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# **Development of Postharvest Handling Technology for Tropical Tree Fruits**

**A workshop held in Bangkok, Thailand, 16–18 July 1992**

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Australian Centre for International Agricultural Research  
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# Introduction to Workshop

G.J. Johnson\*

THIS workshop was an activity of ACIAR Project No. 8844, 'The development of postharvest handling technology for selected tropical tree fruits'. The project was undertaken by collaborating research teams at the Queensland Department of Primary Industries in Australia, and the Department of Agriculture, the Thailand Institute of Scientific and Technological Research, and Chiang Mai and Kasetsart universities in Thailand.

Cooperative research provided the opportunity to maximise benefits per research dollar expended, by reducing duplication of effort and providing additional 'fruit season' time within the life of a research program. Longer term benefits will accrue from the training and equipping of research scientists and facilities and from fostering government-to-government and researcher-to-researcher good will. Benefits and scientific breakthroughs can arise from the synergy of cooperative research sharing ideas, information, skills and pieces of the puzzle being solved.

## Growth in Tropical Fruit Production

Tropical fruit production in Australia is emerging as a significant component of horticulture industry earnings. Interest has generally been greater in the tropical Asian rather than the tropical American fruits. Important commodities include mango and lychee, with a smaller rambutan industry also developing.

In Southeast Asia, market-driven production of these seasonal staples has been increasing (on cleared forest land and rice paddy) to supply export markets in Singapore, Malaysia, Hong Kong, Japan, the Middle East and, to a lesser extent, Europe, to siphon off seasonal production peaks that were previously lost due to wastage or sold at low prices on domestic markets. Export marketing has helped to stabilise (and increase) local commodity prices and has increased foreign currency earnings.

Major fruit commodities produced in Thailand are durian, longan, mango, lychee, and pineapple, the last not covered by ACIAR Project 8844. Postharvest losses substantially reduce returns in domestic and export marketing, accounting for an estimated 25% of horticultural produce in Thailand (Table 1). Losses are due to over-ripening, high temperature spoilage, and disease, mechanical and physiological deterioration.

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**Table 1.** Estimated postharvest losses of fruit and vegetables

Country	%
Indonesia	20–25
India	25–35
Malaysia	15–20
Philippines	40
Sri Lanka	30
Thailand	25–30

Source: FAO 1987–88.

In contrast to temperate fruit (and banana and pineapple) that have been extensively studied for a century, tropical fruits have been under-researched, and possess inherently different biological attributes which reduce storability as compared with temperate fruits. The Thai and Australian tropical fruit industries are developing in (relatively speaking) an information vacuum. ACIAR projects are seeking to overcome this deficiency.

ACIAR Project 8844 developed the technology to improve and maintain post-harvest tropical fruit quality in Thailand and Australia — ensuring increased returns to the tropical fruit industries, enhancing reputations as reliable producers of quality fruit and encouraging market stability for the project beneficiaries.

With increasing prosperity in Asia, the potential markets for tropical fruit within the region are huge: Japan, and in time, Korea and China. The markets forged by Thai produce should create a niche that can be partly filled by Australian fruit during the northern hemisphere off-season. Together, Australia and Thailand (and other ASEAN countries) can work towards producing and supplying quality-assured tropical fruit to international markets.

In Australia, the major postharvest problems affecting fruit are: rapid ripening, disease and sap burn in mango, and fruit browning and disease in lychee. Another significant problem is the need to expand the market niche for mangoes, lychees and other tropical fruit, particularly in southern Australia where fruit consumption patterns are based on 'European' temperate fruit, so that consumers regard tropical fruit (other than bananas and pineapples) as 'exotic', seasonal and luxury commodities. By contrast, tropical fruit are staples throughout much of Asia. In both Australia and Thailand, growers and marketers need to acquire the skills and experience for supplying consumers in more distant markets with quality-assured products. ACIAR Project 8844 aimed to develop the handling procedures that are a component of quality-assurance schemes.

## Research Activities

Research in Project 8844 was a mix of determining basic information about tropical fruit biology, storage requirements, pathogen biology and control, and the

development and implementation of postharvest technology, and reflected the project partner needs. In Australia, sap burn and storage of mangoes, stem end rot (disease) biology, postharvest dip technology, storage and browning of lychee, and rambutan storage received priority. In Thailand, studies on the establishment and confirmation of maturity indices, pathogen biology, storage requirements, modelling of modified atmospheres, waxing of durian and the development of SO<sub>2</sub> slow-release technology for lychee were investigated.

