of appressorium formation while spores in the 100% CO_2 treatment did not produce appressoria at all (Table 4).

Our results showed that CO_2 pulsing delayed mycelial growth and spore germination temporarily, but when returned to normal air these functions recovered very quickly, as reported in other fungi (Manners 1966; Wells and Uota 1970). In the case of appressorium formation, past studies have shown 20 and 50% CO_2 induced appressorium formation, however appressoria did not always produce infection pegs (Griffin 1994). In our experiment, the treatment using 100% CO_2 inhibited appressorium formation, which may lead to a reduction in disease symptom development on the skin of treated fruit.

After separation by thin layer chromatography, the compound showing the greatest antifungal activity was at Rf 0.21 in extracts from the peel of mango from all treatments. The inhibition zone at Rf 0.21 was larger in the 24-hour CO_2 treatments than in the control, suggesting that this treatment produced the highest amount of the putative antifungal compound (Fig. 3). The inhibition zone at Rf 0.21 of 20% CO_2 treated for 48 hours was smaller than that of 20% CO_2 treated for 24 hours as presented in Figure 4. This result did not

Table 1. Diameter (mm) of *Colletotrichum gloeosporioides* colonies after treatment with carbon dioxide compared with normal air (control) for 24 hours, and subsequent transfer to normal air at 25°C. Means within columns with different letters are significantly different at the 5% level (least significant difference test).

Treatment	Time (days)			
	0	3	5	7
Control	15.0	43.2 ^{a}	63.5 ^{p}	78.4 ^{x}
20% CO_2	15.0	39.5 ^{ab}	61.7 ^{p}	78.3 ^{x}
50% CO_2	15.0	35.5 ^{b}	56.7 ^{p}	74.2 ^{xy}
100% CO_2	15.0	18.1 ^{c}	31.7 ^{q}	52.9 ^{z}

Table 2. Germ tube length (mm) of germinated *C. gloeosporioides* spores under 20, 50 or 100% CO_2 compared with air (control) at various periods. Means within columns with different letters are significantly different at the 5% level (least significant difference test).

Treatment	Time (hours)						
	4	6	8	10	12	14	16
Control	0.000	0.016	0.038^{a}	0.070^{d}	0.098^{p}	0.126^{s}	0.157^{w}
20% CO_2	0.000	0.000	0.015^{b}	0.028^{e}	0.050^{q}	0.084^{t}	0.112^{x}
50% CO_2	0.000	0.000	0.014^{b}	0.024^{e}	0.024^{q}	0.056^{u}	0.074^{y}
100% CO_2	0.000	0.000	0.000^{c}	0.000^{f}	0.000^{r}	0.000^{v}	0.000^{z}

Table 3. Germination of *C. gloeosporioides* spores (%) under 20, 50 and 100% CO_2 for various periods of time compared with normal air (control). Means within columns with different letters are significantly different at the 5% level (least significant difference test).

Treatment	Time (hours)						
	4	6	8	10	12	14	16
Control	0	77 ^{a}	95 ^{d}	99 ^{i}	99 ^{m}	100 ^{p}	100 ^{x}
20% CO_2	0	0 ^{b}	84 ^{e}	97 ^{i}	99 ^{m}	100 ^{p}	100 ^{x}
50% CO_2	0	0 ^{b}	53 ^{f}	88 ^{j}	92 ^{m}	99 ^{p}	100 ^{x}
100% CO_2	0	0 ^{b}	0 ^{g}	0 ^{k}	0 ^{n}	0 ^{q}	0 ^{y}

Note: percentage of spore germination under 100% CO_2 for 24 hours was still 0% but after transfer to normal air increased to 95–100% within 8 hours.

Table 4. Appressorium formation (%) of *C. gloeosporioides* spores under 20, 50 or 100% CO_2 for various periods compared with normal air (control). Means within columns with different letters are significantly different at the 5% level (least significant difference test).

Treatment	Time (hours)						
	4	6	8	10	12	14	16
Control	0	0	0	0^{b}	7^{d}	17^{q}	22^{y}
20% CO_2	0	0	0	3^{a}	14^{e}	17^{q}	21^{y}
50% CO_2	0	0	0	3^{a}	16^{e}	28^{r}	31^{z}
100% CO_2	0	0	0	0^{b}	0^{c}	0^{p}	0^{x}

Note: spores under 100% CO_2 did not produce appressoria even after transfer to normal air for 16 hours.

match the pattern of disease development on the skin of mango fruit. These preliminary results show that there is at least one compound which occurs naturally in the peel of 'Nam Doc Mai' mango which has antifungal action.

In conclusion, the main effect of CO_2 pulsing on postharvest decay of 'Nam Doc Mai' mango appears to be a delaying of *C. gloeosporioides* spore germination and mycelial growth, which leads to slower symptom development in comparison to the control. Moreover, higher CO_2 levels can delay the ripening process. The potential for use of naturally occurring antifungal compound(s) to assist in the delay of postharvest decay symptoms requires further investigation.

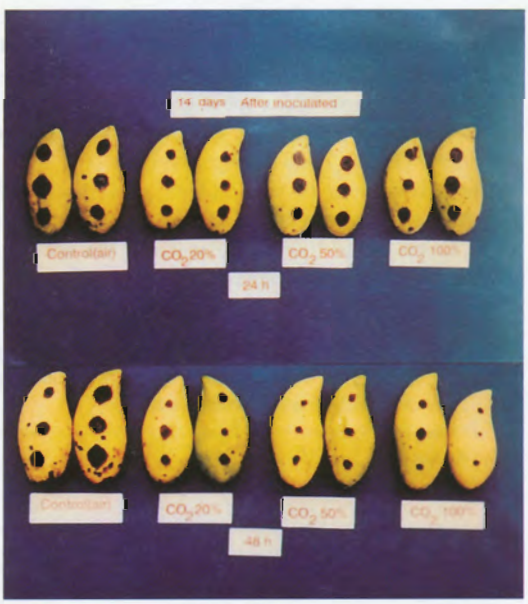

Figure 2. Anthracnose disease symptoms of 'Nam Doc Mai' mango subjected to various treatments (as labelled on the photograph) and kept at 25°C for 14 days.

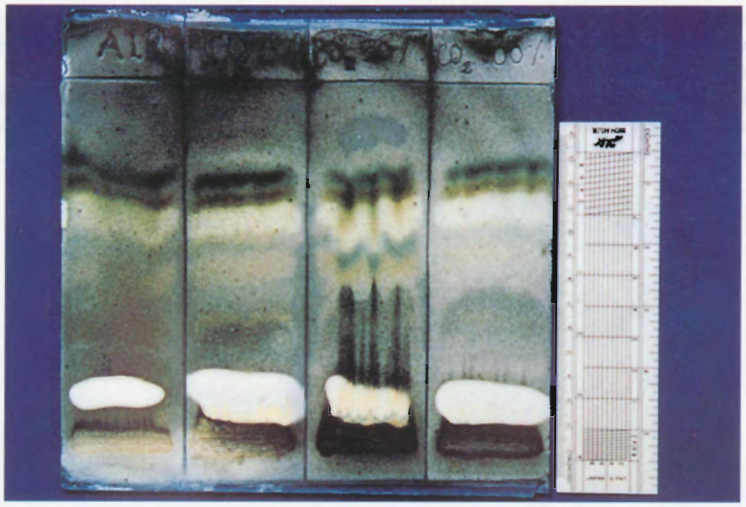

Figure 3. Inhibition zone (arrow; Rf 0.21) of the extracts from 'Nam Doc Mai' mango peels treated with (L–R) 20, 50 or 100% carbon dioxide for 24 hours compared with the control (normal air).

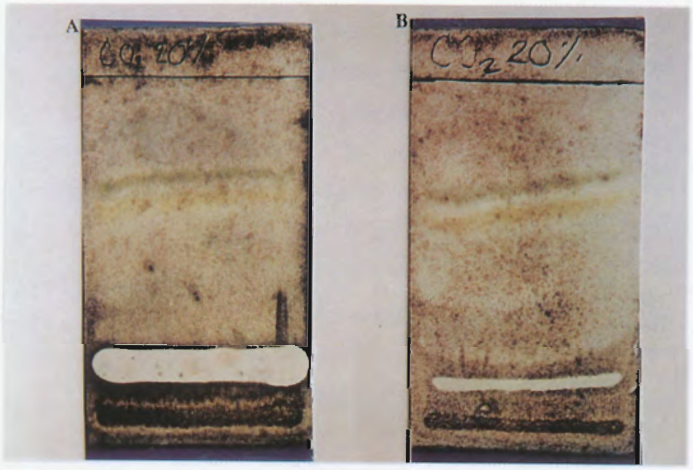

Figure 4. Comparison of inhibition zones (Rf 0.21) between the extracts from mango peel from the treatments with 20% CO_2 for (A) 24 hours or (B) 48 hours.

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Quality of 'Kensington' Mangoes after Short Duration Exposure to High Carbon Dioxide Concentrations

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Abstract

To determine the effects of pre-storage carbon dioxide (CO_2) treatment on 'Kensington' mango quality, fruit from two production districts were exposed to 20, 40 or 60% CO_2 for 1–8 days in a non-flowing system. A single comparative flowing treatment of 40% CO_2 for three days was also included. Fruit were ripened without delay, or after storage for four weeks at 10°C, and fruit quality assessed.

Total disease levels were not reduced by CO_2 treatment. Fruit pulsed with 60% CO_2 were sometimes firmer and greener during storage, and after storage had higher *Rhizopus* spp. and lower *Colletotrichum* spp. infections and more CO_2 injury (observed as a grey skin discoloration) than controls. Carbon dioxide treated fruit ripened without storage softened 0–2.5 days faster than controls. Non-stored fruit treated under the continuous flow system had higher titratable acidity (TA), were less ripe and lower in eating quality, firmer during storage and, in one locality, had higher injury levels after storage than fruit from the corresponding non-flowing CO_2 treatment. The treatments associated with reduced disease levels or increased storage potential also produced greater CO_2 injury.

Further investigations on CO_2 pulsing treatments that extend storage life with negligible CO_2 injury are required to determine the full potential for this treatment with 'Kensington' mango.

EXPOSURE to high carbon dioxide (CO_2) concentrations for short periods (pulsing) soon after harvest has extended storage life and frequently improved quality at outturn in a number of fruit. Lonsdale (1993) reduced anthracnose after storage at 11°C for 4 weeks by exposing hot water-dipped (50°C for 5 min), waxed 'Keitt' mangoes to a 30% CO_2, 15% oxygen (O_2) atmosphere for 24 hours. Similar treatments of 24 and 48 hours under 20% CO_2 did not reduce disease levels. A 10-day pulse of 20% CO_2 at the beginning of controlled atmosphere (CA) storage delayed softening and the loss of titratable acidity in apples (Couey and Olsen 1975) and pears (Couey and Wright 1977), while 12% CO_2 for 2–4 weeks prolonged storage life of pears under modified atmospheres (Wang and Mellenthin 1975). In avocado, Allwood and Cutting (1994) found that 10 and 20% CO_2 pulsing for 2 days delayed ripening of 'Fuerte' avocados after removal from storage at 3.5°C, but not with storage at 5.5°C. Pulsing with CO_2 before cold storage delayed ripening of 'Hass' avocado (20% for 7 days, Veloz et al. 1991). 'Fuerte' avocados treated with 15–30% CO_2 for 3 days (Truter and Eksteen 1987) and 20–25% CO_2 for 3 days (Eksteen and Truter 1985) had reduced internal disorders and chilling injury and improved fruit quality after cold storage, but this was not as effective as CA storage. Intermittent pulsing with 20% CO_2 for a total of 6 days within a 3-week storage period delayed senescence of 'Hass' avocado at 12°C and reduced chilling injury and fungal growth at 4°C (Marcellin and Chaves 1983).

Various techniques for application of pulsing treatments have been used. Lonsdale (1993) flushed the containers with gases to obtain the appropriate gas concentrations and then sealed the containers for the duration of the treatment. Hatton et al. (1972) added

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gases at intervals during pulsing to maintain the desired CO_2 levels, and Marcellin and Chaves (1983) alternated exposure of 'Hass' avocados to 20% CO_2 for 2 days and normal air for 5 days during the entire storage period. The pulsing temperatures were often not indicated in the above reports, but were assumed to be at the temperatures used for subsequent storage.

The research reported here investigated the potential for CO_2 pulsing to reduce disease and improve storage life in 'Kensington' mango.

Methods

Mango fruit (*Mangifera indica*, cultivar 'Kensington') were harvested from two growing regions of Australia: Katherine in Northern Territory (NT) on 5/11/95 and Nambour in southeastern Queensland (SEQ) on 29/1/96. About 120 and 240 fruit (450–500g fruit mass) were harvested, desapped on site and transported to the laboratory by air (overnight) and road (same day) from NT and SEQ, respectively.

The following day fruit were labelled and weighed. Non-flowing pulsing treatments of 20% CO_2 for 2, 4 and 8 days, 40% CO_2 for 1.5, 3, and 6 days, and 60% CO_2 for 1, 2, and 3 days were then applied by placing fruit in 12 separate 30-L plastic barrels at 10°C, flushing with CO_2 to displace air until reaching the desired CO_2 concentrations, then sealing the containers. Atmospheres were set to ±1% of the desired CO_2 concentration as measured by gas chromatography. Control fruit were placed in similar barrels supplied with normal air at a flow rate of 1 L/min for the treatment duration. One flowing pulse treatment was included where fruit were continuously flushed with 40% CO_2 at 1 L/min for 3 days. Barrels were flushed with air at the end of the individual treatments until all pulsing treatments were completed. Atmospheres were monitored using a Bishop 701 Gas Analyser to determine the time taken for the CO_2 concentrations to return to the levels of the controls.

After 8 days all fruit were placed in cartons. All NT fruit, and ten fruit per treatment from the SEQ trial were then stored at 10°C for a total storage time of 4 weeks (including the pulsing treatment time). Individual fruit quality was assessed at intervals during storage and then after ripening for 9 days at 22°C. The remaining 10 fruit per treatment from the SEQ trial were ripened at 22°C after the 8-day treatment period, and assessed for fruit quality at eating soft.

Fruit firmness was measured by hand using a scale from 6 (hard), 2.5 (eating soft) to 1 (over soft), corresponding to 45, 5 and 1 Newton (N) as measured with an Instron model 1122 (8mm hemispherical plunger and 2 mm penetration into the fruit). The shelf life of non-stored fruit was calculated as the days from harvest to eating soft (ES). Skin colour was estimated as the percentage of the skin with green colour. Fruit were rated for disease using the scale of 0 (no disease), 1 (1–5% external fruit surface affected), 2 (6–15%), 3 (16–30%), 4 (31–60%) or 5 (61–100%). In the NT trial, disease was categorised as stem-end rots (caused generally by *Dothiorella* spp.), body rots (caused mainly by *Colletotrichum* spp.) and *Rhizopus* spp. In the SEQ trial, the severity of these diseases on the side of the fruit and at the stem end was rated as above. Pathogen identity was confirmed by culturing sections from the lesion margin onto streptomycin-amended potato dextrose agar (PDA). Carbon dioxide injury was identified as a discoloration of the skin, and ranged from light grey to very dark grey/brown with a slightly sunken appearance when very intense. It was rated as % grey = (percentage of fruit surface area affected) × (intensity)/5. Intensity was rated as 1 = no grey to 5 = intense grey colour.

Five non-stored SEQ fruit per treatment were rated by a taste panel for ripeness (1 = extremely unripe to 9 = extremely ripe) and general acceptability (GA; 1 = dislike extremely to 9 = like extremely). Samples were also analysed for total soluble solids (TSS) using an Atago 3T refractometer corrected to 20°C, and titratable citric acid content (TA) by titrating with a Mettler DL20 autotitrator to pH 8.2 with 0.1N sodium hydroxide (NaOH).

Angular transformation was applied to all percentage data before analysis, and back-transformed means presented. All data was subjected to one-way analysis of variance (ANOVA) and where a significant F test was obtained, pairwise comparison of treatment means was used to give least significant differences. All statistical testing was conducted at P=0.05.

Results

After two days, CO_2 levels within the sealed barrels were about 6% above the initial concentrations. The CO_2 concentrations returned to normal atmospheric levels 13 hours after termination of the pulsing treatment and the start of air flushing.

Non-stored fruit

Shelf life was reduced by up to 2.1 days at higher CO_2 concentrations, but the response was not consist-

ent with CO_2 concentration and duration (Table 1). Pulsing had no effect on percentage green colour or disease levels at eating soft, but grey injury levels increased significantly with 40% CO_2 for 6 days and 60% CO_2 for 3 days.

The eating quality (general acceptability) of the control fruit was not significantly different to treated fruit (Table 1). The TSS decreased with increasing pulsing duration under 40% and 60% CO_2 atmospheres. There was little treatment effect on TA and ripeness, except 40% CO_2 flowing treated fruit were more acid, less ripe and had slightly lower eating quality then fruit from the similar, non-flowing treatment.

Stored fruit

After two and three weeks storage of SEQ and NT fruit, respectively, the loss of green colour was delayed in some pulsing treatments compared to controls (Table 2). However, the percentage of green on the skin was similar to controls by week 4, and there were no commercially significant treatment differences after nine days at 22°C.

Fruit from SEQ pulsed with 60% CO_2 for all durations, 40% for 1.5 days, and the flowing treatment were firmer than controls after 2 weeks storage, but showed no difference by 3 weeks. Northern Territory fruit treated with 60% CO_2 for 2 days and 40% for 4 days were still firmer than controls at removal from storage. No differences were detectable after ripening.

In NT fruit, disease was generally low, no *Colletotrichum* spp. were observed and other diseases were not significantly different from controls (data not presented). In SEQ fruit, total disease levels were not reduced by pulsing, but *Colletotrichum* spp. was reduced with 60% CO_2 (Table 2). *Dothiorella* spp. stem infections were reduced in the 40% flowing treatment, but side infections were increased with 60% for 1 day. Fruit from the 40% CO_2 flowing treatment had less stem-end infections but more side infections, and had higher levels of grey injury than those from the corresponding non-flowing treatment. All other treatments had either similar or higher disease levels than controls.

Carbon dioxide injury was sometimes present immediately after pulsing, but the intensity increased with storage. Levels were greater in treated fruit from SEQ (up to 11%) than from NT (up to 4%) and generally increased with treatment severity (Table 2). Fruit from most of the 60% CO_2 treatments in both trials, and the SEQ flowing treatment had higher injury than controls.

Discussion

The temporary delay in skin colour changes and softening during storage of some treated mangoes is similar to the response of 'Hass' avocado (Marcellin and Chaves 1983; Veloz et al. 1991). This may indicate that more severe pulsing treatments have the potential to increase storage life. However, further work is needed to confirm this, as the effect was somewhat inconsistent with region and treatment intensity.

The increase in CO_2 injury during storage is consistent with the results of Hatton et al. (1972) on grapefruit, who also described similar CO_2 injury and suggested that later-harvested fruit sustained more injury. This may partially explain the higher injury levels in SEQ fruit compared to earlier harvested NT fruit, although other pre-harvest factors (production, location, etc.) may also have affected susceptibility to injury.

Lonsdale (1993) did not report any injury after treatment of 'Keitt' mangoes with 30% CO_2 + 15% O_2. The different results in the current investigation may be due to the inclusion of a waxing and hot dipping pre-treatment by Lonsdale (1993), or cultivar and fruit growing conditions. The lack of effect of pulsing on total disease reduction in both SEQ and NT regions is similar to results of Eksteen and Truter (1985) and Truter and Eksteen (1987) in avocado.

The lower eating quality of non-stored fruit from the flowing treatment may be due to their higher TA, suggesting that this treatment delayed the reduction in TA during ripening to a greater extent than fruit firmness. The differing fruit quality results from 40% CO_2 flowing and non-flowing treatments indicates that the method of CO_2 application may be crucial, and this needs to be considered in future research. Careful description of the treatment application method is also required.

The results presented indicate that the lower CO_2 concentrations used do not provide adequate disease control or extend storage life of 'Kensington' mango. The only CO_2 treatments showing potential to reduce disease levels and extend storage life also caused CO_2 injury (mainly 60% CO_2). Thus, under the conditions tested, there appears to be little commercial potential for CO_2 pulsing to increase shelf or storage life or reduce disease in 'Kensington' mango. Other methods of CO_2 application need to be investigated to determine the potential for this treatment to extend storage life. Alternating CO_2 exposure with air during storage may have potential.

Table 1. The quality of 'Kensington' mango fruit from southeastern Queensland following carbon dioxide (CO_2) pulsing at differing concentrations and durations, and ripening at 22°C to eating soft (GA = general acceptability taste parameter rated from 1=dislike extremely to 9=like extremely, Ripeness rated from 1=extremely unripe to 9 = extremely ripe, TSS = total soluble solids, TA = titratable citric acid). Means followed by the same letter within rows are not significantly different at P=0.05.

% CO_2	Control		20%			40%			60%			40% flowing
Days pulsing			2	4	8	1.5	3	6	1	2	3	3
Shelf life (days)	18.8^{ab}	19.2^{a}	18.2^{abc}	17.1^{cd}	18.6^{abc}	19.2^{a}	16.4^{d}	18.0^{abcd}	17.5^{bcd}	17.1^{cd}	17.1^{cd}	17.3^{bcd}
Injury(%)	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	0.0^{c}	2.2^{b}	0.0^{c}	1.0^{bc}	11.3^{a}	0.2^{bc}
GA (1–9)	5.7^{de}	6.6^{abc}	6.5^{abc}	6.3^{abc}	6.7^{abc}	6.1^{cde}	7.1^{a}	6.2^{bcd}	6.4^{abc}	6.3^{bcd}	6.9^{ab}	5.4^{e}
Ripeness(1–9)	5.5^{ab}	5.5^{ab}	5.3^{b}	5.9^{a}	5.4^{ab}	5.7^{ab}	5.1^{b}	5.5^{ab}	5.3^{ab}	5.5^{ab}	5.1^{b}	4.1^{c}
TSS (%)	15.2^{abc}	15.0^{abc}	14.7^{bcd}	14.9^{bcd}	15.2^{abc}	15.0^{abc}	14.1^{de}	13.7^{e}	15.8^{a}	15.4^{ab}	14.3^{cde}	14.1^{de}
TA (%)	0.16^{c}	0.17^{bc}	0.15^{c}	0.15^{c}	0.16^{bc}	0.16^{bc}	0.17^{bc}	0.20^{b}	0.16^{bc}	0.16^{bc}	0.17^{bc}	0.25^{a}

Table 2. The quality of 'Kensington' mango fruit from the Northern Territory and southeastern Queensland (SEQ) following carbon dioxide (CO_2) pulsing at differing concentrations and durations, and storage for a total of 4 weeks at 10°C and ripening for 9 days at 22°C. Firmness was rated on a scale from 6 (hard), 2.5 (eating soft) to 1 (over soft). Disease was rated such that 0=no disease, 1 = 1–5% external fruit surface affected, 2 = 6–15%, 3 = 16–30%, 4 = 31–60% and 5 = 61–100%. The percentage grey injury on SEQ fruit was measured at storage removal. Means followed by the same letter within rowsare not significantly different at P = 0.05.

% CO_2	Control		20%			40%			60%			40% flowing
Days pulsing			2	4	8	1.5	3	6	1	2	3	3
Northern Territory												
Injury (%)	0.0^{cd}	0.0^{d}	0.0^{cd}	0.0^{d}	0.0^{cd}	0.0^{cd}	0.0^{cd}	1.0^{bc}	0.0^{d}	4.0^{a}	3.0^{ab}	0.0^{cd}
Firmness 3 weeks (6–1)	5.7^{bc}	5.3^{e}	5.4^{de}	5.6^{cd}	5.5^{cde}	5.6^{cd}	5.8^{ab}	5.7^{abc}	5.7^{bc}	5.9^{a}	5.9^{ab}	5.5^{cde}
Firmness 4 weeks (6–1)	5.1^{cde}	4.9^{e}	5.0^{de}	5.0^{e}	5.0^{de}	5.0^{e}	5.2^{bcd}	5.4^{a}	5.1^{cde}	5.3^{ab}	5.2^{abc}	5.0^{de}
Green skin at 3 weeks (%)	68.0^{c}	80.0^{bc}	88.0^{ab}	88.0^{ab}	81.0^{bc}	87.0^{ab}	91.0^{ab}	91.0^{ab}	91.0^{ab}	95.0^{a}	94.0^{a}	90.0^{ab}
Southeastern Queensland												
Injury (%)	0.0^{c}	0.0^{c}	1.0^{bc}	2.0^{bc}	2.0^{bc}	0.0^{bc}	1.0^{bc}	1.0^{bc}	11.0^{a}	3.0^{abc}	10.0^{a}	5.0^{ab}
Firmness at 2 weeks (6–1)	5.7^{cd}	5.6^{cd}	5.9^{abc}	5.9^{abc}	5.7^{bcd}	6.0^{a}	5.8^{abcd}	5.8^{abcd}	6.0^{ab}	6.0^{ab}	6.0^{ab}	6.0^{a}
Green skin at 2 weeks (%)	96.0^{b}	96.0^{b}	100.0^{a}	100.0^{a}	98.0^{ab}	100.0^{a}	100.0^{a}	99.0^{ab}	100.0^{a}	100.0^{ab}	100.0^{a}	100.0^{a}
Total disease (0–5)	4.3^{a}	4.0^{a}	4.5^{a}	4.4^{a}	4.5^{a}	4.2^{a}	4.3^{a}	4.0^{a}	4.8^{a}	4.4^{a}	4.8^{a}	4.7^{a}
Dothiorella spp. at stem (0–5)	1.8^{abc}	2.1^{ab}	2.1^{ab}	1.4^{abcd}	0.7^{bcd}	2.8^{a}	2.1^{ab}	1.5^{abcd}	0.4^{cd}	0.5^{cd}	0.8^{bcd}	0.0^{d}
Dothiorella spp. on sides (0–5)	0.7^{cd}	0.0^{d}	0.2^{d}	0.7^{cd}	1.1^{bcd}	1.1^{bcd}	1.3^{bcd}	2.3^{ab}	2.5^{ab}	1.2^{bcd}	2.0^{abc}	3.2^{a}
Colletotrichum spp. (0–5)	3.2^{a}	2.1^{abc}	1.9^{abcd}	1.5^{bcde}	2.2^{ab}	1.6^{bcde}	2.0^{abcd}	1.8^{bcde}	0.7^{de}	0.8^{cde}	0.6^{e}	0.9^{bcde}
Rhizopus spp. (0–5)	1.3^{bc}	1.0^{bcd}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	0.0^{d}	1.2^{bc}	1.7^{b}	2.8^{a}	0.3^{cd}

High nitrogen pulsing reduced chilling injury and delayed softening of 'Fuerte' avocado (Pesis et al. 1994), but resulted in unacceptable injury in 'African Pride' custard apple (Smith and Meiburg, unpublished data). Further research should also investigate the influence of growing conditions and maturity.

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Bagging of Mango (*Mangifera indica* 'Keitt') Influences Fruit Quality and Mineral Composition

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Abstract

Bagging fruit during growth is used commercially in a number of countries to improve fruit appearance. However, it may also reduce attractive colour because of the exclusion of light, and interfere with fruit calcium accumulation. To investigate this, mango fruit were bagged at various times during fruit growth, and quality and mineral concentration of the ripe fruit determined.

Anthracnose and stem-end rot (SER), caused by *Colletotrichum* and *Dothoriella* spp., respectively, were reduced by bagging, with longer bagging times generally resulting in reduced disease. Bagging increased the percentage area of skin with yellow colour at the eating soft stage. The percentage of skin with red colour, and its intensity, decreased with increasing duration of bagging. Fruit calcium concentrations were reduced by bagging for 56 days, but not by longer bagging times (82–131 days). Other quality attributes were generally unaffected by bagging.

These results indicate that bagging can improve fruit quality through reduction in disease, and this benefit outweighs the negative effects of bagging on skin colour of the 'Keitt' cultivar.

FRUIT bagging during fruit growth is used in Japan for the production of high quality, unblemished fruit (Kitagawa et al. 1992) and wrapping of fruit in newspaper is used in several Asian countries for fruit fly control. Bagging is also being used on a limited scale with late season mango cultivars in Queensland, where fruit are harvested in March for the higher priced markets. While some benefits (e.g. reduction in physical damage) might be expected, there can also be negative effects on quality, such as reduced red skin colour (Arakawa 1991). We investigated bagging of 'Keitt' mango to identify the potential for improving fruit quality.

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Fruit were bagged with white paper bags at about 100 days before harvest on two orchards, and on a third orchard at 131, 105, 82, 56 and 31 days before harvest. Fruit were harvested when mature, and the fruit quality assessed following ripening at 22°C. All bagging treatments increased the percentage of the skin area with yellow colour at the eating soft stage (Fig. 1A). The percentage of the skin with red colour, and its intensity, decreased with increasing duration of bagging. Bagging also reduced the red colour in apple (Arakawa 1991), and is associated with a reduction in anthocyanin production (Saure 1990).

Anthracnose and stem-end rot (SER), caused by *Colletotrichum* and *Dothiorella* spp. respectively, were reduced by bagging (Fig. 1B). This was also observed in a previous season (data not presented). Stem-end rot severity continued to decline with increasing bagging duration, but there was no further consistent reduction in anthracnose severity with bagging durations longer than 56 days. This reduction in anthracnose incidence and severity would have been

partly through a reduction in exposure of fruit to disease propagules. The negligible additional benefit of bagging for longer than 56 days suggests that fruit infection occurred mainly after 56 days from harvest in this experiment, even though infection can occur at any time during fruit development in association with rain (Johnson et al. 1992). *Dothiorella* spp. are endophytes and infect fruit by growth through the inflorescence and peduncle during fruit development (at least in the 'Kensington Pride' cultivar; Johnson et al. 1992). Therefore, the mechanism by which bagging reduced SER is less clear.

Fruit calcium concentrations were reduced by bagging for 56 days or less in the 1994/95 trial, but not by longer bagging times (82 to 131 days; Fig. 1C). The percentage dry matter was higher, and days to ripen fewer, in bagged fruit from only one of the trials. Fruit mass, flesh colour, total soluble solids, acidity, and eating quality were generally not affected by bagging.

These results indicate that bagging can improve fruit quality through reduction in disease. This benefit could outweigh the negative effects of bagging on skin colour in the 'Keitt' cultivar.

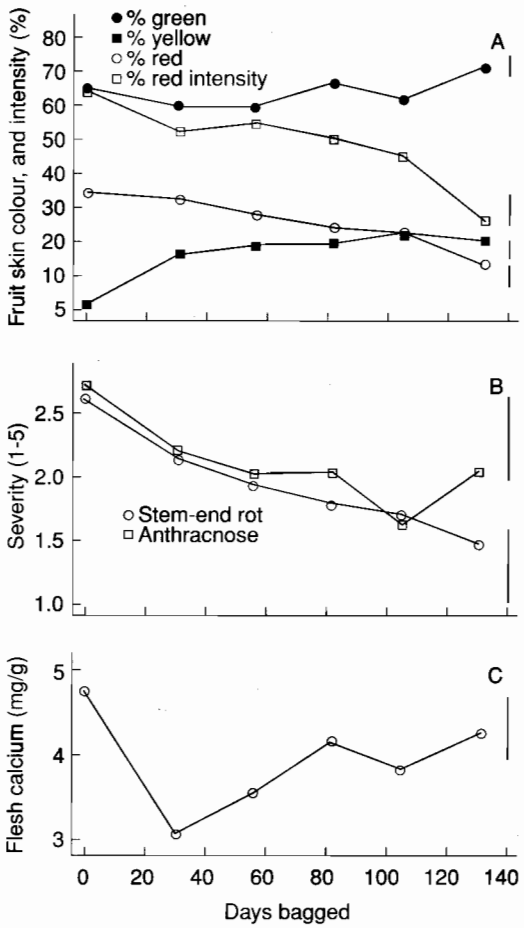

Figure 1. Relationships for 'Keitt' mango between the number of days fruit are bagged before harvest and (A) skin colour at eating soft (expressed as percentage of skin surface area covered with green, yellow or red colour. Red intensity is from 0 [no red] to 100 [intense red]); (B) disease severity (1 = no disease, 2 = 1–5%, 3 = 6–15%, 4 = 16–30% and 5 = 31–100% surface area affected) of anthracnose and stem-end rot, 27 days after harvest; and (C) flesh calcium concentration at eating soft. Vertical bars represent least significant difference (LSD; P = 0.05) for each adjacent data set line. After Hofman et al. 1997.

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Effect of Fruit Bagging, Fruit Position, Cultivar, and Postharvest Treatment on Postharvest Diseases of Mangoes

S. Sangchote*

Mango fruit harvested from the upper (2.5–3.0 m above the ground) and lower (0.5–1.0 m above the ground) position of trees about 3 m tall showed the same levels of anthracnose incidence at 97% but severity differed (20.9 and 30.9 %, respectively). Fruit harvested from the inside versus the outside of the tree canopy showed no difference in anthracnose disease incidence. Fruit inside the canopy had higher stem-end rot incidence than outside. Direction of fruit on the tree had no effect on anthracnose and stem-end rot incidence. In a study made over a two-year period, 98.5% and 5.5% of unbagged 'Palmer' mango fruit had incidence of anthracnose and stem-end rot, respectively. In these fruit, disease severity was 36.2 and 14.3%, respectively. In contrast, bagged fruit had anthracnose and stem-end rot incidence of 26%, 3.5% and disease severity of 1.7 and 15.7%, respectively.

Fruits of mango cultivars 'Haden', 'Alphonso', 'Tommy Atkins', and 'Palmer' were investigated for their susceptibility to postharvest diseases which were infected naturally. 'Alphonso' was the most susceptible to stem-end rot (*Lasiodiplodia theobromae*) while 'Haden', 'Palmer', and 'Tommy Atkins' were more susceptible to anthracnose. Dipping fruits in hot ethanol at 51°C for 5 minutes reduced disease severity from 57.3 to 19.6%.

MAJOR fruit diseases of mangoes are anthracnose (*Colletotrichum gloeosporioides*) and stem-end rot (*Lasiodiplodia theobromae*, *Dothiorella* spp., *Phomopsis mangiferae*) (Sangchote 1987). Anthracnose infection of the fruit starts in the field. Terapawa et al. (1982a) reported that preharvest spraying with benomyl (Benlate 50% W.P.) at a rate of 0.5 g/L from flowering to harvest, at 15-day intervals, could reduce anthracnose incidence by 40–50%. However, spraying in the field with this chemical induced resistant strains (Vichitranon 1982). Another practice used to improve fruit quality is fruit bagging, which has been successful in reducing the incidence of anthracnose and stem-end rot (Terapawa et al. 1982b). Postharvest treatment of mango fruit by dipping in 500 ppm benomyl at at 52°C for 5 minutes, followed by 250 ppm prochloraz at ambient temperature for 30 seconds, was an effective control against disease (Sangchote 1989). Because of public health concerns, however, use of chemicals has had to be limited. Therefore, alternative treatments are sought, especially those which are non-chemical. In this paper, preharvest factors affecting postharvest diseases, including fruit position, fruit bagging, varietal susceptibility, and a postharvest ethanol treatment, are investigated.

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Materials and Methods

Fruit position

Mango trees at Petchaboon Experiment Station (Petchaboon province, Thailand), about 3 m high, were used in this experiment. Fruit at the mature green stage were harvested from 10 trees from two positions on the tree: (i) lower (0.5–1.0 m above the ground) and (ii) upper (2.5–3.0 m), using 100 fruit from each position (25 fruit/replication). Fruit were ripened at room temperature and disease incidence (% of diseased fruit) and severity (% area affected on the fruit on average) were checked.

Mango trees at Pakchong Experimental Station (Nakorn Rachasima province, Thailand) were used to

Table 1. Disease incidence (% of fruit affected) and severity (% area of fruit affected, on average) of mango fruit harvested from upper and lower positions on the tree. Means in columns followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test (DMRT).

Position of the fruit	Disease incidence		Disease severity	
	Anthracnose	Stem-end rot	Anthracnose	Stem-end rot
Lower part of the tree	97[a]	11[a]	30[a]	28[a]
Upper part of the tree	97[a]	8[a]	20[b]	24[a]

investigate the effect of two other fruit positions—inside or outside the plant canopy—on postharvest diseases. One hundred fruit/position (25 fruit/replication) were harvested, then ripened at room temperature and disease incidence checked.

Direction of fruit on the tree

Mature green mango fruit were harvested from the north, south, east, and west sides of the trees from Petchaboon Experiment Station using 100 fruit/direction (25 fruit/replication). Fruit were ripened at room temperature and disease incidence checked.

Fruit bagging

Mango trees of the cultivar 'Palmer' were used. One month after flowering, each fruit was bagged with a clear plastic bag (polyethylene) with eight holes (0.5 mm in diameter) for ventilation. Bagged and unbagged fruit were harvested at maturity, 100 fruit for each treatment (25 fruit/replication), ripened at room temperature, and disease incidence and severity checked. This experiment was conducted over a 2-year period.

Varietal susceptibility

Mango fruit of 'Alphonso', 'Haden', 'Palmer', and 'Tommy Atkins' cultivars were investigated for their comparative susceptibility to natural infection by postharvest pathogens using 100 fruit/cultivar. Fruit were harvested at the mature green stage, ripened at room temperature, and checked for disease incidence and severity.

Postharvest treatments

Mango fruit were harvested at the mature green stage, inoculated with *Colletotrichum gloeosporioides* at a concentration of 4000 spores/mL, incubated in a moist chamber for 24 hours, then dipped in ethanol at concentrations of 10, 20, 30, 40% at 51°C for 5 minutes using 50 fruit/treatment. Fruit were ripened at room temperature and checked for anthracnose severity.

Results

Fruit position

Mango fruit harvested from the lower part of the tree, 0.5–1.0 m above the ground, showed anthracnose disease incidence of 97% with an average disease severity of 30.9%. Fruit in upper positions (2.5–3.0 m) also showed the same incidence, but severity was lower (20.9%). Fruit from the lower position showed stem-end rot incidence of 11% with an average severity of 28.2%. Fruit from the upper position had stem-end rot incidence and severity of 8% and 24.4%, respectively (Table 1). Fruit harvested from inside or outside the plant canopy showed no difference in anthracnose disease incidence. However, fruit from inside the canopy had higher incidence of stem-end rot than from outside (Table 2).

Table 2. Postharvest disease incidence (% of fruit affected) on ripened mango fruit harvested from different positions on the tree. Means in columns followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Position within plant canopy	Disease incidence	
	Anthracnose	Stem-end rot
Inside	28[a]	28[a]
Outside	28[a]	12[b]

Direction of fruit on the tree

Direction of fruit on the tree had no significant effect on incidence of anthracnose and stem-end rot (Table 3).

Fruit bagging

Bagging of fruit during maturation gave a significant reduction in anthracnose incidence and severity.

However this benefit was not significant for stem-end rot. Unbagged, ripened 'Palmer' mango fruit had 98% anthracnose and 5% stem-end rot incidence, and disease severity was 36 and 14%, respectively. In contrast, bagged fruit had incidence of anthracnose and stem-end rot of 26% and 3%, and disease severity was 2 and 16%, respectively (Table 4).

Varietal susceptibility

Comparison of mango cultivars showed that 'Haden', 'Palmer', and 'Tommy Atkins' had a high incidence of anthracnose (100, 100, and 88.4%, respectively) with varying degrees of severity, whereas 'Alphonso' exhibited no symptoms. In contrast, 'Haden', 'Palmer', and 'Tommy Atkins' showed no stem-end rot but 'Alphonso' had a stem-end rot incidence of 39% and severity of 14% (Table 5).

Postharvest treatments

Dipping fruit in hot 40% ethanol at 51°C for 5 min reduced anthracnose severity on ripened fruit from 54% to 20% as compared with untreated fruit (Table 6).

Table 3. Disease incidence (% of fruit affected) in mango fruit harvested from different directions on the tree and ripened at room temperature (~28°C). Means in columns followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Direction	Disease incidence	
	Anthracnose	Stem-end rot
North	100[a]	22[a]
South	100[a]	24[a]
West	95[a]	18[a]
East	100[a]	18[a]

Table 6. Anthracnose disease severity (% area of fruit affected, on average) on mango fruit treated with water or ethanol at different concentrations at 51°C for 5 minutes and ripened at room temperature (~28°C). Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Treatment	Disease severity
Hot water	30[bc]
10% ethanol	28[b]
20% ethanol	34[c]
30% ethanol	27[b]
40% ethanol	20
Untreated	54[d]

Table 4. Disease incidence (% of fruit affected) and severity (% area of fruit affected, on average) of unbagged and bagged mango fruit harvested and ripened at room temperature (~28°C). Means in columns followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Treatment	Disease incidence (%)		Disease severity (%)	
	Anthracnose	Stem-end rot	Anthracnose	Stem-end rot
Unbagged fruit	98.5[a]	5.5[a]	36.2[a]	14.3[a]
Bagged fruit	26.0[b]	3.5[a]	1.7[b]	15.7[a]

Table 5. Disease incidence and severity of mango fruit of different cultivars harvested and ripened at room temperature (~28°C). Means in columns followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Cultivar	Disease incidence (%)		Disease severity (%)	
	Anthracnose	Stem-end rot	Anthracnose	Stem-end rot
'Alphonso'	0.0	38.7	0.0	13.5
'Haden'	100.0[a]	0.0	53.3[a]	0.0
'Palmer'	100.0[a]	0.0	41.0[a]	0.0
'Tommy Atkins'	88.4[a]	0.0	20.0[b]	0.0

Discussion

These experiments indicated that position on the tree had no effect on anthracnose incidence, however disease severity was higher in fruit from lower positions on the tree than from higher up. Trees used in this experiment were about 3 m high so the difference in distance between the fruit of the lower part and the upper part was not great. Mango fruit used were harvested in the rainy season. Furthermore, in the field used in this trial, anthracnose also infected leaves. Increasing anthracnose in mango is positively correlated with amount of rainfall and environmental conditions after rain episodes that favour infection (Fitzell et al. 1984; Dadd et al. 1991). Mango fruit on the lower part of the tree were under conditions which favoured infection for a longer period than those higher up the tree. Therefore it is consistent that disease severity on these fruit was greater.

Comparison of mango cultivars revealed differences in susceptibility to anthracnose and stem-end rot in the four cultivars tested which could be considered when choosing the most suitable cultivars to use in particular situations.

The usual purpose of fruit bagging is to prevent fruit fly infestation. However, our experiment showed that bagging also interferes with fruit fungal infection. Mangoes are subject to various leaf, fruit, and blossom diseases which cause major losses to the industry (Lonsdale 1992). Control of these diseases has been achieved by preharvest spraying (Pelser and Leser 1989). Our study indicates that fruit bagging is an additional practice which could reduce these diseases. Postharvest dipping of the fruit in ethanol is another treatment worthy of further investigation in this regard.

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Effect of Locality, Irrigation, and Paclobutrazol on Quality of 'Hass' Avocado

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Abstract

To test the effects of locality and/or cultural practices on 'Hass' avocado quality, fruit were harvested from orchards in different locations in southeastern Queensland at three stages of maturity (approximately 24, 29 and 34% dry matter [DM]). Fruit were either ripened at 22°C or stored at 7°C for 3 weeks before ripening. Fruit shape and mass, and days to eating soft varied with production location. The days to eating soft declined with later harvests in fruit from most, but not all, locations. Anthracnose severity was either not affected by harvest, or increased with later harvests in seasons where higher severity occurred.

In a second series of experiments over two seasons, seven-year-old trees were re-watered to field capacity when tensiometer readings reached –20, –40 or –70 kPa. Tree yield increased with more irrigation, due mainly to increased fruit number. The differences in yield between trees increased with less irrigation. There was little effect on fruit quality or fruit mineral concentrations.

In a third series of experiments, seven-year-old 'Hass' trees were either fertilised with 1 kg per tree of urea at panicle emergence, foliar sprayed with 2.5 g/L (a.i.) paclobutrazol (as Cultar®) at mid-bloom, both urea and paclobutrazol treatments applied, or untreated. Tree yield over three seasons was not affected by the treatments, but paclobutrazol treatments increased average fruit size and the number of fruit in the larger size categories. There was little treatment effect on fruit quality.

In all three experiments, there were significant positive correlations between fruit calcium concentrations and days to eating soft, and negative correlations with anthracnose severity. There were weaker correlations with fruit magnesium and potassium. This indicated a large influence of individual trees on fruit mineral concentrations and quality, and the potential to increase the level and consistency of quality through better selection and use of clonal rootstocks. Under Australian conditions, this is likely to produce better gains in productivity and quality than further refinement of other cultural practices.

SUCCESSFUL marketing of horticultural products is facilitated by having predictable and consistent product quality. Postharvest performance of fruit can be influenced by both cultural and climatic factors (Monselise and Goren 1987; Hofman and Smith 1994), and fruit mineral composition, particularly calcium, can play a major role (Ferguson 1980). An understanding of the effects of production factors and cultural practices on quality will improve predictability of fruit response to postharvest handling.

This paper reports some of the results obtained from a research program aimed at understanding the interactions between production location, irrigation and growth regulators (paclobutrazol) on the quality of 'Hass' avocado fruit.

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Materials and Methods

Effect of production location/cultural practices

Five uniform 6 to 12-year-old 'Hass' avocado trees were selected from each of five commercial orchards in southeastern Queensland between latitudes 25–27.5°S (see Table 1 for locations). Twenty (1993) or 40 (1994) fruit of 250–300 g mass each were harvested over two seasons at early, mid and late maturity (approx. 24, 29 and 34% flesh dry matter [DM] respectively) from the northern aspect of the canopy, at 1.5 to 3 m above ground and 0 to 0.5 m into the canopy, and immediately dipped in 0.55 mL/L Sportak® for 30 sec. For each harvest, 10 fruit per tree were ripened at 22°C under humidified, ethylene-free air in ventilated containers, and the remainder stored at 7°C for 3 weeks before ripening at 22°C.

Irrigation

Seven-year-old 'Hass' avocado trees growing on clay loam soil (krasnozem) were selected in a commercial orchard at Childers (southeastern Queensland, latitude 25°S). The climate is described as warm, humid, subtropical with a mean annual rainfall of 1000 mm in a summer/wet, winter/dry pattern. Trees were irrigated over two cropping cycles to field capacity using under-tree micro-sprinklers when tensiometer readings at 30 cm soil depth reached –20, –40 or –70 kPa soil matric potential (Ψ_s). Ten uniform trees per row were used, with each row receiving one of the irrigation treatments.

Twenty (1994) or 36 (1995) fruit of average size were harvested from each tree, as described above, and immediately dipped in 0.55 mL/L Sportak® for 30 sec. Ten (1994) or 12 (1995) fruit per tree were ripened as above, and the remainder stored at 7°C for 3 or 5 weeks, or at 2°C for 5 weeks, then ripened at 22°C as above.

Paclobutrazol

The experimental trees and orchard site were similar to those for the irrigation experiments. The following treatments were applied to each of five single tree replications, in a randomised block design:

1. untreated control (standard commercial practice),
2. urea, soil applied at the rate of 30% of annual nitrogen (1 kg/tree) at panicle emergence,

Table 1. Effect of production location in southeastern Queensland (characterised by the maximum average temperatures in January), on fruit characteristics of 'Hass' avocado fruit at harvest. Data presented are means of 180 fruit from three harvests in each of two seasons. Means for location followed by the same letter are not significantly different when separated by least significant difference at P = 0.05.

Location	Max. temp. January (°C)	Mass (g)	Length: diameter ratio	Shape	Skin texture	Skin thickness	Flesh colour[a]
Childers	30.4	256 [b]	1.35 [a]	oval	rough	thick	3.5 [bc]
Nambour	28.2	257 [b]	1.40 [abc]	oval	rough	thick–very thick	2.9 [a]
Gatton 1A	31.0	287 [d]	1.39 [abc]	oval–short pyriform	relatively smooth	medium thick	4.0 [d]
Gatton 1B	31.0	271 [c]	1.43 [bcd]	oval–short pyriform	relatively smooth	medium thick	3.9 [d]
Gatton 2	31.0	246 [a]	1.45 [cd]	oval–short pyriform	relatively smooth	thin	4.2 [d]
Maleny	26.3	302 [e]	1.36 [ab]	pyriform	rough	medium thick–thick	3.5 [bc]
Toowoomba 1A	27.1	317 [f]	1.47 [d]	pyriform	relatively smooth	thin–medium thick	3.6 [c]
Toowoomba 1B	27.1	315 [f]	1.47 [d]	pyriform	relatively smooth	thin–medium thick	3.4 [b]

[a] Flesh colour index: 1 = light cream; 2 = cream; 3 = creamy yellow; 4 = yellow; and 5 = very yellow.

3. paclobutrazol (as Cultar®), sprayed at the rate of 2.5 g/L (a.i.) at full bloom, and
4. treatments 2 and 3 combined.

Fruit measurements, sampling and harvest procedures were as above.

Fruit quality

Fruit softness was measured by gentle hand pressure, and the days to eating soft recorded. Ethylene evolution was quantified daily by measuring the ethylene concentration in 1 mL of air from the ventilated containers, using gas chromatography. The number of fruit achieving full black skin colour at eating soft was recorded. The severity of anthracnose (caused by *Colletotrichum* spp.) and internal flesh disorders was rated as the percentage of the longitudinally cut surface area affected. The percentage dry matter (% DM) and mineral concentrations (by wet digestion and inductively coupled plasma emission spectroscopy) were determined on equatorial sections of fruit flesh.

Results

Production location

Fruit from the cooler production locations (Maleny and Toowoomba) had a higher fruit mass, higher length:diameter ratio (except between Maleny and Childers) and a smoother and thinner skin than those from warmer production areas (Childers; Table 1). There was no consistent difference in flesh colour with climate.

Later-harvested fruit generally ripened more quickly, but this was not consistent for all production locations (Fig. 1). There was no obvious relation between production climate and days to eating soft.

The percentage of the fruit that attained full black skin colour at eating soft differed with production location, but there was no obvious interaction with production climate (Table 2). There was no noticeable effect of maturity on skin colour, but stored fruit generally had a greater percentage with full black skin at eating soft than fruit ripened directly after harvest.

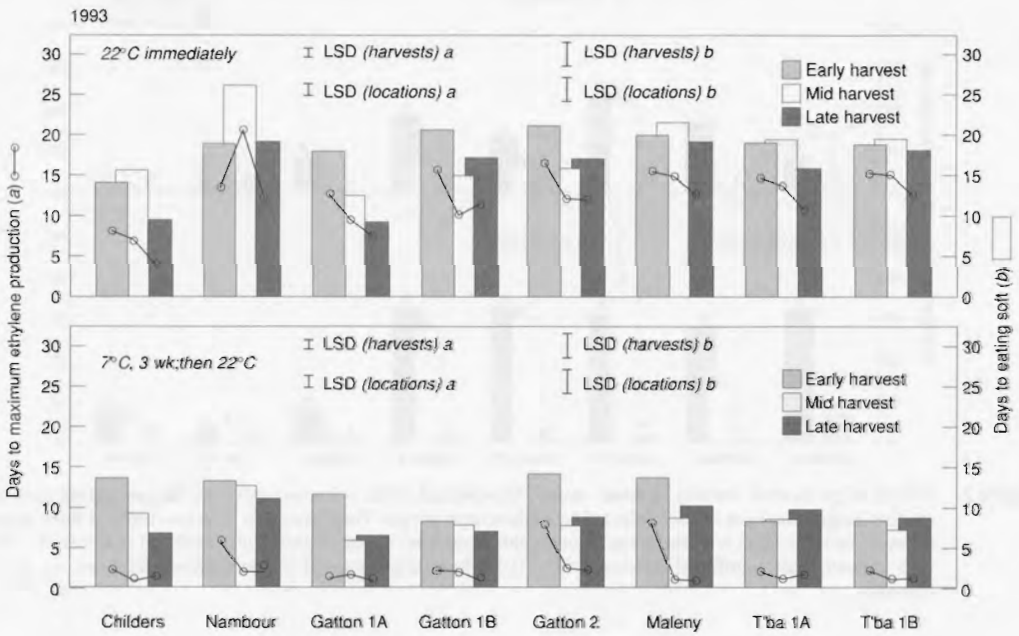

Figure 1. Effects of production location in southeastern Queensland (T'ba = Toowoomba) on the days from harvest, or removal from storage, to maximum ethylene production or eating soft of 'Hass' avocado fruit harvested at three stages of maturity in 1993. Vertical bars indicate the significant difference between harvests (same location) or between locations (same harvest) at least significant difference (LSD) = 0.05. LSDa is for comparing days to maximum ethylene production, and LSDb for days to eating soft.

Table 2. Effects of production locality in south eastern Queensland on the percentage of 'Hass' avocado fruit achieving full black skin colour at eating soft at three stages of maturity in 1993. Fruit were either ripened immediately after harvest (non-stored), or stored at 7°C for 3 weeks before ripening at 22°C (stored). Means in rows (between harvests) within each season followed by the same letter, or in columns (between locations) followed by the same second letter, are not significantly different when separated by least significant difference at P = 0.05. Data were arcsine transformed for analysis, and the back-transformed data presented.

Location	Percentage of fruit attaining full dark skin colour at eating soft					
	Non-stored			Stored		
	Early harvest	Mid harvest	Late harvest	Early harvest	Mid harvest	Late harvest
Childers	90 ^{a,c}	90 ^{a,c}	95 ^{a,b}	100 ^{b,c}	0 ^{a,a}	87 ^{b,a}
Nambour	90 ^{b,c}	40 ^{a,ab}	87 ^{b,b}	100 ^{a,c}	88 ^{a,b}	100 ^{a,a}
Gatton 1A	40 ^{a,ab}	40 ^{a,ab}	29 ^{a,a}	90 ^{a,bc}	70 ^{a,b}	76 ^{a,a}
Gatton 1B	20 ^{a,a}	60 ^{b,abc}	67 ^{b,ab}	50 ^{a,a}	70 ^{a,b}	83 ^{a,a}
Gatton 2	40 ^{a,ab}	25 ^{a,a}	59 ^{a,ab}	60 ^{a,ab}	90 ^{a,b}	89 ^{a,a}
Maleny	70 ^{a,bc}	70 ^{a,bc}	60 ^{a,ab}	100 ^{a,c}	100 ^{a,b}	100 ^{a,a}
Toowoomba 1A	40 ^{a,ab}	50 ^{a,ab}	63 ^{a,ab}	100 ^{a,c}	90 ^{a,b}	93 ^{a,a}
Toowoomba 1B	40 ^{a,ab}	60 ^{a,abc}	73 ^{a,b}	60 ^{a,ab}	90 ^{ab,b}	100 ^{b,a}

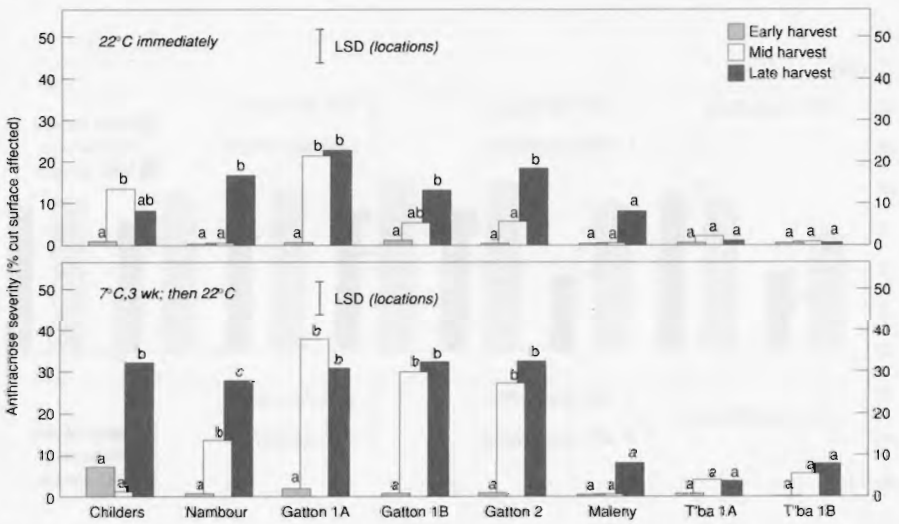

Figure 2. Effects of production locality in south eastern Queensland (T'ba = Toowoomba) on the percentage area of the median longitudinal cut surface affected by anthracnose in ripe 'Hass' avocado fruit harvested at three stages of maturity in 1993. Bars with the same letter (within locations) are not significantly different at P = 0.05. Vertical bars indicate least significant difference (P = 0.05) for comparisons of means between locations (at the same harvest).

Anthracnose severity generally increased with later harvests in fruit from the warmer production areas (Childers and Gatton) in 1993 (Fig. 2). In 1994, this effect was less obvious in the warmer areas because of lower disease severity, but was stronger in fruit from Maleny and Toowoomba (data not presented).

Fruit mineral concentrations varied with production location, and calcium (Ca) concentrations decreased with fruit maturity (data not shown). The days to eating soft was positively correlated to flesh Ca concentration, and negatively correlated to potassium (K) (Table 3). Fruit with higher Ca or magne-

Table 3. Correlation coefficients (r) between fruit calcium (Ca), potassium (K) and magnesium (Mg) concentrations and quality of 'Hass' avocado fruit for fruit harvested from different localities in southeastern Queensland in 1993 at early, mid and late maturity. Fruit were either ripened immediately after harvest (non-stored), or stored at 7°C for 3 weeks before ripening at 22°C (stored). Data were combined from the six orchards ('*' and '**' indicates significance at the 0.05 and 0.01 level, respectively; ns = not significant).

Fruit characteristic	Storage	Variables		
		Ca	K	Mg
Days to eating soft	Non-stored	0.351 **	–0.328 **	0.061 ns
	Stored	0.547 **	–0.079 ns	–0.071 ns
Anthracnose severity	Non-stored	–0.236 **	0.373 **	–0.285 **
	Stored	–0.221 **	0.398 **	–0.312 **
Mesocarp discoloration severity	Stored	–0.009 ns	–0.140 *	–0.048 ns

sium (Mg), or lower K concentrations, had lower anthracnose severity.

Irrigation

Tree yield (yearly and cumulative over two seasons), was significantly higher when trees were more frequently irrigated (–20 kPa compared to –70 kPa), due mainly to a doubling in fruit number per tree (Table 4). Annual tree yield (t/ha) was improved by 50% with more frequent irrigation (–20 kPa). Reduced irrigation (–70 kPa) increased the variation in yield and average fruit mass between individual

Table 4. Effects of irrigation at different soil matric potentials (kPa) on tree yield, fruit number per tree, and average mass of 'Hass' avocado fruit. Means in rows followed by the same letter are not significantly different when separated by least significant difference at P = 0.05.

Parameter	Mean			Variance		
	–20 kPa	–40 kPa	–70 kPa	–20 kPa	–40 kPa	–70 kPa
1994						
Fruit no./tree	461.0[a]	279.0[a]	238.0[a]	45447	58234	87192
Yield (kg/tree)	81.0[b]	44.0[ab]	40.0[a]	1417	1098	2239
Fruit mass (g)	179.0[a]	185.0[a]	170.0[a]	427	1426	2327
1995						
Fruit no./tree	1038.0[b]	739.0[ab]	474.0[a]	66839	100886	185689
Yield (kg/tree)	148.0[b]	109.0[ab]	80.0[a]	2009	1646	4971
Fruit mass (g)	141.0[a]	155.0[ab]	180.0[b]	245	376	701
Cumulative 2 seasons						
Fruit no./tree	1499.0[b]	1018.0[ab]	712.0[a]			
Yield (kg/tree)	229.0[b]	153.0 [ab]	120.0[a]			
Fruit mass (g)	160.0[a]	170.0[a]	181.0[b]			
Yield (t/ha)[z]	47.6	31.9	24.9			

[z] No statistical comparison made due to the data being derived from a single value for each treatment.

trees as shown by higher variance (Table 4), with a greater number of trees having low yields (Fig. 3). Most trees showed alternative bearing ('off' year in 1994 and 'on' year in 1995), but the pattern in yield between trees was similar in the two years.

Irrigation did not affect days to eating soft in fruit ripened immediately, or following 7°C storage for 3 weeks (Table 5). However, fruit with –70 kPa irrigation ripened 1.3 days faster than those with –20 kPa irrigation following 5 weeks storage at 7 and 2°C in 1995. There was no significant effect of irrigation on disease severity.

Irrigation had no effect on fruit mineral concentrations. However, there were strong positive correlations between flesh Ca and Mg, and days to eating soft, and a negative correlation with K in 1995 (Table 6). Also, for individual trees, fruit with higher flesh Ca or Mg concentrations, or lower K concentrations, had lower anthracnose severity.

Paclobutrazol

Total tree yield of the treated trees was not significantly different to the controls (Table 7). However, urea+paclobutrazol increased the average fruit mass

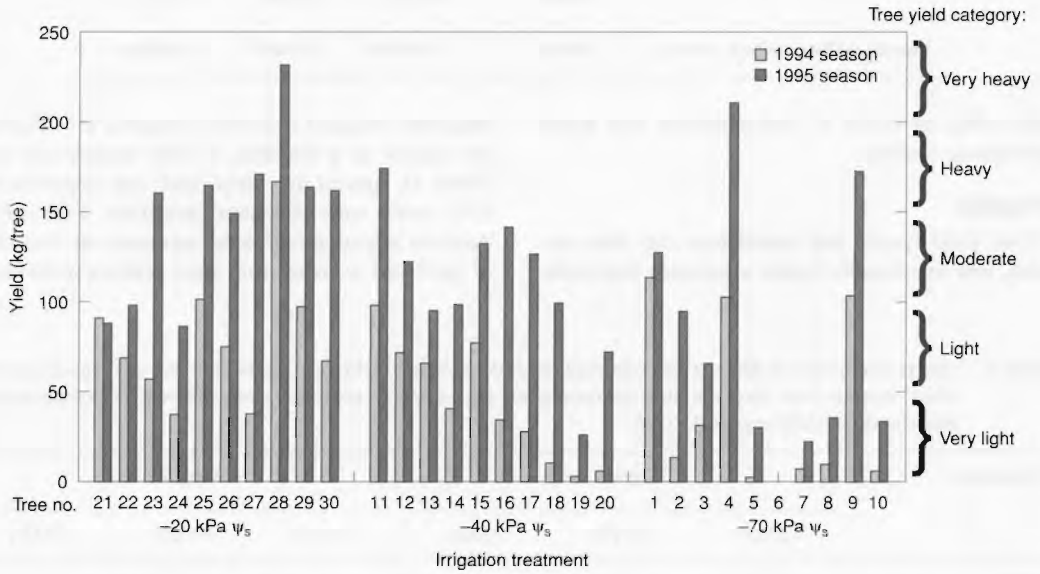

Figure 3. Effects of irrigation at different levels of soil matric potential (Ψs) on individual 'Hass' avocado tree yield in 1994 and 1995.

Table 5. Effect of irrigation at different soil matric potentials (kPa) on days from harvest or storage removal to eating soft of 'Hass' avocado fruit. Fruit were either ripened immediately after harvest (non-stored), or cold stored before ripening at 22°C (stored). Means in columns within a season followed by the same letter are not significantly different when separated at least signficant difference at P = 0.05.

Irrigation treatment	Days to eating soft				
	1994		1995		
	Non-stored	Stored 7°C, 3 weeks	Non-stored	Stored 7°C, 5 weeks	Stored 2°C, 5 weeks
–20 kPa	20.2 [a]	8.5 [a]	20.7 [a]	8.7 [b]	10.6 [b]
–40 kPa	19.4 [a]	8.6 [a]	18.9 [a]	8.8 [b]	10.3 [ab]
–70 kPa	18.5 [a]	8.2 [a]	18.3 [a]	6.8 [a]	9.3 [a]

Table 6. Correlation coefficients (r) between fruit calcium (Ca), magnesium (Mg) and potassium (K) concentrations and postharvest quality of 'Hass' avocado fruit from the irrigation experiments for individual trees. Fruit were either ripened immediately after harvest (non-stored), or cold stored before ripening at 22°C (stored). ('*' and '**' indicates significance at the 0.05 and 0.01 level respectively; ns = not significant).

Parameter	Mineral		
	Ca	Mg	K
Days to eating soft			
1994			
Non-stored	0.585 *	0.229 ns	–0.489 ns
Stored, 7°C for 3 weeks	0.779 **	0.735 **	–0.382 ns
1995			
Non-stored	0.783 **	0.512 *	–0.848 **
Stored, 7°C for 5 weeks	0.875 **	0.672 **	–0.565 *
Stored, 2°C for 5 weeks	0.843 **	0.431 ns	–0.680 **
Severity of anthracnose			
1994			
Non-stored	–0.795 **	–0.616 *	0.354 ns
Stored, 7°C for 3 weeks	–0.621 *	–0.773 **	–0.073 ns
1995			
Non-stored	–0.877 **	–0.595 *	0.776 **
Stored, 7°C for 5 weeks	–0.812 **	–0.825 **	0.598 *
Stored, 2°C for 5 weeks	–0.637 *	–0.689 **	0.457 ns

Table 7. Effects of foliar paclobutrazol and urea application on tree yield, fruit number per tree, and average fruit weight of 'Hass' avocado fruit. Mean values in rows with different letters are significantly different when separated by least significant difference at P = 0.05.

Parameter	Treatment			
	Control	Urea alone	paclobutrazol alone	Urea + paclobutrazol
Yield (kg/tree)				
1994	109.1 [a]	96.5 [a]	133.5 [a]	98.3 [a]
1995	147.3 [a]	171.7 [a]	147.9 [a]	116.5 [a]
1996	117.7 [ab]	79.2 [a]	130.9 [ab]	188.8 [b]
Average fruit weight (g)				
1994	196.0 [a]	208.0 [ab]	213.0 [ab]	233.0 [b]
1995	202.0 [ab]	194.0 [a]	215.0 [ab]	238.0 [b]
1996	190.0 [a]	204.0 [a]	223.0 [ab]	243.0 [b]

in two of the three seasons, due to a greater number of fruit in the larger size categories. The average fruit mass from the urea+paclobutrazol treatment was 18% higher than those from the untreated controls.

The treatment effect on days to eating soft (without storage) was slight, with a significant increase noted only in 1994 with paclobutrazol treatment (Table 8). The days from storage removal to eating soft was not affected by any treatments (data not presented).

Flesh mineral concentrations were generally not affected by treatment. Calcium concentration and (Ca+Mg)/K were positively correlated to days to eat-

Table 8. Effects of foliar paclobutrazol and urea application on days from harvest to eating soft of 'Hass' avocado fruit ripened immediately at 22°C. Mean values in rows (within each season) with different letters are significantly different when separated by least significiant difference at P = 0.05.

Year	Treatment			
	Control	Urea alone	paclobutrazol alone	Urea + paclobutrazol
1994	17.0 [a]	17.8 [ab]	19.6 [c]	19.2 [bc]
1995	17.2 [ab]	16.6 [ab]	17.8 [b]	16.0 [a]
1996	15.6 [ab]	15.0 [a]	16.4 [b]	15.6 [ab]

Table 9. Correlation coefficients (r) between fruit calcium (Ca), magnesium (Mg) and potassium (K) concentrations and postharvest quality of 'Hass' avocado fruit from the paclobutrazol experiments for individual trees. Fruit were either ripened immediately after harvest (non-stored), or cold stored before ripening at 22°C (stored). '*' and '**' indicates significance at the 0.05 and 0.01 level respectively. (ns = not significant).

Parameter	Ca	Mg	K	Ca+Mg/K
1994 season: days to eating soft				
Non-stored	0.737 **	0.532 ns	–0.196 ns	0.838 **
Stored, 7°C for 3 weeks	0.534 *	0.270 ns	–0.086 ns	0.533 *
1995 season: days to eating soft				
Non-stored	0.689 **	0.406 ns	–0.491 *	0.660 **
Stored, 7°C for 5 weeks	0.711 **	0.561 *	–0.306ns	0.569 **
Stored, 2°C for 5 weeks	0.538 *	0.314 ns	–0.206 ns	0.360 ns
Mesocarp discoloration				
Stored, 7°C for 5 weeks	–0.586 **	-0.294 ns	0.663 **	–0.686 **
Stored, 2°C for 5 weeks	–0.650 **	-0.602 **	0.370 ns	–0.606 **
Severity of anthracnose				
Non-stored	–0.639 **	–0.310 ns	0.273 ns	–0.436 ns
Stored, 7°C for 5 weeks	–0.687 **	–0.466 *	0.736 **	–0.848 **
Stored, 2°C for 5 weeks	0.646 **	0.492 *	–0.462 *	0.675 **

ing soft and negatively correlated to severity of mesocarp discoloration and anthracnose (Table 9). Some correlations existed between fruit Mg and K and postharvest quality in 1995.

Discussion

Significant effects of production locality and/or cultural practices on quality have been noted in many fruits (e.g. Blanpied et al. 1987; Rowell 1988).

A reduction in shelf life with increasing maturity has been observed in avocado by Cutting et al. (1992), and in mango by Hofman et al. (these proceedings). Strong correlations between avocado flesh Ca concentration and days to ripe have been noted by Witney et al. (1990) and Cutting et al. (1992), and in other fruit (Hofman and Smith 1994).

Skin colour of 'Hass' is an important commercial consideration in Australia, since consumers often assume that 'Hass' fruit are ripe only when the skin is full black in colour (Ledger and Barker 1995). Thus, a delay in skin colour development relative to flesh softening may result in over-ripe fruit and a reduction in quality. The present results indicate that skin colour can be affected by production factors. These pro-

duction factors may include increased light exposure improving red colour in peach (Génard and Bruchou 1992), and excess nitrogen increasing green skin colour in mango (McKenzie 1994). However, there is no indication of which production factors are important in determining 'Hass' skin colour.

Disease was the major factor determining fruit quality after storage in these experiments. The generally increased severity in more mature fruit may partially result from a longer exposure time of fruit to inoculum with later harvests, and reduced physiological 'vigour' of the more mature fruit.

Our experiments identified that irrigation affected 'Hass' yield, but there are conflicting reports in the literature in this regard. Lahav and Kalmar (1983) found little effect of irrigation on yield, fruit size, or growth rate of 'Hass' between dry (crop factor of 0.39) and wet (crop factor of 0.54) treatments in Israel. The initial results of Faber et al. (1995) also indicated little effect of irrigation on 'Hass' yield, but they noted that the variability between individual tree yields was increased with reduced irrigation. Stottlemyer et al. (1994) found no effect of irrigation on fruit mineral concentrations, but Bower (1985) noted higher Ca concentrations in fruit from trees irrigated at –55 kPa than those from trees at –35 and –80 kPa. However, it is difficult to make accurate comparisons between these investigations, since irrigation treatment, soil type, rainfall, and tree characteristics were different.

Cultar® has increased avocado yields in previous studies (Wolstenholme et al. 1990; Whiley et al. 1991), but not in all cases (Cutting and Bower 1990; Symons and Wolstenholme 1990). Kremer-Köhne et al. (1991) found that a yield increase in 'Fuerte' only occurred in a low-yield ('off') year. An increase in the percentage of larger fruit was also found by Symons and Wolstenholme (1990), which is desirable in reducing the small size problem in 'Hass'. The negligible effect of paclobutrazol on other aspects of fruit quality indicates that only production and yield effects need to be considered in the practical evaluation of this treatment.

The significant correlations between fruit mineral concentrations and fruit quality were based on representative fruit samples from individual trees, which indicates that individual tree characteristics can have an important effect on fruit quality. Thus, both the level and consistency of quality can be improved through reducing tree-to-tree variability in fruit minerals. All trees used in the above experiments were grown on seedling rootstocks, and important rootstock effects on scion mineral concentrations have been noted in avocado (Embleton et al. 1962). It is likely that greater advances in fruit quality can be made by the selection and adoption of clonal rootstocks with the ability to influence fruit mineral concentrations for optimum fruit quality, rather than further refinement of current cultural practices.

Acknowledgments

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Partial Pressure Infiltration of Mango Fruit with Dye to Reveal the Potential Xylem Pathway for Stem-end Rot Infection

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Abstract

Harvested 'Kensington' mango fruit were infiltrated with food dye under partial pressure during fruit development and ripening in order to trace the xylem system in the fruit and seed. Vascular tracing was undertaken as part of a study into fruit infection by the stem-end rot fungus *Dothiorella dominicana*. Fruit with peduncles cut to 1.5 cm were immersed in 'Brilliant Blue' dye solution in a vacuum desiccator for 1 minute. A partial pressure of –33 kPa was then applied and maintained for 5 minutes. The pressure was released and the fruit kept submerged for another 4.5 minutes before being removed and washed in deionised water. Fruit were then dissected and examined for dye penetration. In the first experiment, dye infiltrated into the xylem of the pedicel, peduncle, fruit flesh, endocarp, funicule, and testa of green fruit harvested from 6 weeks after flowering until maturity, but never into the embryo. Failure of the dye to infiltrate into the embryo of green fruit suggests that mango fruit xylem is not connected directly to the embryo. In contrast, infiltration into ripe fruit was minimal, with dye infiltrating only the pedicel. In a second experiment, mature fruit were harvested and subsamples partial pressure infiltrated with dye every other day from harvest until they became overripe. Infiltration in green mature fruit was as extensive as described for the previous experiment, but again decreased for both harvested ripening fruit and for fruit which commenced ripening on the tree. This observation confirmed the results of the previous experiment, in that mango xylem pathways apparently remain continuous only until the fruit ripen. It was also observed that as fruit ripened the funicule dried out from the seed end first. This observation suggests that as the fruit ripen the seed disconnects from the fruit vascular system. It was confirmed by microscopic examination of squash mounts of infiltrated tissue that the infiltration pathway was xylem tissue cells. In further work, the proposition that stem-end rot fungi progress along the xylem will be examined.

STEM-END rot (SER) of mangoes caused by *Dothiorella dominicana* (Dd) (syn. *Fusicoccum aesculi*) is a serious postharvest disease of mango, particularly in situations where anthracnose is well controlled (Johnson et al. 1993). It causes a rapidly growing, watery lesion beginning at the stem end of the fruit as it ripens, and which engulfs the entire fruit within days. Optimising strategies for the control of stem-end rot is somewhat dependent on determination of the infection pathway. Johnson et al. (1992) established that some SER infection may result from endophytic colonisation of the fruit pedicel and peduncle, infecting the fruit as it ripens. Other infection sources may be inoculum produced in infected twigs or tree litter (Johnson et al. 1989). The way in which Dd subsequently colonises the peduncle and the fruit itself is undocumented. Xylem vessels are a possible pathway that the pathogen may utilise, as described for *Botry-*

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osphaeria dothidea (the teleomorph of Dd) in birch trees (McPartland and Schoeneweiss 1984). We hypothesise that the endophytic Dd harboured in fruit pedicels colonises the fruit xylem vessels after harvest. Accordingly, we have traced the mango fruit xylem system by partial pressure infiltration of dye with a view to further investigation of this potential infection pathway.

Methods

Experiment A

Fruit were harvested from a commercial orchard at Childers in south eastern Queensland, Australia, at 3-week intervals from 6 weeks after flowering until maturity. Fruit were bought back to the Brisbane laboratory, weighed, and their diameter measured prior to partial pressure infiltration.

Experiment B

Fruit were picked from Lismore in northern New South Wales, Australia, sprung fruit were removed and the remainder were hot water dipped (Sangchote 1989). Fruit dry matter at harvest was 13.05% ± 0.30%. Subsample sets of fruit were partial pressure infiltrated every other day from harvest to over-ripe. Changes in the colour and firmness of the fruit were also recorded on these days. Colour was scored on a 1 to 5 scale of 1 = 100% of fruit surface area green, 2 = 75% green, 3 = 50% green, 4 = 25% green and 5 = 100% yellow. Colour was also measured objectively with a Minolta CR-200 colorimeter. Hue angle (H°) values are presented. Firmness was based on a manual scale of 1 = hard, 2 = sprung, 3 = slightly soft, 4 = eating soft and 5 = over soft, and also measured with a digital tomato firmness tester (DFT).

On day 1, some of the fruit were found to be sprung and showed softening and coloration at the distal 'nose' of the fruit. Six of these fruit and six hard green fruit (with a firmness score of 1) were partial pressure infiltrated on day 1 in order to compare infiltration patterns of fruit considered to have commenced ripening on the tree with mature green fruit which commenced ripening after harvest.

Partial pressure infiltration method

Fruit peduncles were freshly cut 1.5 cm long and immersed in Brilliant Blue dye solution (8g/L) in a vacuum infiltrator for 1 min. A partial pressure of –40 kPa was applied, adjusted to –33 kPa and maintained for 5 min. The partial pressure was then released and the fruit kept immersed for another 4.5 min. They were then removed and rinsed with deionised water, dissected and examined for dye penetration.

Results

Experiment A

Dye infiltrated into the xylem of the pedicel, peduncle, fruit flesh, endocarp, funicule and testa of green fruit harvested from 6 weeks post flowering until maturity, but never into the embryo (Figs. 1, 2). These fruit ranged from 13 to 82 mm in diameter. Failure of the dye to infiltrate into the embryo of green fruit suggests that mango fruit xylem is not connected directly to the embryo. In contrast, dye infiltration into ripe fruit was

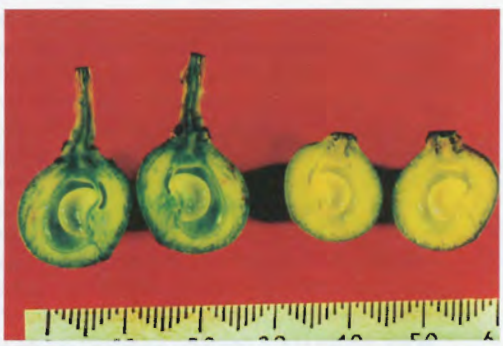

Figure 1. Mango fruit harvested 6 weeks after flowering and with peduncle length of >1.0 cm (left) or <0.5 cm (right), showing distribution of Brilliant Blue dye after partial pressure infiltration.