In addition, the commercial implementation of SO<sub>2</sub> treatments for longan, lychee and green coconut in Thailand for export markets emerged from ACIAR-supported research. In Australia, a quality assurance scheme for mangoes is being developed by QDPI extension staff and the International Business Development Centre (University of Queensland), utilising the results of research supported by ACIAR and local industry.

### **Highlights — Thai Program**

A feature of the project was support for export trials for Thai produce, monitoring the implementation of project developed technology within a commodity handling and marketing system. The project supported three outturn trials: the export of waxed durian by sea to Hong Kong, the SO<sub>2</sub> fumigation and consignment of longan by road from Chiang Mai to Singapore and the evaluation of SO<sub>2</sub> slow-release pads on lychee sent by sea to Europe.

- The durian trial showed that while waxing reduced fruit splitting, skin colour development was retarded, and flesh tainting was detected in some samples. However, the results showed sufficient promise for some exporters to adopt the treatment.
- The longan trial enabled documentation of SO<sub>2</sub> residue samples, a requirement for legal registration of the treatment, and highlighted deficiencies associated with hydro-cooling and in-transit ice cooling systems. The treatment was widely accepted by longan exporters.
- SO<sub>2</sub> slow-release pads for lychee performed well in trial shipments to Europe, and their commercial potential appears promising.

The successful implementation of SO<sub>2</sub> fumigation technology for lychee and longan in Thailand prompted assessment of the methods for the Australian lychee industry.

### **Highlights — Australian Program**

Highlights of the Australian program included:

- an SO<sub>2</sub>-dilute hydrochloric acid treatment to prevent browning, control disease and fix skin colour in lychee;
- indications that storage temperatures below the current recommendation (13°C), together with close control of storage atmosphere composition, may prove to be the elusive combination required for successful extension of shelf life in mango;

- demonstration of a novel infection process for stem end rot pathogens in mango which could be exploited to achieve successful non-chemical control in mango and many other fruits, including citrus (which currently depends on a 2,4-D treatment) and avocado; and
- the development of a mechanical de-sapper and other techniques to reduce packing-shed sap burn.



# Effects of Fruit Maturity at Harvest on Disease Development in Lychee During Storage

C. Sittigul, U. Sardsud, V. Sardsud and T. Chaiwangri\*

## Abstract

At harvest, lychee fruit of three maturity levels (M3 [31–60% surface red], M4 [31–90% surface red] and M5 [91–100% surface red]) were either treated with hot benomyl (500 ppm at 52°C for 2 minutes) and air dried, or left untreated prior to PVC wrapping in punnets, and storage at 5°C. The fruits were assessed weekly between 2 and 5 weeks after harvest.

Browning of the pericarp and disease development were absent from fruit of M4 and M5 maturity levels 2 weeks after harvest, while > 50% skin browning had developed on the M3 fruits. Browning had appeared on all fruits by 3 weeks after harvest. Similar levels and spectra of fungi were recorded on fruits of the three maturity levels, and were more prevalent in fruits that were not dipped in hot benomyl. *Cladosporium* sp. and *Fusarium* sp. were the most commonly detected fungi. Treatment in hot benomyl appeared to give greater control of side lesions than stem-end lesions.

LYCHEE (*Litchi chinensis* Sonn.) is native to southern China. The fruit is round with a diameter of about 30 mm. The surface of the pericarp has an attractive red colour. Inside is a single seed surrounded by film translucent white flesh. The pericarp turns brown and fruit deteriorate rapidly after harvest. In storage many organisms grow on fruit. These organisms cause fruit to decay and became unmarketable. Recently, cold storage and fungicidal treatment of lychee to expand the shelf life of the fruit after harvest have been investigated. The objectives of this study were to: (1) determine the relationship between stage of maturity at harvest and fruit decay factors; (2) evaluate hot benomyl treatment and cold temperature storage for controlling contaminate fungi; and (3) determine the genera of contaminate fungi after intervals of storage times.

## Materials and Methods

Lychee (*Litchi chinensis* Sonn.) fruit cv. Hong Huay were selected for the study of disease development during storage. At harvest, choice of fruit maturity (M3 [31–60% surface red], M4 [31–90% surface red] and M5 [91–100% surface red]) was considered as a

treatment in the experiment. A second treatment, dipping fruit into benomyl solution at 52°C and concentration of 500 ppm for 2 minutes, was also considered. After treatment, 20 fruit of each treatment were kept in foam punnets prior to wrapping with PVC film. Punnets were stored in a temperature and moisture controlled chamber at 5°C and relative humidity of 90–95% for 0, 2, 3, 4 and 5 weeks. All treatments were replicated two times. The study thus involved 60 punnets.