Figure 2. Harvested hard green mature mango fruit, showing distribution of Brilliant Blue dye after partial pressure infiltration.

minimal, with dye only infiltrating the pedicel of ripe fruit (Fig. 3).

Maximum dye infiltration was achieved when peduncles were left long (i.e. at least 1 cm in length). In fruit with short stems (< 0.5 cm), dye infiltrated only the pedicel (Fig. 1). This difference was due to blocking of the xylem by sap released on cutting the pedicel. Desapping fruit by cutting stems under running water and allowing sap to drain for 1 hour increased infiltration, but the infiltration was still not as consistent or extensive as that in fruit with longer stems.

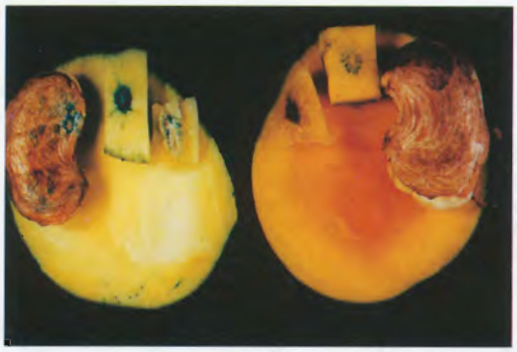

Figure 3. Distribution of Brilliant Blue dye in harvested unripe, mature (left) and eating soft (right) mango fruit after partial pressure infiltration. Dye penetrated into the testa in unripe fruit, but only into the pedicel of ripe fruit.

Experiment B

In the second experiment, infiltration was observed to be limited to the xylem. This was seen as blue staining of xylem tissue on the pedicel surface, in cross sections of flesh vascular bundles and in microscopically examined squash mounts of flesh and funicle vascular tissue. Phloem and lactifers remained unstained.

Fruit which commenced ripening on the tree showed the same difference in infiltration patterns as harvested ripening fruit, in that hard green fruit had extensive infiltration and healthy funicules, while sprung fruit had more shrivelled funicules and less dye infiltration in flesh vascular tissue, endocarp vascular tissue, funicule and testa (Table 1).

Dye infiltration in green mature fruit was as extensive as described for the previous experiment, and again decreased as fruit ripened (Table 1). There was little difference in infiltration between fruit on days 1 to 4, but by day 7 there was no infiltration into the fruit flesh and funicule in more than half the fruit, into the endocarp in nearly all fruit and into the testa in all fruit. This changing infiltration pattern can be related to changes in funicule form and function. The funicule is the vascular connection from the fruit to the testa which surrounds the seed. In hard green fruit, the funicule was 'healthy' in appearance. As fruit ripened, the funicle shrivelled and dried from the end closest to the seed (Table 1).

Discussion

Partial pressure dye infiltration of ripening mango fruit revealed several aspects of the xylem 'behaviour' during ripening. Minimal infiltration of the dye under partial pressure into ripe fruit suggests that mango xylem pathways only remain continuous until the fruit reach eating soft. Apparent decomposition of the xylem system may be associated with the softening of fruit flesh with ripening, and possibly with leakage of cellular contents (e.g. organic acids) into the xylem. It is interesting that the dye infiltrated only the xylem. Lactifers are also 'hollow tunnels' in the fruit flesh, but seemingly lack the characteristics of xylem which allows partial pressure infiltration, or may be blocked with latex. Drying out of the funicule from the seed end suggests that the seed disconnects itself from the fruit vascular system as fruit ripen, when supply of water and photosynthates to the seed would presumably cease. Failure of dye to infiltrate into the embryo of green fruit indicates that mango fruit xylem is probably not connected directly to the embryo.

The findings reported above support our hypothesis that the xylem could be the pathway for fruit and seed infection by stem-end rot pathogens. The xylem remains continuous until the fruit is ripe, by which time SER lesion development has begun. The separation of the seed from the fruit by the drying of the funicule may not, however, affect the passage of the fungus into the seed, as the tissue connection remains continuous even though the functional xylem does not. The delay in SER symptom expression from inoculation when fruit are green, to lesion development when fruit are ripe is not, however, explained by the change in the xylem. Rather it may be due to the breakdown of antifungal compounds in the fruit flesh which in turn allows flesh colonisation by Dd. It now remains to partial pressure infiltrate mango fruit inoculated with Dd when green mature and match the dye infiltration throughout ripening to disease progression, and also to histologically examine naturally

Table 1. Colour and firmness changes, and dye infiltration of subsamples of mango fruit harvested mature green and partial pressure infiltrated with Brilliant Blue dye every second day until fruit were oversoft (Experiment B). On Day 1 fruit which had commenced ripening on the tree were also infiltrated. Values describing dye infiltration are percentage incidence.

Parameter	Day 1		Day 2	Day 4	Day 7	Day 9
	Hard green	Sprung				
Colour and firmness measures						
Colour score	1.00	1.00	1.00	1.17	2.83	3.67
Minolta hue angle (H°)	94.48	82.47	95.20	90.24	85.15	79.05
Firmness score	1.00	2.00	1.33	2.00	3.50	4.83
DFT deformation (mm)	0.35	0.65	0.37	0.63	1.05	1.57
Dye infiltration (% incidence)						
Peduncle	100	100	100	100	100	50
Pedicel	100	100	100	100	75	67
Flesh vascular tissue	100	67	83	100	42	43
Funicule stem end	100	66	92	100	33	0
Funicule seed end	100	17	100	90	33	9
Testa	100	0	90	70	0	0
Seed	0	0	0	0	0	0
Papery integuments	0	0	0	0	0	0
Endocarp interior	17	17	55	39	9	0
Endocarp exterior vascular	67		67	75	9	9
Funicule condition (% incidence)						
100% healthy	100	0	83	60	63	17
50% shrivelled	0	66	17	40	20	25
100% shrivelled	0	33	0	0	17	58
Stem-end funicule shrivelled	–	–	34	42	58	92
n (number of fruit)	6	6	12	12	12	12

infected fruit to determine whether this potential infection pathway is indeed that which Dd utilises.

Acknowledgments

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Fruit Rots of Mangosteen and Their Control

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Abstract

Mangosteen collected from different production areas in eastern and southern Thailand had many kinds of fruit rot fungi associated with them, including *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Phomopsis* sp., *Gliocephalotrichum bulbilium*, and *Pestalotiopsis* sp. *Lasiodiplodia theobromae* was the main pathogen in both areas. The incidence of *Pestalotiopsis* sp. on different parts of sound fruits, such as stem ends, bracts, and stylar ends, was rather high, but it was a minor pathogen. *Lasiodiplodia theobromae* and *Phomopsis* sp. were also found on these parts of the fruit but at low incidence. Dipping the fruits in 1000 ppm thiophanate-methyl for 3 minutes reduced the incidence of disease by 20% as compared with untreated fruits.

MANGOSTEEN is grown mainly in eastern Thailand, including the Chantraburi, Rayong, and Trad regions, and in southern districts including Nakorn Sri Thammarat, Surat Thani, and Chumporn. Mangosteen has a short storage life due to fruit rot diseases. Infected fruits become hard and rotten inside. These diseases are caused by *Lasiodiplodia theobromae*, *Phomopsis* sp., and *Pestalotiopsis flagisettula*. Preharvest spraying with copper oxychloride 86–88% (a.i.) at a rate of 70 g/20 L at 14-day intervals does not reduce fruit rot (Terapawa et al. 1980a,b). Thiabendazole was an effective fungicide against these fungi when it was tested directly on the fungi (Terapawa et al. 1980a, b), however it showed no significant effect in reducing fruit rot when used as preharvest spray or postharvest dip (Terapawa et al. 1986; Terapawa et al. 1988). Little is known about the aetiology of mangosteen pathogens. In this paper, some aspects of fruit infection of mangosteen pathogens and postharvest treatments are reported.

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Materials and Methods

Postharvest diseases of mangosteen and their incidence

Mangosteen were collected from 12 locations in eastern and seven locations in southern Thailand. About one hundred fruit were collected from each location. Fruit were packed in plastic baskets lined with newspaper and held at 25°C for 2 weeks. They were then examined for disease and the causal organisms isolated by tissue transplanting. Small pieces of tissue from diseased fruit were cut and surface sterilised with 1% sodium hypochlorite, then plated on potato dextrose agar (PDA) and incubated at 25°C for 7 days. Mycelium was transferred to a PDA slant and maintained for future study.

Isolation of the causal organisms of mangosteen fruit rot

Two hundred mangosteen fruit were collected from both the eastern and southern parts of Thailand and divided into two samples from each area (100 fruit/sample) for the following experiments.

1. Tissues were cut from the stem end, middle part of four bracts, and stylar end of the fruit and isolated for the causal organisms by tissue transplanting.

2. Fruit were packed in a plastic basket with a newspaper liner. Diseased fruit were examined after 2 weeks.

Postharvest dips

One hundred fruit were dipped in one of three chemicals—benomyl, thiophanate-methyl, or iprodione—at a concentration of 1000 ppm for 3 minutes then packed in plastic trays, covered with plastic bags, and stored at 25°C for 2 weeks. Percentages of diseased fruits were checked and the causal organisms isolated.

Results

Postharvest diseases of mangosteen and their incidence

After two weeks storage, the mangosteen fruit had various diseases including *Lasiodiplodia* fruit rot, *Phomopsis* fruit rot, *Pestalotiopsis* fruit rot, *Gliocephalotrichum* fruit rot, and anthracnose. Details of these diseases follow.

Phomopsis *fruit rot*

Diseased fruit turned hard, and were light-brown in colour. *Phomopsis* sp. was the causal organism. Pycnidia in the rind of the affected area eventually broke through the epidermis and produced white conidia. The conidia were hyaline, ellipsoid, of dimensions 2.5 × 6.4 µm (Fig. 1).

Pestalotiopsis *fruit rot*

Diseased fruit became hard and the infected area turned light-pink. *Pestalotiopsis* sp. was the causal organism. The fungus produced acervuli in the infected area. Acervuli were globose to lenticular and ruptured the epidermis by a pore which became wide and irregular. Conidia emerged in black columns and gradually became effused over the fruit surface. Conidia were fusiform to slightly clavate, five-celled, 6.4 × 19.3 µm, three median cells olivaceous, apical and basal cell hyaline, three apical appendages hyaline (Fig. 2).

Lasiodiplodia *fruit rot*

Diseased fruit became hard and dark-coloured. *Lasiodiplodia theobromae* was the causal organism. The diseased area was grey to black, fluffy with abundant mycelium, with pycnidia also produced immersed in the epidermis, they later became erumpent, simple or grouped, and ostiolate. Conidia extruded in a black mass, initially unicellular, hyaline, subovoid, thin-walled and becoming uniseptate, cinnamon to brown, often longitudinally striate, dimensions 11.6 × 27.7 µm as the conidia mature (Fig. 3).

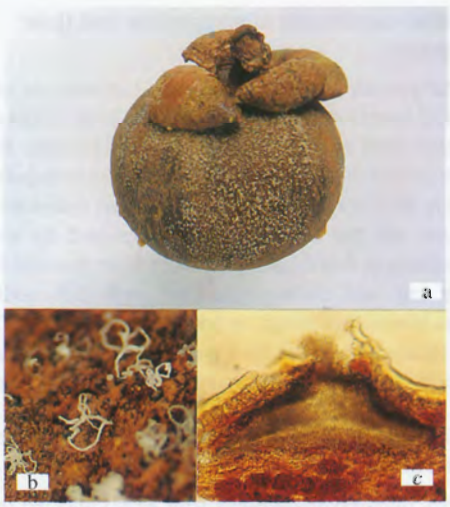

Figure 1. *Phomopsis* fruit rot of mangosteen. a. Diseased fruit. b. Pycnidia are immersed in the rind of diseased area and have produced white conidial masses. c. Pycnidia and conidia of *Phomopsis* sp. (bar = 100 µm).

Figure 2. *Pestalotiopsis* fruit rot of mangosteen. a. The diseased fruit. b. Acervuli have ruptured epidermis of the rind and conidia have emerged in a black column. c. Acervulus and conidia of *Pestalotiopsis* sp. (bar = 100 µm).

Anthracnose

Infected areas became hard and turned light-brown in colour. *Colletotrichum gloeosporioides* was the causal organism. It produced acervuli in the infected area. Acervuli were rounded to elongated, subepidermal, and disrupted the outer epidermal cell walls of host. Conidia were hyaline, cylindrical, aseptate, dimensions 3.4 × 13.1 µm (Fig. 4).

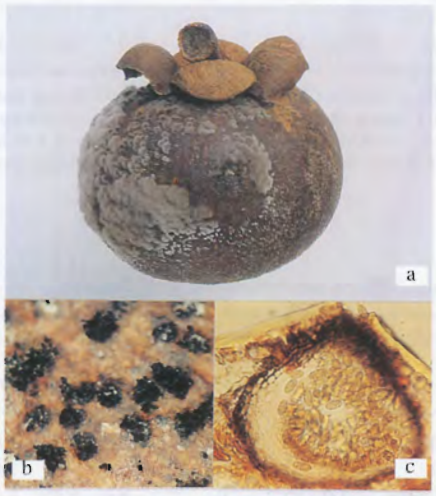

Figure 3. *Lasiodiplodia* fruit rot of mangosteen. a. Diseased fruit. b. Pycnidia are immersed in the epidermis of the rind and have extruded black masses of conidia. c. Pycnidia and conidia of *Lasiodiplodia theobromae* (bar = 100 µm)

Figure 4. Anthracnose of mangosteen. a. Diseased fruit. b. Acervuli have disrupted the outer epidermis of the rind. c. Acervulus and conidia of *Colletotrichum gloeosporioides* (bar = 100 µm).

Gliocephalotrichum *fruit rot*

Epidermal tissue of diseased fruit became swollen, and turned light-pink in colour. *Gliocephalotrichum bulbilium* was the causal organism. It produced conidia and conidiophores subepidermally, disrupting the outer epidermal cell wall of the host. Conidia were produced in groups at the end of conidiophore. They were one-celled, cylindrical to oblong, of dimensions 3.3 × 8.5 µm (Fig. 5).

L. theobromae was the most common fungus infecting mangosteen from eastern and southern Thailand. However, other fungi including *Phomopsis* sp., *C. gloeosporioides, Pestalotiopsis* sp., and *G. bulbilium* were also found (Tables 1 and 2).

Isolation of organisms causing mangosteen fruit disease

The fungi *Pestalotiopsis* sp., *Phomopsis* sp. and *L. theobromae* were isolated from different parts of fruit including the stem end, bracts, and stylar end. *Pestalotiopsis* sp. was most abundant in sound fruit collected from the eastern and southern regions, while *L. theobromae* and *Phomopsis* sp. ranked second and third in frequency (Tables 3 and 4).

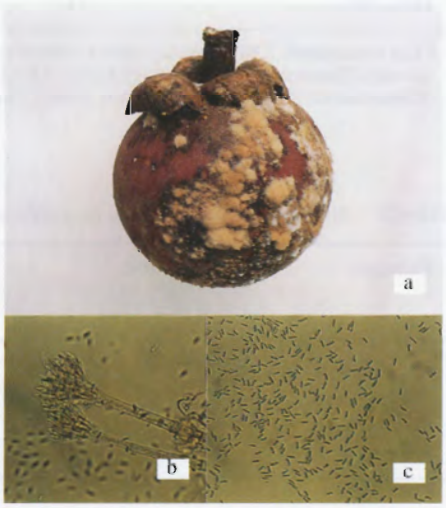

Figure 5. *Gliocephalotrichum* fruit rot of mangosteen. a. Diseased fruit. b. Conidiophores (bar = 100 µm). c. Conidia of *Gliocephalotrichum bulbilium* (bar = 10 µm)

Table 1. Abundance of diseased fruit found in samples collected from different locations in eastern Thailand.

Pathogen	Percentage of diseased fruit from different locations[a]											
	1	2	3	4	5	6	7	8	9	10	11	12
Lasiodiplodia theobromae	10.2	19.3	45.7	17.7	18.0	18.0	15.3	30.7	41.6	45.7	30.7	16.0
Colletotrichum gloeosporioides	1.0	0.0	0.9	1.1	4.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0
Hard fruit	4.1	5.2	15.0	15.0	7.0	6.0	8.6	13.0	7.9	9.7	6.9	13.0

[a] Key to locations: 1. 47 Maung district, Chantraburi province; 2. 62 Maung district, Chantraburi province; 3. 39 Maung district, Chantraburi province; 4. 59 Maung district, Chantraburi province; 5. 31/1 Maung district, Chantraburi province; 6. 23 Maung district, Chantraburi province; 7. 41 Klung district, Chantraburi province; 8. 31/2 Klung district, Chantraburi province; 9. Kao Saming district, Trad province; 10. Tamai district, Chantraburi province; 11. Klang district, Rayong province; 12. 28 Maung district, Chantraburi province

Table 2. Abundance of diseased fruit found in samples collected from different locations in southern Thailand.

Pathogen	Percentage of diseased fruit from different locations[a]						
	1	2	3	4	5	6	7
Lasiodiplodia theobromae	16.0	19.0	30.0	41.0	23.0	22.0	25.0
Colletotrichum gloeosporioides	0.0	0.0	1.0	0.0	2.0	3.0	1.0
Pestalotiopsis sp.	4.0	2.0	14.0	2.0	1.0	7.0	6.0
Phomopsis sp.	5.0	4.0	2.0	0.0	0.0	3.0	2.0
Gliocephalotrichum bulbilium	0.0	18.0	9.0	0.0	0.0	0.0	0.0
Dothiorella sp.	0.0	0.0	1.0	0.0	2.0	0.0	0.0
Hard fruit	15.0	19.0	13.0	5.0	2.0	9.0	2.0

[a] Key to locations: 1. 61/4 Lansaka district, Nakorn Sri Thammarat; 2. Chawang district, Nakorn Sri Thammarat; 3. 57 Langsuan district, Chumporn province; 4. Prom Keeree district, Nakorn Sri Thammarat; 5. Lansaka district, Nakorn Sri Thammarat; 6. Tha Chana district, Surat Thani; 7. Langsuan district, Chumporn.

Table 3. Abundance of fungi isolated from different parts of the fruit collected from Rayong province (eastern Thailand).

Pathogen	Stem end	Bracts				Stylar end	Fruit rot
		1	2	3	4		
Pestalotiopsis sp.	82.5	92.5	90.0	80.0	65.0	87.5	0.0
Phomopsis sp.	7.5	7.5	7.5	7.5	5.0	15.0	2.0
L. theobromae	12.5	5.0	2.5	7.5	7.5	10.0	15.0
Dothiorella sp.	0.0	2.5	2.5	2.5	2.5	0.0	1.0

Table 4. Abundance of fungi isolated from different parts of the fruit collected from Nakorn Sri Thammarat (southern Thailand).

Pathogen	Stem end	Bracts				Stylar end	Fruit rot
		1	2	3	4		
Pestalotiopsis sp.	57.1	68.1	72.5	61.5	74.7	71.4	1.8
Phomopsis sp.	5.5	10.9	12.0	10.9	12.0	0.0	4.0
L. theobromae	7.7	18.7	26.4	16.5	16.5	30.8	17.7
C. gloeosporioides	14.3	1.0	0.0	0.0	2.2	0.0	0.0

Table 5. Abundance of fruit rot and fungi isolated from fruit rot of mangosteen after chemical dipping (1000 ppm) for 3 minutes and storage at 25°C for 2 weeks.

Chemical	Fruit rot (%)	Fungi related to fruit rot (%)[a]			
		L	Ph	Pes	Col
Control	26	18	3	3	2
Benomyl	11	4	3	2	2
Iprodione	11	7	2	2	0
Thiophanate-methyl	6	4	2	0	0

[a] L = *Lasiodiplodia theobromae*, Ph = *Phomopsis* sp., Pes = *Pestalotiopsis* sp., Col = *Colletotrichum gloeosporioides*.

Postharvest dips

Postharvest treatment by dipping fruit in one or other of the three chemicals at 1000 ppm for 3 minutes indicated that thiophanate-methyl was the most effective in reducing fruit rot, from 26% to 6% (Table 5).

Discussion

Fruit rot of mangosteen is caused mainly by *L. theobromae*. However, it is difficult to detect at the initial stage of infection because no symptoms are produced. In this study, we found that fruit infected by the fungi initially hardened. Later, the causal organisms could be identified by microscopic examination of their fruiting bodies. The fungi causing fruit rot were found in the stem end, bracts, and stylar end of the sound fruit. *Pestalotiopsis* sp. had the highest incidence, but caused no problem in the stored fruit. It causes a common leaf disease of mangosteen (Terapawa et al. 1988). *Lasiodiplodia theobromae* and *Phomopsis* sp. were less common than *Pestalotiopsis* sp. They are serious postharvest pathogens of fruit, especially *L. theobromae* because it has a wide host range. For example, it also causes diseases on rambutan (Farungsang et al. 1994). The sources of inoculum and the infection process of this fungus need further study.

Treating fruit with thiophanate-methyl at 1000 ppm for 3 minutes could reduce disease incidence by 20%. This treatment may be useful for marketing of fresh fruit which are to be stored for some time. However, if the fruit are to be frozen this treatment is unnecessary because the disease has insufficient time to develop.

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Pre- and Postharvest Infection of Rambutan by Pathogens and Effects of Postharvest Treatments

S. Sangchote*, U. Farungsang* and N. Farungsang†

Immature and mature rambutan fruit collected from different production areas in eastern and southern Thailand showed different levels of fruit rot. The main pathogen of immature fruit was *Pestalotiopsis* sp., compared with *Pestalotiopsis* sp. and *Greeneria* sp. in mature fruit after 2 weeks storage. *Phomopsis* sp., *Colletotrichum gloeosporioides*, and *Gliocephalotrichum bulbilium* were minor pathogens. Fungi infected both the spinterns and rind of the fruit, with the rind being the more important site of infection for all except *Phomopsis* sp. and *Pestalotiopsis* sp., which had a higher occurrence in the spinterns. The effect on the incidence of postharvest diseases in rambutan fruit was investigated with regard to storage temperature, storage with volatile spices, and treatment with a range of fungicides. Rambutan stored at 15°C for 10 days had no disease and good fruit quality. Addition of *Amonum krervanh* or *Eugenia caryophyllus* at 1% (w/w) to the storage environment of rambutan fruit significantly reduced disease incidence. Dipping fruit in fungicides after harvest was not an effective control measure.

RAMBUTAN (*Nephelium lappaceum* L.) is grown in eastern and southern Thailand. The main production areas include Chantraburi, Rayon, Trad, and Prachin Buri in the east, and Surat Thani, Nakorn Si Thammarat, and Chumporn in the south. Rambutan has a rather short shelf life with browning of the rind occurring after 4–5 days at ambient temperature (Watson 1988). Furthermore, it is attacked by many fungi causing fruit rot, including *Lasiodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Phomopsis* sp., *Gliocephalotrichum bulbilium*, and *Greeneria* sp. (Farungsang et al. 1994). Control of rambutan diseases using postharvest dips has been unsatisfactory (Saenyoung and Visarathanonth 1985; Mohamed et al. 1988; Farungsang et al. 1994). In this paper, further investigations are made into the incidence and aetiology of infection by pathogens of rambutan, and the effects of three postharvest treatments.

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Materials and Methods

Pre- and postharvest infection by rambutan pathogens

Immature and mature rambutan fruit were collected from eastern Thailand at five locations in Prachin Buri, two in Chantraburi, and two in Trat. In the south, fruit were collected at two locations in Nakorn Si Thammarat, and three in Surat Thani. Fifty fruit were collected at each location and pathogens isolated using a tissue transplanting method. Small pieces of tissues from immature fruit were cut, surface sterilised with 1% sodium hypochlorite, and plated onto potato dextrose agar (PDA). The colonies were counted and examined after 7 days of incubation at 25°C.

Mature rambutan fruit were packed in baskets and covered with a plastic bag. After 2 weeks, diseased fruit were checked and the causal organisms isolated as described above.

Infection of immature fruit in the spintern and rind

Immature rambutan fruit from Nakorn Si Thammarat were collected and the pathogens isolated from the

rind and spintern by tissue transplanting. Four pieces of tissue from spintern and rind were cut from the middle part of the fruit at opposite directions to each other. Rind and spintern tissue was cut from the same area on each fruit. Tissue samples were surface sterilised with 1% sodium hypochlorite and plated on PDA. The colonies were counted and examined after 7 days of incubation at 25°C.

Effect of storage temperature on postharvest diseases

Rambutan fruit were packed in foam trays (8 fruit/tray), wrapped with plastic film, and stored at 15, 20, 25, or 30°C (5 trays/temperature). After 10 days, fruit rot incidence was checked.

Effects of volatiles from spices on postharvest diseases

Rambutan fruit were packed in foam trays (8 fruit/tray). Ground spices including *Amomum krervanh* Pier, *Cinnamomum bejolghota* Sweet, *Cuminum cyminum* L., *Eugenia caryophyllus* (Spr.) Bull. & Harr., *Diospyros decandra* Lour., and *Piper chaba* Hunt. were wrapped in blotting paper and added to the fruit packages at a rate of 1% w/w of fruit (5 trays/spice). Trays were kept at 25°C for 10 days, then disease incidence was checked.

Efficacy of fungicidal postharvest dips

Rambutan fruit were treated with one of six chemicals—benomyl, iprodione, thiabendazole, prochloraz, thiophanate-methyl, or tricyclazone—at a concentration of 500 ppm. The fruit were dipped in the chemical for 3 minutes using 40 fruit/chemical. Fruit were then packed in foam trays, wrapped with plastic film (8 fruit/tray), and stored at 25°C. After 10 days, proportions of diseased fruit were checked.

Results

Pre- and postharvest infection by rambutan pathogens

Rambutan fruit from eastern and southern Thailand were infected with similar pathogens including *Pestalotiopsis* sp., *Phomopsis* sp., *L. theobromae*, *G. bulbilium*, and *C. gloeosporioides*, however the levels of infection by these pathogens varied (Tables 1–3). In fruit from the east, *Pestalotiopsis* sp. was the principal pathogen of immature fruit. *Pestalotiopsis* sp. was also predominant in mature fruit which had been stored at 25°C for 2 weeks, except for fruit from Prachin Buri province, in which *Greeneria* sp. was most common (Tables 1 and 2). In the south, *Pestalotiopsis* sp. and *Greeneria* sp. were the main pathogens of immature fruit. In mature fruit, after 2 weeks storage, *Greeneria* sp. was predominant (Table 3).

Table 1. Incidence (%) of fungal species isolated from (A) immature rambutan fruit and (B) mature fruit after storage at 25°C for 2 weeks, which were collected from different locations in eastern Thailand.

Pathogens	Locations[a]			
	1	2	3	4
A. Immature fruit				
Pestalotiopsis sp.	100	94	100	87
Greeneria sp.	1	0	1	1
Phomopsis sp.	0	0	0	0
Lasiodiplodia theobromae	1	0	0	0
Gliocephalotrichum bulbilium	0	1	0	1
B. Mature, stored fruit				
Pestalotiopsis sp.	88	60	80	60
Greeneria sp.	12	16	12	28
Phomopsis sp.	0	4	0	0
Lasiodiplodia theobromae	4	8	32	0
Gliocephalotrichum bulbilium	0	4	0	4

[a] Key to locations: 1. 50 Khao Saming district, Trat province; 2. Khlung district, Chantraburi province; 3. 41 Khlung district, Chantraburi; 4. 100 Khao Saming district, Trat province.

Infection of immature fruit in the spintern and rind

Observation of pathogens in the rind and spinterns of immature fruit indicated that rambutan pathogens could infect at both these sites (Table 4). The rind was more commonly infected for most pathogens except for *Phomopsis* sp. and *Pestalotiopsis* sp., which had a higher incidence in the spintern.

Table 2. Incidence (%) of fungal species isolated from (A) immature rambutan fruit, and (B) mature fruit stored at 25°C for two weeks, collected from different locations in Phachin Buri province, eastern Thailand.

Pathogens	Locations[a]				
	1	2	3	4	5
A. Immature fruit					
Pestalotiopsis sp.	95	97	91	97	92
Greeneria sp.	0	0	0	0	0
Phomopsis sp.	0	0	0	0	0
Lasiodiplodia theobromae	1	0	0	2	0
Gliocephalotrichum bulbilium	5	2	0	0	1
B. Mature, stored fruit					
Pestalotiopsis sp.	12	24	16	12	20
Greeneria sp.	76	88	52	12	76
Phomopsis sp.	16	4	12	28	8
Lasiodiplodia theobromae	0	4	12	4	0
Gliocephalotrichum bulbilium	0	0	0	0	0
Colletotrichum gloeosporioides	0	0	0	4	4

[a] Key to locations: 1. 80/1 Maung district; 2. 5/2 Maung district; 3. 35 Maung district; 4. 82 Srimahapho district; 5. 361/3 Srimahapho district.

Table 3. Incidence (%) of fungal species isolated from (A) immature rambutan fruit and (B) mature fruit after storage at 25°C for 2 weeks, collected from different locations in southern Thailand.

Pathogens	Locations[a]				
	1	2	3	4	5
A. Immature fruit					
Pestalotiopsis sp.	49.0	19.7	23.2	32.2	36.3
Greeneria sp.	23.6	46.8	26.1	39.7	29.5
Phomopsis sp.	3.4	7.0	24.6	3.4	11.6
Lasiodiplodia theobromae	0.0	0.7	2.1	2.1	5.5
Gliocephalotrichum bulbilium	15.1	4.2	10.5	9.5	2.7
Colletotrichum gloeosporioides	0.5	0.7	0.0	0.7	0.7
B. Mature, stored fruit					
Pestalotiopsis sp.	17.0	10.4	10.3	3.2	14.8
Greeneria sp.	55.0	55.8	37.1	68.4	51.6
Phomopsis sp.	7.0	1.2	8.2	3.2	9.9
Lasiodiplodia theobromae	7.0	4.6	20.6	6.3	8.8
Gliocephalotrichum bulbilium	9.0	23.3	23.7	17.9	11.0
Colletotrichum gloeosporioides	0.0	0.0	0.0	0.0	0.0

[a] Key to locations: 1. 37/1 Phrom Khiri district, Nakorn Si Thammarat province; 2. 67/1 Phrom Khiri district, Nakorn Si Thammarat province; 3. Wiang Sa district, Surat Thani province; 4. 58/3 Ban Na San district, Surat Thani province; 5. 26/1 Ban Na San district, Surat Thani province.

Effect of storage temperature on postharvest diseases

Rambutan fruit stored at 15°C for 10 days showed no disease symptoms and had good appearance. Diseases started to develop at 20°C and increased dramatically at higher temperatures (Table 5).

Effects of volatiles from spices on postharvest diseases

Incorporating ground spices into the storage environment of rambutan fruit had varying results (Table 6). Inclusion of *Eugenia caryophyllus* or *Amomum krervanh* significantly reduced disease incidence from 56.3% (control) to 40.6 and 43.7%, respectively.

Efficacy of fungicidal postharvest dips

Treating the fruit by dipping in one of six chemicals at 500 ppm for 3 minutes did not not significantly reduce disease incidence as compared to untreated (control) fruit (Table 7).

Discussion

These experiments indicated that infection by a range of pathogens causing fruit rot of rambutan began in the field. Fruit at immature stages could be infected, as also reported by Visarathanonth and Ilag (1987), however development of disease was greater after harvest. Pathogens infected the pedicel, stem-end, rind, and spintern, but produced only small lesions on the immature or just harvested fruit. Problems arose when fruit were kept for longer periods, a situation similar to that found for mango postharvest diseases (Sangchote et al. 1994). Storage of rambutan fruit at 15°C successfully suppressed disease development. Postharvest dips of rambutan fruit in fungicides were ineffective in reducing disease. Saenyoung and Visarathanonth (1985) reported similar results for use of fungicides in controlling rambutan fruit rot. The use of spices in the storage environment might be a feasible alternative to fungicides, but reduction of inoculum in the field by preharvest sprays and management is necessary.

Table 4. Incidence (%) of pathogens isolated from spintern and rind which were cut from the same areas of immature rambutan fruit.

Pathogen	Rind	Spintern
Colletotrichum gloeosporioides	7.1	3.3
Greeneria sp.	42.9	38.8
Pestalotiopsis sp.	12.7	17.6
Phomopsis sp.	2.9	21.2
Gliocephalotrichum bulbilium	7.1	1.2
Lasiodiplodia theobromae	11.4	4.7
Others	15.7	12.9

Table 5. Incidence of fruit rot disease in rambutan fruit stored at different temperatures for 10 days. Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Storage temperature (°C)	Disease incidence (%)
15	0.0[a]
20	0.9[a]
25	74.9[b]
30	74.9[b]

Table 6. Incidence of fruit rot disease in rambutan packed with small packages of spice (1% w/w) and kept at 25°C for 10 days. Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Spice	Disease incidence (%)
Amomum krervanh	43.7[a]
Cinnamomum bejolghota	62.5[c]
Cuminum cyminum	65.6[c]
Eugenia caryophyullus	40.6[a]
Diospyros decandra	62.5[c]
Piper chaba	53.1[b]
Without spice	56.3[b]

Table 7. Incidence of disease in fruit after treatment with one of a range of chemicals at 500 ppm for 3 minutes and storage at 25°C for 10 days.

Chemical	Disease incidence (%)
Control	72.5[a]
Benomyl	87.5[a]
Iprodione	72.5[a]
Prochloraz	65.0[a]
Thiabenbadazole	65.0[a]
Thiophanate-methyl	87.5[a]
Tricyclazone	77.5[a]

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Bagging of Lychee Fruit to Reduce Postharvest Disease

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Abstract

Lychee fruit cv. 'Hong Huay' were bagged at 45 days after anthesis (defined here as 75% of flowers on a panicle open). Bags of four different materials were used: (i) clear polyethylene, (ii) clear polypropylene, (iii) remay, and (iv) newspaper, and there was an unbagged control. Fruit were harvested when ripe (107 days after anthesis), pre-cooled, packaged in foam trays which were then wrapped in polyvinyl chloride film, and stored at either 5–6°C or 22–25°C. All treatments stored at 22–25°C had high levels of browning and disease after 5 days. Fourteen genera of fungi were isolated from these fruit. The predominant fungal genera were *Trichothecium*, *Fusarium*, and *Aspergillus*. Disease levels at 5°C were much lower, even after 25 days storage. Thirty-two genera of fungi were isolated from fruit stored at this temperature, predominantly *Cladosporium*, *Phoma*, *Fusarium*, and *Nigrospora*. Pathogenicity tests of eleven genera of fungi on lychee fruit 'Hong Huay', 'Kim Jeng', and 'Chakrapat' confirmed the pathogenicity of *Botryodiplodia*, *Colletotrichum*, *Glomerella*, *Mycosphaerella*, *Phoma*, *Phomopsis*, and *Trichothecium*. The incidence of most pathogenic fungi was reduced by bagging. Remay bags were found to be superior to other bag types.

LYCHEE (*Litchi chinensis* Sonner) is native to south eastern China. The tree is long-lived and there are many cultivars. The fruit has a tough dry reddish peel or shell, and inside is a single seed surrounded by firm, translucent white flesh. Lychees are being grown as a commercial crop in subtropical Asia, Hawaii, South Africa, and Australia. Fruit browning and rotting are the most significant causes of loss and can make the fruit unsaleable. The diseases affecting lychee after harvest are generally not apparent during harvest and packing. They develop as the fruit reach the end of their postharvest life. Fungi are the most common cause of decay of lychee. Prasad and Bilgrami (1969, 1974) described 11 fungal pathogens of lychee fruit in India. These included nine virulent pathogens (*Aspergillus* spp., *Colletotrichum gloeosporioides*, *Cylindrocarpon tonkinense*, *Lasiodiplodia theobromae*, and *Pestalotiopsis* sp.) and two mild pathogens (*Penicillium lilacinum* and *Fusarium* sp.) Other fungi which have been isolated from diseased lychee include *Geotrichum candidum* (Tandon and Tandon 1975), *Stemphylium* and *Fusarium* spp. (Roth 1963; Tongdee et al. 1982).

Subsequently, Fitzell and Coates (1995) reported several pathogenic fungi causing postharvest diseases of lychee in Australia, including *Alternaria alternata*, *Colletotrichum* spp., and *Phomopsis* sp. which are most commonly isolated from diseased lychee fruit. Other fungi involved include *Phoma* sp., *Pestalotiopsis* sp., *Fusarium* sp. and *Curvularia* sp. Control of postharvest diseases may be achieved by preventing infection, eradicating infection, or delaying symptom development so that the fruit can be marketed and consumed before disease appears. Fruit bagging (Kitagawa et al. 1992) is one method which has the ability to control postharvest disease of fruit. The objective of this study was to find optimal material(s) for bagging lychee fruit in order to control postharvest disease.

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Materials and Methods

Preparation of healthy lychee fruit

Panicles of lychee 'Hong Huay' with about 75% of their flowers opened (henceforth referred to as 'anthesis') were tagged in February 1994. Forty-five days after anthesis the fruit clusters were bagged. Five bagging materials were assessed in the trial: (1) clear polyethylene (PE), (2) clear polypropylene (PP), (3) remay, (4) newspaper and (5) unbagged (control). The PE and PP bags were punctured with numerous holes and bag ends cut off. The ripened lychee fruits (107 days after anthesis) of each treatment were harvested and immediately put in plastic bags to retain the fruit skin quality. All fruits were pre-cooled in an airconditioned room at 22–25°C for three hours. The stem ends of lychee fruit in each treatment were cut and healthy and uniform fruits were selected. Ten lychee fruits per tray were placed in 5 × 9 × 1 inch foam trays with a cavity for each fruit. The trays were wrapped in polyvinyl chloride (PVC) film to retain moisture. In these experiments there were 95 lychee fruit trays in total: 20 fruit trays for each treatment, except for bagging with PP, for which there were 15 trays.

Occurrence of lychee fruit browning and rotting after harvest

The experiments were set up as two trials.

Trial 1

Four lychee fruit trays from each bagging treatment (three trays from bagging with PP) were stored in an airconditioned room at 22–25°C. After storage for 5 days the skin of the fruit in each treatment was examined.

Trial 2

Sixteen lychee fruit trays from each bagging treatment (12 trays from bagging with PP) were stored in a cold-storage room at 5–6°C and 90–95% relative humidity. Four lychee fruit trays from each treatment (three trays from bagging with PP) were randomly sampled after storage for 25, 30, 35, and 40 days. At each removal, the fruit skin browning and rotting of each unit of fruit were scored on a scale of 0–5: 0 = no fruit browning and rotting; 1 = 1–10%; 2 = 11–25%; 3 = 26–50% ; 4 = 51–75%; 5 = 76–100%. Treatment means using this disease severity rating were used to produce a series of bar graphs.

Isolation of the fungi associated with fruit browning and rotting

Trial 1

Each lychee fruit was checked and fungal genera present were identified over a period of 3–14 days.

Trial 2

Forty fruit samples of each treatment were taken for the isolation of associated fungi. Four pieces of tissue, each 5 × 5 mm, were taken from browning and rotting areas of each fruit and sterilised by immersion in 0.525% sodium hypochlorite for 3 minutes. The samples of fruit skin were transplanted and cultured on half-strength PDAS (potato dextrose agar supplemented with 100 mg/L streptomycin sulfate), with four pieces per petri dish. All petri dishes were incubated for 3–5 days under fluorescent light + near ultraviolet light on a 12 hour illumination cycle to encourage sporulation. Fungal growth was observed and the genera identified. The non-sporulating isolates were subcultured on PCA (potato carrot agar) in culture tubes to stimulate sporulation. The percentage frequency and abundance of each fungal genus were calculated using Equations 1 and 2 below (Prasad and Bilgrami 1969).

Pathogenicity tests

Ten genera of fungi were isolated from fruit browning and rotting: *Acremonium*, 2 isolates; *Cladosporium*, 1 isolate; *Colletotrichum*, 1 isolate; *Glomerella*, 2 isolates; *Lasiodiplodia*, 1 isolate; *Mycosphaerella*, 2 isolates; *Nigrospora*, 2 isolates; *Phoma*, 4 isolates (2 of them *Phoma epicoccina*) *Phomopsis*, 2 isolates; and *Trichothecium* 1 isolate.

$$\text{Percentage frequency} = \frac{\text{No. of observations in which a genus appeared}}{\text{Total no. of observations}} \times 100 \quad (1)$$

$$\text{Percentage abundance} = \frac{\text{Total no. of colonies of a species from all observations taken together}}{\text{Total no. of colonies}} \times 100 \quad (2)$$

They were inoculated onto unwounded ripened lychee fruit cv. 'Hong Huay', 'Kim Jeng', and 'Chakrapat' fruit after harvest. Five lychee fruit from each cultivar were used per isolate in this experiment. Fruit were immersed in hot water at 50°C for 2 minutes before inoculation to control latent infections (Coates et al. 1993). Mycelial discs 6 mm in diameter from 7-day PDA cultures were inverted over the unwounded surface of each fruit on a flat site and close to the stem end. Comparable fruit were inoculated with PDA as controls. The trays containing inoculated fruit were put into 19 × 28 × 11.5 cm clear plastic boxes together with 50 mL sterilised water. After closing the cover, each box was put into a polyethylene plastic bag to keep moist. The fruit were incubated under the same conditions as for isolation of fruit rot fungi described above, and tested for infection 8 days after inoculation. Fungi from the affected fruits were re-isolated with half-strength PDAS, in order to complete the application of Koch's postulates for assessing the source of disease.

The experiments were conducted on lychee fruit trees in an experimental orchard and in a laboratory at Chiangrai Horticultural Research Center between October 1994 and July 1996.

Results

Occurrence of the fruit browning and rotting after harvest

Trial 1

High levels of fruit browning and rotting developed in all treatments after 5 days storage at 22–25°C. Disease severity ratings were scored at 5, 5, 5, 4.9, and 5 for the fruit bagged with PE, PP, remay, newspaper and unbagged (control), respectively (Fig. 1).

Trial 2

Figure 2 shows levels of fruit browning and rotting after storage at 5–6°C for 25, 30, 35 and 40 days. The disease severity after storage for 25 days was low and rose slowly as the fruit were stored for longer. After storage for 25 days (Fig. 2A), the average of disease severity rating was the lowest at 0.48 in fruit bagged with newspaper bags and slightly increased in unbagged (control) and fruit bagged with remay bags. Fruit bagged with PP and PE bags had higher levels of browning and rotting at 0.87 and 0.88, respectively.

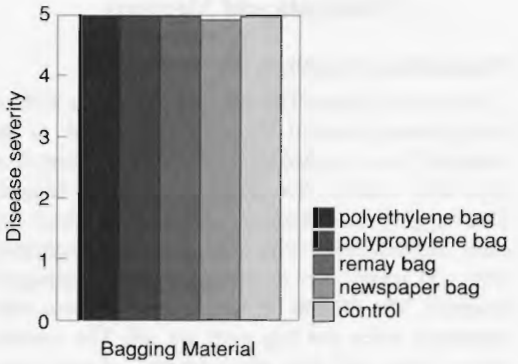

Figure 1. Average severity of browning and rotting in bagged lychee fruit (cv. 'Hong Huay') stored at 22–25°C for 5 days. Fruit were scored on a scale of 0–5: 0 = no fruit browning and rotting; 1 = 1–10%; 2 = 11–25%; 3 = 26–50% ; 4 = 51–75%; 5 = 76–100%.

After storage for 30 days (Fig. 2B), disease severity was rated at a similarly low level for the unbagged (control; 0.80), remay bagged (0.82) and newspaper bagged (0.85) treatments. Fruit bagged with PE bags had rating of 1.05, with PP bagging causing the highest level of browning and rotting at 1.30.

After storage for 35 days (Fig 2C), the average disease severity rating was the lowest at 1.05 in fruit bagged with remay bags and rose slightly in fruit bagged with newspaper bags, unbagged (control), and PE bags, in that order. Fruit bagged with PP bags had the highest level of browning and rotting at 1.60.

After storage for 40 days (Fig. 2D), the average of disease severity rating was the lowest at 1.13 in fruit bagged with remay bags and slightly increased in unbagged (control), fruit bagged with PE bags, and newspaper bags, in that order. Fruit bagged with PP bags had the highest level of browning and rotting at 2.20.

Isolation of fungi associated with fruit browning and rotting

Trial 1

There were 14 genera of fungi, a yeast, and two unidentified fungal isolates associated with lychee fruit after storage at 22–25°C for 3–14 days. The percentage frequency and percentage abundance of each genus of fungi from fruit bagged in the in five different treatments are shown in Table 1. The predominant fungal genera in all bagging treatments

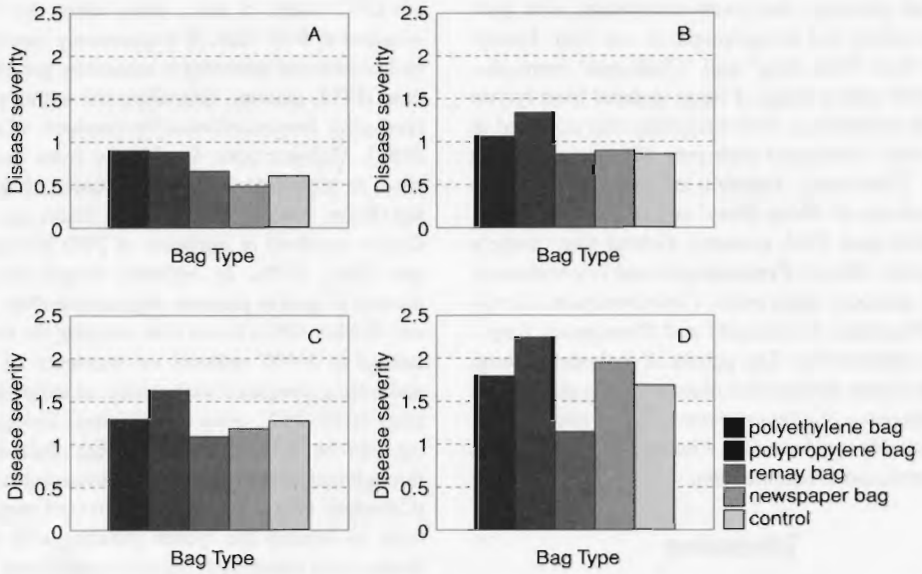

Figure 2. Average severity of browning and rotting in bagged lychee fruit (cv. 'Hong Huay') stored at 5–6°C for (A) 25, (B) 30, (C) 35 or (D) 40 days. Fruit were scored on a scale of 0–5: 0 = no fruit browning and rotting; 1 = 1–10%; 2 = 11–25%; 3 = 26–50% ; 4 = 51–75%; 5 = 76–100%.

excluding the unbagged control were *Trichothecium*, *Fusarium*, and *Aspergillus*, with the average percentage frequency of 33.30, 24.99, and 10.59, respectively, and the average percentage abundance of 30.0, 23.75, and 11.88, respectively.

Trial 2

There were 32 genera of fungi and four unidentified fungal isolates identified from lychee fruit after storage at 5–6°C for 25–40 days. In addition there was a small proportion of fruit with no fungal growth. The percentage frequency and percentage abundance of each fungal isolate derived from the five bagging treatments are shown in Table 2. The predominant fungal genera from all treatments excluding unbagged (control) were *Cladosporium*, *Phoma* (predominantly *P. epicoccina*), *Fusarium* and *Nigrospora* with the average percentage frequency of 88.13, 47.56, 15.58, and 10.63, respectively, and the average percentage abundance of 33.49, 28.91, 9.61, and 6.51, respectively.

Pathogenicity tests

Pathenogenicity tests were conducted on the fungi suspected on being pathogens of lychee fruit. Three cultivars of lychee were inoculated with 10 genera of fungi isolated from lychee fruit cv. 'Hong Huay'. The results shown in Table 3. The lychee fruit cv. 'Hong Huay' showed fruit rot symptoms when inoculated with nine of the fungal genera as well as pure PDA (control), cv. 'Kim Jeng' by seven fungal genera and pure PDA (control), and cv. 'Chakrapat' by eight fungal genera, with symptoms not clear for *Cladosporium*. The fungal pathogens causing lychee fruit browning and rotting in this study were *Colletotrichum*, *Glomerella*, *Lasiodiplodia*, *Mycosphaerella*, *Phoma* (*P. epicoccina*), *Phomopsis* and *Trichothecium*.. However, *Trichothecium* was a weakly parasitic fungi. Cheema and Jayarajan (1972) reported a fruit rot of sweet orange caused by *Trichothecium roseum*.

The results of re-isolating the fungi from fruit of lychee 'Hong Huay', 'Kim Jeng', and 'Chakrapat' showing fruit rot symptoms following inoculation with fungal isolates are shown in Table 4. The percentage frequency and percentage abundance of *Lasiodiplodia* re-isolated from lychee fruit rot symptom inoculated with *Lasiodiplodia* were 100%. Finally, the percentage frequency and percentage abundance of *Trichothecium* re-isolated from lychee fruit rot symptom inoculated with *Trichothecium* isolate 1 were 23.33 and 12.38, respectively. Lychee 'Hong Huay' is so prone to fruit browning, owing to a very

thin fruit pericarp, that even inoculation with pure PDA (control) led to symptoms in our trial. Hence, lychee fruit 'Kim Jeng' and 'Chakrapat' were also inoculated with a range of fungi isolated from lychee fruit for comparison. Fruit browning also occurred in 'Kim Jeng' inoculated with pure PDA (control) but not in 'Chakrapat'. Isolation of fungi from lychee fruit pericarp of 'Hong Huay' and 'Kim Jeng' inoculated with pure PDA (control) yielded four (namely *Aspergillus*, *Mucor*, *Pestalotiopsis* and *Trichoderma*), and six (namely *Aspergillus*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Glomerella* and *Phomopsis*) fungal genera, respectively. The genera of pathogenic fungi causing lychee postharvest disease in this study were *Cladosporium*, *Colletotrichum*, *Glomerella*, *Lasiodiplodia*, *Mycosphaerella*, *Phoma* (*P. epicoccina*), *Phomopsis* and *Trichothecium*.

Discussion

In this study it was shown that the incidence of lychee fruit browning and rotting after bagging with all materials occurred rapidly at ambient temperatures of 22–25°C within 5 days, even when the fruit were wrapped in PVC film. It is commonly considered that lychee pericarp browning is caused by polyphenol oxidase (PPO) activity, degrading the anthocyanins and producing brown-coloured by-products (Tan and Li 1984). Moisture loss, specifically from the pericarp, plays an important role in the initiation of skin browning (Kuhn 1962; Underhill 1989). There are numerous factors involved in initiation of PPO activity (Mayer and Harel 1979). In addition, fungal enzymes are thought to lead to pigment degradation (Nip 1988; Lee and Wicker 1991) In our trial, keeping the fruit in cold storage at 5–6°C reduced the incidence of browning and rotting compared with storage at ambient temperatures of 22–25°C, even after 25 days storage. Browning can be delayed by controlling fruit desiccation through increasing humidity and lowering temperature (Campbell 1959). Under long-term cool storage conditions (4 weeks) the lychee pericarp will eventually brown even under high moisture conditions. Polyphenol oxidase may be further involved in this process because of its close association with whole fruit senescence and interaction with fungal enzymes.

Table 1. The percentage frequency (%f) and percentage abundance (%a) of fungi observed on lychee fruit 'Hong Huay' after storage at 22–25°C for 3–14 days following fruit bagging with different materials (PE = polyethylene, PP = polypropylene).

Fungi	Treatments									
	PE		PP		Remay		Newspaper		Control	
	%f	%a	%f	%a	%f	%a	%f	%a	%f	%a
Acremonium	0.0	0.0	0.0	0.0	0.0	0.0	2.86	2.50	3.85	2.50
Alternaria	0.0	0.0	0.0	0.0	2.08	2.50	0.0	0.0	0.0	0.0
Arthrinium	0.0	0.0	0.0	0.0	0.0	0.0	2.86	2.50	0.0	0.0
Aspergillus	17.65	22.50	0.0	0.0	10.42	12.50	14.28	12.50	0.0	0.0
Chaetomium	0.0	0.0	0.0	0.0	0.0	0.0	2.86	2.50	0.0	0.0
Cladosporium	1.96	2.50	4.17	2.50	12.50	15.00	5.71	5.00	3.85	2.50
Colletotrichum	0.0	0.0	4.17	2.50	14.58	17.50	0.0	0.0	19.23	12.50
Curvularia	0.0	0.0	0.0	0.0	2.08	2.50	2.86	2.50	0.0	0.0
Fusarium	19.61	25.00	20.83	12.50	16.67	20.00	42.86	37.50	34.61	22.50
Macrophoma	0.0	0.0	0.0	0.0	0.0	0.0	2.86	2.50	19.23	12.50
Penicillium	19.61	25.00	0.0	0.0	12.50	15.00	0.0	0.0	0.0	0.0
Phoma	0.0	0.0	0.0	0.0	2.08	2.50	2.86	2.50	0.0	0.0
Phomopsis	1.96	2.50	0.0	0.0	0.0	0.0	14.28	12.50	0.0	0.0
Trichothecium	35.29	45.00	70.83	42.50	27.08	32.50	0.0	0.0	3.85	2.50
Yeast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.69	5.00
Dark sterile mycelium	3.92	5.00	0.0	0.0	0.0	0.0	2.86	2.50	7.69	5.00
White sterile mycelium	0.0	0.0	0.0	0.0	0.0	0.0	2.86	2.50	0.0	0.0

Table 2. The percentage frequency (%f) and percentage abundance (%a) of fungi isolated from lychee fruit cv. 'Hong Huay' after storage at 5–6°C for 25–40 days following fruit bagging with different materials (PE = polyethylene, PP = polypropylene).

Fungi	Treatments									
	PE		PP		Remay		Newspaper		control	
	%f	%a	%f	%a	%f	%a	%f	%a	%f	%a
Acremonium	0.94	0.55	1.46	1.83	0.31	0.21	0.47	0.30	0.16	0.08
Alternaria	2.19	1.29	2.08	1.18	1.72	1.13	3.44	2.20	2.34	1.24
A. longisima	0.63	0.37	0.42	0.24	0.31	0.21	0.47	0.30	0.63	0.33
Arthrinium	0.31	0.18	0.21	0.12	0.31	0.21	0.47	0.30	0.16	0.08
Aspergillus	0.16	0.09	0.21	0.12	2.34	1.54	0.16	0.10	0.0	0.0
Botryotrichum	0.0	0.0	0.0	0.0	0.16	0.10	0.0	0.0	0.0	0.0
Chaetomium	0.47	0.28	0.0	0.0	0.47	0.31	0.0	0.0	0.0	0.0
Cladosporium	60.94	35.94	63.33	35.85	47.34	31.20	48.44	30.97	49.69	26.37
Colletotrichum	2.50	1.47	2.92	1.65	2.03	1.37	3.13	2.00	16.09	8.54
Curvularia	3.13	1.84	2.71	1.53	1.25	0.82	2.19	1.40	3.13	1.66
Cylindrocarpon	0.0	0.0	0.83	0.47	0.0	0.0	0.47	0.30	0.0	0.0
Diplodia	0.0	0.0	0.0	0.0	0.47	0.31	0.0	0.0	0.0	0.0
Drechslera	0.16	0.09	0.0	0.0	0.0	0.0	0.47	0.30	0.0	0.0
Fusarium	14.06	8.29	14.17	8.02	17.66	11.64	16.41	10.49	26.25	13.93
Gloeosporium (?)	1.41	0.83	0.21	0.12	0.31	0.21	0.47	0.30	0.16	0.08
Glomerella	1.09	0.65	1.88	1.06	3.28	2.16	2.81	1.80	10.47	5.56
Lasiodiplodia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.16	0.08
Leptosphaerulina	0.0	0.0	0.0	0.0	0.16	0.10	0.0	0.0	0.0	0.0
Macrophoma	0.16	0.09	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Mucor	2.03	1.20	0.42	0.24	0.31	0.21	0.63	0.40	2.19	1.16
Mycosphaerella	0.0	0.0	0.21	0.12	0.47	0.31	0.63	0.40	0.63	0.33
Nectria	0.0	0.0	0.21	0.12	0.0	0.0	0.0	0.0	0.0	0.0
Nigrospora	11.25	6.64	10.63	6.01	10.16	6.69	10.47	6.69	16.41	8.71
Penicillium	0.78	0.46	0.0	0.0	1.72	1.13	0.31	0.20	0.61	0.08
Periconia	0.47	0.28	0.42	0.24	2.03	1.33	0.31	0.20	0.31	0.17
Pestalotiopsis	1.25	0.74	2.50	1.42	1.72	1.13	1.88	1.20	5.63	2.99
Phaeotrichonis	0.0	0.0	0.0	0.0	0.16	0.10	0.16	0.10	0.0	0.0
Phoma	4.70	2.76	5.42	3.07	9.84	6.49	12.81	8.19	8.75	4.64
P. epicoccina	51.10	30.14	57.71	32.66	40.32	26.57	41.10	26.28	31.72	16.83
Phomopsis	3.91	2.30	1.04	0.59	1.72	1.13	4.84	3.10	10.63	5.64
Pithomyces	0.0	0.0	0.0	0.0	0.47	0.31	0.0	0.0	0.0	0.0
Torula	0.0	0.0	0.0	0.0	0.0	0..0	0.0	0.0	0.16	0.08
Trichoderma	0.0	0.0	0.21	0.12	0.16	0.10	0.31	0.20	0.31	0.17
Trichothecium	2.66	1.57	5.83	3.30	2.50	1.65	2.66	1.70	0.0	0.0
Brown sterile mycelium	0.94	0.55	0.0	0.0	0.47	0.31	0.31	0.20	0.16	0.08
Dark sterile mycelium	1.09	0.65	0.63	0.35	0.63	0.41	0.0	0.0	0.47	0.25
Grey sterile mycelium	0.0	0.0	0.42	0.24	0.0	0.0	0.0	0.0	0.16	0.08
White sterile mycelium	0.63	0.37	0.21	0.12	0.47	0.31	0.0	0.0	0.0	0.0
No growth	0.78	0.46	0.21	0.12	0.47	0.31	0.31	0.20	1.25	0.66

Recent work by Huang et al. (1990) reported increased PPO activity in the pericarp after 29 days storage at 4°C, and the reports of Nip (1988) and Lee and Wicker (1991) support the possible involvement of the enzyme under long-term storage conditions.

Isolation of fungi from lychee fruit pericarp found that fungi from a number of genera, including yeast and unidentified genera, were present in the pericarp of lychee cv. 'Hong Huay'. The range of fungal genera identified in this study agrees with reports of Fitzell and Coates (1995) and Prasad and Bilgrami (1969, 1974). In addition, a number of other fungi were isolated including *Acremonium*, *Arthrinium*, *Botryotrichum*, *Cladosporium*, *Gloeosporium(?)*, *Glomerella*, *Mycosphaerella*, *Periconia* and *Trichothecium*. Follow up using pathogenicity tests showed eight genera of fungi causing lychee postharvest disease in this study—*Cladosporium*, *Colletotrichum*, *Glomerella*, *Lasiodiplodia*, *Mycosphaerella*, *Phoma*, *Phomopsis* and *Trichothecium* which met the criteria defined in Koch's postulates.The re-isolation of a number of fungal genera from the fruit of 'Hong Huay' and 'Kim Jeng' inoculated with pure PDA (control) indicates that the heat sterilisation treatment applied in this experiment was not sufficient to eradicate the naturally occurring fungi from the fruit.

Our study into the bagging of lychee fruit in the orchard to reduce postharvest disease using five different bagging materials (clear polyethylene, clear polypropylene, remay, newspaper or unbagged [control]) showed that the incidence of most pathogenic fungi was reduced by bagging, although the incidence of some fungi was increased. Remay bags were found to be superior to other bag types. Bagging is one option for controlling postharvest disease of lychee in an overall effort to reduce the use of fungicides in postharvest disease control.

Table 3. Pathogenicity tests 8 days after inoculation of lychee fruit 'Hong Huay', 'Kim Jeng', and 'Chakrapat' with fungi isolated from lychee fruit rot cv. 'Hong Huay' (= no symptom, + = low disease incidence, ++ = moderate disease incidence, +++ = high disease incidence and ++++ = very high disease incidence, ND = not determined).

Fungi	Isolate no.	Disease incidence		
		'Hong Huay'	'Kim Jeng'	'Chakrapat'
Acremonium	1	+++	–	–
Acremonium	2	+++	–	–
Botryodiplodia	1	++++	++++	++++
Cladosporium	1	+++	–	+
Colletotrichum	1	+++	++	++
Glomerella	1	+++	++	++
Glomerella	2	+++	++	+
Mycosphaerella	1	+++	+	+
Mycosphaerella	2	ND	ND	+
Nigrospora	1	–	–	–
Nigrospora	2	+++	–	–
Phoma	1	+++	+	+
Phoma	2	+++	+	+
Phoma epicoccina	1	+++	+	+
P. epicoccina	2	+++	+++	++
Phomopsis	1	+++	+++	++
Phomopsis	2	+++	+++	+++
Trichothecium	1	+++	++	–
Control				

Table 4. Fungi re-isolated from lychee fruit 'Hong Huay', 'Kim Jeng' and 'Chakraprat' which had been inoculated with fungi originally isolated from lychee fruit of cv. 'Hong Huay' showing symptoms of browning and rotting (% f = percentage frequency, % a = percentage abundance, 0 = could not be isolated, ND = not determined)

Isolates	Isolated fungi	Cultivars					
		Hong	Huay	Kim	Jeng	Chakrapat	
		%f	%a	%f	%a	%f	%a
Botryodiplodia -1	*Botryodiplodia*	100.0	100.0	100.0	100.0	100.0	100.0
Cladosporium - 1	*Cladosporium*	ND	ND	ND	ND	30.00	19.69
Colletotrichum - 1	*Colletotrichum*	65.00	54.17	100.0	83.33	100.0	90.69
Glomerella - 1	*Glomerella*	100.0	90.91	55.00	52.83	90.00	75.00
Glomerella - 2	*Glomerella*	85.00	68.00	85.00	73.91	100.0	80.00
Mycosphaerella - 1	*Mycosphaerella*	0.0	0.0	40.00	20.51	56.00	41.18
Mycosphaerella - 2	*Mycosphaerella*	ND	ND	ND	ND	16.00	15.38
Phoma - 1	*Phoma*	70.00	29.97	65.00	33.33	43.75	46.67
Phoma - 2	*Phoma*	40.00	20.51	60.00	50.00	25.00	20.00
Phoma epicoccina -1	*P. epicoccina*	0.0	0.0	0.0	0.0	25.00	16.13
Phoma epicoccina -2	*P. epicoccina*	10.00	8.7	50.00	24.39	48.00	42.86
Phomopsis - 1	*Phomopsis*	65.00	56.52	95.00	86.36	27.00	40.91
Phomopsis - 2	*Phomopsis*	55.00	37.93	75.00	62.50	40.00	28.57
Trichothecium - 1	*Trichothecium*	0.0	0.0	10.00	7.14	60.00	30.00
Control	*Aspergillus*	50.00	31.58	50.00	21.74	ND	ND
	Colletotrichum	0.0	0.0	45.00	19.57		
	Curvularia	0.0	0.0	25.00	10.87		
	Fusarium	0.0	0.0	60.00	26.09		
	Glomerella	0.0	0.0	35.00	15.22		
	Mucor	50.00	31.58	0.0	0.0		
	Pestalotiopsis	8.33	5.26	0.0	0.0		
	Phomopsis	0.0	0.0	15.00	6.52		
	Trichoderma	50.00	31.58	0.0	0.0		

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Biological Control of Anthracnose in Tropical Fruit

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Abstract

Epiphytic bacteria and yeasts isolated from mango and avocado fruit and leaf surfaces were screened for inhibition of the anthracnose pathogen, *Colletotrichum gloeosporioides*. Isolates were initially screened using a mango leaf disk assay which was developed specifically for this purpose. Leaf disks excised from glasshouse-grown mango seedlings were immersed in boiling water for 30 seconds prior to treatment with various combinations of the pathogen and the candidate microorganisms. Surface sporulation by *C. gloeosporioides* following incubation of leaf disks for 7 days was used as a measure of pathogen inhibition. Isolates which repeatedly suppressed pathogen sporulation were selected for evaluation on detached mango fruit.

A number of isolates reduced sporulation of *C. gloeosporioides* on mango leaf disks, including *Bacillus* sp. 359 which had previously been shown to be effective against avocado anthracnose. This isolate also suppressed lesion development in detached mango fruit inoculated with *C. gloeosporioides*. On the basis of these results, a trial to evaluate the field performance of *Bacillus* sp. 359 was conducted on a lychee orchard in southeastern Queensland. Monthly field applications of *Bacillus* sp. 359 to developing 'Kwai May Pink' lychee fruit significantly reduced the incidence of postharvest side lesions (which were caused predominantly by *Colletotrichum* sp. and *Phomopsis* sp.) in fruit which were stored at 20°C for 1 week, although were not as effective as a fungicide treatment which was tested for comparison.

ANTHRACNOSE caused by *Colletotrichum* spp. is the most serious postharvest disease of tropical fruit worldwide. Control of this disease is primarily by fungicides, applied both before and after harvest. In most countries the use of fungicides is becoming increasingly restricted, particularly in the case of those fungicides applied postharvest. Research is now firmly focused on developing non-chemical alternatives to fungicides for the control of postharvest diseases. Already there has been success with biological control of postharvest diseases in various tropical fruit crops using naturally-occurring phylloplane microorganisms. In South Africa, various *Bacillus* spp. have been shown to control anthracnose in avocado (Korsten et al. 1991a), mango (Korsten et al. 1991b; Korsten et al. 1992) and lychee (Korsten et al. 1993). Koomen and Jeffries (1993) also demonstrated biological control of mango anthracnose using an isolate of *Pseudomonas* sp. In Australia, Stirling et al. (1995) reported biological control of avocado anthracnose using selected bacteria (mostly *Bacillus* spp.) and yeasts isolated from avocado fruit and leaf surfaces.

The aim of the study reported here was to further investigate the potential of biological control for the suppression of anthracnose in tropical fruit, with particular emphasis on the development of a rapid assay for screening candidate microorganisms. While screening candidate microorganisms on detached fruit would be the ideal process for selection of effective antagonists against anthracnose, the cost and time involved would severely limit the number of organisms which could be tested in this way. In addition, the highly seasonal nature of fruit availability

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would further limit the usefulness of the method. For these reasons, it was decided to develop a leaf disk assay for initial screening of candidate microorganisms. Large numbers of organisms could then be rapidly screened all year round and isolates showing promise could be evaluated in more detail on fruit.

Materials and Methods

Isolation of candidate microorganisms

Bacteria and yeasts were isolated from the surface of mango leaves and fruit peel. Leaf segments or peel disks were blended in 20 mL of phosphate buffered saline (PBS, pH 7.2) containing 0.01% (v/v) Tween-80 in a Stomacher Lab-Blender 80 (Seward Medical Co.) for 3 minutes. Samples were then serially diluted in PBS and 0.2 mL aliquots plated on either half-strength tryptic soy agar (Difco) amended with cycloheximide (75 µg/mL) (Stirling et al. 1995) or yeast extract-malt extract (Lodder 1970) acidified with 0.75% (v/v) 1M hydrochloric acid (HCl) to pH 3.8 (Stirling et al. 1995). Isolates were purified and stored in sterile distilled water until required.

Development of a leaf disk assay for screening candidate microorganisms

Glasshouse-grown 'Kensington Pride' mango plants were used in all studies. Disks (10 mm diameter) cut from individual mango leaves were surface sterilised with 70% ethanol, inoculated with *Colletotrichum gloeosporioides* (using a 25µL drop of a 5×10^5 conidia/mL suspension) and incubated at 25°C in petri dishes lined with moist filter paper. Seven days after inoculation, leaf disks were submerged individually in vials containing 1 mL of sterile distilled water and shaken at 2000 rpm on an orbital shaker for 30 minutes to dislodge conidia. Conidia were then counted using a haemocytometer. A standard replication of six leaf disks was used in this and all subsequent trials.

As levels of conidial production were found to be very low using this method, a further trial was conducted with the aim of enhancing infection levels. This was done by inducing tissue damage in the leaf disks either by treatment with the herbicide paraquat (Cerkauskas and Sinclair 1980) or immersion in boiling water. For the herbicide treatment, leaf disks were immersed in paraquat (2 mL/L Gramoxone®) for 30 seconds, washed and inoculated with *C. gloeosporioides* 24 hours later, and assessed 7 days after inoculation. For the hot water treatment, leaf disks were immersed in boiling water for 30 seconds, inoculated immediately with *C. gloeosporioides* and assessed 7 days after inoculation. For each treatment, two isolates of *C. gloeosporioides* (1-13 and 1-5) were tested.

The effect of leaf age on sporulation of *C. gloeosporioides* on mango leaf disks was also investigated. Leaves of four different stages ranging from new flush red leaves (stage 1) to old leaves (stage 4) were sampled. For each leaf stage, one disk was excised from each of six leaves. Disks were immersed for 30 seconds in boiling water, inoculated with *C. gloeosporioides* (isolate 4-20), incubated and assessed as previously described.

On the basis of results obtained with these preliminary experiments (see Results section), it was decided to avoid the use of new flush red leaves and to standardise on leaf stage sampled in subsequent trials. It was also decided to immerse leaf disks in boiling water rather than paraquat. To screen potential antagonists, therefore, leaf disks were immersed in boiling water for 30 seconds, dipped in a suspension of the test isolate for 60 seconds, inoculated with *C. gloeosporioides* 24 hours later, incubated for 7 days and then sampled for measurement of conidial production. In some tests, leaf disks were inoculated with *C. gloeosporioides* first and then dipped in the test isolate 24 hours later. Bacteria and yeasts isolated from mango fruit and leaves were tested in this way, along with isolates from avocado previously shown to be antagonistic to *C. gloeosporioides* (Stirling et al. 1995). Isolates of *C. gloeosporioides* from both mango and avocado were used as the test pathogens.

Effect of timing of antagonist application

An experiment was conducted to determine the effect of timing of antagonist application on suppression of *C. gloeosporioides* on mango leaf disks. Isolate 359 (*Bacillus* sp.) from avocado was chosen for this experiment as it suppressed *C. gloeosporioides* consistently on mango leaf disks.

Mango leaf disks were immersed in boiling water for 30 seconds, and dipped in a suspension of isolate 359 either 24 hours prior to inoculation with *C. gloeosporioides*, immediately prior to inoculation, or up to 72 hours after inoculation. *Colletotrichum gloeosporioides* isolates 1-5 ex mango (1×10^6 conidia/mL) and 23691 ex avocado (1×10^6 conidia/mL) were used. Inoculated leaf disks were incubated at 25°C for 7 days and assessed as previously described. Data were analysed by analysis of variance and treatment means were compared using the least significant difference (LSD) test at $P = 0.05$.

Evaluating the performance of promising antagonists on detached mango fruit

Antagonists which showed promise in the leaf disk test were selected for evaluation on detached 'Kensington Pride' mango fruit. Three separate experiments were conducted using fruit harvested from the Burdekin, Childers and Mareeba (all Queensland, Australia). For the first two experiments, Burdekin and Childers fruit were surface sterilised and inoculated with 25 µL droplets of a *C. gloeosporioides* conidial suspension (1×10^6 conidia/mL) within 24 hours of harvest. Inoculated fruit were incubated at 25°C under high humidity for 24 hours, and then dipped in a suspension containing the test antagonist. Ten fruit were used per treatment. After air-drying, fruit were stored at 25°C. For the third experiment, Mareeba fruit were surface sterilised, dipped in a suspension containing the test antagonist, inoculated with *C. gloeosporioides* (1×10^6 conidia/mL) and incubated as previously described. The diameter of anthracnose lesions which developed within the inoculated areas on the fruit surface was recorded. Data were analysed by analysis of variance and treatment means were compared using the LSD test at P = 0.05.

Field application of isolate 359 to lychee 'Kwai May Pink'

The following treatments were applied to six randomly selected 'Kwai May Pink' lychee trees:

1. Water (control)
2. Diluted broth (control)
3. Isolate 359 in diluted broth (biocontrol treatment)
4. Octave/Dithane (chemical treatment)

Each treatment was applied to a single tagged branch on each of the six trees. In order to minimise confounding effects of tree position when analysing results, each treatment was applied to branches positioned at different locations on each of the 6 trees. The water and diluted broth (in which isolate 359 was grown) controls were used to eliminate any effect of spraying water or broth onto the leaves. The chemical treatment was used so that a comparison could be made between fungicides and the biocontrol agent. Sprays were applied approximately once a month. The concentration of viable bacterial cells in each 359 spray suspension was determined. Leaves were sampled both before and after spraying to monitor the survival of 359 on leaf surfaces.

Fruit were harvested early February, packed into plastic punnets and then stored at either 5 or 20°C. Fruit were assessed for postharvest disease (incidence and severity of side lesions) at either 1 week (20°C) or 4 weeks (5°C) after harvest. The cause of disease lesions was determined by isolation onto potato dextrose agar.

Results and Discussion

Development of a leaf disk assay for screening candidate microorganisms

Table 1 compares different methods for pre-treating mango leaf disks. When leaf disks received no treatment prior to inoculation, levels of sporulation by *C. gloeosporioides* 7 days later were very low. This was particularly the case with isolate 1-13. Immersion in boiling water prior to inoculation greatly increased levels of sporulation, although not to such an extent as pre-treatment with paraquat. Although paraquat worked well in enhancing infection, later studies showed that some of the candidate microorganisms were sensitive to paraquat in vitro. For this reason, immersion in boiling water was selected as the standard technique.

Table 1. A comparison of mango leaf disk pre-treatments: effect on sporulation of *Colletotrichum gloeosporioides*.

Pre-treatment	Sporulation of *C. gloeosporioides* no. of conidia × 10^5	
	Isolate 1-13	Isolate 1-5
None	0.7	3.4
Immersion in boiling water	8.8	8.8
Immersion in paraquat	14.0	16.8

The effect of leaf age on sporulation of *C. gloeosporioides* on mango leaf disks is shown in Table 2. Sporulation increased steadily with increasing leaf age. On the basis of these results, it was decided to avoid the use of very young leaves in all subsequent work.

Table 3 shows the effect of various yeasts and bacteria isolated from mango on sporulation of *C. gloeosporioides* on leaf disks. Many isolates were tested but not listed in the table because of their lack of inhibition of *C. gloeosporioides*. Two bacterial isolates, M12 and M19, performed particularly well.

Table 2. The influence of leaf age on sporulation of *Colletotrichum gloeosporioides* on mango leaf disks.

Leaf stage	Sporulation of *C. gloeosporioides* no. of conidia × 10^6
stage 1 (new flush)	0.1
stage 2	0.4
stage 3	1.0
stage 4 (old)	1.4

Table 3. Effects of antagonists from mango (applied 24 hours after pathogen inoculation) on sporulation of *Colletotrichum gloeosporioides* on mango leaf disks.

Antagonist	Type of antagonist	% reduction in the number of conidia produced[a]
M 12	bacterium	94.3
M 19	bacterium	90.0
M 60	yeast	72.7
M 74	bacterium	71.4
M 58	yeast	63.6
M 55	yeast	63.6
M 48	yeast	58.3
M 53	yeast	50.0
M 40	bacterium	42.9
M 50	yeast	23.1
M 64	yeast	11.1

[a] Relative to the control where no antagonist was applied

The results obtained from screening bacteria and yeast isolates from avocado are shown in Table 4. These isolates have previously been shown to be antagonistic to *C. gloeosporioides* from avocado (Stirling et al. 1995). Many of these isolates were tested on the mango leaf disks but only the most effective antagonists were listed in the table. In almost every instance, inhibition of *C. gloeosporioides* was greater when the antagonist was applied 24 hours prior to inoculation rather than 24 hours after inoculation. Despite this, it was surprising to find that most of the isolates listed in Table 4 suppressed *C. gloeosporioides* to some extent when applied either 24 hours before or 24 hours after inoculation. Perhaps

Table 4. Effect of antagonists from avocado on sporulation of *Colletotrichum gloeosporioides* on mango leaf disks.

Antagonist	Time of antagonist application			
	24 hours before pathogen		24 hours after pathogen	
	Times successful/ times tested	Average reduction in sporulation[a] (%)	Times successful/ times tested	Average reduction in sporulation[a] (%)
Bacteria				
330	2/3	48.0	4/4	37.0
359	11/15	56.2	12/14	47.1
632	5/7	61.9	3/5	31.6
638	4/6	52.5	1/4	17.1
677	4/5	63.6	3/5	51.6
933	3/4	57.8	1/2	9.7
Yeasts				
268	4/4	40.8	3/5	16.9
274	2/3	43.5	3/5	50.1
373	5/5	73.2	3/7	48.0
468	4/4	80.9	2/4	26.5
711	4/4	20.8	0/4	–
Q34	4/4	56.2	2/6	22.5

[a] Relative to the control where no antagonist was applied

this indicates that the antagonists are capable of both preventing infection (i.e. reducing spore germination and/or appressoria formation) and reducing infection once it has become established (i.e. reducing penetration and subsequent development of infection hyphae). Alternatively, and probably more likely, infections on leaf disks are taking longer to establish than 24 hours. This would explain why some suppression of *C. gloeosporioides* was still occurring even when antagonists were not applied until 24 hours after inoculation.