Fruit of each punnet were scored for quality. After cold storage for 2, 3, 4 and 5 weeks, the colour of the pericarp was scored on a scale 0–10:0 = no browning; 10 = 100% of pericarp area was brown. Ten fruit samples of each week were taken for the isolation of any fungal contaminants. Four 3 × 3 mm pieces of tissues from stem end, pericarp and fleshy part were transplanted and cultured on potato dextrose agar (PDA). The growth and species of fungi were observed and identified.

## Results

After the punnets of lychee fruit were stored in the cold condition for 2 weeks, browning was observed on the skins of both nondipped and dipped M3 fruit. No sign of browning developed on M4 and M5 fruit. Interestingly, in the second week, fungicide did not prolong the attractive colour of fruit skin; conversely

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**Table 1.** Browning scores of the pericarp of lychee fruit harvested at three maturity levels<sup>a</sup> and treated with hot benomyl<sup>b</sup> and held in punnets covered with PVC film for 5 weeks at 5°C; 0 = red, 10 = very brown.

Treatment	Week/mean score			
	2	3	4	5
M3, no dip	1	4.5	7.5	9.5
M3, dipped in hot benomyl	5	6.5	8.5	10.0
M4, no dip	–	4.5	7.0	7.5
M4, dipped in hot benomyl	–	6.0	7.5	8.0
M5, no dip	–	6.5	7.5	7.5
M5, dipped in hot benomyl	–	7.0	8.0	8.0

<sup>a</sup>M3, 31–60% surface red (at harvest); M4, 61-90% surface red; M5, 91-100% surface red.

<sup>b</sup>500 ppm, 52°C, 2 minutes.

it seemed to accelerate the pericarp becoming browner compared to nontreated fruit (Table 1).

In the third, fourth and fifth weeks of all three maturity levels, the fruit treated with hot benomyl turned browner than the control treatments. Moreover, in the last week of observation, fruit of M3 were completely brown (Table 1).

Isolations of organisms were prepared from fruit samples after storage in cold conditions for 0, 2, 3, 4

and 5 weeks. The following organisms were detected: *Botryodiplodia* sp., *Cladosporium* sp., *Fusarium* sp., *Penicillium* sp., *Pestalotia* sp., unknown with white and grey mycelium colony, bacteria and no growth. In M3 fruit, *Cladosporium* sp., and *Cladosporium* sp. growing together with *Fusarium* sp. were predominant among the fungi detected in the nontreated fruit (Table 2). However, when benomyl-treated fruit were examined, the frequencies of fungi detected were less

**Table 2.** Frequency of isolation of fungi obtained from stem end (S), pericarp (P) and fleshy part (F) of M3 maturity level<sup>a</sup> of lychee fruit without fungicidal treatment and stored at 5°C for 5 weeks.

Fungus	Storage time														
	0 week			2 weeks			3 weeks			4 weeks			5 weeks		
	S	P	F	S	P	F	S	P	F	S	P	F	S	P	F
<i>Botryodiplodia</i> sp.	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–
<i>Cladosporium</i> sp.	–	3	6	2	2	4	3	3	4	1	5	5	3	5	4
<i>Fusarium</i> sp.	–	4	2	–	–	–	–	4	1	–	–	–	–	–	–
<i>Penicillium</i> sp.	–	–	–	–	–	–	–	–	–	–	–	2	–	–	–
<i>Pestalotia</i> sp.	–	–	–	–	–	–	–	–	–	–	–	–	–	3	–
<i>Cladosporium</i> sp. + <i>Fusarium</i> sp.	10	–	–	8	8	–	7	–	–	8	3	1	7	2	–
<i>Fusarium</i> sp. + <i>Penicillium</i> sp.	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–
Unknown (white and grey colony)	–	–	–	–	–	4	–	–	4	–	–	–	–	–	–
No growth	–	–	1	–	–	2	–	–	1	–	–	2	–	–	4
Bacteria	–	3	1	–	–	–	–	3	–	–	–	–	–	–	2
Total	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

<sup>a</sup>31–60% surface red at harvest.

**Table 3.** Frequency of isolation of fungi obtained from stem end (S), pericarp (P) and fleshy part (F) of M3 maturity level<sup>a</sup> of lychee fruit dipped in hot benomyl at 500 ppm, 52°C, 2 minutes and then stored at 5°C for 5 weeks.