Effect of timing of antagonist application

Table 5 shows the effect of timing of antagonist isolate 359 application on suppression of *C. gloeosporioides* on mango leaf disks. When isolate 359 was applied either 24 hours prior to inoculation with a mango isolate of *C. gloeosporioides* or up to 48 hours after inoculation, sporulation was reduced in comparison to the control which was not treated with the antagonist at any time. When the antagonist was applied 72 hours after inoculation, there was no significant reduction in sporulation. A different result was obtained using an avocado isolate of *C. gloeosporioides* (Table 5). Only when isolate 359 was applied 24 hours before or at the same time as inoculation with *C. gloeosporioides* was there any significant reduction in sporulation. This supports the theory that isolate 359 is acting by preventing infections of *C. gloeosporioides* from becoming established.

Table 5. Effect of timing of antagonist (isolate 359) application on sporulation of *Colletotrichum gloeosporioides* on mango leaf disks. Means followed by the same letter do not differ significantly at P=0.05 using the least significant difference test.

Time of 359 application	Sporulation of *C. gloeosporioides* no. of conidia × 10^5	
	1-5 (ex mango)	23691 (ex avocado)
24 h before pathogen	1.42 b	1.54 c
0 h before pathogen	0.75 b	2.29 bc
24 h after pathogen	1.46 b	6.67 a
48 h after pathogen	1.38 b	6.46 a
72 h after pathogen	7.67 ab	5.29 ab
Control (no 359 applied)	10.60 a	6.04 a

Evaluating the performance of promising antagonists on detached mango fruit

The best performing antagonists isolated from mango fruit were evaluated for the suppression of anthracnose lesions on detached mango fruit (Tables 6a and 6b). Three of the four bacteria tested significantly reduced the diameter of anthracnose lesions when applied to fruit 24 hours after inoculation, with isolate M19 giving the greatest level of suppression (Table 6a). None of the yeasts significantly reduced lesion diameter (Table 6b), although it should be noted that the concentration of yeast cells in dip suspensions was generally much lower than that of the bacteria in the first experiment. It is also worth noting that most of the yeasts did reduce lesion diameter even though these reductions were not statistically significant.

The efficacy of isolate 359 from avocado was compared to that of isolate M19 from mango in Table 7.

Table 6. Diameter of anthracnose lesions in mango fruit 'Kensington Pride' inoculated with *Colletotrichum gloeosporioides* 24 hours before dipping in selected antagonists from mango (cfu = colony forming units). Means followed by the same letter do not differ significantly at P=0.05 using the least significant difference test.

Antagonist	Type of antagonist	Concentration of antagonist in dip (cfu/mL)	Lesion diameter (mm)
(a) Experiment 1 (Burdekin fruit)			
Water (control)		0	3.20 a
M 12	bacterium	3.1×10^8	2.70 a
M 19	bacterium	3.0×10^8	0.23 c
M 40	bacterium	3.0×10^7	1.23 b
M 74	bacterium	9.4×10^8	1.63 b
(b) Experiment 2 (Childers fruit)			
Water (control)		0	3.98 a
M 48	yeast	4.5×10^5	3.70 a
M 50	yeast	1.6×10^6	2.68 a
M 53	yeast	1.7×10^5	1.95 a
M 55	yeast	not recorded	2.38 a
M 60	yeast	4.0×10^6	1.84 a
M 64	yeast	3.9×10^6	2.00 a
M 74	bacterium	6.0×10^7	1.87 a

Table 7. Severity of anthracnose lesions (rated on a 0–3 scale where 0 = nil and 3 = severe) in 'Kensington Pride' mango fruit dipped in selected antagonists prior to inoculation with *Colletotrichum gloeosporioides*. Means followed by the same letter do not differ significantly at P=0.05 using the least significant difference test.

Antagonist	Lesion severity 14 days after treatment	Lesion severity 15 days after treatment
Water (control)	2.64 a	2.86 a
359 (ex avocado)	1.86 b	2.00 b
M19 (ex mango)	1.93 b	2.29 ab

Both isolates, which were applied at the same time as the pathogen, significantly reduced anthracnose lesion severity at 14 days after inoculation, although only isolate 359 did so at 15 days after inoculation.

Field application of isolate 359 to lychee 'Kwai May Pink'

Table 8 shows the effect of monthly field applications of isolate 359 on the incidence and severity of postharvest side lesions in 'Kwai May Pink' lychees. The chemical treatment (Octave + Dithane) significantly reduced both the incidence and severity of postharvest side lesions in fruit stored at 20°C for 1 week. The biological treatment (isolate 359) reduced the incidence but not the severity of these lesions in fruit stored at 20°C. Neither treatment reduced disease in fruit stored at 5°C for 4 weeks.

The survival of isolate 359 on the surface of lychee leaves was found to be poor in the early stages of the

Table 8. Effect of monthly field applications of isolate 359 on the incidence and severity of postharvest side lesions in 'Kwai May Pink' lychee fruit stored at either 5 or 20°C. Means followed by the same letter do not differ significantly at P = 0.05.

Treatment	Severity of postharvest side lesions (% area affected)		Incidence of postharvest side lesions (% fruit affected)	
	5°C (4 weeks)	20°C (1 week)	5°C (4 weeks)	20°C (1 week)
Water (control)	18.18 a	10.82 a	97.80 a	88.98 a
Broth (control)	23.83 a	11.53 a	99.44 a	90.30 a
Isolate 3S9 (biological treatment)	20.97 a	8.05 ab	99.99 a	74.70 b
Octave + Dithane (chemical treatment)	15.80 a	3.07 b	99.44 a	51.05 c

Table 9. The cause of postharvest side lesions in lychee fruit 'Kwai May Pink' stored at 5 and 20°C.

Treatment	% of side lesions caused by individual pathogens				
	Colletotrichum	*Alternaria*	*Phomopsis*	*Dothiorella*	Other
(a) 5°C					
Water (control)	39	42	12	0	7
Broth (control)	20	66	9	2	3
Isolate 359	16	62	16	2	4
Octave + Dithane	16	66	12	2	4
(b) 20°C					
Water (control)	73	0	18	5	4
Broth (control)	39	0	24	27	10
Isolate 359	35	4	23	29	9
Octave + Dithane	75	6	12	0	7

experiment (i.e. after the second and third sprays). This probably resulted in reduced efficacy of the treatment.

The causal agents of postharvest side lesions are shown in Tables 9a and 9b. *Alternaria* sp. was the predominant cause of lesions in fruit stored at 5°C, whereas *Colletotrichum* spp. were more important in fruit stored at 20°C. This may at least partly explain the variable efficacy of the chemical and biological treatments in fruit stored at the two different temperatures.

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Control of Postharvest Diseases of Mango Using Yeasts

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Abstract

The biological control potential of eleven yeast isolates against *Lasiodiplodia theobromae* was investigated. The yeasts were screened for their ability to reduce spore germination, mycelial growth, and the severity of disease caused by *L. theobromae* on mango fruit. Fruit were sprayed with yeasts at a rate calculated to produce about 1×10^5 colony forming units/cm^2, then challenged with the pathogen at 1.5×10^3 conidia/cm^2. *Candida tropicalis* (IFRPD 6010) reduced spore germination of *L. theobromae* by 18%. Fruit lesion diameter was reduced from 5.4 to 1.1 cm. Increasing nutrient supply on the fruit surface with 0.5 or 1.0% sucrose did not increase competitiveness of *C. tropicalis* against *L. theobromae*. The eleven yeasts were also tested against *Colletotrichum gloeosporioides*. It was shown that *Torulopsis glabata* (IFRPD 6035) and *Pichia membranaefaciens* (IFRPD 6031) could reduce disease incidence. *T. glabata* (IFRPD 6035) was the most promising yeast in this case.

STEM-END rot caused by *Lasiodiplodia theobromae* is an important disease of mango in Thailand. Infection occurs at the stem end and through wounds on the fruit. The disease is often controlled by dipping the fruit in hot benomyl at 55°C for 5 minutes. However, some importing countries such as Japan have a nil residue tolerance (Dezman et al. 1986). Furthermore, postharvest application of fungicides is of general concern to public health. Therefore, alternative treatments need to be developed, such as the use of of an antagonistic microorganisms. Many papers have reported success in control of pathogens using antagonistic yeasts, such as *Penicillium expansum* (Janisiewicz et al. 1997) and *Penicillium digitatum* (McGuire 1994). These antagonistic yeasts were mostly isolated from fruit or plant surfaces. Some antagonistic yeasts may produce antibiotics which are unacceptable for use on fruit. Consequently, in this paper edible yeasts were tested to evaluate their antagonistic properties toward *L. theobromae* on mango fruit.

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Materials and Methods

Sources of yeasts and preparation of yeast suspension

Yeasts, *Saccharomyces cerevisiae*—'Burgundy', 'Montrachet', and 'Champagne'— were obtained from the Central Laboratory and Greenhouse Complex, Kasetsart University, Kamphaengsaen Campus. Other yeasts, *Candida tropicalis* (IFRPD 6010), *Candida utilis* (IFRPD 6014), *Hansenula saturnus* (IFRPD 6027), *Pichia membranaefaciens* (IFRPD 6031), *Torulopsis glabrata* (IFRPD 6035), *Saccharomyces rouxii* (IFRPD 6103), *Saccharomyces chervalieri* (IFRPD 6107), and *Rhodotorula* sp. (IFRPD 6032) were obtained from the Quality Control Division, Institute of Food Research and Product Development (IFRPD), Kasetsart University. They were cultured on potato dextrose agar (PDA) plates and incubated at 25°C for 4

days. A suspension was then made by adding 4 mL of distilled water to each plate and scraping the surface of the colony with a glass rod. Yeast suspensions were filtered through cheesecloth.

Preparation of *L. theobromae* inoculum

Lasiodiplodia theobromae was isolated from diseased mango fruit displaying stem-end rot symptoms by tissue transplanting. Small pieces of diseased tissue were cut from fruit, disinfected with 1% sodium hypochlorite for 5 minutes, plated on PDA, and incubated at 25°C for 2 days. The mycelium from the developing colony was transferred to fresh PDA for maintenance (stock culture).

a. *Preparation of mycelium of* L. theobromae. Mycelium from the stock culture of *L. theobromae* was transferred to a fresh PDA plate and incubated at 25°C for 4 days. The mycelium near the margin of colony was cut with a cork borer (0.5 cm in diameter). Mycelial disks were used for the procedures which follow.

b. *Preparation of* L. theobromae *conidia*. Mangosteen fruit were inoculated on wounded areas with the mycelial disks of *L. theobromae* and incubated in a moist chamber (98–100% relative humidity [RH]) at 25°C for 2 days. It was further incubated at the same temperature for 2 weeks at normal RH (ca 60–65%). Conidia of *L. theobromae* produced on the fruit surface were brushed off, collected in a jar, then dried in silica gel. These conidia were used for experiments which follow.

Antagonist screening

a. *Effect of antagonistic yeasts on mycelial growth of* L. theobromae. Glass slides were coated with water agar (1% agar and 1% dextrose), then sprayed with yeast suspension. Cell counts, determined by dilution plating, were 1×10^5 colony forming units (cfu)/cm^2. Mycelial disks of *L. theobromae* were placed on these slides at a distance of 0.5 mm from the area of yeast spraying, incubated at 98–100% RH and 25°C for 24 hours, then the length of mycelium was measured.

b. *Effect of antagonistic yeasts on conidial germination of* L. theobromae. Glass slides were coated with water agar and sprayed with yeast suspension as described above, followed by spraying with conidial suspension at concentration of 1.5×10^3 conidia/cm^2. Conidial germination was checked after incubation at 25°C for 24 hours.

c. *Effect of antagonistic yeasts on disease severity caused by* L. theobromae *on mango fruit*. Yeast suspensions were applied to the stem end of mango by dipping. Fruit were then inoculated in the same area by dusting with *L. theobromae* conidia. Fruit were incubated at 98–100% RH and 25°C for 24 hours and further incubated at 25°C for 4 days. Disease severity was measured when the fruit were ripe.

In another experiment, two wounds were made with a needle on the side of healthy mango fruit. Yeast suspensions were sprayed onto wounded areas at a rate of 4×10^2 cfu/cm^2, followed by spraying of a conidial suspension of *L. theobromae* at 10^4/cm^2. Fruit were incubated at 98–100% RH and 25°C for 24 hours and then further incubated at 25°C for 5 days. Sizes of lesions developed on the fruit were measured when the fruit were ripe.

Test of antagonistic properties

A suspension of the most promising yeast as ascertained from initial testing, *Candida tropicalis*, was applied to mango fruit, followed by *L. theobromae*, as above. After 24 hours of incubation, conidia of *L. theobromae* were washed from the wounded area with water and checked for germination and germ tube growth. The other set of fruit was also checked for the extent of symptoms.

Candida tropicalis was checked for production of toxic metabolites or antibiotics. It was streaked on PDA and a mycelial disk of *L. theobromae* placed beside the yeast. After 7 days of incubation, the inhibition zone between these organisms was examined.

Electron microscopy. Mango fruit were either sprayed with the selected yeast or unsprayed, followed by inoculation with *L. theobromae* as described above. Tissues (0.25 cm^2) from sprayed areas were cut and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2. After fixation, tissues were washed in the same buffer, dehydrated through a graded series of ethanol including 10, 20, 30, 50, 70, 90, 100, and 100% for 15 minutes in each concentration and dried in a critical point dryer. Specimens were mounted on stubs, coated with gold, and examined with a Joel JSM–T20 scanning electron microscope.

Effect of nutrients on the fruit surface and antagonistic properties of yeast

Mango fruit were sprayed with 1% sucrose, then sprayed with the selected yeast before inoculation with *L. theobromae* by dusting conidia on the stem end. Fruit were incubated at 98–100% RH and 25°C for 24 hours and further incubated at 25°C for 4 days at normal RH (ca 60–65%). Disease severity was measured at fruit ripening.

Results

Screening of antagonistic yeasts against *Lasidiplodia theobromae*

Screening of 11 yeasts against *L theobromae*, based on reduction of stem-end rot severity on mango fruit, indicated that *Candida tropicalis* (IFRPD 6010) was the most promising yeast (Table 1). Further testing of *C. tropicalis* showed that this yeast was not pathogenic toward mango and supported initial disease suppression findings (Table 2).

Table 1. Stem-end rot severity of mango fruit treated (stem end) with different yeasts followed by inoculation with *Lasiodiplodia theobromae* and ripening at room temperature (ca 28°C). Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test (DMRT).

Yeast	Disease severity (% area of individual fruit affected)
Untreated	39.0a
Saccharomyces cerevisiae 'Burgundy'	45.0a
Saccharomyces cerevisiae 'Montrachet'	42.0a
Saccharomyces cerevisiae 'Champagne'	41.0a
Saccharomyces cerevisiae (IFRPD 6103)	44.0a
Saccharomyces cerevisiae (IFRPD 6107)	47.0a
Candida tropicalis (IFRPD 6010)	14.0c
Candida utilis (IFRPD 6014)	29.0b
Hensenula saturnus (IFRPD 6027)	35.0ab
Pichia membranefacians (IFRPD 6031)	28.0b
Rhodotorula sp. (IFRPD 6032)	50.0a
Torulopsis glabrata (IFRPD 6035)	32.0b

Test of antagonistic properties

Candida tropicalis was further tested on mango to investigate its mechanism of action against *L. theobromae*. Conidial germination was reduced from 51 to 29%. There was a lesser effect on germ tube length. Lesion diameter on yeast-treated fruit was reduced from 5.4 to 1.1 cm as compared with untreated fruit (Table 3; Fig. 1).

Table 2. Stem-end rot severity (%) of mango fruit treated (needle wounding) with *Candida tropicalis*, inoculated with *Lasiodiplodia theobromae* and ripened at room temperature (~28°C). Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test

Treatment	Disease severity (% area of individual fruit affected)
Untreated	1.0a
C. tropicalis only	0.0a
L. theobromae only	33.0c
C. tropicalis/*L. theobromae*	15.5b

Table 3. Conidial germination, germ tube length of germinated conidia, and disease severity of *Lasiodiplodia theobromae* on mango with or without prior treatment with *Candida tropicalis* (IFRPD 6010) yeast.

Treatment	Conidial germination (%)	Germ tube length (μm)	Lesion diameter (cm)
L. theobromae only	51	71.8	5.4
C. tropicalis/ *L. theobromae*	29	60.9	1.1

Candida tropicalis showed no antibiotic production on potato dextrose agar and no inhibition zone was produced between *C. tropicalis* and *L. theobromae*. Populations of the yeast on wounded areas were increased as compared to unwounded areas. Germinated conidia of *L. theobromae* were surrounded by high populations of the yeast.

Effect of nutrients on the fruit surface

Supplementing nutrient levels on the fruit surface using 1.0% sucrose increased the severity of disease caused by *L. theobromae* and could not improve the competitiveness of *C. tropicalis* against *L. theobromae* (Table 4).

Screening of antagonistic yeasts against *Colletotrichum gloeosporioides*

Eleven yeasts were tested against *Colletotrichum gloeosporioides*. It was shown that *Torulopsis glabrata* (IFRPD 6035) could reduce disease incidence (Table 5). Further testing of the more promising yeasts showed *T. glabrata* to tbe the most effective (Table 6).

Table 4. Stem-end rot severity (%) of mango fruit treated with 1% sucrose, *Candida tropicalis* and then inoculated with *Lasiodiplodia theobromae* and ripened at room temperature (~28°C). Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Treatment	Disease severity (% area of individual fruit affected)
C. tropicalis only	0.0a
1% sucrose/*C. tropicalis*	0.0a
L. theobromae only	31.0b
1% sucrose/*L. theobromae*	41.0c
1% sucrose/*C. tropicalis*/ *L. theobromae*	28.0b

Table 5. Anthracnose severity (%) of mango fruit inoculated with *Colletotrichum gloeosporioides*, treated with different yeasts and ripened at room temperature (~28°C). Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Yeast	Disease severity (% area of individual fruit affected)
Untreated	56.6abc
Saccharomyces cerevisiae 'Burgundy'	56.6abc
Saccharomyces cerevisiae 'Montrachet'	46.6bcd
Saccharomyces cerevisiae 'Champagne'	31.6cd
Saccharomyces cerevisiae (IFRPD 6103)	81.6a
Saccharomyces cerevisiae (IFRPD 6107)	51.6bcd
Candida tropicalis (IFRPD 6010)	65.0ab
Candida utilis (IFRPD 6014)	56.6abc
Hensenula saturnus (IFRPD 6027)	83.3a
Pichia membranefacians (IFRPD 6031)	41.6bcd
Rhodotorula sp. (IFRPD 6032)	42.0bcd
Torulopsis glabrata (IFRPD 6035)	25.8d

Table 6. Anthracnose severity (%) of mango fruit inoculated with *Colleototrichum gloeospoioides*, treated with different yeasts and ripened at room temperature (~28°C). Means followed by the same letter are not significantly different at the 5% level using Duncan's multiple range test.

Treatment	Disease Severity (% area of individual fruit affected)
Untreated	12.8a
C. gloeosporioides/ *Pichia membranefacians*	12.1a
C. gloeosporioides/ *Torulpsis glabrata*	8.7a
C. gloeosporioides/ *Saccharomyces cerevisiae* 'Champagne'	31.6b

Discussion

This experiment showed that the yeast *C. tropicalis* has the most potential for further development as a postharvest biological control agent against *L. theobromae*. *Candida tropicalis* grows fast so it can successfully compete with *L. theobromae* for nutrients in the wounded area of the fruit. An advantage of this yeast is that it does not appear to produce toxic metabolites or antibiotics. Furthermore, it is already used as a source of protein in stockfeed so public acceptance might be easier to obtain compared with other antagonistic microorganisms (IFRPD, personal communication). Another *Candida* species, *C. guilliermondii*, has already been developed for use as an antagonistic microorganism (Wilson and Chalutz 1989; Chalutz and Wilson 1990). It also seems to act by competing with *Pencillium* for nutrients. Droby et al. (1989) have also mentioned *Candida* spp. as potential biocontrol agents of postharvest diseases of apples. In our study, *Torulpsis glabrata* also showed promise as a biological control agent against pathogenic fungi, however its mode of action needs to be assessed. It may be possible to apply a combination of effective yeasts onto fruit to gain maximum benefit.

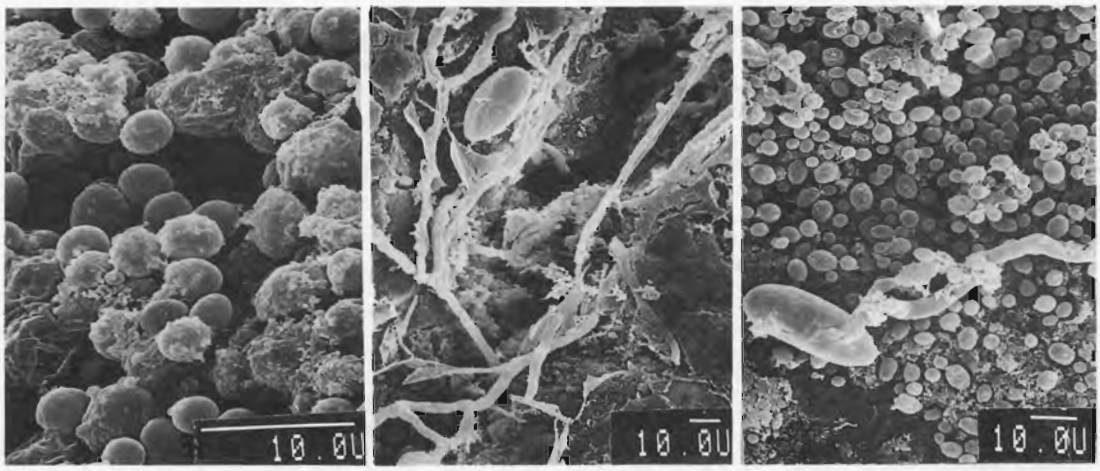

Figure 1. Growth of *Candida tropicalis* (left); *Lasiodiplodia theobromae* (centre), and *L. theobromae* and *C. tropicalis* (right) on wounded areas of mango fruits (bar = 10 µm).

Acknowledgments

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Biological Control of Postharvest Fruit Rot (*Greeneria* sp.) in Rambutan with Phylloplane Yeasts

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Abstract

An enrichment technique led to the successful isolation of phylloplane yeasts from rambutan. They were enriched for 15–20 hours in highly acidified glucose yeast peptone broth (GYPB) before dilution plating. Some 1124 isolates of phylloplane yeasts, mainly facultative anaerobes, were obtained. In vitro, antagonistic yeasts caused swelling of germ tubes of germinating conidia and a reduction in conidial germination. Further screening of antagonistic yeasts against *Greeneria* sp. (isolate 052-02-02) using a rambutan leaf disk assay showed that 17 of 105 selected yeasts (from 1124 isolates) were highly effective. Eight of the 17 isolates were tested against rambutan fruit rot naturally infected with *Greeneria* sp. The most effective antagonistic yeasts reduced disease incidence by 23%. Three of these were then tested for their antagonistic properties. Washed yeast cells and the supernatant liquid obtained after centrifugation using GYPB as a washing medium were tested against *Greeneria* sp. (isolate 052-02-02) using leaf disk and agar assays. Antagonistic properties were obtained both from yeast cells and the supernatant. Treated *Greeneria* sp. showed abnormal colonies, suppressed acervular formation, and little or no conidial formation.

POSTHARVEST fruit rot is a serious problem for long-term storage and transportation of rambutan. One of the most significant causal pathogens is the fungus *Greeneria* sp. (Gn), which represented 50% of disease incidence during 1990–1993. Gn has been mistaken for *Colletotrichum gloeosporioides,* another fungus which causes fruit rot, however the two species can be quite readily distinguished. Gn produces healthy, well-sporing colonies which are olive-green to dark sepia in colour and is restricted in its host range. In contrast, *C. gloeosporioides* produces pinkish conidial masses and has a wide host range. Furthermore, the *Glomerella cingulata* stage was found in *C. gloeosporioides* but never in Gn (Farungsang et al. 1992, 1994). It was identified clearly by Dr John Alcorn of Queensland Department of Primary Industries (QDPI), Australia in 1996.

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Various control measures which have been consistently used on rambutan, such as hot water or chemical treatments, have proven unsatisfactory for control of Gn. In addition, Gn shows resistance to high doses of benomyl (Farungsang and Farungsang 1992; Farungsang et al. 1992). In the study presented here, biological control is investigated as a possible alternative to these treatments.

The way in which most bacteria are effective as biological control agents is through the production of antibiotics. Hence their use as biological control agents is not possible, as it is undesirable to have antibiotics associated with edible products. Yeasts, however, often do not produce antibiotics as their mode of action. Some common yeast isolates obtained from the phyllosphere can function as biological control agents on plant surfaces (Fokkema and Lorbeer 1974). Another yeast has been suggested as a biological control agent in cheese (Wilson and Chalutz 1989). Attention is now focused on the use of yeasts for non-toxic, antibiotic-free biological control. Isolation of phylloplane yeasts is difficult because they represent only a small proportion of the

microflora in relation to a large population of highly competitive, fast growing bacteria. They can not be isolated unless an enrichment technique is used (Beech and Davenport 1971). The facultative anaerobic nature of the yeasts could be advantageous to their significance in the biological control of postharvest pathogens (Wilson and Pusey 1985).

This paper presents effective techniques for the isolation and screening of phylloplane yeasts, as well as investigating some of the antagonistic activity displayed by the yeast isolates obtained, in relation to the control of postharvest fruit rot in rambutan caused by Gn.

Materials and Methods

Isolation of phylloplane yeasts and test of their facultative anaerobic nature

Flushing leaves, inflorescences and fruit at various stages of development were collected from rambutan canopies. Dew was also collected from the canopies. These materials were shaken in highly acidified GYPA (10g glucose, 2g peptone, 0.5g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$ and 0.5g yeast extract per litre) for 15–20 hours at pH 3.7, in order to enrich a small population of accompanying yeasts in the mixture. Isolation of the yeasts was then achieved on GYPA plates by flooding with 0.5mL of a 1% peptone dilution.

The facultative anaerobic character of the obtained yeast isolates was tested by stab-inoculation using semisolid, acidified GYPA (in test tubes) overlayed with and without paraffin oil, for anaerobic and aerobic testing, respectively. Test tubes were incubated for 24–48 hours at 13 or 25°C. A total of 316 isolates were tested.

Observation of antagonistic effect on conidial germination of *Gn*

An intermixture of yeast cells and a Gn conidial suspension was spread onto potato carrot agar (PCA), then conidial germination was observed microscopically after an 8-hour incubation period. Forty-five facultative anaerobic yeast isolates were tested against three Gn isolates.

Screening of antagonistic yeasts using a leaf disk assay

Newly mature rambutan leaf disks ('Rongrien') were prepared following Farungsang et al. (these proceedings). Inoculations were prepared as intermixtures of yeast isolates (cultured on glucose yeast peptone broth [GYPB] for 15–20 hours) and GYPB conidial suspensions of Gn (isolate 052-02-02). A 20 mL drop of inoculum was put onto the prepared leaf disk. Fruiting body and total conidial production by the fungus were assessed on five replications after a 6-day incubation period. Fruiting body formation was rated on a 0–4 scale where 0=none, 1=1–25%, 2=26–50%, 3=51–75% and 4=>75%. A total of 105 yeast isolates were identified as potentially suitable candidates for biological control.

Determination of antagonistic potential of yeast isolates on detached rambutan fruit

Detached rambutans of healthy appearance were thoroughly soaked in 15–20-hour GYPB cultures of tested yeast isolates, drained, then covered with polyethylene wrap and left on the laboratory shelf. Incidence of disease by natural field infections was determined by tissue transplanting soon after rot symptoms were observed. Fungal species identified were Gn, *C. gloeosporioides* (Cg), *Gliocephalotrichum bulbilium* (Gb), *Lasiodiplodia theobromae* (Lt), *Pestalotiopsis* sp. (Pe), and *Phomopsis* sp. (Po). Eight highly effective yeast isolates screened by the leaf disk assay were tested, with six non-antagonistic isolates used for comparison. A GYPB-only treatment was conducted as a control. Forty rambutans were assigned to each treatment.

Determination of the active consituents of the potential antagonists

A mixture of 3 antagonistic isolates which were completely successful in controlling Gn activity in the leaf disk assay (neither fruiting body nor conidium was formed by Gn) was used for this experiment. Washed cells and culture filtrates obtained from 15–20-hour GYPB cultures of the selected isolates were tested against Gn (isolate 052-02-02) through both leaf disk and agar assays. Washed cells were resuspended by 1 absorbance (turbidity of the cell suspension) adjusted at 660 nm with GYPB. For the leaf disk assay, washed cells or culture filtrates (1:1 v/v), together with a Gn conidial suspension (10^6 conidia/mL in GYPB), were inoculated as an intermixture (1:1 v/v) onto newly matured 'Rongrien' rambutan leaf disks. For the agar assay, hyphal tips from an advancing Gn colony were inoculated onto potato dextrose agar (PDA) supplemented with washed cells (0.1% resuspension) or culture filtrates (20% v/v).

Results and Discussion

Acidified glucose yeast peptone broth medium was very effective in suppressing competition from bacteria and stimulating development of the yeasts. A total of 1124 isolates of phylloplane yeasts were obtained between 1993–1995 from orchards located in eastern and southern Thailand. Of these, 316 isolates tested were shown to be facultative anaerobes growing at 13 and 25°C (Table 1, Fig. 1).

Table 1. Facultative anaerobic test of the collected phylloplane yeasts. ($+O_2$ = stabbed medium without paraffin oil overlay [aerobic condition], $-O_2$ = stabbed medium with paraffin oil overlay [anaerobic condition]). Total number of isolates tested was 316.

Growth ability	Number of isolates			
	13°C		25°C	
	$+O_2$	$-O_2$	$+O_2$	$-O_2$
Excellent	73	87	68	56
Good	229	193	236	153
Medium	11	31	11	81
No growth	3	5	1	26

Typical characteristics of Gn are shown in Fig. 2. Forty-five facultative anaerobes showed an aggressive inhibitory effect on conidial germination of Gn, with germination rates of 5–30% in contrast to an average of 50.3% in the control (Table 2). In addition, swelling of the germ tubes was observed upon germination and after 24 hours growth. Mycelia with a rough surface and with short hyphal cells, or exhibiting abnormal branching, were seen (Fig 3).

Table 2. Antagonistic effect of facultative anaerobes on conidial germination of *Greeneria* sp.

Antagonistic rate	Conidial germination percentage rank[a]	Number of yeast isolates
Very aggressive	5–10	9
Moderately aggressive	11–20	28
Aggressive	21–30	8
Control	50.3	

[a] Average of the three Gn isolates tested

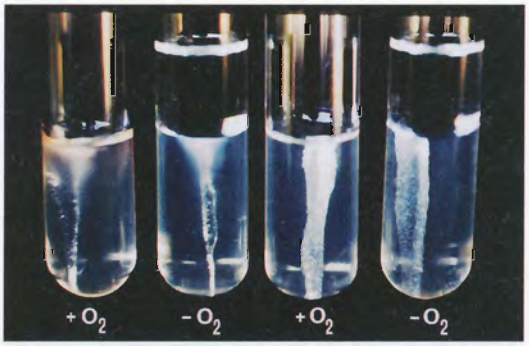

Figure 1. Comparison of the phylloplane yeast isolates tested in acidified semisolid glucose yeast peptone broth with ($-O_2$) and without ($+O_2$) paraffin oil overlay, showing their facultative anaerobic nature.

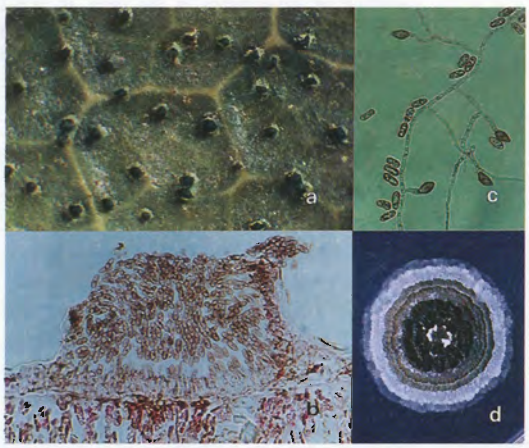

Figure 2. *Greeneria* sp. characteristics: acervuli on rambutan leaf disk (a), a paraffin section (10 mm) of an acervulus (b), appressoria (c) and colony on potato dextrose agar (d).

The leaf disk assay identified 47 antagonistic isolates from 105 candidates screened, as judged by a reduction in both fruiting bodies and total conidia/disk formed by the pathogen. Among these, 17 isolates proved to be highly effective antagonists, with a fruiting body formation rating of <1.25 and total conidia/disk rates of 25×10^5 conidia, compared to the control (2.6 and 95×10^5, respectively) (Table 3). Three of these isolates showed complete control with neither fruiting body nor conidium formation. Development of Gn on the leaf disks was not inhibited by the tested yeast isolates if the conidial suspension was prepared in sterilised water or 1% peptone water, rather than nutrient broth.

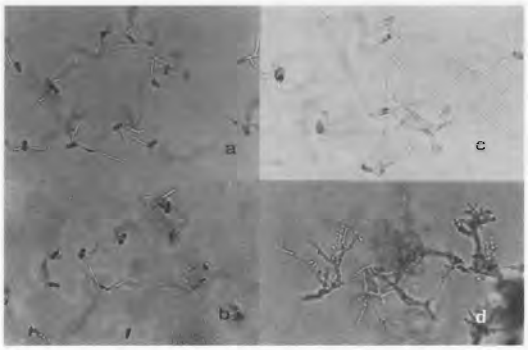

Figure 3. Antagonistic effect on conidial germination of *Greeneria* sp. shown by tested yeast isolates. Normal appearance (control) of germ tubes at the time of initial germination (a) and after 24 hours (c). In the treated samples there is swelling of germ tubes at the time of initial germination (b), and mycelia with a rough surface and with short hyphal cells or abnormal branching seen after 24 hours (d).

Direct testing on detached rambutan fruit showed antagonistic potential in all of the eight most highly effective isolates identified in the leaf disk assay. They showed an average of 34.8% disease incidence caused by naturally infected Gn and 4.7% disease-free rambutan fruit, whereas the non-antagonists and the control showed an average of 52.8 and 51.8% Gn disease incidence, respectively, and no disease-free fruit at the end of 8 days (Table 4). The most effective antagonist (isolate 94-04-01) resulted in the lowest Gn disease incidence of 28% and the highest proportion of disease-free fruit (10%). All of the fruit in the non-antagonistic and control treatments were decayed by natural infections within 5–7 days. Similar results were reported for postharvest disease control in mango (Coates et al. 1996).

These results indicate that the leaf disk assay is very effective in successfully identifying potentially useful isolates. The advantage of being able to use the leaf disk assay to screen for non-antibiotic producing antagonists is that the assay is very simple, economical, and quick. Conversely, screening directly on rambutan fruit is time consuming, costly, and only possible during a limited season.

The effect of the antagonists on the other pathogenic fungi tested was varied (Fig. 4). Incidence of disease caused by Cg, Pe, and Po was lowered, but increased for Lt and the minor pathogen Gb. There was an inverse correlation between disease incidence caused by Gn compared to Lt (or Gb) under our experimental conditions which suggests that there may be natural competition for infection between these two rambutan pathogens (see also Farungsang et al. 1994). It would be useful to undertake further screening to identify an antagonistic isolate which may be able to offer broad spectrum control.

Table 3. Screening of antagonistic yeasts through the leaf disk assay.

Antagonistic rank	Number of isolates	Fruiting body formation level[a]	Total conidia/ disk ($\times 10^5$)
Completely effective	3	0	0
Highly effective	14	<1.25	1–25
Effective	36	1.25–2.25	26–60
Non-antagonist	52	>2.25	>60
Control		2.6	95

[a] Scale of 0–4 used of 0=none, 1=1–25%, 2=26–50%, 3=51–75% and 4=>75%.

Table 5. Determination of the active component of the effective antagonistic yeasts through use of the leaf disk assay.

Treatment	Component	FBL[a]	TC/D[b] ($\times 10^4$)
94-04-01 (yeast)	Washed cell	0.0	0.0
	Culture filtrate	0.7	9.5
	Cell culture	0.0	0.0
95-01-17 (yeast)	Washed cell	1.45	31.0
	Culture filtrate	1.05	14.0
	Cell culture	0.35	3.0
95-02-05 (yeast)	Washed cell	1.35	25.0
	Culture filtrate	0.65	4.0
	Cell culture	0.35	3.0
052-02-02 (Gn)		1.8	51.5

[a] Fruiting body formation level as a percentage of leaf disk area occupied by fruiting bodies, using a scale of 0–4 of 0=none, 1=1–25%, 2=26–50%, 3=51–75% and 4=>75%.
[b] Total conidia/disk.

Table 4. Potential of the selected antagonists for control of *Greeneria* fruit rot caused by natural field infection on detached rambutan fruit. The proportion of disease free fruit was measured at the end of the 8-day experiment (Cg = *Colletotrichum gloeosporioides*, Gn = *Greeneria* sp., Gb = *Gliocephalotrichum bulbilium*, Lt = *Lasiodiplodia theobromae*, Pe = *Pestalotiopsis* sp., Po = *Phomopsis* sp., GYPB = glucose yeast peptone broth).

Antagonistic candidate	Effect on Gn in leaf disk assay		Disease incidence by natural infection (%)						Disease-free fruit (%)
	FBL[a]	TC/D[b]	Cg	Gn	Gb	Lt	Pe	Po	
Highly effective antagonists									
39-47-2	1.0	0.26	0	33.5	6	34	6	11.0	5
39-52-3	0.8	0.9	0	39.5	1	33	2	15.0	5
39-77-4	0.5	0.54	0	31	6	39	3	7.5	5
39-82-1	1.2	0.86	0	38	0	54.5	2	13.0	2.5
39-84-1	1.2	0.86	3	38	1	40	3	9.0	0
39-87-2	0.8	1.7	0	34	0	43.5	1	6.0	7.5
94-04-01	0.0	0.0	0	28	4.5	46	2	7.0	10
94-04-11	0.7	0.9	1	36	8.5	31	4	14.5	2.5
Average				34.8	3.4	40.1	2.9	10.4	4.7
Non-antagonists									
39-67-2	2.0	9.6	0	50	0	41	1.5	22.0	0
39-87-1	2.4	8.1	0	45	1.5	37	6	12.5	0
39-88-2	2.2	9.0	0	62.5	3	25	5	9.5	0
94-03-16	2.2	11.3	0	50	6	34	1.5	9.5	0
94-03-20	2.5	10.5	3	47	1.5	39	4.5	12.5	0
94-04-04	2.3	9.3	0	62	1.5	23	3	12.5	0
Average			0.5	52.8	2.3	33.1	3.6	13.1	0
Greeneria sp.	2.5–3.1	8.6–1.42							
GYPB	–	–	0	55.0	1	31	5	15.5	0
Untreated			1	48.5	2.5	38	2	12.0	0
Average			0.5	51.8	1.8	34.5	3.5	13.8	0

[a] Fruiting body formation level using a scale of 0–4 of 0=none, 1=1–25%, 2=26–50%, 3=51–75% and 4=>75%.
[b] Total conidia/disk ($\times 10^6$).