Fungus	Storage time														
	0 week			2 weeks			3 weeks			4 weeks			5 weeks		
	S	P	F	S	P	F	S	P	F	S	P	F	S	P	F
<i>Botryodiplodia</i> sp.	-	-	-	-	-	-	3	-	-	-	-	-	-	-	4
<i>Cladosporium</i> sp.	3	-	-	-	-	-	-	-	-	6	-	-	1	9	-
<i>Fusarium</i> sp. (1)	3	-	-	3	1	-	4	-	-	-	-	-	-	-	-
<i>Fusarium</i> sp. (2)	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
<i>Pestalotia</i> sp.	2	1	-	5	-	-	2	1	-	-	-	-	-	1	-
<i>Cladosporium</i> sp. + <i>Fusarium</i> sp. (1)	2	-	-	-	-	-	-	4	-	1	-	-	9	-	-
Unknown (white and grey colony)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
No growth	-	5	10	2	9	10	-	5	10	3	10	10	-	-	6
Bacteria	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

<sup>a</sup>31-60% surface red at harvest.

and higher frequencies of no growth were obtained (Table 3). Similar patterns of fungal organisms as in M3 fruit were also found in M4 and M5 fruit (Tables 4, 5, 6 and 7). Results indicate that units treated with hot benomyl were generally in better condition in terms of contaminated fungi than those not treated (Tables 8 and 9). In non-treated M3 and M5 fruit, higher numbers of *Cladosporium* sp. grew on the fleshy part than on the pericarp and stem end of the fruit (Table 8).

## Discussion

Stage of maturity of lychee fruit at harvest appeared to be related to the browning of the pericarp when fruit were kept at 5°C. The pericarp of early-harvested fruit turned brown sooner than that of the late-harvested fruit. Hot benomyl treatment produced some damage to the pericarp of fruit at all stages of maturity. The results of this investigation agreed with the work of Scott et al. (1982), in which they mention

**Table 4.** Frequency of isolation of fungi obtained from stem end (S), pericarp (P) and fleshy part (F) of M4 maturity level<sup>a</sup> of lychee fruit without fungicidal treatment and stored at 5°C for 5 weeks.

Fungus	Storage time														
	0 week			2 weeks			3 weeks			4 weeks			5 weeks		
	S	P	F	S	P	F	S	P	F	S	P	F	S	P	F
<i>Cladosporium</i> sp.	1	6	3	-	1	6	3	2	5	2	5	7	4	8	-
<i>Fusarium</i> sp.	7	2	-	-	-	-	-	-	-	1	2	2	-	-	-
<i>Cladosporium</i> sp. + <i>Fusarium</i> sp.	2	2	7	10	8	-	7	8	-	7	3	-	6	2	-
Unknown (white and grey colony)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9
No growth	-	-	-	-	1	4	-	-	5	-	-	1	-	-	1
Total	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

<sup>a</sup>61-90% surface red at harvest.

**Table 5.** Frequency of isolation of fungi obtained from stem end (S), pericarp (P) and fleshy part (F) of M4 maturity level of lychee fruit dipped in hot benomyl at 500 ppm, 52°C, 2 minutes and then stored at 5°C for 5 weeks.

Fungus	Storage time														
	0 week			2 weeks			3 weeks			4 weeks			5 weeks		
	S	P	F	S	P	F	S	P	F	S	P	F	S	P	F
<i>Cladosporium</i> sp.	-	-	-	1	3	1	-	3	-	2	4	2	2	8	6
<i>Fusarium</i> sp.	5	-	-	3	-	-	5	1	-	8	4	2	2	-	-
<i>Pestalotia</i> sp.	2	2	-	-	-	-	4	-	-	-	-	-	-	-	-
<i>Cladosporium</i> sp. + <i>Fusarium</i> sp.	-	-	-	3	1	-	-	-	-	-	-	4	-	-	-
<i>Cladosporium</i> sp. + <i>Pestalotia</i> sp.	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Unknown (white and grey colony)	-	-	-	-	-	-	-	-	-	-	-	-	5	2	3
No growth	2	6	10	3	6	9	1	6	10	-	2	2	1	-	1
Bacteria	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

<sup>a</sup> 61–90% surface red at harvest.

that when fruit were exposed to water or benomyl at 56°C for 4 minutes, serious damage of the skin occurred and pigment was leached from the fruit. However, in a different treatment, they obtained a contrary result and concluded that after 8 days of storage, a benomyl dip (0.05% at 52°C for 2 minutes) followed by packing the fruit in punnets and overwrapping with PVC film controls rotting, browning and weight loss at temperatures of 20–30°C.

Snowdon (1990) wrote that storage of lychee fruit at 5°C for a month may result in chilling injury. She also concluded that the red colour is best retained at 7 to 10°C. In our case, the cold temperature kept a good skin colour on M4 and M5 fruit with and without dipping in hot benomyl, for at least 14 days. Lychee deteriorate rapidly after harvest. At ambient temperatures of 20–30°C, browning of the pericarp can occur within 24 hours of harvest (Scott et al.