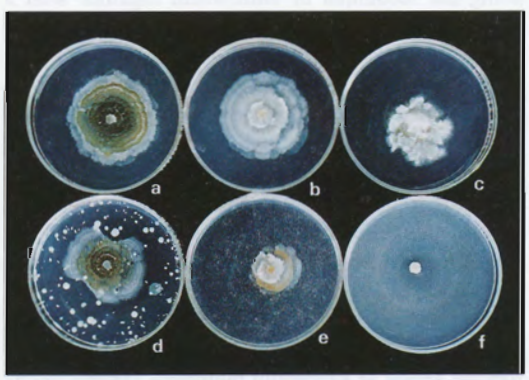

Figure 4. Antagonistic effect of the tested yeast isolates on Gn development grown on potato dextrose agar. Typical dark-sepia, well sporing colony (a), atypical colonies resulting from yeast culture filtrate being supplemented into the medium (b and c), fungal colonies affected by development of the tested yeasts showing atypical radial growth and scattered spot development (d), suppressed fruiting body formation (e), and completely inhibited fungal colony achieved by full extension of the yeast (f).

Table 6. Determination of the active part of the effective antagonistic yeasts on potato dextrose agar (PDA) (cell = washed cell, filtrate = culture filtrate, n/a = not applicable, as there was no growth of the fungus).

Yeast isolate	Determined part	Effect on colony		Effect on sporulation		Development of yeast
		Size[a]	Appearance	Amount	Appearance	
94-04-01	Cell	41.0	Lobe-normal	Abundant	Normal	Scattered spots
	filtrate	51.0	Normal	Abundant	Normal	–
95-01-17	Cell	0.0	n/a	n/a	n/a	Full extension
	filtrate	0.0	n/a	n/a	n/a	Full extension
95-02-05	Cell	0.0	n/a	n/a	n/a	Full extension
	filtrate	43.6	Lobe	Rare-less	Normal	–
PDA	–	48.0	Normal	Abundant	Normal	–

[a] Diameter (mm)

A dramatic decline in the total conidia/disk formed by Gn, as shown in Table 5, demonstrated that the active constituent which results in the antagonistic effect is present in both washed cells and culture filtrates. However, the severity of the effect was reduced in the separate components compared to the original isolate conditions. The agar assay showed that antagonistic behaviour was greater using washed cells compared to culture filtrates. Radial growth of Gn was suppressed completely by the aggressive development of yeast cells (Table 6). In the presence of washed cells or culture filtrates of antagonistic yeast isolates, Gn colonies were characteristically lobe-edged, in addition to having reduced or rare sporulation (Fig. 4).

Conclusions

Isolation of phylloplane yeasts was very successful using the technique of enrichment together with a highly acidified broth medium. The obtained yeast isolates showed some antagonistic effect on the germination and development of Gn The leaf disk assay was found to be a simple, adaptable method for the screening of non-antibiotic antagonists. A sharp decline in disease incidence caused by naturally infected Gn as well as a significant increase of disease-free fruit resulted from protection provided by the highly effective antagonistic yeast isolates identified by the leaf disk assay. Antagonistic effects of the yeast isolates were caused by both cells and metabolites. We propose that the mode of action of the phylloplane yeasts as successful biological control agents is, at least in part, through competition for nutrients. In future studies we aim to improve the precision and effectiveness of screening through the leaf disk assay as well as focus on identification of the effective antagonists and their modes of action.

Acknowledgments

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Factors Affecting the Colonisation of Mango by *Dothiorella dominicana* (syn. *Fusicoccum aesculi*) : a Model System

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Abstract

Seed transmission of *Dothiorella dominicana* (= *Fusicoccum aesculi*) was achieved by heat disinfesting mango fruit then inoculating them at the stem-end with a mycelial plug. Fruit were incubated and the seeds extracted when symptoms of stem-end rot (SER) began to appear (5–20% decay). Seeds were (in mango) removed from the endocarp and surface sterilised in sodium hypochlorite before planting. Seeds in soil were held in a glasshouse or growth chamber at 20–28°C until seedlings emerged then assayed at various intervals.

Seed-to-seedling transmission was demonstrated for *Dothiorella dominicana* as well as *D. aromatica*, *Lasiodiplodia theobromae*, *Phomopsis mangiferae* and *Colletotrichum gloeosporioides*. Transmission was demonstrated in the polyembryonic mango cultivar 'Kensington' and the monoembryonic cultivar 'Tommy Atkins'. Colonisation occurred mainly in the hypocotyl zone but in some cases extended up into the seedling stem.

Transmission of SER pathogens (*D. dominicana* but also *Phomopsis mangiferae*) to seedlings of 'Kensington' mangoes was affected by:

- SER severity at seed extraction—High seed mortality occurred when seed were extracted from fruit with more than 10% decay.
- Pathogen virulence—Seed viability, seedlings per seed and seedling size were reduced when fruit were inoculated with more virulent isolates of *D. dominicana*.
- Fruit ripening (decline in constitutive resistance)—Seed from fruit allowed to ripen 7 days before inoculation with SER pathogens had lower viability than seed of fruit of the same consignment which were inoculated within 24 hours of harvest (mature green). Also, seedling number and vigour were reduced.
- Soil type/soil microflora—Transmission of *D. dominicana* and *P. mangiferae* was lower in steam pasteurised sand/peat (UC) mix than in commercial potting mix (high organic matter UC/potting mix combined. In UC mix, more frequent/extensive colonisation of seedlings by *Talaromyces flavus* and *Trichoderma viride* may have interfered with or obscured confirmation of transmission of SER fungi.

STEM-END rot diseases caused by anamorphs of *Botryosphaeria* spp. (*Dothiorella dominicana* Pet. & Cif. (= *Fusicoccum aesculi* Corda), *Lasiodiplodia theobromae* (Pat.) Griff. & Maubl., and other *Dothiorella* spp.) and *Phomopsis mangiferae* Ahmad cause serious losses in mangoes during postharvest storage and marketing. Johnson et al. (1991,1992, 1993) reported that infection of mangoes by stem-end rot pathogens occurred by endophytic (symptomless) colonisation of the peduncle during fruit development, or by contamination of the cut end of the pedicel with soil-borne inoculum at harvest.

The mango (*Mangifera indica* L.) tree is a perennial evergreen tree growing up to 40 m tall, and usually producing one crop of fruit per year in late spring or summer. The fruit is a fleshy drupe containing a single, large seed (Whiley 1984). Two general types

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of mango are recognised; polyembryonic types from Southeast Asia, and monoembryonic cultivars from India, from which most commercial cultivars have been derived. The former have one to six apomictic, and one zygotic embryo, while the latter have one zygotic embryo per seed (Whiley 1984). Superior cultivars are propagated by grafting, although polyembryonic varieties show little variation in progeny from seed.

While intra-tree spread of stem-end rot pathogens could be facilitated by endophytic inoculum, the mode of inter-tree and inter-orchard spread of the pathogens has not been documented. Davis (1987) confirmed that seedborne infections of *C. gloeosporioides* could facilitate regional and inter-continental spread of anthracnose of *Stylosanthes* pasture legumes. Pandey and Dwivedi (1987) reported *Lasiodiplodia theobromae* and *C. gloeosporoides* as seed borne pathogens of guava, and seed transmission of endophytic fungi has been documented for other hosts (Rayner 1929; Hayden and Maude 1992; Maximay et al. 1992). Seed transmission would provide a means of inter-continental dissemination for mango fruit pathogens, and may have potential as an experimental technique to produce seedling lines symptomlessly infected with particular fungi. In the work reported here, seed transmission of mango fruit rot pathogens has been examined (Johnson et al. 1993).

Materials and Methods

Seed transmission

Seed was extracted from mango fruit that were ripe and either unaffected by decay, or showing stem-end decay due to natural infection or inoculation with a particular fruit rot pathogen. Unless indicated otherwise, the mangoes were 'Kensington', a polyembryonic variety which is the main commercial cultivar grown in Australia (Whiley 1984). Fruit were immersed in hot water at 55°C for 5 minutes before inoculation, to control quiescent infections already present on fruit (Johnson et al. 1990). Fruit were inoculated by inverting on the stem-end wound a disc of mycelium from a potato dextrose agar culture of the test fungus. Before seed extraction, fruit were allowed to decay until 5–20% of the fruit had been destroyed.

The seeds were surface sterilised by immersion in sodium hypochlorite (10%) after extraction from the fruit flesh and endocarp. Seeds were germinated either in the laboratory or in the glasshouse.

Laboratory germination

Seeds were placed on sterile cotton wool moistened with sterile distilled water inside sterile incubation chambers (clear plastic food storage containers that had been washed with sodium hypochlorite (10%) and allowed to air dry in a biosafety cabinet). The chamber lids were kept loose to allow air exchange, and the chambers stored in darkness at 22°C until the seedlings appeared, or the seeds decayed. When germination was apparent, the chambers were held in the light on the laboratory bench.

Glasshouse germination. Seeds were planted in commercial potting mix consisting of pulverised bark and other materials in styrofoam fruit boxes. The medium was kept well watered and held in the glasshouse until seedlings appeared. As seeds of 'Kensington' mangoes are polyembryonic, more than one seedling emerged from each seed. Seedlings were removed from the containers once the first growth-flush leaves of the largest seedling had changed from bronze-brown (immature) to dark-green (mature) (Joubert 1991). The seedlings were separated, photocopied to record growth stage and sampling positions, and segments excised at the positions indicated in Table 1.

The segments were surface sterilised in 70% alcohol (for 60 seconds), then sodium hypochlorite (10% for 3 minutes), and finally 70% alcohol (for 30 seconds) (Johnson et al. 1992). Tissue was excised from segments as described by Johnson et al. (1992) and plated onto Difco® potato dextrose agar (PDA) with added streptomycin sulfate (40 mg/L). Assay plates were incubated at 25°C, and examined after 7 to 14 days to assess the extent and composition of the endophytic fungal colonisers. Pathogenicity of selected isolates (*Pestalotiopsis mangiferae, Colletotrichum gloeosporioides,* and *Dothiorella dominicana*) from seedlings of healthy seed was demonstrated by wound inoculating heat treated mango fruit (Johnson et al. 1990).

Seeds from fruit free of decay symptoms (healthy seed) from two sites, and seeds from one site of fruit inoculated with either *D. dominicana, C. gloeosporioides, L. theobromae* or *Phomopsis mangiferae* were raised in the laboratory (22°C). Healthy seeds from both sites, and seeds from one site of fruit inoculated with *P. mangiferae* or *D. dominicana*, was raised in commercial potting mix in the glasshouse (18–26°C). Seeds of healthy fruit from additional sites were germinated in the laboratory and assayed as described where possible.

Seeds from fruit of the monoembryonic cultivar 'Kent' showing a range of stem-decay symptoms (20–40% decay) resulting from natural infections were surface sterilised, planted in UC mix (Baker 1957), and raised in the glasshouse. Immediately before seed extraction, isolations were made from lesions on the fruit, and a record kept of seed × decay lesion lineage. Seedlings from all plantings were assayed as described.

Mature seed assays

Seed was extracted from the endocarp of Kensington mangoes showing various levels of stem-end decay resulting from postharvest inoculation with *D. dominicana*. Tissue was excised from various parts of the seed—the funiculus at the pedicel and hilum ends, the inner and outer integuments, healthy and unhealthy (discoloured) endosperm and the embryo tissue, sterilised and plated onto PDA (Johnson et al. 1992).

Effect of stem-end rot severity at seed extraction

To determine the effect of isolate vigour and fruit age (vitality) on seed transmission, mango fruit were inoculated with strains shown previously to colonise fruit at different rates. In addition, fruit from the same source and harvest were either inoculated within 48 hours of harvest or at 7 days after harvest. The fruit were incubated in humid conditions for 24 hours and then stored at 23°C until individual fruit were ca 20% decayed. Seed viability and transmission of inoculated fungi were then assessed in steam pasteurised sand–peat by the methods described above, with assessments being made 3 months after planting.

Soil type/soil microflora

To determine if soil type affected the endophytic transmission of *D. dominicana* or its impact on seed viability, seed from healthy and inoculated fruit was planted in (a) freshly steam sterilised sand–peat (UC) mix, (b) commercial potting mix (CPM) or (c) a 50:50 mix of the two soils. Seed viability and the incidence and recovery of *D. dominicana* were recorded.

Results

Seed transmission, laboratory studies

Seedling production from individual seed ranged from one to six, with three to four on average (Table 1). The seedlings appeared healthy and showed no visible symptoms of colonisation by fungi. Generally, all seedlings from one seed were colonised by the same one or two fungi. *Pestalotiopsis mangiferae* and *Penicillium* sp. were detected most frequently from seedlings of healthy fruit (Table 2), with the seedling stem at the hypocotyl zone and the tap-root yielding fungi most frequently, and the shoot tip least frequently (Table 1). *Phomopsis mangiferae* was the predominant fungus isolated from hypocotyl segments of the ten seedlings that emerged from only two seeds (Table 2), and *D. dominicana* was recorded from some seedlings that emerged from two seeds from another site (data not shown).

Most seed planted in enclosed containers in the laboratory failed to germinate when they were from fruit with stem-end decay arising from inoculation with *D. dominicana* or *Phomopsis mangiferae*. Tissue segments taken from the few seedlings that did emerge yielded *D. dominicana* or *Phomopsis mangiferae*, and symptomless colonisation was detected at all assay points. All seed from fruit inoculated with either *C. gloeosporioides* or *Lasiodiplodia theobromae* failed to germinate, and decayed, when enclosed in containers in the laboratory.

Seed transmission, glasshouse studies

Seedlings from seed of healthy fruit yielded a variety of fungi not familiar as pathogens or saprophytes of mango. Detection frequency decreased with increasing distance from the roots (Tables 1 and 2). Emergence of seedlings from seed from inoculated fruit was lower than that from seed of healthy fruit. Assays of seedlings grown from fruit inoculated with *Phomopsis mangiferae* or *D. dominicana* yielded the respective test fungus almost exclusively. Seedlings from ''Kent' mangoes affected by naturally contracted stem-end decay were colonised by the fungi associated with the corresponding stem-end decay lesion, including *L. theobromae, C. gloeosporioides* and *D. dominicana* (Table 4).

Mature seed assays

When stem-end rot had destroyed about 8% or less of fruit at the time of seed extraction, *D. dominicana* was isolated from seed parts less frequently than when fruit were more extensively decayed at the time of seed extraction. Embryo and healthy endosperm yielded *D. dominicana* least frequently (Table 3).

Table 1. Incidence of detection of endophytic fungi in tissue segments of mango seedlings raised from either seed extracted from healthy, ripe fruit from (i) Bowen or (ii) Brisbane Markets, and planted in either (a) sterilised, enclosed plastic containers in the laboratory, or (b) commercial potting mix in styrofoam trays in the glasshouse

Seed source	Incidence (%) in segments a to e					
	Root (a)	Hypocotyl (b)	Stem base (c)	Mid-stem (d)	Apex (e)	Seedlings per seed
Bowen						
(a)	36.4	45.5	57.6	21.2	9.1	3.56
(b)	79.5	86.4	65.9	27.3	11.4	4.60
Markets						
(a)	38.2	51.3	48.8	16.3	6.0	4.00
(b)	91.7	63.9	50.0	17.8	2.8	3.89

Table 2. Fungi detected as endophytic colonisers from mango seedlings raised from seed extracted from ripe, healthy fruit from (i) Bowen or (ii) Brisbane Markets and planted in either (a) sterilised, enclosed plastic containers in the laboratory, or (b) commercial potting mix in styrofoam trays in the glasshouse

Fungus	Root (a)	Hypocotyl (b)	Stem base (c)	Mid-stem (d)	Apex (e)
(a) Fungus incidence (%) in tissue segments a to e averaged across fruit source: laboratory-grown seedlings					
Pestalotiopsis mangiferae	13.23	22.05	22.05	5.89	8.82
Penicillium sp.	11.76	13.23	8.82	7.35	4.41
Colletotrichum gloeosporioides	1.47	0.0	0.0	1.47	0.0
Dothiorella dominicana	0.0	0.0	0.0	0.0	0.0
Aspergillus niger	2.94	2.94	2.94	0.0	0.0
Phomopsis mangiferae	1.47	7.35	0.0	1.47	0.0
Fusarium sp.	7.35	7.35	7.35	1.47	1.47
Other	0.0	1.47	1.47	0.0	2.49
(b) Fungus incidence (%) in tissue segments a to e averaged across fruit source: glasshouse-grown seedlings					
Fungus A	7.4	17.7	17.7	5.9	1.5
Fungus C	16.2	23.5	13.2	1.5	2.9
Fungus E	5.9	2.9	2.9	1.5	0.0
Aspergillus niger	16.2	4.4	4.4	1.5	0.0
Other	16.6	17.7	20.6	4.4	2.9

Table 3. Incidence of detection of *Dothiorella dominicana* in seed tissue parts at planting

Seed part	Detection incidence (%) from fruit with stem-end rot severity of:	
	1 to 8%[a]	8 to 15%[b]
Pedicel	66.7	100.0
Upper funiculus	83.3	88.9
Lower funiculus	66.7	77.9
Outer integument	66.7	88.9
Inner integument	66.7	88.9
Healthy endosperm	16.7	0.0
Necrotic endosperm	66.7	88.9
Embryo	0.0	22.2

[a] Mean of 6 seed; [b]Mean of 9 seed.

Table 4. Fungi detected as endophytic colonisers of 'Kent' mango seedlings raised in U.C. type soil mix in styrofoam trays in the glasshouse from seed extracted from ripe fruit from the Northern Territory showing a variety of disease lesions. Fungus incidence (%) is tissue segments a to e[a] of seedlings sampled 30 days after planting (incidence of fungi associated with stem-end lesions shown in parenthesis)

Fungus	Root a	Hypocotyl b	Stem base c	Mid-stem d	Apex e
Pestalotiopsis mangiferae	8.3	8.3	8.3	0.0	16.7
Colletotrichum gloeosporioides (41.6)	0.0	8.3	0.0	0.0	0.0
Dothiorella domincana (8.3)	8.3	8.3	8.3	8.3	8.3
Lasiodiplodia theobromae (8.3)	0.0	0.0	8.3	8.3	0.0
Trichoderma viride	58.4	41.7	33.3	16.7	0.0
Other	8.3	0.0	8.3	8.3	0.0
Nil	41.7	33.3	41.7	57.3	90.9

[a] Tissue segments are as indicated in Figure 2

Pathogen virulence

Seed viability, seedlings per seed and seedling size were reduced when fruit were inoculated with more virulent isolates of *D. dominicana* (data not shown). Seedling colonisation by virulent isolates was more extensive (Fig. 1).

Fruit ripening

Seed from fruit allowed to ripen 7 days before inoculation with SER pathogens had lower viability than seed of fruit of the same consignment which were inoculated within 24 hours of harvest (mature green) (Fig. 2). As well, seedling number and vigour were reduced.

Soil type/soil microflora

Seed transmission of *D. dominicana* was more extensive in inoculated seed planted in commercial potting mix (CPM) and the 50:50 CPM:UC mix than in UC mix alone (Fig. 3). More extensive colonisation by *T. flavus* of seedlings in UC mix (Fig. 4) may have interfered with transmission.

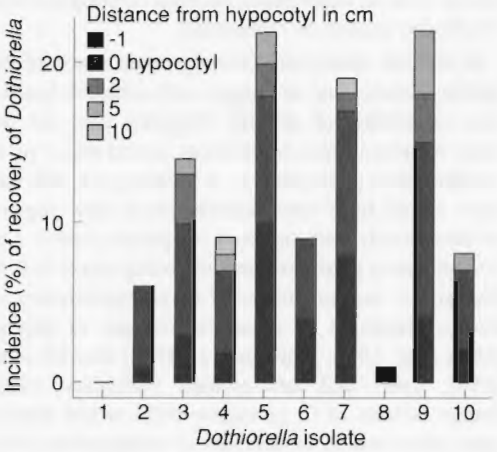

Figure 1. Effect of *Dothiorella* isolate virulence on the extent of colonisation of 'Kensington' mango seedlings 6 weeks after planting. 1 = control (uninoculated); 2–10 = inoculated with *Dothiorella* spp. isolates as follows: 2. *D. dominicana* 23300, ex avocado SER; 3. *D. dominicana* 23348, ex mango SER; 4. *D. mangifera* 23350, ex mango SER; 5. *D. mangifera* 23396 ex mango fruit rot; 6. *Fusicoccum cajani* 23401, ex mango fruit rot; 7. *F. cajani* 23491 ex mango SER; 8. *D. dominicana* 23556, ex mango fruit rot; 9. *D. aromatica* (*Fusicoccum luteum*) 23597, ex mango wood rot. Stack graph shows recovery (incidence) at assay points from 1 cm below to 10 cm above the hypocotyl (0).

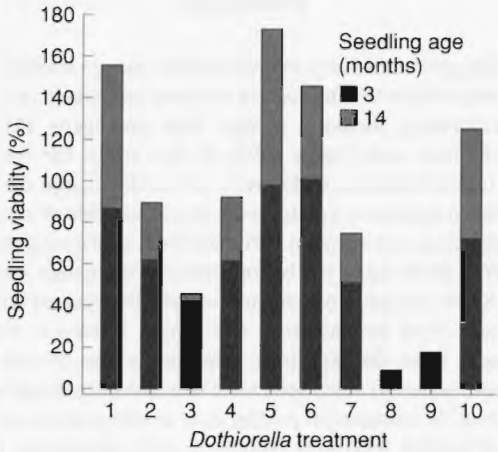

Figure 2. Effect of *Dothiorella* isolate virulence and fruit vitality on viability of mango seedlings grown from fruit inoculated 2 and 7 days after harvest. Seedlings assayed either 3 or 14 months after planting. Treatments 2–5: seed from fruit inoculated 2 days after harvest; treatments 7–10: seed from fruit from the same farm/harvest inoculated 7 days after harvest; treatments 1 and 6: uninoculated fruit from 2 and 7 day inoculations. Pathogen isolates: treatments 2 and 7, *D. dominicana* 23300; treatments 3 and 8, *D. dominicana* 23301; treatments 4 and 9, *D. dominicana* 234031; treatments 5 and 10, *D. aromatica* 23597.

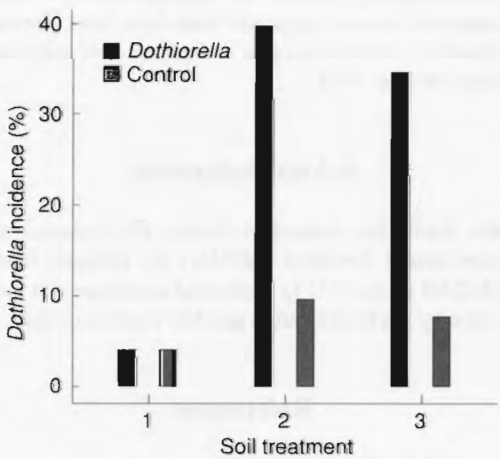

Figure 3. Recovery of *D. dominicana* from the hypocotyl of seedlings grown from inoculated (Doth) and uninoculated (Con) seed planted in 1. UC mix, 2. commercial potting mix (CPM), or 3. 50:50 UC:CPM, and assayed within 2 months of planting.

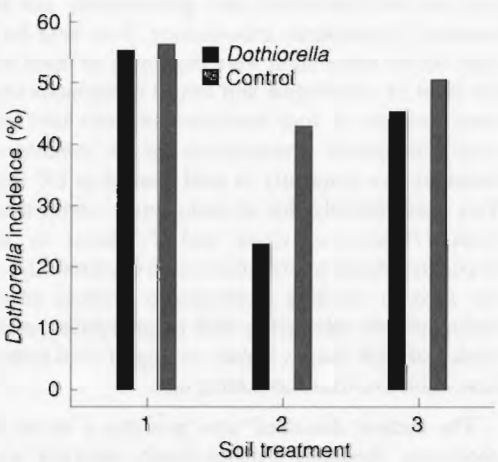

Figure 4. Recovery of *T. flavus* from the hypocotyl of seedlings grown from inoculated (Doth) and uninoculated (Con) seed planted in 1. UC mix, 2. commercial potting mix (CPM), or 3. 50:50 UC:CPM, and assayed within 2 months of planting.

Discussion

The genera of fungi recovered from mango seedlings raised from healthy seed in enclosed containers in the laboratory included mango fruit pathogens (MP) (Johnson and Coates 1993). In this study, the fungi were recorded as endophytes of healthy mango seedlings, and some isolates were shown capable of causing stem-end decay in harvested fruit, confirming that fruit decay fungi can be transmitted with mango seed. A low incidence of transmission of MP occurred with seed from healthy fruit, and a high incidence with seed from diseased fruit. Seed decay and mortality also occurred when seed were selected from decaying fruit. In commercial potting mix, seedlings from seed of healthy fruit were colonised more extensively by fungi not usually recorded as parasites or saprophytes of mango. The non-mango fungi (NMF) probably arose from bark and other organic debris in the non-sterile potting mix. Emergence of seedlings from seed infected with *D. dominicana* or *Phomopsis mangiferae* was higher in the glasshouse, suggesting that conditions in the germination chambers favoured the pathogens to the detriment of the host. Sinclair (1993) noted the greater risk of seedling mortality when soybean seed infected with *Phomopsis longicolla* T.W. Hobbs were raised in moist rolled paper towels rather than in sand.

We have also shown that isolate virulence, fruit age and soil type affect seed germinability and the amount of endophytic transmission. Fruit held for 7 days before inoculation were beginning to ripen and the level of constitutive anti-fungal compounds may have declined c.f. fruit inoculated 48 hours after harvest. Endophytic transmission of *D. dominicana* occurred less frequently in seed planted in UC mix. This was probably due to antagonistic competition from *Trichoderma viride* and *T. flavus* in the hypocotyl region where colonisation occurred. Use of the seed to seedling technique to produce plants endophytically infected by SER fungi requires care in choice of SER fungus isolate, timing of seed extraction, choice of fruit and potting mix.

The method described here provides a means of producing seedlings symptomlessly infected with decay fungi and isolates of choice. Infected seedlings could be used to examine the effects of variables such as water stress (Johnson et al. 1992) on colonisation of mango stem tissue by *D. dominicana*, and to elucidate the histopathology of the natural infection in both stressed and unstressed material. The effect of factors such as temperature on seed/seedling survival would also need to be considered.

In the late nineteenth century, when much of the primary distribution of mango seed occurred, healthy fruit (a product of modern fungicide use) did not exist. Almost all fruit would have carried one or more common fruit pathogens. As a consequence, MP isolates would have been introduced to new regions simultaneously with the seed (or potted plants). Co-dissemination partly explains the comparative lack of diversity in mango isolates of *C. gloeosporioides* ex mango compared to those ex avocado or papaya (Mills et al. 1992; Johnson et al. 1993; Hayden et al. 1994). Inter- and intra-orchard variability within mango isolates of *C. gloeosporioides* would depend upon commonality or diversity of seed/seedling budwood sources. The lack of diversity in *Colletotrichum musae* (Berk. & Curt.) Arx. from banana in a limited number of isolates (Hayden et al. 1994) could also reflect the greater chance of inter-generational transmission of fungi as mycelium or hyphae in hosts that are vegetatively propagated.

The establishment of pathogen-free mango orchards (PFO), especially in remote areas, may be possible by careful production of planting material using tissue cultured, pathogen-tested root-stocks and budwood. Such a process would assume that the excluded organisms (MF) provided no ecological benefit to mango trees. Benefits, including repellance of bark-boring beetles and nutrient recovery from senescent leaves, twigs and roots have been demonstrated or mooted for other endophyte–host combinations (Wilson 1993).

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The Effect of Commercial Pruning on Colonisation of Mango by Endophytic *Dothiorella* Species

A.W. Cooke*, A. van der Kruyssen† and G.I. Johnson§

Abstract

Ten 'Kensington Pride' mango trees in a commercial orchard in southeastern Queensland were used to determine if mechanical orchard pruning ca. 11 months before harvest reduces endophytic colonisation of new growth by *Dothiorella* spp., or results in a decrease in postharvest stem end rot in fruit during storage.

Five trees were pruned following normal commercial practice and five were left unpruned. Old and young growth of stems were assayed to assess the extent of endophytic colonisation by *Dothiorella* at the time of pruning, and again during fruit development when fruit were pea-sized. During the second assessment, fruit pedicel tissue was also assayed. Subsequently, mature fruit were harvested and stored at 23°C for 18 days before assessing the incidence and severity of stem-end rot.

The stem assays at the time of pruning indicated that *Dothiorella* spp. was recovered more frequently from older wood. There were no significant differences between levels of *Dothiorella* spp. isolated from pruned and unpruned stems assayed when fruit were pea-sized. The incidence and severity of stem-end rot were also similar in fruit harvested from both unpruned and pruned trees.

Introduction

STEM-END rot (SER) caused by *Dothiorella* spp. and *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl. (anamorphs of *Botryosphaeria* spp.), as well as by *Phomopsis mangiferae* Ahmad and other fungi, is a serious postharvest disease of mango. The main cause of SER is considered to be *L. theobromae* in tropical Asia and *Dothiorella dominicana* Petrak & Cif in Australia (Ridgway 1989).

Recent research suggests that SER fungi occur endophytically in mature stem tissue, and asymptomatically colonise new growth flushes, moving along the inflorescence, pedicel and into the fruit (Johnson et al. 1992). The aim of this study was to investigate the effect of pruning on endophytic colonisation of mango stem tissue by the major SER fungi. It has been hypothesised that strategic pruning to promote growth flushes may reduce fruit infection by the SER fungi (Johnson 1994).

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Materials and Methods

Ten mango trees 'Kensington Pride' were selected in a single row in a 15-year-old orchard near Gin Gin in southeastern Queensland (latitude 25°S). Five trees were commercially pruned within one month after harvest using mechanical pruning machinery, and five left unpruned. The trial was laid out in a systematic design (i.e. pruned, unpruned, pruned, unpruned, etc.).

Assessment of endophytic colonisation of stem tissue by *Dothiorella* spp. was undertaken twice: at commercial pruning and during fruit development (pea size). Samples of mature fruit were also taken for assessment of SER lesion development.

When fruit were pea size, stems bearing fruitlets were collected from the north side only, as there was little fruit set on the south. Sampled stems were approximately 500 mm long, which included a number of growth flushes. Isolations were made at the junction of each growth flush. Isolations were made from all trees (two stems from each tree, five isolation points per stem) using a modified version of the triple sterilisation technique (Johnson et al. 1992) to detect endophytic fungi. Two trays of fruit from the north side of each tree were harvested when mature, then de-sapped, stored and ripened at 22°C for 20 days. Disease assessments (incidence and severity of fruit affected) were assessed at regular intervals throughout the ripening period, but incidence data for day 18 only are presented. Fungi associated with the lesions were identified by isolation onto potato dextrose agar.

Data were analysed by analysis of variance. For the assessment of stem endophyte colonisation at the time of pruning, data were analysed as a factorial (pruned versus unpruned; young wood versus old wood) using tree position as a co-variate. It was necessary to analyse the data in this way due to highly significant variation in levels of *Dothiorella* incidence along the row. For the assessment of stem endophyte colonisation in pea-sized fruit, data were analysed as a completely randomised design, since both tree position and incidence of *Dothiorella* at the time of pruning were non-significant as co-variates. In the case of fruit SER assessments, data were analysed as a completely randomised design using incidence of *Dothiorella* at the time of pruning as a co-variate. For all data sets, percentage incidence data were initially arcsine transformed, but since the residual plot and the normal probability plot for the untransformed data were satisfactory and the conclusions from ANOVA were the same as for the transformed ANOVA, only untransformed data are presented. Treatment means were compared using the LSD test at $P < 0.05$.

Results

The levels of *Dothiorella* spp. recovered from mango stems and harvested fruit are shown in Figures 1-4. Incidence data only is presented.

At commercial pruning, *Dothiorella* spp. were isolated more frequently from unpruned than pruned trees (Fig. 1) and older wood than in younger wood (Fig. 2) ($P = 0.05$). There were no significant differences between the recovery of *Dothiorella* spp. from unpruned and pruned trees at 36 weeks after pruning (Fig. 3), nor were there any differences between fruit stem end rot levels 18 days after harvest (Fig. 4).

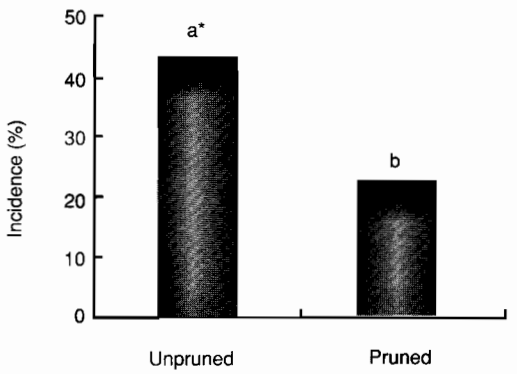

Figure 1. Incidence of endophytic *Dothiorella* spp. isolated from mango stem tissue at the time of commercial pruning. *Treatment means represented by different letters differ significantly at $P = 0.05$.

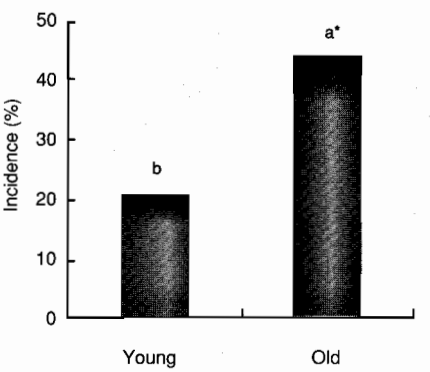

Figure 2. Incidence of *Dothiorella* spp. isolated from young and old mango stem tissue at the time of commercial pruning. *Treatment means represented by different letters differ significantly at $P = 0.05$.

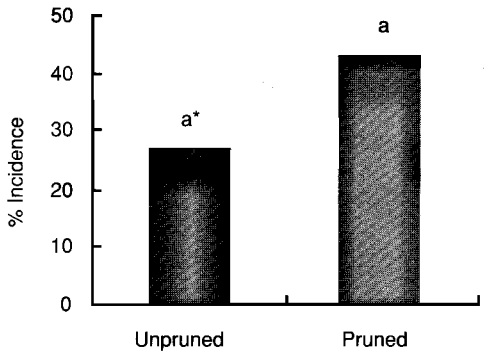

Figure 3. Incidence of endophytic *Dothiorella* spp. isolated from young wood and pedicel tissue (second assessment 36 weeks after commercial pruning). *Treatment means represented by different letters differ significantly at P = 0.05.

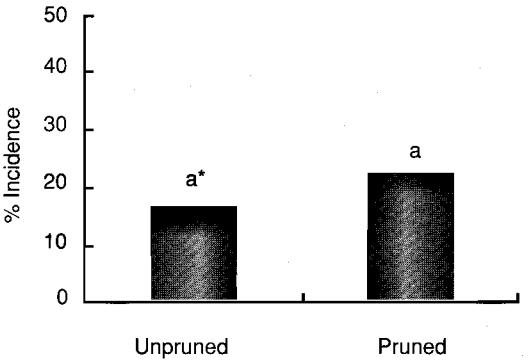

Figure 4. Incidence of stem end rot caused by *Dothiorella* spp. in mango fruit harvested from pruned and unpruned trees. Fruit were stored for 18 days at 23°C. *Treatment means represented by different letters differ significantly at P = 0.05.

Discussion

Johnson et al. (1992) demonstrated that infection of mango fruit occurred before harvest by endophytic colonisation of pedicel tissue by SER fungi present in previous growth flushes. The aim of this study was to determine whether pruning to promote growth flushing would reduce inoculum in stem tissue from which new seasonal infloresences emerged. No significant differences in inflorescence colonisation were detected in our study. While we were not able to demonstrate a benefit from pruning, further work may be necessary to determine whether other factors — such as water stress or stored carbohydrates (McPartland and Schoeneweiss 1984), or copper spray effects on colonisation by SER fungi might override any beneficial effects of pruning.

Acknowledgments

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Factors Affecting the Consistency of Leaf Disk Assays

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Abstract

Factors likely to affect the consistency of leaf disk assays were investigated. They included plant species, variety, leaf age, and variation amongst *Greeneria* sp. isolates. Conidial suspensions of various isolates of *Greeneria* sp. and *Colletotrichum gloeosporioides* were inoculated onto leaf disks and the extent of both fruiting body and conidial formation evaluated. The results showed that the development patterns of these factors were indicative of the fungus involved.

GREENERIA sp. (Gn) is one of the most important fruit rot pathogens of rambutan. A leaf disk assay was developed by R. Davis (Coates et al. 1996) which we hoped would be applicable to the screening of non-antibiotic-producing antagonists against rambutan postharvest fruit rot caused by naturally infected Gn. In vitro screening on agar media is not possible as it favours growth of antibiotic-producing antagonists which are undesirable for use with edible products (Droby et al. 1989; Droby and Chalutz 1991; McLaughlin and Wilson 1992; Wilson et al. 1993). Procedures which involve direct screening on the fruit potentially increase postharvest wound infection (Janisiewicz and Roitman 1988; Wilson and Chalutz 1989; Chalutz and Wilson 1990). In addition, direct screening using rambutan fruit is complicated by the morphology of the fruit, interference by many natural field infections, and a limited season in which fruit is available.

Before the leaf disk assay could be used consistently for antagonist screening the desired context, it was necessary to determine factors affecting the assay. These factors included the infection capability and intraspecific variation in colonisation of *Greeneria* sp. on both mango and rambutan leaf disks, variation in fruiting body formation as well as sporulation by *Greeneria* sp. on the leaf disks obtained from mango and rambutan of different cultivars, and the effects of leaf age. Isolates of *Colletotrichum gloeosporioides* (Cg) were tested for comparison, as fruit rot caused by *Greeneria* sp. previously attributed to *Colletotrichum*-like-fungus (CLF), before being correctly identified by Dr John Alcorn (Queensland Department of Primary Industries) in 1996 (see paper by Farungsang et al., p. 113, these proceedings).

Initial experiments showed that Gn colonies failed to thrive through subculturing, exhibiting poor sporulation and atypical colonies. Consequently, variations to the protocol were tested to identify techniques suitable for (i) good sporulation and (ii) maintenance of healthy Gn isolates allowing consistent retrieval for use in leaf disk assays.

Materials and Methods

Fresh leaves of mango ('Keow-savoy', 'Nam Dok Mai', and 'Lin-ngoo-how') or rambutan ('Rong-rian' and 'Si-chom-poo') were surface disinfected with 70% ethanol, and 15 mm diameter leaf disks were excised. These were immersed for 15 seconds in boiling sterilised water, blotted, then placed in sterile moist cham-

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bers for further treatment. Inoculation was made using a 20 µL drop of a 10^6 conidia/mL suspension obtained from well-sporing Gn or Cg cultures. The results were evaluated by measurement of the resultant lesion area, the extent of fruiting body formation, and the total conidia/disk formed by the fungi in five replications. Lesion area was assessed as the percentage of leaf disk area occupied by a lesion extension. Fruiting body formation was rated into five levels based on the percentage of leaf disk area occupied by fruiting bodies (0 = none, 1 = 1–25%, 2 = 26–50%, 3 = 51–75% and 4 = >75%, respectively). Infection ability on fresh leaf disks (the 30 second immersion in boiling sterilised water was omitted) by the isolates of Gn was investigated using mango leaf disks.

Mango leaves of four orders on the same flush shoot (orders 1–4 from the flush basal position: L-1 = newly mature, soft, sap-green colour; L-2 = nearly mature, soft, moss-green; L-3 = young, late flushing, olive-green; L-4 = young, full-sized, flushing, very soft and delicate, developing the olive-green colour) were tested to determine any leaf age effect. Rambutan leaves which are nearly mature or newly mature were assessed similarly.

An evaluation of the fungal culture protocol was made by continuous subculturing of the tested Gn isolates onto established potato dextrose agar (PDA) culture medium series. Success of preservation was assessed via colonised plant tissue by inoculating the tested isolates into sterilised, chopped 'Rong-rian'rambutan leaves. Well colonised tissue in which the fungus produced fruiting bodies were then stored in an incubator at 15°C.

Results and Discussion

Leaf infection by Gn has never been observed naturally but in this study could be induced artificially. It presented as fruiting body formation and sporulation without lesion development on the inoculated leaf disks. As shown in Table 1, variation between Gn isolates was very significant, as indicated by the amount of conidial formation on both mango and rambutan leaf disks. Differences between mango cultivars were also marked (Table 1). In rambutan, there was little difference between 'Rong-rian' and 'Si-chom-poo' cultivars overall, but there was considerable variation in production of total conidia/disk amongst Gn isolates on 'Rong-rian' leaf disks (Table 2).

Where fresh mango leaves (no boiling water immersion) were used, no infection by Gn was observed but natural infection by Cg did occur as identified by characteristic pinkish conidial masses. Note that fresh leaf disks, owing to their natural senescence, were unable to remain viable long

Table 1. Intraspecific variation amongst Greeneria sp. (Gn) isolates as determined by the leaf disk assay using three cultivars of mango (MK = 'Keow-savoy', MN = 'Nam Doc Mai', ML = 'Lin-ngoo-how').

Gn isolate	Lesion area (%)			Fruiting body formation level				Total conidia/disk ($\times 10^5$)			
	MK	MN	ML	MK	MN	ML	Average	MK	MN	ML	Average
143-02-06	0	0	0	3.9	4.0	3.7	**3.87**	44.25	36.38	27.13	**32.58**
149-02-08	0	0	0	4.0	3.5	4.0	**3.83**	27.50	32.88	49.13	**36.50**
167-02-08	0	0	0	2.78	3.6	3.7	**3.36**	15.13	26.13	19.0	**20.08**
168-02-08	0	0	0	4.0	3.95	4.0	**3.98**	18.13	32.88	45.88 3	**2.29**
188-02-09	0	0	0	4.0	3.9	4.0	**3.97**	31.75	36.50	43.63	**37.29**
192-02-09	0	0	0	3.5	3.75	3.7	**3.65**	20.25	46.63	47.13	**38.0**
202-02-09	0	0	0	4.0	4.0	3.8	**3.93**	28.38	46.13	22.38	**32.29**
206-02-09	0	0	0	4.0	4.0	4.0	**4.0**	27.25	44.0	42.50	**37.91**
237-02-09	0	0	0	4.0	4.0	4.0	**4.0**	34.13	37.25	28.0	**33.13**
238-02-02	0	0	0	3.6	2.4	3.7	**3.23**	8.38	11.25	13.75	**11.13**
154-02-15	0	0	0	2.15	2.3	3.3	**2.58**	26.25	12.38	8.25	**15.63**

enough to complete the experiment. The colonisation of leaf disks by Gn might be saprophytic.

No difference was found in either fruiting body formation level or total conidia/disk when leaf disks were obtained from the green leaves (orders 1–3) were used (Table 3). For rambutan, there was no significant difference between the upper (nearly mature leaf) and the lower (newly mature leaf) (Table 4).

Comparison of Gn and Cg using the leaf disk assay provided further support for the identification of Gn as distinct from Cg, in addition to those previously described (see Farungsang et al. 1998). Table 5 shows that colonisation by Cg was always associated with an increase in lesion area in both mango and rambutan, however this did not occur with Gn infection (see also Coates et al. 1996). In addition, the two fungal genera were also opposite in host preference. Intraspecific variation in colonisation between mango and rambutan isolates of Cg was also detected (Table 5).

The poor correlation between the amount of fruiting body and conidia formation depended on the time of colonisation and interaction between the fungus and substrate.

The investigation into culture protocol demonstrated that potato dextrose agar (PDA) was a suitable medium for use in initial fungal culturing, i.e. when the fungus was freshly isolated from its host. However, for subsequent subculturing, it is necessary to supplement the basic medium with rambutan leaf extract (10% v/v) to ensure recovery of healthy, well sporing colonies. Colonies of Gn grown on an agar slant declined very rapidly, but isolates could be preserved under water for up to a year. It was possible to maintain the isolates for at least one year using this technique, as proven by successful colonisation of host tissue at the end of that period. This technique was simple in relation to both material preparation and retrieval and also allowed for mass production of the fungal isolates. In addition, the technique was able to be applied to dried specimens. Retrieval could be achieved on PDA or rambutan leaf extract supplemented PDA.

Conclusions

Screening of antagonistic yeasts against Gn can be evaluated by means of the amount of both fruiting body and total conidia/disk formed by the fungus through the leaf disk assay. There is noticeable intraspecific variation between Gn isolates, as well as differences caused by the plant cultivar used for the leaf disks. Age of leaves used (nearly or newly mature of the same flush) did not have a significant effect on fungal colonisation. The 30-sec boiling sterilised water immersion step was critical, not only for a senescence-shock, but also for elimination of naturally infected leaf micro-organisms. Leaf infection by Gn was achieved artificially. Intergeneric variation between Gn and Cg was very clear.

Addition of 10% v/v rambutan leaf extract supplemented into PDA resulted in typical well sporing *Greeneria* sp. colonies. Maintenance of colonised host tissue was a successful and convenient technique for handling and long-term survival of *Greeneria* sp.

Acknowledgments

Grateful thanks are expressed to Dr John Alcorn of the Queensland Department of Primary Industries (QDPI), Australia, for the identification of *Greeneria* sp., and to the cooperative research program being sponsored by ACIAR.

Table 2. Effect of rambutan cultivar on colonisation by *Greeneria* sp. (Gn) on leaf disks (LA = lesion area, FBL = fruiting body formation level [on a scale of 0–4 representing percentage of leaf disk area occupied by fruiting bodies, levels being 0, 1–25, 26–50, 51–75, and >75%, respectively], TC = total conidia/disk).

Gn isolate	'Rong-rian'			'Si-chom-poo'		
	LA (%)	FBL	TC ($\times 10^5$)	LA (%)	FBL	TC ($\times 10^5$)
261-02-08	0	4.0	7.60	0	3.85	13.0
300-02-08	0	4.0	12.55	0	3.85	12.2
278-02-09	0	3.9	18.10	0	3.85	13.3
Average	0	3.97	12.75	0	3.85	12.87

Table 3. Effects of (i) mango leaf age (L-1 = newly mature, soft, with sap-green colour; L-2 = nearly mature, soft, with moss-green colour; L-3 = young, late flushing with olive-green colour; L-4 = young, full size upon flushing, very soft and delicate, approaching olive-green in colour; leaf order starting from the flush basal position). and (ii) use of fresh leaf disks (non-senescence-shock assay, i.e. the step of 30 second immersion in boiling sterilised water was omitted) on colonisation by *Greeneria* sp. (Gn).

Gn isolate	Lesion area (%)[a]	Normal leaf disk assay										Non-senescence-shock assay
		Fruiting body formation level					Total conidia/disk (10^5)					
		L-1	L-2	L-3	L-4	Average	L-1	L-2	L-3	L-4	Average	
129-02-06	0	4.0	4.0	4.0	2.9	3.73	53.4	40.1	48.8	42.9	46.3	Development of naturally infection by *C. gloeosporioides*
155-02-15	0	3.8	4.0	4.0	4.0	3.95	39.5	46.1	35.5	36.9	39.5	
156-02-15	0	4.0	4.0	3.7	3.25	3.74	41.1	27.5	24.2	24.1	29.2	
206-02-09	0	4.0	4.0	4.0	4.0	4.0	41.5	39.8	49.6	28.4	39.8	
Average	**0**	**3.95**	**4.0**	**3.93**	**3.54**		**43.8**	**38.4**	**39.5**	**33.4**		

[a] Average of all of the four leaf orders

Table 4. Effect of rambutan leaf order on colonisation by *Greeneria* sp. (Gn) on leaf disks (LA = lesion area, FBL = fruiting body formation level [on a scale of 0–4 representing percentage of leaf disk area occupied by fruiting bodies, levels being 0, 1–25, 26–50, 51–75, and >75%, respectively], TC = total conidia/disk).

Gn isolate	Upper leaf[a]			Lower leaf[b]		
	LA (%)	FBL	TC ($\times 10^5$)	LA (%)	FBL	TC ($\times 10^5$)
261-02-08	0	4.0	10.85	0	3.85	9.85
300-02-08	0	3.9	12.65	0	3.95	12.10
278-02-09	0	3.9	17.95	0	3.85	13.45
Average	0	3.93	13.82	0	3.88	12.87

Table 5. Colonisation by Greeneria sp. (Gn) and *Colletotrichum gloeosporioides* (Cg) on 'Rong rian' rambutan (RR) and 'Keow-savoy' mango (MK) leaf disks.

Fungal isolate	Lesion area (%)		Fruiting body formation level		Total conidia/disk (10^5)	
	RR	MK	RR	MK	RR	MK
Gn						
052-02-02	0	0	3.25	2.0	43.50	68.50
336-02-08	0	0	2.60	2.45	76.50	9.50
339-02-08	0	0	3.26	2.50	83.50	126.50
Average	**0**	**0**	**3.02**	**2.32**	**67.83**	**68.17**
Cg-rambutan isolates						
051-01-03	10	21	0.75	1.60	5.50	17.0
108-01-06	10	45	1.0	1.85	17.0	80.5
341-01-08	10	20	0.35	1.60	9.0	35.0
Average	**10**	**28.7**	**0.70**	**1.68**	**10.50**	**44.17**
Cg-mango isolates						
CK 3-2	10	24	0.6	1.95	0.50	32.50
CN 6-5	10	22	2.35	1.20	9.0	17.0
PK 1-5	10	25	4.5	2.0	2.0	30.50
Average	**10**	**23.7**	**2.5**	**1.72**	**3.83**	**26.67**

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Effects of Fruit Bagging During Growth on Calcium Concentrations in 'Sensation' and 'Kensington' Mangoes

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Abstract

Fruit bagging during growth is practised on some fruit crops in order to optimise fruit quality through reduced levels of damage associated with a range of 'insults', including pathogen infection. The practice of bagging mango fruit is becoming increasingly widespread in Australia. High fruit calcium levels are also associated with better fruit quality, as judged by increased postharvest longevity and reduced levels of physiological disorders. Since calcium moves in the xylem, it is possible that fruit bagging may have a negative impact on fruit calcium levels because the bag may constitute an additional barrier to transpirational water loss. In order to check for possible deleterious effects, the influence of bagging 'Sensation' and 'Kensington' mangoes with paper or plastic bags on skin and flesh calcium concentrations was investigated. Bagging had no significant effect on either fruit skin or flesh calcium concentrations, or on fruit size. However, bagging in plastic led to fruit 'shrivelling' during postharvest shelf life evaluation; presumably through interference with normal cuticle and/or lenticel development. Bagging in paper, however, is likely to enhance fruit visual quality, without risk of either calcium deficiency or cuticle formation-associated disorders.

FRUIT bagging during growth and development on the tree is widely practised in Asia, and is increasingly practised in Australia (S. Penny, personal communication). Bagging of banana bunches, for example, is widespread. Individual fruit, such as guavas and mangoes, are also bagged because of their high unit value. Bagged fruit are protected to varying degrees, depending on the duration of bagging and the nature of the bags, from pest attack and diseases (Johnson and Sangchote 1994). They are also protected from radiation (sunburn), mechanical damage (e.g. branch-rub) and contamination (e.g. dirt).

The calcium content of fruit is of general importance in terms of postharvest quality (Hofman and Smith 1994; Yuen 1994). Fruit with higher calcium contents tend to have longer shelf life, enhanced resistance to disease and lower incidence of physiological disorders. Thus, additional calcium is often supplied to crops, such as apples, during fruit set and development. This calcium may be applied as fertilizer either to the soil and/or on the leaves and fruit (i.e. 'foliar application'). Supplementary calcium can also be provided by postharvest treatment (e.g. dip, partial pressure infiltration).

Mango is one of the most important fruit crops in the world in terms of social significance, tonnage harvested (Buchanan 1994) and commercial value. However, being a tropical crop subject to chilling injury at low storage and transport temperatures (Kader 1992), postharvest losses of mango can be relatively high. Losses to postharvest diseases, such as anthracnose and stem-end rot (Sommer 1992b), are a particularly obvious problem. Loss to disease is related to a number of factors, which include the level of inoculum and the susceptibility of the fruit to decay (Sommer 1992a; Johnson and Sangchote

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1994). Bagging of mango fruit can potentially reduce the level of airborne inoculum, and thus reduce loss to pathogens such as *Colletotrichum* spp., which cause anthracnose disease (Coates and Gowanlock 1994). On the other hand, bagging might reduce the passive transpirational flux of calcium into fruit, thereby adversely affecting fruit resistance to disease. That is, reduced calcium levels might shorten the green life period, thereby bringing forward disease expression associated with quiescent or latent infection by anthracnose and stem-end rot pathogens. Similarly, lower cell wall and membrane calcium concentrations may favour rapid disease development. Additionally, reduced calcium may predispose mango fruit to 'physiological' disorders, such as jelly seed, stem-end cavity and soft nose (Hofman and Smith 1994).

'Kensington' and 'Sensation' are two of the most economically important mango varieties grown in Australia. 'Kensington' is a relatively green-skinned, strongly flavoured variety which was selected in Australia (Alexander 1987). 'Sensation' is a highly coloured (red and yellow skin), sweet variety which was bred in Florida. The aim of the work reported herein was to determine the effects of bagging 'Kensington' or 'Sensation' mangoes on fruit calcium concentration and on postharvest characteristics.

Materials and Methods

Plant material

Preharvest bagging of 'Kensington' and 'Sensation' mango (*Mangifera indica*) fruit was undertaken on farms near Gatton and Nambour, respectively, in southeastern Queensland. They were harvested when judged to be commercially mature by the farmers.

Bagging treatments

Developing mango fruit were bagged in either opaque white, waterproofed 'T20' paper bags obtained from Palmwoods Farm and Garden Supplies (Palmwoods, Queensland) or opaque white polyethylene bags obtained from Beaver Plastics Pty. Ltd. (Coopers Plains, Queensland). Both types of bags were designed and made for the purpose of fruit bagging. The 'Kensington' and 'Sensation' fruit were bagged 41 and 50 days prior to harvest, respectively. Unbagged 'control' fruit were also tagged at the time of bagging.

Assessments

Fruit dry matter content and fruit mass (size) were assessed at harvest. Dry weight (DW), for calculation of percentage dry matter (% DM), was obtained by oven drying at 60°C to constant weight. Skin and flesh calcium concentrations were determined separately. Oven dried tissue samples were ashed in a muffle furnace and the residue dissolved in 1 M hydrochloric acid (HCl). Calcium was measured by atomic absorption spectroscopy, and data are presented as mg/g DW. Shelf life at 25°C and 70% relative humidity (RH) was determined for each fruit individually as the time (days) until a hand firmness rating of soft (eating ripe) was recorded for three consecutive days. Fruit weight (water) loss during shelf life evaluation was monitored gravimetrically, and the data expressed as mg/g initial fresh weight (FW)/day. Data were statistically evaluated by analysis of variance. In tables, means within data blocks and with the same lower case letter against them do not differ significantly according to least significant difference (LSD; P=0.05).

Results and Discussion

Fruit development

Bagging treatments had no significant effect on either fruit size or % DM of 'Kensington' or 'Sensation' fruit (Table 1). The average harvest weights for 'Kensington' and 'Sensation' fruit were 368 and 387g, respectively. The average DM contents at harvest were 13.8 and 15.1%, respectively.

Skin and flesh calcium concentrations

There were no significant effects of bagging treatments on either fruit skin or flesh calcium concentrations (Table 2). For 'Kensington' fruit, the overall average skin calcium concentration was 3.45 mg/g DW, approximately four times the average flesh calcium concentration of 0.87 mg/g DW. For 'Sensation' mango, the average skin calcium concentration of 1.62 mg/g DW was approximately three times that of the flesh at 0.5 mg/g DW.

Fruit shelf life and water loss

Significant differences in shelf life between bagging treatments were obtained (Table 3). Bagging in plastic bags resulted in shortened shelf life, as compared to unbagged control fruit and those bagged in paper, for both 'Kensington' (about 20% shorter) and

Table 1. Fruit mass and dry matter (DM) content of unbagged and bagged 'Kensington' and 'Sensation' mangoes harvested at commercial maturity (n = number of fruit per treatment). Means within data blocks and with the same lower case letter against them do not differ significantly (least significant difference; P=0.05).

Treatment	Parameter at harvest	
	Weight (g)	DM (%)
Kensington	(n = 30)	(n = 60)
Unbagged	363 a	14.2 a
Paper bagged	368 a	13.7 a
Plastic bagged	372 a	13.6 a
Sensation	(n = 5)	(n = 45)
Unbagged	380 a	14.9 a
Paper bagged	385 a	15.7 a
Plastic bagged	396 a	14.6 a

Table 2. Skin and flesh calcium concentrations (mg/g dry weight) for unbagged and bagged 'Kensington' and 'Sensation' mango fruit harvested at commercial maturity (n = number of fruit per treatment). Means within data blocks and with the same lower case letter against them do not differ significantly (least significant difference; P=0.05).

Treatment	Skin	Flesh
Kensington	(n = 5)	(n = 5)
Unbagged	3.90 a	1.05 a
Paper bagged	3.44 a	0.84 a
Plastic bagged	3.01 a	0.71 a
Sensation	(n = 5)	(n = 5)
Unbagged	1.53 a	0.55 a
Paper bagged	1.61 a	0.45 a
Plastic bagged	1.73 a	0.51 a

Figure 1. Appearance of control (unbagged), paper-bagged and plastic-bagged 'Sensation' mango fruit during ripening following harvest at commercial maturity.

'Sensation' (about 45% shorter) fruit. The reduction in shelf life could be explained on the basis of accelerated water loss from fruit bagged in plastic (Table 3). For both 'Kensington' and 'Sensation' mangoes, the rates of water loss associated with bagging in plastic were increased approximately 1.85 fold.

Increased rates of postharvest water loss are known to speed mango fruit ripening (Joyce et al. 1997). However, the almost doubling in the rate of water loss associated with enclosure in plastic bags resulted in fruit shrivelling (Fig. 1) and failure to ripen to an edible state.

Conclusions

The absence of any significant differences between bagging treatments in terms of fruit weight (Table 1), fruit % DM (Table 1), fruit skin calcium concentration (Table 2) and fruit flesh calcium concentration

(Table 2) indicated that bagging should not have had any detrimental effect on total fruit calcium content. Thus, it follows that bagging is unlikely to shorten fruit shelf life and/or to predispose mango fruit to disease or calcium-related 'physiological' disorders (see Introduction)

Table 3. Postharvest shelf life and water loss rate during ripening for unbagged and bagged 'Kensington' and 'Sensation' mango fruit (n = number of fruit per treatment). Means within data blocks and with the same lower case letter against them do not differ significantly (least significant difference; P=0.05).

Treatment	Shelf life (d)	Water loss (mg/g fresh weight/day)
Kensington	(n = 10)	(n = 10)
Unbagged	14.3 a	7.00 a
Paper bagged	14.3 a	8.19 a
Plastic bagged	11.6 b	14.2 b
Sensation	(n = 15)	(n = 15)
Unbagged	15.7 a	10.4 a
Paper bagged	16.1 a	11.4 a
Plastic bagged	8.7 b	20.3 b

On the other hand, bagging in plastic is clearly undesirable because postharvest fruit water loss and water loss-associated disorders (e.g. skin shrivelling) are enhanced (Table 3, Plate 1). It is evident from these observations that pre-harvest bagging with plastic bags during fruit development somehow interferes with 'normal' cuticle and/or lenticel formation. This effect presumably occurs because a low vapour pressure deficit environment (i.e. high relative humidity) exists within the virtually water vapour impermeable low density polyethylene bag. The use of perforated or spun bonded plastic films for bag manufacture may obviate this problem.

In future applied research, plastic bags made of different polymers and with varying degrees of perforation should be evaluated. Our results also suggest that plastic bags might be used as a tool in more fundamental studies investigating chemical (e.g. composition) and physical (e.g. thickness) aspects of mango fruit cuticle and lenticel formation and function. The failure of plastic bags to have any influence on fruit calcium levels, whilst having large effects on the epidermal characteristics of mango fruit, can be explained by accumulation of calcium early in fruit development. However, the total absence of any measurable effect over 6 or 7 weeks prior to harvest could warrant more detailed investigation, since calcium movement into fruit by way of active calcium transport or osmotically driven water (plus calcium) redistribution may not have been given due evaluation in scientific research to date.

More detailed accounts of the experiments overviewed herein have been prepared for publication in the Australian Journal of Experimental Agriculture (Joyce et al. 1997; Beasley et al. 1998).

Acknowledgments

The authors gratefully acknowledge ACIAR for providing the operating funds which allowed this work to be undertaken. D.C.J. and A.J.S. are also grateful to the CSIRO Division of Horticulture, which was our employer organisation at the time these experiments were conducted. ACIAR Project 9313 management support from Dr Greg Johnson and Dr Lindy Coates is acknowledged, as is advice to D.R.B. from Dr David Simons.

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Proline Does Not Accumulate in Water-stressed 'Kensington' Mango Leaves

M.J. Gosbee*, D.C. Joyce†, P.A. Joyce§ and G.I. Johnson¶

Abstract

Proline is an amino acid that accumulates in the tissues of many plant species when water stressed. In this study, leaf discs of mango cv. 'Kensington' were water stressed by floating them on polyethylene glycol MW 6000 solutions providing a range of water potentials from 0 to –3.6 MPa. Proline accumulation was determined after 24 hours. Barley cv. 'Prior' leaf segments, which are known to accumulate proline under water stress, were included as 'controls' in this experiment. Relative water contents of stressed leaf tissue of both species were similar, falling to 69% at –3.6 MPa. Proline concentrations in barley leaf segments reached a maximum of 1440 µg/g dry weight (DW) at –3.6 MPa, which amounted to a four-fold increase in proline over turgid tissue levels. In contrast, proline levels in mango were highest at 0 MPa, being 57 µg/g DW, and actually decreased slightly as water potential fell. Thus, water stress imposed for 24 hours to a level of –3.6 MPa did not induce proline accumulation in mango leaf discs. Mango tissue may need to experience lower water potentials to induce proline accumulation. Alternatively, it is possible that either no stress-induced metabolite or a different metabolite (e.g. glycine betaine) is produced.

PROLINE is an amino acid which accumulates in cells of many plant species under stress (Aspinall and Paleg 1981). During water stress and other environmental stresses (including temperature and salinity stresses) proline accumulates to many times the concentration in non-stressed tissue. Accumulation increases with the severity of the stress and the length of the stress period. Proline accumulation offers several possible benefits to the plant. It increases the cell solute concentration and hence osmotic concentration under dehydrated conditions, and thus draws water into the cells where it is needed. It is an easily available form of reduced nitrogen and can be rapidly converted to glutamic acid (a key amino acid) when stress is relieved. In addition, proline increases the solubility and protects the structure of proteins present in the cell (Aspinall and Paleg 1981).

As part of studies on the effect of water stress on colonisation of mango by the stem-end rot pathogen *Dothiorella dominicana* we sought to measure proline accumulation as a potential means of quantifying stress in mango.

Materials and Methods

Mango leaves were harvested from field-grown, non-stressed, mature 'Kensington' trees. Non-stressed barley cv 'Prior' plants were grown in a glasshouse. The youngest, healthy, fully expanded leaves were harvested from both mango and barley plants. Mango leaf discs of 1 cm diameter were taken with a cork borer and pooled in a Petri dish for random division into various treatments. Barley leaves were cut into 1.5 cm long segments using a razor blade and were pooled and assigned to treatments in a

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similar fashion. Three replicates of 10–15 leaf discs or segments per replicate were used for both species at each water potential level.

Water Stress Treatments

Polyethylene glycol MW 6000 (PEG) was used to create solutions of different water potentials. The appropriate amount of PEG required was calculated as in Steuter et al. (1981). Actual solution water potentials were measured with a Wescor CR7 psychrometer system (Table 1). The positive water potential obtained for deionised water is a result of the straight line equation used to calculate water potential. Leaf explants were floated stomatal side down (abaxial side for mango, either side for barley) on PEG at each prescribed water potential for a period of 24 hours in a covered Petri dish under dim light at 23°C. They were then blotted dry and their fresh weight was measured. Discs from each replicate were then floated on deionised water for 4 hours in the dark, blotted dry and weighed for the turgid weight. The segments were dried at 60°C for 48 hours, and the dry weight was recorded. Relative Water Content (RWC) was calculated as:

$$RWC = \frac{(\text{fresh weight} - \text{dry weight})}{(\text{turgid weight} - \text{dry weight})} \times 100$$

Replicate stressed tissue samples of 30 leaf discs were frozen in liquid nitrogen, freeze dried and stored at –20°C until used for proline determination.

Proline measurement

Proline was extracted and quantified using the method described by Joyce (1982), which was based on that of Singh et al. (1973). Freeze dried samples (0.05 g) were homogenised in 5 mL 'MCW solution' (12 methanol:5 chloroform:3 distilled water) at room temperature, then vortexed and centrifuged until the supernatant was clear. The supernatant was removed and transferred to a boiling tube with glass beads. Five mL of fresh ninhydrin solution (3 mL glacial acetic acid: 2 mL 5 M orthophosphoric acid and 125 mg ninhydrin) and 5 mL glacial acetic acid was added to the tube and the solution heated in a boiling bath for 45 minutes. After cooling to room temperature, the mixture was vortexed with 5 mL volume of toluene, and diluted further with toluene if necessary until the ninhydrin product concentration fell within the range of the standard curve. Optical density of ninhydrin reaction product dissolved in the toluene layer was read at 520 nm.

Proline standard curves were similarly prepared in duplicate for 0–100 µg proline, and proline concentrations were determined by comparison to the standard curve. Calibration curves were established using linear regression. Recovery was tested by spiking samples with 50 µg of proline and calculating the proportion recovered (viz. 95 and 92%).

Results

Relative water content

The RWCs of the two species were similar at all water potential levels, except at –2 MPa, where barley had a RWC of 86% and mango had 77% (Fig. 1A). Both tissue types appeared very desiccated at a RWC of 69% which occurred at –3.6 MPa.

Proline accumulation

Proline accumulation in barley increased to a maximum of 1440 µg/mg dry weight (DW) at –3.6 MPa. This amounted to a four-fold increase in proline concentration over turgid tissue. In contrast, proline levels in mango were highest at 0 MPa, being 57 µg/mg.

Table 1. Water potentials of mango and barley leaf explants floated on polyethylene glycol MW 6000 (PEG) solutions in order to create a range of water potentials.

Prescribed water potential (MPa)	Concentration of PEG (g/1000 g H_2O)[a]	Measured water potential (MPa)[b]	Prescribed water potential (MPa)	Concentration of PEG (g/1000 g H_2O)[a]
0	0	0.067	0	0
–0.5	139.7	–0.048	–0.5	139.7
–1.0	250.2	–0.616	–1.0	250.2
–2.0	447.9	–1.920	–2.0	447.9
–3.0	629.7	–3.635	–3.0	629.7

[a] From Steuter et al. 1981

[b] Measured with a Wescor CR7 psychrometer

They showed hardly any change as water potential and RWC decreased (Fig. 1B). A RWC of 69% is low, and should have initiated stress metabolism in mango as it did in barley. Variation between replicates was low in mango but high in barley. However, the difference between proline accumulation in the two species was clear.

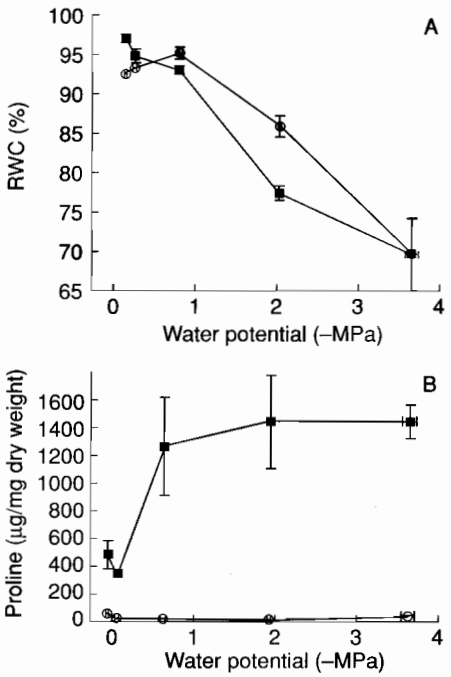

Figure 1. Relative water content (RWC) (A) and proline accumulation (B) in mango (○) and barley (■) leaf explants floated on polyethylene glycol MW 6000 solution for 24 hour. Water potentials were measured with Wescor CR7 psychrometers. Bars are standard errors of means.

Conclusion

Proline did not accumulate in mango. Accordingly, it is not a useful measure of water stress in mango. The water stress levels imposed in this experiment (–3.6 MPa for 24 hours) may not have been harsh enough to induce accumulation in leaf discs. However, a study of drought-stressed 2-year-old potted mango trees by Pongsomboon et al. (1992) showed that the critical point where non-reversible tissue damage occurred was at a leaf water potential of –3.45 MPa and a RWC of 77%. While proline accumulation was not measured in their experiment, these stress levels described are within the range of those achieved in our experiment which did not promote proline accumulation. The former measurements, however, were made on intact leaves stressed over a 44-day period, which may influence stress metabolism. Thus, mango may not accumulate proline in response to water stress. Perhaps a different metabolite (e.g. glycine betaine) is produced. Alternatively, no metabolite is produced, and mangoes resist water stress by other means.

Acknowledgment

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Modified Atmosphere Modelling

P. Boon-Long*

WORK on modified atmosphere (MA) modelling of mango has focused on further refining the model developed in a previous project, and on presenting the results in a form which is easier to use.

The main aim of this work is to develop a mathematical model which will predict the atmospheric conditions (levels of oxygen [O_2]and carbon dioxide[CO_2]) inside a sealed film package of fruit, given the type of fruit and maturity index, the type of film, and the ratio between the film area and the fruit mass. The result should help fruit handlers to design better MA packages to preserve quality and extend the storage life of fruit.

In the previous project, a set of correlations was obtained to predict respiration rates of 'Nam Dok Mai' mango. A computer model was then developed to calculate the required film parameters (permeability and area) for a given fruit mass necessary for the atmosphere (levels of O_2 and CO_2) inside the sealed package to reach a certain level deemed optimal for storage. The model could also describe the changes in O_2 and CO_2 levels inside the package with time. However, to use the model a computer and some knowledge of programming were needed.

In the present work, the respiration correlations have been recalculated and refined, and the model used to generate a set of graphs for 'Nam Dok Mai' mango which show the relationships between O_2 concentration inside the package, the fruit mass, the film permeability and film area, and time after storage. These parameters can be read from the graphs, eliminating the need for a computer.

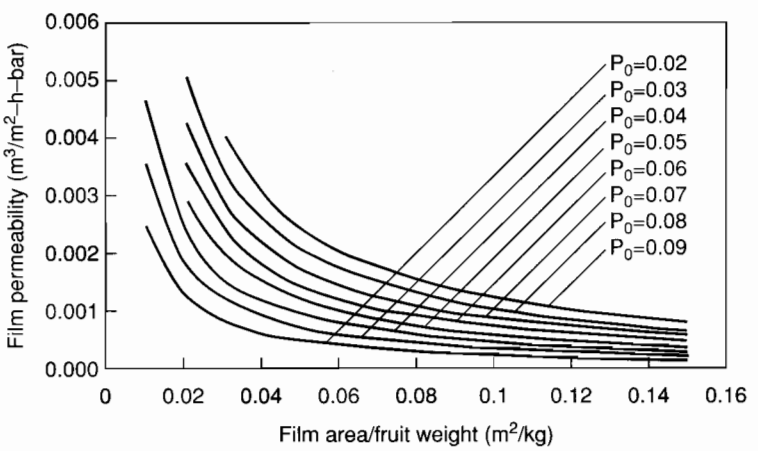

Figure 1. Partial pressure of oxygen (p_o) as calculated from film permeability versus film area per fruit weight, at equilibrium.

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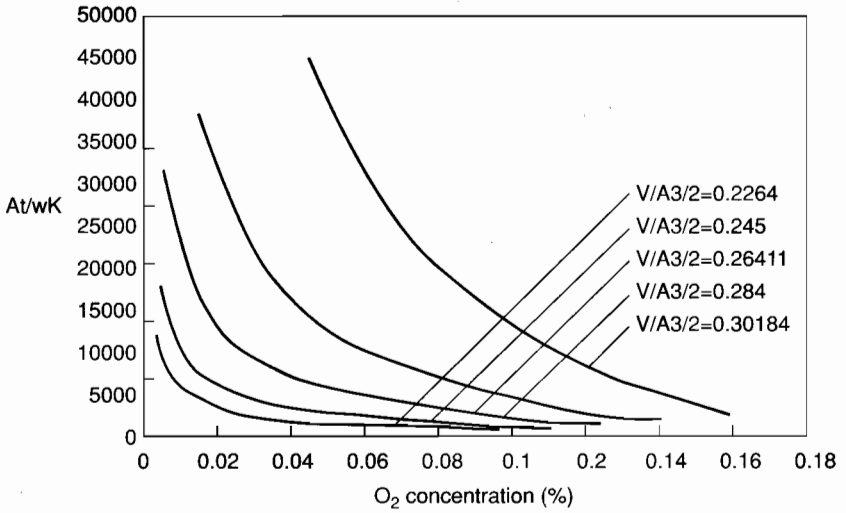

Figure 2. Plot of film area × time/fruit weight × film permeability versus oxygen (O_2) concentration, at equilibrium.

Figure 1 shows the film permeability (K) versus the film area (A) per fruit weight (w), and the resulting partial pressure of oxygen (P_o), *at equilibrium.* This graph may be used in two ways: *either* the film permeability and the required level of O_2 are known, and it is necessary to find out how much film area is needed for the given fruit mass, *or* the film area/fruit weight ratio and the required level of O_2 are known, and it is necessary to find out the permeability of film needed.

Figure 2 introduces *time* into the prediction. The graph will, in addition to the equilibrium results of Fig. 1, estimate the time (t) needed for the atmosphere inside the sealed package to reach the previously determined equilibrium level. The time required is a function of the free volume (V) inside the package, in addition to the other parameters already present in Fig 1.

Both graphs are valid for 'Nam Dok Mai' mango , harvested at 120 days after full bloom, in the north of Thailand. Three film area/fruit weight ratios were used, with four replications, and gas sampling over a period of three days, giving a total of about 300 data points for fitting.

Endophytic Fungi in Longan

V. Sardsud*, C. Sittigul*, U. Sardsud†, P. Chantrasri§, and S. Promin*

Abstract

Fungi were isolated from shoots, young fruit panicles, and various points on the fruit of longan (*Dimocarpus longana* Lour. 'Daw'). Many kinds of fungi were found from the various tissues, which had been triple sterilised. These fungi are expected to be in plant tissues and to penetrate the fruit before it is harvested, resulting in fruit damage after harvest.

Four different species of fungi frequently isolated were selected for endophytic study. *Lasiodiplodia* sp., *Pestalotiopsis* sp., *Fusarium* sp. and *Curvularia* sp. inoculated on young longan shoots. The results showed that these fungi were able to invade the shoot tissues after inoculation. *Lasiodiplodia* sp. was the most virulent pathogen and was able to spread from the point of inoculation to the tip of the shoots. Many shoots died or displayed retarded growth as a result of the pathogen.

These fungi were also inoculated at the stem end of longan fruit. After inoculation for one week, seeds of inoculated fruits were planted in sterilised sand. The results showed that fewer seedlings were obtained from fruit inoculated with *Lasiodiplodia* sp. Root, stem, petiole, and apical meristem tissues of the young seedling with two pairs of true leaves were then examined for the presence of endophytic fungi. They were found to contain the four species of fungi originally inoculated.

LONGAN (*Dimocarpus longana* Lour 'Daw') is one of the most important fruits in Thailand. The estimated planting area is 31 855 ha, about 60% of which is in the northern part of the country. Large volumes of the fresh fruit are exported, mainly to Hong Kong, Singapore, Malaysia, Canada, and some European countries. Some canned and dried fruits are also exported. Longan fruit have a short shelf life of 2–3 days at ambient temperatures of 25–32°C. Fruit rot after harvest is among of the most important diseases of longan. It is caused by some fungal species, and results in economic losses. These fungi might come from both of the surface area and inner tissue at various parts of longan trees. Normally, the fruit can be stored at 5°C for 15–20 days at a relative humidity of 85–90%, but rot if stored for longer periods. Nachaiweing (1994) found the fungal species *Curvularia* sp., *Fusarium* sp., *Lasiodiplodia* sp., and *Pestalotiopsis* sp. in the inner tissues of the longan shoots. Further knowledge of the sources of fungi that attack longan fruit during storage will be useful for selecting a suitable control measure.

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Materials and Methods

Isolating of fungi in shoot, panicle and fruit

Samples of shoots, young fruit panicles, and mature fruit were collected and triple sterilised. Various parts, as shown in Figure 1, were then cut into small pieces and plated on potato dextrose agar medium. Fungi grown were tested for pathogenicity on fruit after harvest. Three sets of experiments were carried out to detect the fungi that caused rot disease by inoculating the fungi onto the stem ends.

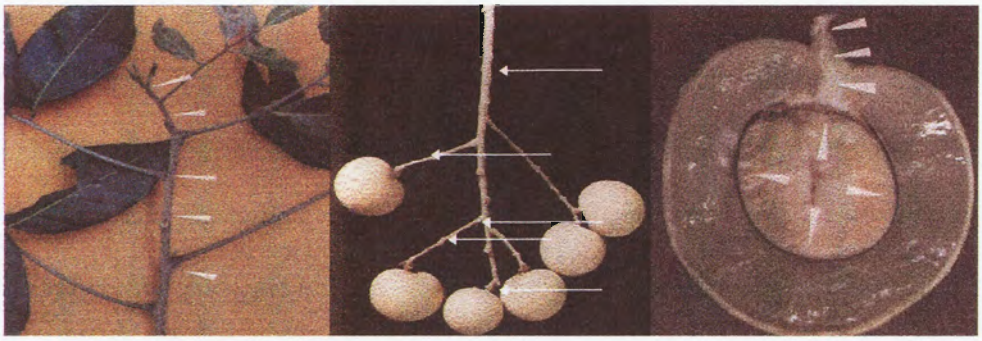

Figure 1. Isolation points on shoot, panicle, stem end, and seed

Figure 2. Inoculation with mycelium plug on the young shoot.