**Table 6.** Frequency of isolation of fungi obtained from stem end (S), pericarp (P) and fleshy part (F) of M5 maturity level<sup>a</sup> of lychee fruit without fungicidal treatment and stored at 5°C for 5 weeks.

Fungus	Storage time														
	0 week			2 weeks			3 weeks			4 weeks			5 weeks		
	S	P	F	S	P	F	S	P	F	S	P	F	S	P	F
<i>Botryodiplodia</i> sp.	-	-	8	-	-	-	-	1	1	-	-	-	-	-	2
<i>Cladosporium</i> sp.	2	2	-	3	1	7	2	2	3	-	2	9	1	1	3
<i>Fusarium</i> sp.	6	1	-	-	-	-	-	-	4	-	-	1	9	2	1
<i>Cladosporium</i> sp. + <i>Fusarium</i> sp.	2	7	-	7	9	-	8	7	-	10	7	-	-	4	-
<i>Pestalotia</i> sp.	-	-	-	-	-	-	-	-	-	-	1	-	-	1	1
Unknown (white and grey colony)	-	-	-	-	-	-	-	-	-	-	-	-	-	2	3
No growth	-	-	2	-	-	3	-	-	2	-	-	-	-	-	-
Total	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

<sup>a</sup> 91–100% surface red at harvest.

**Table 7.** Frequency of isolation of fungi obtained from stem end (S), pericarp (P) and fleshy part (F) of M5 maturity level<sup>a</sup> of lychee fruit dipped in hot benomyl at 500 ppm, 52°C, 2 minutes and then stored at 5°C for 5 weeks.

Fungus	Storage time														
	0 week			2 weeks			3 weeks			4 weeks			5 weeks		
	S	P	F	S	P	F	S	P	F	S	P	F	S	P	F
<i>Botryodiplodia</i> sp.	-	-	-	-	-	-	4	4	-	-	4	-	-	-	-
<i>Cladosporium</i> sp.	2	-	-	3	3	1	-	-	1	2	-	2	5	5	2
<i>Curvularia</i> sp.	-	-	-	-	-	-	-	-	1	-	-	-	-	-	4
<i>Fusarium</i> sp.	-	2	-	5	2	-	6	-	-	4	-	-	-	-	-
<i>Pestalotia</i> sp.	1	-	-	-	-	-	-	-	-	1	-	-	-	-	-
<i>Cladosporium</i> sp. + <i>Fusarium</i> sp. (1)	4	-	-	-	3	-	-	-	-	-	4	4	-	5	-
Unknown (white and grey colony)	-	-	-	-	-	-	-	-	-	-	-	4	4	-	3
No growth	3	8	10	2	2	9	-	6	8	3	2	-	1	-	1
Total	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

<sup>a</sup>91-100% surface red at harvest.

**Table 8.** Frequency of isolation of fungi obtained from stem end (S), pericarp (P) and fleshy part (F) of M3, M4 and M5 maturity levels<sup>a</sup> of lychee fruit without fungicidal treatment and stored at 5°C for 5 weeks. Isolations were done on weeks: 0, 2, 3, 4 and 5.

Fungus	Maturity level								
	M3			M4			M5		
	S	P	F	S	P	F	S	P	F
<i>Botryodiplodia</i> sp.	-	2	-	-	-	-	-	1	11
<i>Cladosporium</i> sp.	9	18	23	10	22	21	8	8	22
<i>Fusarium</i> sp.	-	8	3	8	4	2	15	3	6
<i>Penicillium</i> sp.	-	-	2	-	-	-	-	-	-
<i>Pestalotia</i> sp.	-	3	-	-	-	-	-	2	1
<i>Cladosporium</i> sp. + <i>Fusarium</i> sp.	40	13	1	32	23	7	27	34	-
<i>Fusarium</i> sp. + <i>Penicillium</i> sp.	1	-	-	-	-	-	-	-	-
Unknown (white and grey colony)	-	-	8	-	-	9	-	2	3
No growth	-	-	10	-	1	11	-	-	7
Bacteria	-	6	3	-	-	-	-	-	-
Total	50	50	50	50	50	50	50	50	50

<sup>a</sup>M3, 31-60% surface red (at harvest); M4, 61-90% surface red; M5, 91-100% surface red.

**Table 9.** Frequency of isolation of fungi obtained from stem end (S), pericarp (P) and fleshy part (F) of M3, M4 and M5 maturity levels<sup>a</sup> of lychee fruit dipped in hot benomyl at 500 ppm, 52°C, 2 minutes and then stored at 5°C for 5 weeks.

Fungus	Maturity level								
	M3			M4			M5		
	S	P	F	S	P	F	S	P	F
<i>Botryodiplodia</i> sp.	3	–	4	–	–	–	4	8	–
<i>Cladosporium</i> sp.	10	9	–	5	18	9	12	8	6
<i>Fusarium</i> sp.	10	1	–	23	5	2	15	4	–
<i>Curvularia</i> sp.	–	–	–	–	–	–	–	–	5
<i>Pestalotia</i> sp.	9	3	–	6	2	–	2	–	–
<i>Cladosporium</i> sp. + <i>Fusarium</i> sp.	12	4	–	3	1	4	4	12	4
<i>Cladosporium</i> sp. + <i>Pestalotia</i> sp.	–	–	–	1	–	–	–	–	–
Unknown (white and grey colony)	–	–	–	5	2	3	4	–	7
No growth	6	29	46	7	20	32	9	18	28
Bacteria	–	4	–	–	2	–	–	–	–
Total	50	50	50	50	50	50	50	50	50

<sup>a</sup> M3, 31–60% surface red (at harvest); M4, 61–90% surface red; M5, 91–100% surface red.

1982). The finding of this experiment is that storage at 5°C will keep the lychee fresh for more than two weeks.

Fungal cultures of *Cladosporium* sp., *Fusarium* sp., and *Cladosporium* sp. growing together with *Fusarium* sp. predominated among the fungi obtained on fruit after storage for up to 5 weeks. The finding of this test indicates that the use of hot benomyl treatment could reduce the frequencies of contaminate fungi on fruit during storage. Bunches of fruit could be separated into single fruits, dipped in hot benomyl (0.05% at 52°C for 2 minutes), then packed in punnets and over-wrapped with a suitable plastic film. With this treatment, fruit should be better able to withstand shipment by surface transport to more distant markets.

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# Postharvest Physiology and Storage of Rambutan: a Review

T.J. O'Hare\*

## Abstract

Rambutans (*Nephilium lappaceum* L.) rapidly deteriorate after harvest unless proper handling techniques are employed. Appearance can be maintained if moisture loss (largely from the spinterns) is minimised (95% r.h.) and the fruit are refrigerated (7.5–12.5°C, depending on cultivar). Postharvest pulp quality and pathogen development generally are not problems under refrigeration until after fruit have become visually unacceptable. Under the above storage conditions, rambutans have a shelflife of approximately 10–16 days. Other techniques of extending shelf life have been trialled, including enhanced carbon dioxide atmospheres which can extend shelf life to 18–20 days. Further research into maintaining appearance of rambutans is needed before shelf life can be extended.

THE rambutan can vary in colour from pink to deep crimson, with yellow or yellow–orange varieties also existing (Watson 1988). The skin is covered in hair-like protuberances (spinterns) which, depending on variety, can be coloured similarly to the skin or remain green. The edible portion of the fruit is a fleshy, translucent-white sarcotesta which arises from a paper-like integument surrounding a single oblong seed (Tongumpai 1980). In 'freestone' cultivars, the sarcotesta and integument come freely away from the seed, while in 'clingstone' cultivars they are more difficult to separate (Watson 1988).

Rambutans, unlike lychees, require a tropical climate for cultivation (Popenoe 1920; Almeyda et al. 1979). Although originating in West Malaysia and Sumatra, the rambutan is now grown widely in Southeast Asia, as well as Sri Lanka, Australia, Central America, Equatorial Africa and Malagasy (Popenoe 1920; Mendoza et al. 1972; Watson 1984; Delabarre 1989). The continuing spread of this crop to other countries, together with its increasing popularity in foreign markets has led to an increased research effort into rambutan cultivation and storage. As with many tropical fruit, the rambutan has a short postharvest life and without correct handling will

deteriorate quickly after harvest (Mendoza et al. 1972). This paper reviews the current knowledge concerning the postharvest physiology and storage of rambutan.

## Harvest Maturity

Rambutan is nonclimacteric (Mendoza et al. 1972; Leong 1982) and will not continue to ripen once removed from the tree. Consequently, fruit must be harvested when they have reached a suitable eating quality and visual appearance. Wanichkul and Kosiyachinda (1982) reported that fruit are of acceptable appearance between 16 and 28 days after colour-break when the skin and spinterns are brightest and most evenly coloured. Although the pulp may be acceptable outside this period, the fruit is often unmarketable due to the poor colour of the skin. Overripe fruit also have drier, cloudier and firmer pulp than normal fruit and have been described as 'puffy' due to the development of an air cavity between the pulp and the skin (Kosiyachinda et al. 1987).