Figure 3. Inoculation with mycelium plug on the stem end of longan fruit.

In the first set, the fruit were stored at 5°C immediately after inoculation with the fungi. In the second set, they were stored at 5°C, beginning 24 hours after inoculation, and the third set was stored at room temperature (28±2°C) after being inoculated with fungi. The occurrence and progress of disease were checked by observing changes in the fruit skin.

Internal spread of some fungi in longan shoot

Four genera of fungi, *Lasiodiplodia* sp., *Fusarium* sp., *Curvularia* sp. and *Pestalotiopsis* sp., were inoculated on young longan shoots (Fig. 2). At 15 days after inoculation, the shoots were collected, cut into pieces, triple surfaced sterilised, and plated onto acidified PDA to observe the growth of fungi from plant tissues.

Seed transmission of some fungi in longan

The four genera of fungi were also used to inoculate the stem end on attached longan fruit for one week before harvest (Fig. 3). After harvest, isolations were made by plating out parts of embryo and endosperm of longan seed onto acidified PDA.

Some seeds of inoculated fruit were planted in sterilised sand and kept in a glasshouse. Then, root, stem, petiole, and apical meristem tissues of young seedlings (Fig. 4) were sampled and triple sterilised before plating onto acidified PDA.

Results

Fungi in the shoot, panicle, and fruit

Fourteen species of fungi were isolated from the shoot before budding, 13 from the panicle during flowering, 11 from the panicle at the early stage of fruiting, 9 from the stem end, and 5 species from the seed of the fruit after harvest. Many fungi of longan were isolated from tissues inside the shoot, panicle, stem end, and seed (Table 1). *L. theobromae* and *Pestalotiopsis* sp. were frequently detected. They were also found in seed. Under conditions of natural infection no symptom development was observed.

The results of pathogenicity studies showed that all species, especially *Lasiodiplodia theobromae* and *Pestalotiopsis* sp., caused early fruit browning in longan fruit in all three experimental conditions. Fruit in the first set (5°C storage immediately after inoculation) rotted within 22 days, those in the second set (5°C storage 24 hours after inoculation) rotted in 18 days, and those in the third set (storage at room temperature) rotted within 6 days. Fruit from the third set were rotten 4 days earlier than control.

Internal spread of some fungi in longan shoot

The results showed that these fungi were able to invade the shoot tissues (Table 2; Fig. 5). *Lasiodiplodia* sp. was the fungus most frequently isolated from the shoots.

Table 1. The genera of fungi were detected from various parts of longan tree which plant tissues had been triple sterilised before isolation.

Genera of fungi	Isolation parts of longan tree				
	The shoots before budding	The panicles during blooming	The panicles at the early stage of fruiting	The stem end	The seeds
Alternaria sp.	+	+	+	–	–
Aspergillus flavus	–	–	–	+	–
Aspergillus niger	+	+	–	+	–
Botrytis sp.	+	+	+	–	–
Colletotrichum sp.	+	–	+	+	+
Cladosporium sp.	–	+	+	–	–
Chaetomium sp.	–	–	+	–	–
Curvularia sp.	+	+	+	+	+
Fusarium sp.	+	+	+	+	+
Lasiodiplodia	+	+	+	+	+
Nigrospora sp.	+	+	+	–	–
Pestalotiopsis sp.	+	+	+	+	+
Rhizopus sp.	–	–	–	+	–
Sphaeropsidaceae	+	+	–	–	–
Deuteromycetes	+	+	+	+	–
Deuteromycetes	+	+	–	–	–
Deuteromycetes	+	+	–	–	–
Deuteromycetes	+	–	–	–	-
Total	14	13	11	9	5

Table 2. Percentage of fungi isolated from each distance of young longan shoot at 15 days after inoculation.

Pathogen inoculated	Fungi isolated	Distance (cm) away from inoculation point[a]									
		0.2	0.4	0.6	0.8	1.0	1.5	2.0	2.5	3.0	3.5
None	*Lasiodiplodia* sp.	80	80	40	60	20	20	40	80	60	80
	Pestalotiopsis sp.	0	0	0	0	0	0	0	0	0	0
	Curvularia sp.	0	0	0	0	0	0	0	0	0	0
	Fusarium sp.	0	0	0	0	0	0	0	0	0	0
Fusarium sp.	*Lasiodiplodia* sp.	40	20	40	40	60	40	80	80	80	60
	Pestalotiopsis sp.	0	0	0	0	0	0	0	0	0	0
	Curvularia sp.	0	0	0	0	20	0	0	0	0	0
	Fusarium sp.	20	20	40	20	40	40	20	20	20	0
Curvularia sp.	*Lasiodiplodia* sp.	0	20	40	0	40	60	80	60	100	100
	Pestalotiopsis sp.	20	20	20	20	0	0	0	20	0	0
	Curvularia sp.	80	40	40	60	40	40	0	20	0	0
	Fusarium sp.	0	0	0	0	0	0	0	0	0	0
Lasiodiplodia sp.	*Lasiodiplodia* sp.	100	100	100	100	100	80	60	80	80	80
	Pestalotiopsis sp.	0	0	0	0	0	0	0	0	0	0
	Curvularia sp.	0	0	0	0	0	0	0	0	0	0
	Fusarium sp.	0	0	0	0	0	0	20	20	20	20
Pestalotiopsis sp.	*Lasiodiplodia* sp.	20	40	40	60	80	80	80	80	100	100
	Pestalotiopsis sp.	80	60	60	20	20	20	0	0	0	0
	Curvularia sp.	0	0	0	20	0	0	0	0	0	0
	Fusarium sp.	0	0	0	0	0	0	20	20	20	20

[a] 5 replicates for each isolation

Table 3. Percentage of fungi isolated from the parts of embryo and endosperm of longan seed after inoculation on the stem end of the fruit for one week.

Treatment	Fungi isolated	Parts from which isolated[a]	
		Embryo	Endosperm
Control	*Lasiodiplodia* sp.	25	25
	Pestalotiopsis sp.	20	25
Inoculated with *Fusarium* sp.	*Fusarium* sp.	60	0
	Pestalotiopsis sp.	0	0
Inoculated with *Lasiodiplodia* sp.	*Lasiodiplodia* sp.	45	90
	Pestalotiopsis sp.	5	0
Inoculated with *Curvularia* sp.	*Fusarium* sp.	0	5
	Pestalotiopsis sp.	10	5
	Curvularia sp.	35	5
Inoculated with *Pestalotiopsis* sp.	*Lasiodiplodia* sp.	10	0
	Pestalotiopsis sp.	75	45

[a] 20 replicates for each isolation

Table 4. Percentage of fungi isolated from various parts of longan seedling.

Pathogen inoculated	Fungi isolated	Parts of seedling*				
		A	B	C	D	E
Control	*Lasiodiplodia* sp.	10	10	60	45	10
	Pestalotiopsis sp.	5	20	30	0	5
	Fusarium sp.	10	5	5	10	5
Pestalotiopsis sp.	*Lasiodiplodia* sp.	20	40	40	15	0
	Pestalotiopsis sp.	15	20	25	10	5
	Fusarium sp.	5	5	5	5	0
	Curvularia sp.	15	5	0	0	0
Lasiodiplodia sp.	*Lasiodiplodia* sp.	50	50	45	10	0
	Pestalotiopsis sp.	25	0	10	0	0
	Fusarium sp.	0	5	0	0	0
	Curvularia sp.	0	0	5	0	0
Curvularia sp.	*Lasiodiplodia* sp.	35	35	45	15	10
	Pestalotiopsis sp.	5	15	5	0	0
	Fusarium sp.	0	0	0	5	0
	Curvularia sp.	.10	15	20	0	0
Fusarium sp.	*Lasiodiplodia* sp.	35	35	30	5	0
	Pestalotiopsis sp.	0	0	10	5	0
	Fusarium sp.	15	0	0	0	0
	Curvularia sp.	0	0	10	0	0

[a] 20 replicates for each isolation

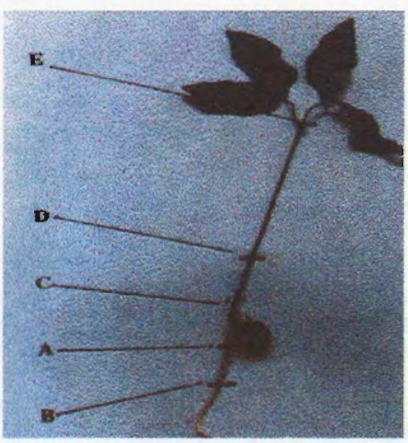

Figure 4. Isolation points on various points of young seedling

It was also the most virulent pathogen, able to spread from the point of inoculation to the tip of the shoots (Fig. 6). Many shoots died or displayed retarded growth as a result of the pathogen invasion.

Seed transmission of some fungi in longan

The numbers of fungi isolated from various parts of the embryo and endosperm are shown in Table 3. The fruit-rot fungi can invade inside the stem end through the seed (Fig. 7). A smaller number of seedlings was obtained from fruit inoculated with *Lasiodiplodia* sp. However, it was found that the four species of fungi inoculated were detected from the seedlings of all treatments. They were also frequently isolated from the positions of hypocotyl and radicle (positions A, B, C and D) (Table 4).

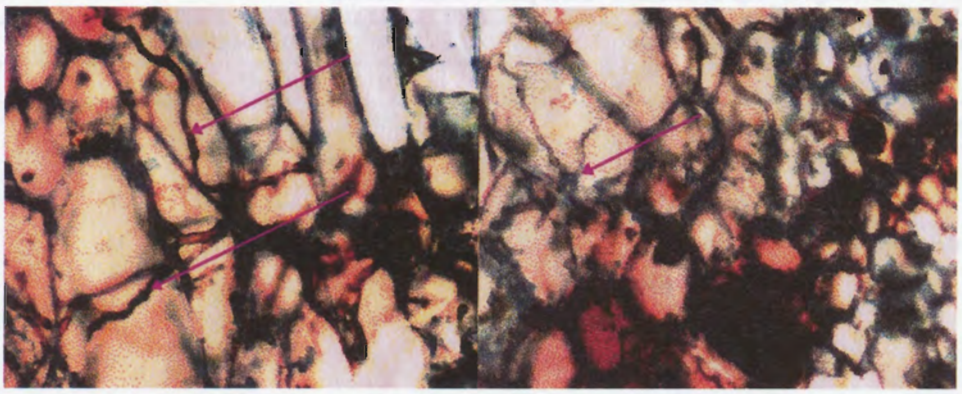

Figure 5. The hyphae of *Lasioderma theobromae* inside the shoot tissue of longan

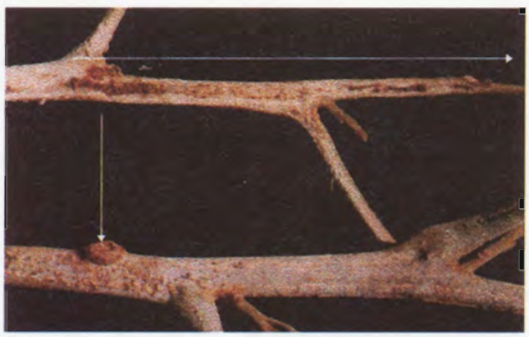

Figure 6. *Lasiodiplodia* sp. is the most virulent pathogen of longan, able to spread from the inoculation point to the tip of the shoot

Figure 7. Symptoms developing on stem end, flesh, and seed of longan fruit following artificial inoculation with various fruit rot fungi

Discussion and Conclusion

Many of the fungi isolated were found to be organisms endophytic on the longan tree. *Lasiodiplodia* sp., *Pestalotiopsis* sp., *Fusarium* sp. and *Curvularia* sp. were the most frequently isolated from shoot, panicle, stem end and seed. These fungi are recognised as causing fruit rot in longan after harvest. On most of the fruits, Dennis (1993) noted that, for most of these fruits, decay during storage was caused by fungi that were already present on the surface or inside the fruit before harvest. The results of this work agree those of Johnson et al. (1994) who found that *Lasiodiplodia theobromae* (the causal agent of stem-end rot of mango) and *Pestalotiopsis* sp. were endophytic fungi.

Acknowledgment

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Diurnal Water Potential Changes and Avocado Fruit Ripening

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Abstract

The influence of water deficit on ripening time of avocado fruit was investigated. Avocados with different xylem water potentials (ψ_x) were obtained by harvesting fruit diurnally. The ψ_x of fruit and leaves from the northern side of the trees were measured immediately after harvest using a large pressure chamber. The water potential was subsequently measured hourly over 25 hours. Fruit harvested at night between 8 pm and 5 am had a significantly higher ψ_x (–0.16 MPa) than those harvested during the day between 7 am and 3 pm (–0.3 MPa). When subsequently held under the same conditions, avocado fruit of lower ψ_x ripened a day earlier than those of higher ψ_x. Studies of fruit tissue water relations may provide further insight into the relationship between water potential and ripening time.

WATER deficit in harvested fruit and vegetables is undesirable because consumers perceive it as loss of freshness. The more severe the water deficit, the less likelihood there is of a sale. The postharvest technologist understands that water deficit-induced wilting is due to continued transpiration coupled with loss of water supply from the parent plant (Ben-Yehoshua 1987; Sastry et al. 1978; Kays 1991). Severe water deficit is termed water stress (Kays 1991). In contrast to qualitative descriptions, quantification of fruit water relations provides more insight into plant organ responses to different levels of water deficit.

Detailed understanding of fruit responses to water loss and, in particular, quantitative knowledge of the level of water deficit at which the fruit begin to deteriorate should benefit both science and industry. This knowledge would provide a valuable contribution towards devising approaches to retard deterioration induced by water loss. Industry, armed with such knowledge and improved approaches, would in turn be better placed to practise measures to maintain produce quality. However, there is little detailed information on postharvest water relations and their role in ripening and senescence of harvested produce. This paper reports on an investigation into the response of avocados to developing postharvest water deficit.

Relationships between tissue water status and fruit ripening remain ambiguous. It has not been determined whether water loss simply acts as an enhancer (i.e. accelerator) of fruit ripening, or whether it can actually induce or initiate the ripening process. Knowledge of the responses should lead to better understanding of the influence of water loss on ripening avocado fruit and, in turn, to improved postharvest management of the fruit. To investigate this question, water deficit needs to be established in fruit at, or immediately after, harvest. The relationship between water deficit and subsequent ripening of avocado was investigated.

Natural diurnal changes in fruit water status were used in an attempt to obtain a series of differing fruit xylem water potentials (ψ_x). The effects of variation in water status as influenced by positions on the trees

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and diurnal changes were investigated. Diurnal changes in fruit diameter have been reported in apple (Jones and Higgs 1985), citrus (Elfving and Kaufmann 1972), and avocado (Schroeder and Wieland 1956). Avocado fruit diameter decreased during the middle of the day and increased during the night (Schroeder and Wieland 1956). However, diurnal changes in actual water relations parameters of mature avocado fruit have not been reported. Accordingly, diurnal changes in avocado fruit water relations from different positions on the tree and different times of day were investigated (experiment 1 and experiment 2). In a subsequent experiment, also aimed at testing the hypothesis that water deficit accelerates fruit ripening (experiment 3), fruit were harvested only predawn and at midday. The effect of different water loss rates during storage on the ripening of these fruit was also investigated.

The pressure chamber offers a convenient way of measuring the ψ_x of harvested organs (Turner 1981). It is simple, fast and reliable (Spomer 1985). Joyce and Shorter (1992) used a small pressure chamber and atypically small avocado fruit in a postharvest fruit water relations study. They reported that film wrapped avocado fruit, which maintained 0.4 MPa higher ψ_x than unwrapped fruit, ripened more slowly. Bower (1984) also used a pressure chamber to measure avocado fruit ψ_x. He found reasonable agreement between ψ_x values obtained this way and those determined psychrometrically on tissue taken from the pedicel immediately adjacent to the fruit.

Materials and Methods

Experiment 1. Diurnal water potential changes and avocado fruit ripening.

Fruit and leaves on the same shoots from the northern side of the canopy of three 'Hass' avocado trees at Mt. Cotton (latitude 27°10'S, longitude 153°12'E, Queensland, Australia) were used for measurement of diurnal ψ_x changes with a pressure chamber on 3–4 April 1993. Fruit and leaf ψ_x were determined hourly. At each measurement time, a fruit from each tree and the first adjacent, fully mature leaf from the same branch were wrapped in film wrap (Glad Wrap®) while on the tree. The fruit were harvested with a 4 cm length of stalk remaining. Measurement of ψ_x was carried out immediately after fruit and leaves were detached from the tree.

Temperature and humidity of the air were recorded at the time of ψ_x measurements with a Vaisala (model HM 34, Finland) humidity and temperature meter. Vapour pressure deficits (VPD) were calculated from the relative humidity (RH) and temperature measurements.

Diurnal changes in fruit diameter were also measured on an additional three representative fruit from each of the three trees referred to above. Fruit diameters were measured hourly with a digital vernier calliper (Mitutoyu, RS 600–800, Japan).

After ψ_x measurements, fruit from each time and tree were labelled, wrapped in film wrap and packed in tray pack boxes. The detached fruit were kept in the shade to avoid heating. After sampling was completed, all harvested fruit were transported to the laboratory in Brisbane. On arrival, fruit were unwrapped, weighed, and assessed for colour and firmness(see below for details of scoring system). Fruit were ripened in a 22°C room.

Assessments

Fruit weight loss. Weight loss rate was calculated on a per day basis from the difference in fresh weight recorded on successive days over 18 days. The average weight loss rate was calculated as percentage of initial weight/day.

Fruit ripening. Ethylene (C_2H_4) production was measured for fruit sealed individually in 900 mL glass jars for 1 h at 22°C. Headspace gas samples were withdrawn with 1 mL plastic syringes for analysis (Saltveit 1982). Ethylene was measured with a GowMac 740P gas chromatograph fitted with an activated alumina column (2.1 mm ID, 2.5 m long, 100/120 mesh) and a H_2/air flame ionisation detector (FID). Injector, oven, and detector temperatures for the FID were 90, 90, and 150°C, respectively.

Fruit softness was determined daily using the following rating score: 1 = hard, 2 = sprung, 3 = slightly soft, 4 = soft (eating ripe), and 5 = very soft (Joyce and Shorter 1994). Mechanical firmness was measured as deformation (mm) using a home-made McGlasson Tomato Firmness Tester (A. Macnish, D. Joyce and A. Shorter, pers. comm.) at a marked location on the fruit, under a 500 g load applied for 1 min. Fruit colour was scored as follows: 1 = green, 2 = <25% coloured, 3 = >25% but <50% coloured, 4 = >50% but <75% coloured, and 5 = >75% coloured. Fruit colour was measured, at a marked position on each fruit, with a Minolta CR-200 Chroma meter, and chromaticity coordinate (a*) values were recorded. Pedicel abscission was recorded as the day when separation occurred under the fruit's own weight.

Statistical design and analysis. Diurnal fruit and leaf ψ_x and fruit ripening times (data not shown) were first analysed at hourly intervals (25 times) and three blocks (trees), using analysis of variance (ANOVA). The experimental design was a randomised complete block. The data on fruit ripening were not significant over 25 h due to small number of replicate fruit (three fruit per hour). These data were then grouped for three time periods; (i) morning (midnight to 7 am), (ii) day (7 am to 3 pm), and (iii) evening (3 pm to midnight). The data were then again analysed by ANOVA, three times by three blocks and group treatment means were compared using Duncan's multiple range test (DMRT). The software program used for these analyses was SAS Statistical Program (SAS 1987).

Experiment 2. Variation of avocado fruit water potential with position on the tree.

Variations in fruit ψ_x at different positions on the tree were investigated in a further effort to identify fruit at different ψ_x. Xylem water potential of a fruit and its neighbouring, fully expanded, mature and undamaged leaves was measured with a pressure chamber. Fruit and leaves were sampled from different positions in three avocado trees on a farm at Grantham (latitude 27°34'S, longitude 152°12'E, Queensland) on 12 to 14 July 1994. Three 10 year old 'Hass' avocado trees from the exposed first row on the eastern side of the orchard were chosen. These trees were fully exposed to morning sunlight. The fruit and leaves were approximately 7 months old.

Fruit and adjacent leaves were sampled from the north, east, south and west of each tree, with or without exposure to sunlight and at four different positions: from the outer canopy at three levels; (i) below 1 m, (ii) 2 to 3 m, or (iii) 4 to 5 m, and (iv) from the inner canopy at 2 to 3 m. Measurements were made for each tree during four different time periods: (i) early morning (7 am to 10 am), (ii) mid morning (10 am to 12 am), (iii) mid afternoon (12 am to 2 pm), and (iv) late afternoon (2 pm to 5 pm). Sun exposure (yes or no) was recorded for fruit at each position and time period.

On each of the three sampling days, the ψ_x of fruit and adjacent leaves at four different positions from each of the three trees were measured. For measurement of ψ_x, the fruit or leaf was enclosed in a plastic bag to prevent moisture loss and ψ_x was measured with a pressure chamber immediately after detachment. The leaf ψ_x measurement was made immediately after the adjacent fruit ψ_x had been measured.

Statistical design and analysis

The experimental design was a 2×4^3 mixed factorial with unequal sample size. The treatment combinations were two levels of exposure to sunlight, four compass orientations (north, south, east and west), four positions within the canopy, and four times of day. Individual trees were treated as blocks. Water potentials of fruit and leaves were compared using the general linear model (GLM) procedure (SAS 1987) and means were compared using DMRT.

Experiment 3. Effect of water deficit at harvest and two postharvest relative humidities on avocado fruit ripening.

Fruit of uniform size and maturity were tagged on trees at a farm at Mt Cotton on 12 August 1994. The tagged fruit were harvested twice on 14 August 1994: predawn (3 am to 5 am) and at midday (12 pm to 2 pm). Each fruit was first enclosed in a polyethylene bag to prevent moisture loss and then harvested with 4 cm pedicel remaining. Fruit were packed into tray pack cartons and transported to the laboratory.

On arrival at the laboratory, the polythene wrap was removed and the fruit from each harvest time were labelled and divided into two groups of 30 before storage at 60% or 90% RH at 20°C. Concentrations of C2H4 and CO2 in the storage rooms (12 m3 at each RH) were monitored daily using the gas chromatograph. An ethylene oxidising scrubber (Purafil®, potassium permanganate on aluminium oxide particles; 1 kg per room) was included to help prevent C2H4 build up in the rooms. Additionally, an air exchange rate of at least 12 air changes per day was maintained to ventilate each room.

Statistical design and analysis

The experiment was arranged as a 2×2 factorial in a completely randomised design. The factors were two different harvest times (predawn and midday) and two storage RHs (~60% RH and ~90% RH). ANOVAs were carried out using SAS (1987) and treatment means were compared using DMRT.

Results

Experiment 1

Diurnal vapour pressure deficits and water potential changes

Water potentials of avocado fruit and leaves tended to follow changes in diurnal VPD (Fig. 1a, b). Water

potentials of fruit and leaves were low when the VPD was high. The opposite trend was true when the atmospheric VPD was low. Water potentials of both fruit and leaves were highest in the morning until 5 am (–0.16 MPa) and rapidly declined thereafter (Fig. 1b). The fruit ψ_x decreased to –0.7 MPa by 10 am and remained at this level until 3 pm. The ψ_x rose between 3 pm and 8 pm to –0.3 MPa and remained high until morning. The leaf ψ_x decreased between 10 am (–0.4 MPa) and 12.30 pm (–1.7 MPa) dropping by about 1.3 MPa, followed by a sharp rise until 3 pm (–0.4 MPa; Fig. 1b).

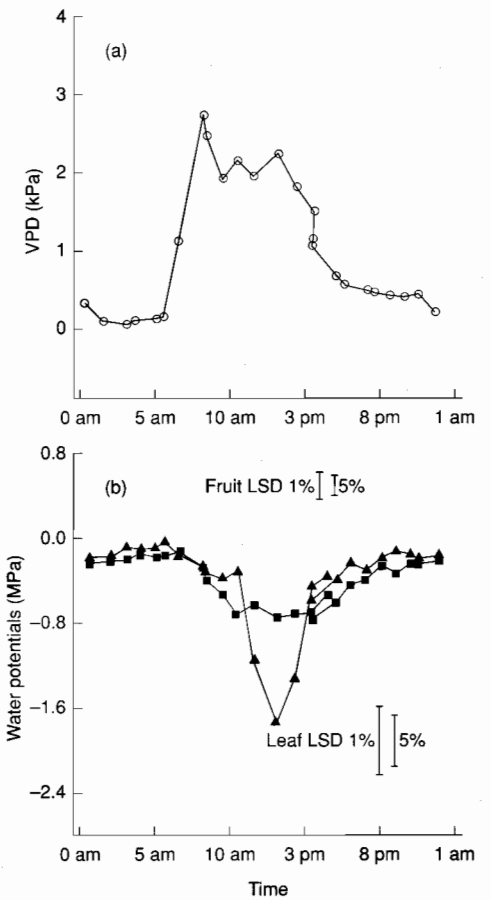

Figure 1. (a) Diurnal vapour pressure deficits (VPD) (○) of air at the time of avocado fruit and leaf water potential measurement on 3–4 April 1993 at Mt. Cotton, Queensland, and (b) water potentials of avocado fruit (■) and leaves (▲) on the northern side of the trees measured at hourly intervals over a 25 h cycle. Vertical bars indicate the least significant difference at 5% and 1% for fruit and leaves.

When the diurnal cycle was divided into three time groups, ψ_x of both fruit and leaves during the 'day' periods (7 am to 3 pm) were significantly lower than those of the 'night' and 'morning' periods (Table 1). This result corresponds to the changes in atmospheric VPD, such that fruit ψ_x were negatively correlated with the VPD of the air (Table 1). Leaf ψ_x appeared to recover to near morning levels during the evening, while fruit ψ_x tended to remain significantly lower than those in the morning (Table 1).

Table 1. Water potentials (ψ_x) of fruit and leaves and atmospheric vapour pressure deficits (VPD) for data pooled as time groupings for morning (midnight to 7 am), day (7 am to 3 pm), and evening (3 pm to midnight).

Time grouping	Fruit ψ_x (MPa)	Leaf ψ_x (MPa)	VPD (kPa)
Morning	–0.17 a	–0.11 b	0.27 c
Day	–0.58 c	–0.70 a	1.99 a
Evening	–0.37 b	–0.25 b	0.50 b

Fruit diameter changes

Fruit diameters were 0.3 mm smaller during the 'day' period compared with those measured during the 'morning' and 'evening' period (Table 2). This is equivalent to a volume change in the order of 1.3%.

Table 2. Fruit diameter for each time grouping for fruit from each avocado tree used for water potential measurements (morning = midnight to 7 am, day = 7 am to 3 pm, evening = 3 pm to midnight).

Time grouping	Fruit diameter (mm)
Morning	68.8 a
Day	68.5 b
Evening	68.8 a

Within columns, means followed by the different letter are significantly different ($P<0.05$) by Duncan's multiple range test.

Fruit weight loss and ripening

Weight loss of fruit harvested at different times of the day did not differ during ripening at 22°C and 75% RH (Table 3). There was no significant difference in ripening time ($P>0.05$) of fruit harvested hourly throughout the day as judged by the ripening parameters used (data not presented). However,

when fruit were grouped into three harvest periods, ripening, as judged by the onset of C_2H_4 production and achieving a fruit softness score of 3, was significantly earlier ($P<0.05$) in fruit harvested during the 'day' period (7 am to 3 pm) than those harvested during the 'morning' (Table 3). Nevertheless, ripening judged by other parameters did not differ significantly ($P>0.05$). However, there was a trend across all the other ripening parameters that ripening occurred earlier in fruit harvested during the 'day' period than during the 'morning' or 'evening' periods (Table 3).

Table 3. Rates of fruit weight loss and ripening parameters of avocado fruit harvested hourly and regrouped into morning (midnight to 7 am), day (7 am to 3 pm), and evening (3 pm to midnight) periods and ripened at 22°C and 75% relative humidity. Softness score 3 = slightly soft, colour score 3 = >25% but <50% coloured, according to scales described in the text.

Parameter	Time period		
	Morning	Day	Evening
Weight loss (%/day)	0.64 a	0.61 a	0.62 a
Days from harvest to			
(i) ethylene onset	12.2 a	11.3 b	12.0 ab
(ii) ethylene peak	14.3 a	13.7 a	14.3 a
Softness score 3	14.6 a	14.2 b	14.6 a
Colour score 3	16.4 a	15.7 a	15.8 a
Pedicel abscission	13.7 a	13.2 a	13.7 a
Deformation	15.6 a	14.8 a	15.1 a
Chroma a*	17.3 a	16.7 a	16.4 a

Within a row, means followed by the same letter are not significant different at $P<0.05$, by DMRT.

Experiment 2

Water potentials of avocado fruit and leaves were not affected by different exposure to sunlight, orientation on the tree and height above the ground, but did vary with time of the day (Table 4). Generally, changes in fruit and leaf ψ_x over time followed similar trends.

Declines in ψ_x from the morning to the lowest level during mid afternoon were recorded for both fruit and leaf (Table 5). However, while the fruit remained low at mid and late afternoon, the leaves regained the high ψ_x found in the morning by late afternoon (Table 5).

Table 4. F-values (summary analysis of variance,) for effects of exposure to sunlight, position on the tree at different orientations and height above the ground, and time of day on fruit and leaf water potentials (ψ_x) (DF = degrees of freedom).

Source of variance	DF	Variance ratio (F)	
		Fruit ψ_x	Leaf ψ_x
Tree	2	0.06 ns	0.08 ns
Exposure	1	0.05 ns	0.98 ns
Orientation	3	0.49 ns	0.38 ns
Level	3	0.11 ns	0.18 ns
Time	3	0.001**	0.001**
Time × orientation	12	0.51 ns	0.62 ns
Orientation × level	9	0.61 ns	0.89 ns

ns = not significant, and ** when Pr < F: > 0.05, and ≤ 0.01, respectively using Duncan's multiple range test

Experiment 3

Vapour pressure deficits at harvest were calculated from the temperature and RH recorded using a Vaisala humidity and temperature sensor. At the initial predawn harvest (3 am), VPD decreased from 0.16 kPa to 0.08 kPa over 2 h. Vapour pressure deficits continued to decrease to 0.06 kPa at dawn (5:30 am) and then to 0.02 kPa at sunrise (5:45 am). Midday temperature was about 10°C higher and the RH was about 40% lower than at predawn. The VPD varied from 1.14 kPa at noon to 1.41 kPa about 2 hours later. Average Ψx of fruit harvested predawn and at midday were –0.16 MPa. and –0.42 MPa, respectively.

Effect of harvest time and subsequent storage on fruit weight loss and ripening

Weight loss from fruit harvested at different times and kept at the same storage RH did not vary significantly ($P>0.05$) (Table 6). Fruit kept at low RH lost weight more rapidly (by about 0.4 % day-1) than those at high RH (Table 6). Fruit harvested at midday softened and coloured about 0.5 day faster than fruit harvested predawn (Table 6). Fruit which were harvested at the same time of the day and were subsequently exposed to low (60 %) RH ripened about 1 day earlier than those exposed to high (90 %) RH, as judged by fruit softness score of 4 and colour score of 4 (Table 6).

Table 5. Effect of exposure to sunlight, orientation and position in the tree (low [below 1 m], middle [2 to 3 m], and upper [4 to 5 m] on the outer canopy and 2 to 3 m in the inner canopy) at 4 times: early morning (7 am to 10 am), mid morning (10 am to 12 pm), mid afternoon (12 pm to 2 pm) and late afternoon (2 pm to 5 pm) on average avocado fruit and leaf water potentials measured with a pressure chamber.

Position of fruit on the tree	No. of observations	Mean water potential (MPa)	
		Fruit	Leaf
Exposure			
Yes	33	–0.41 a	–0.29 a
No	36	–0.37 a	–0.30 a
Orientation			
Northern side	20	–0.36 a	–0.29 a
Southern side	20	–0.46 a	–0.35 a
Eastern side	16	–0.28 a	–0.16 a
Western side	13	–0.42 a	–0.18 a
Position			
Lower-outer	18	–0.35 a	–0.19 a
Middle- inner	16	–0.42 a	–0.29 a
Middle- outer	18	–0.38 a	–0.27 a
Upper-outer	17	–0.43 a	–0.46 a
Time			
Early-morning	17	–0.21 a	–0.15 a
Mid-morning	16	–0.36 b	–0.24 a
Mid-afternoon	21	–0.49 c	–0.50 b
Late-afternoon	15	–0.50 c	–0.25 a

Within the same columns and factors, means followed by different letters are significantly different at P <0.05 by Duncan's multiple range test.

Discussion

The water content of attached avocado fruit was affected by diurnal changes in water potential.

Measured changes in avocado fruit ψ_x due to water loss were associated with fruit shrinkage around midday (Table 2). Fruit diameter decreased by about 0.3 mm at midday equivalent to a volume change of 1.3% (Table 2). A similar decrease in avocado fruit diameter of 0.5 mm at midday was also reported by Schroeder and Wieland (1956). This implies that the maximum water deficit achieved at midday was 1.3% at the ψ_x of –0.6 MPa. Midday shrinkage of avocado tended to be negatively correlated to increased VPD. Similarly, diurnal changes in fruit and leaf ψ_x tended to be negatively correlated with VPD, such that ψ_x of fruit and leaves were low when the VPD was high (Fig. 1a, b, Table 1). The resultant water loss from the fruit to the atmosphere was presumably due in part to transpirational loss. The strong dependence of diurnal fruit shrinkage on environment and its close relationship with leaf ψ_x has been reported in apple by Higgs and Jones (1984). They suggested that changes in plant water status may mediate fruit shrinkage in response to high irradiance.

Table 6. Fruit weight loss and time (days) taken for fruit to reach eating ripe softness (fruit softness score 4) and >75% coloured (colour score 5) for avocado fruit harvested predawn or at midday and subsequently stored at 60% or 90% relative humidity (RH) over 18 days (refer to text for details of fruit softness and colour scales).

Treatment	Treatment (%/day)	Days taken for fruit to reach	
		softness score 4	colour score 5
Effect of time at harvest (n=60)[a]			
Predawn	0.70 a	10.8 a	11.7 a
Midday	0.74 a	10.3 b	11.1 b
Effect of storage RH (n=60)			
High RH	0.52 b	11.2 a	11.8 a
Low RH	0.92 a	9.9 b	11.0 b
Effect of harvest time and storage RH (n=30)			
Predawn-high RH	0.53 a	11.3 a	12.0 a
Predawn-low RH	0.88 b	10.3 b	11.4 b
Midday-high RH	0.52 a	11.0 a	11.7 a
Midday-low RH	0.96 b	9.6 c	10.5 c

[a] Within each group for each effect and within columns, those means followed by different letter are significantly different (P<0.05) by DMRT. n = number of observations per trial.

A method of achieving significant water deficits using the diurnal effects was determined.

Varying initial water deficit levels in avocado can be obtained by harvesting fruit at different times of the day, as the ψ_x of avocado varies diurnally (Fig. 1, Tables 1 and 5). Fruit harvested at midday are at lower ψ_x than those harvested at night or predawn. Significant differences in time to ripen, as indicated by days to onset of the ethylene peak or a fruit softness score of 3 (Table 3), can only be detected by grouping fruit into three broad harvest periods in the diurnal experiment. This suggests that it would be necessary to increase the number of replicate fruit in

order to discern a subtle response of fruit to water deficit. The low replicate number in the diurnal study was necessitated by the time involved in making ψ_x measurements. The time taken to measure three replicate fruit plus three replicate leaf ψ_x was about one hour. The lower the ψ_x, the longer the measurement time. For avocado fruit and leaves with lower ψ_x during midday, measurement required more than 25 minutes per sample since slow release of pressure (0.5 MPa/min) was adopted to avoid potential fruit damage (Turner, 1981). The lowest ψ_x (–0.74 MPa) was found during midday and the highest (–0.13 MPa) during predawn (Fig. 1). In contrast, the ψ_x of the fruit was seemingly not influenced by position on the tree (e.g. height) or exposure to sunlight (Tables 4 and 5). This means that the position of the fruit on the tree may be ignored for the purpose of random sampling of fruit for water status studies.

The magnitude of the diurnal effect on water deficit varied with a number of factors.

The range of the ψ_x measured in experiment 3 between predawn and midday was from –0.2 MPa and –0.4 MPa, respectively. The differences were not as pronounced as in the experiment 1 (Table 1; –0.17 and –0.6 MPa, respectively) and experiment 2 (Table 5; –0.2 MPa and –0.5 MPa, respectively). This was probably due to a combination of several factors including overcast weather and high RH (low VPD) at midday on the harvesting day and the effect of the irrigation regime of different orchards.

Ripening as judged by fruit softness and colour from avocado fruit harvested at midday was advanced by only half a day compared with those harvested at predawn (Table 6). Fruit ripening was further advanced by about a day (Table 6) by subsequently storing fruit, from both harvest times, at low RH. This absence of a pronounced effect suggests the ψ_x differences at the level of these studies (–0.6 MPa and –0.17 MPa) were of little consequence physiologically compared with the effects of subsequent storage conditions.

The diurnal effect on water content could be measured using a modified pressure chamber

The pressure chamber used in the studies was modified to measure ψ_x of an avocado fruit of normal size. The diurnal ψ_x of mature normal size avocado fruit has not been previously reported. The minimum avocado leaf ψ_x measured in the present study was –1.7 MPa at about 1 pm, a time similar to that reported for avocado by Sterne et al., (1977;1978) and Whiley et al. (1986).

By using the pressure chamber to measure ψ_xx, Blanke and Whiley (1995) were able to report on the midday ψ_x of developing 'Hass' and 'Feurte' avocado fruit compared with the leaf ψ_x. This study indicated that there are differences in the way these cultivars respond to water stress during early development. Their results suggest that after an initial development stage the midday ψ_x of the fruit is maintained at a higher (less negative) level than the leaves. As shown in Fig 1b, the ψ_x of the fruit and leaves of mature 'Hass' avocado were quite similar except for around midday when the fruit ψ_x fell by 0.8 MPa compared with the ψ_x at dawn, and the leaf ψ_x fell by 1.6 MPa. Although this difference indicates that the fruit is less affected by diurnal water deficit than the leaves, the fall in ψ_x indicates substantial movement of water from the fruit to the leaves along the ψ_x gradient. This is consistent with the dawn to midday shrinkage of fruit diameter by 0.3 mm, reported in Table 2, equivalent to a loss of 1.3% of the fruit volume. The shrinkage of mature avocado fruit during the middle of the day has been attributed to the reverse flow of water from the fruit to the stem and the leaves (Schroeder and Wieland 1956).

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