Although the rambutan is generally harvested on the basis of its skin colour, flavour should also be at an optimum. Red cultivars do not necessarily reach similar total soluble solids (TSS) at the same intensity of colour (Watson 1988). As the fruit ripens on the tree, TSS increase and titratable acidity (TA) declines (Mendoza et al. 1972; Lee and Leong 1982a; Wanichkul and Kosiyachinda 1982). Consequently, fruit

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harvested too early are acidic and lack sweetness, while fruit harvested too late can be bland. Generally, depending on cultivar, fruit have a TSS and TA concentration in the range of 17–21% and 0.7–5.5 meq/g, respectively, at harvestable maturity (Kosiyachinda et al. 1987).

## Postharvest Storage

Following harvest, the visual appearance of rambutans rapidly declines after 2–3 days if they are left under ambient conditions (Watson et al. 1988). Loss of appearance seems to be largely associated with spintern dehydration (Mendoza et al. 1972; Pantastico et al. 1975; Brown et al. 1985), although colour loss can also occur over the storage period (Lam and Ng 1982; Brown et al. 1985; Mohamed and Othman 1988).

The shelf life of rambutans can be significantly extended by minimising moisture loss and cool-storing fruit. Mendoza et al. (1972) reported that a relative humidity (r.h.) of 95% appeared optimal for storage; higher humidities were conducive to water soaking and decay. Water loss increased with decreasing humidity, although water loss decreased between 75% and 65% r.h., possibly due to the formation of a dry, less permeable epidermal layer. Artificial conditions that approximated a 95% r.h. would be likely to improve fruit storage life.

Mendoza et al. (1972) stored fruit (cvs. Seematjan and Maharlika) in sealed and perforated polyethylene bags at temperatures from 7–41°C. Bags were found to greatly reduce weight loss at all temperatures, although temperatures above 30°C aggravated spintern drying, and spinterns turned brown. Greatest shelf life was reported to be at 10°C, with fruit remaining marketable for 12 days inside sealed bags and 10 days inside perforated bags. Unbagged fruit at this temperature became unmarketable within 4 days. Other researchers have also reported polyethylene bags reduce moisture loss (Lam and Ng 1982; Lee and Leong 1982b; Inpun 1984; Somboon 1984; Brown et al. 1985; Mohamed and Othman 1988).

Artificial skin coatings (e.g. waxes) have generally been reported to be less effective than polyethylene bags in extending storage life of rambutans. Mendoza et al. (1972) reported two waxes to reduce water loss for 2 days (cv. Maharlika) at 27°C, but subsequent transpiration was high. Brown and Wilson (1988) also found two antitranspirants and a wax to have little effect on preventing skin discoloration or water loss at 20°C.

Once moisture loss has been minimised, colour deterioration is the next major storage problem and this can be greatly reduced by refrigeration. Mendoza et al. (1972) reported that 10°C gave the longest shelf life for cv. Maharlika (10–12 days). At higher temper-

atures shelf life was reduced while at lower temperatures (7°C) fruit suffered chilling injury of the skin and spinterns. Harjadi and Tahitoe (1991) also reported chilling injury to cv. Lebakbulus at 7°C. Leong (1982) reported cv. Jit Lee to store best at 10°C, with chilling apparent at lower storage temperatures of 0 and 5°C. Mohamed and Othman (1988), however, stored cv. R7 at 8°C for approximately 13 days (50% unmarketable fruit) without any chilling injury. Similarly, O'Hare and Prasad (1990b) stored cv. R162 at 7.5°C without sign of chilling injury. It is possible that different cultivars may have different tolerances to chilling or may be able to mask symptoms. Inpun (1984) reported chilling injury in cv. Seechompoo after only one day storage at 10°C. With the yellow cultivar R156, chilling symptoms were also quite distinct at 10°C (O'Hare and Prasad, unpublished). By contrast, red cultivars (cvs. Jit Lee and R162) appeared undamaged with only slight darkening of the peel.

Storage atmospheres also have been found to influence the rate of colour loss in rambutans. Fruit (cv. Jit Lee) packaged in sealed polyethylene bags at 10°C have been reported to have increased shelf life (Lee and Leong 1982b). This was possibly due to an enhancement in carbon dioxide (7.5–9.2% CO<sub>2</sub>) generated in the bags during the last week of storage. Mohamed and Othman (1988) investigated the effect of high initial carbon dioxide concentrations on fruit (cv. R7) stored at 8°C. Skin colour was best maintained for fruit stored under 7% CO<sub>2</sub>, with a shelf life of approximately 20 days (50% marketable fruit). Recent study (O'Hare and Prasad 1990a) found that reduced oxygen concentration (3% O<sub>2</sub>) had no significant effect on colour loss of cv. R162, while enhanced carbon dioxide concentrations (9–12% CO<sub>2</sub>) were capable of extending shelf life to approximately 18 days at 10°C. This would suggest that under most modified atmospheres (i.e. generated by fruit in plastic bags) enhanced carbon dioxide, rather than reduced oxygen concentration, is the causal factor in maintaining skin colour.

Various chemical treatments have been applied to rambutans in an effort to extend storage life, but attempts have generally been unsuccessful. Leong (1982) reported that dipping fruit in calcium chloride (CaCl<sub>2</sub>) failed to extend storage life, with high concentrations (above 4%) actually causing skin browning. Ratanachinakorn and Nanthachai (1991) have confirmed these findings and have also reported vacuum infiltration with CaCl<sub>2</sub> to be equally ineffective. Mohamed et al. (1988) reported that CaCl<sub>2</sub> mixed with sodium metabisulfite slightly extended the storage life of fruit held at 8°C, but not at ambient temperature. O'Hare and Prasad (1991b), however, found sodium metabisulfite effective in maintaining spintern colour, but it caused serious skin injury to the body of the fruit.



Ethylene absorbents ( $\text{KMnO}_4$ ) also have been reported to be ineffective in extending shelf life (Sabari and Muhadjer 1982). This is in agreement with trials by O'Hare and Prasad (1991a) who further observed that constant exposure to ethylene (5 ppm) was not detrimental to fruit held at 10°C.

## Postharvest Physiology

Rambutans do not have an upsurge in respiration typical of climacteric fruit. Mendoza et al. (1972) observed a gradual decline in respiration of fruit (cv. Maharlika) stored at 27°C for 4 days. Various researchers (Kosiyachinda, cited in Lam and Kosiyachinda 1987; Agravante and Lizada, cited in Lam and Kosiyachinda 1987), however, have found respiration rate (cvs. Rongrien, Seechompoo and Seematjan) to increase during storage, although this was associated with desiccation and browning of the peel.

Rambutans also have been reported to produce low levels of ethylene after harvest (0.14–2.6  $\mu\text{L/kg/hour}$ ), although this has been associated also with senescent activity rather than ripening (Kosiyachinda, cited in Lam and Kosiyachinda 1987; Agravante and Lizada, cited in Lam and Kosiyachinda 1987). Ethylene also has been recorded after extended storage under refrigeration (5–13°C) in association with pathogen development (O'Hare, unpublished data).

Moisture loss from rambutan fruit occurs largely through the spinterns, which have a stomate density approximately five times greater than the main fruit body (Pantastico et al. 1975). When spinterns are intact, moisture loss is compensated by water translocated from the fruit body. In stored fruit, spinterns have the greatest weight loss, followed by the skin and then the pulp (Prabawati and Laksmi 1983, cited in Lam et al. 1987). Nathiwatthana (1981) further reported that weight loss of stored fruit (cv. Seechompoo) is directly proportional to the number of spinterns on the fruit.

Although skin colour deterioration appears to be one of the major problems with storage, the underlying biochemistry is not well documented. Tangtirit (1982) reported that while fruit are on the tree the concentration of chlorophyll in the peel declines gradually until approximately 13 days after colour break. No study appears to have been made of anthocyanin during fruit development.

Once harvested, skin colour declines and appears to be due to skin browning (Agravante and Lizada, cited in Lam and Kosiyachinda 1987; Lam et al. 1987) and a reduction in anthocyanin content (Paull and Chen 1987). Further studies (O'Hare et al., unpublished data) have indicated that during chilling, anthocyanin decolorises but is not broken down until after an extended period of storage. Browning

appears to be independent of anthocyanin decoloration and occurs in the epidermal region. Brown et al. (1985) reported that skin browning could be minimised by reducing moisture loss of fruit, but that some other factor reduced skin appearance over time. It is possible that this factor may be related to anthocyanin degradation.

The principal sugars present in rambutan (in order of decreasing concentration) are sucrose, glucose and fructose (Popenoe 1920; Wills et al. 1986; Paull and Chen 1987). Changes in pulp sugar levels after harvest have been reported in the literature, although the reports are conflicting. Mendoza et al. (1972) reported cv. Seematjan to increase in total soluble solids (TSS) and decline in starch. Slight increases in TSS have also been reported for cvs. Seechompoo and Rongrien (Somboon 1984) and cv. Lebakbulus (Harjadi and Tahitoe 1991). By contrast, Inpun (1984) and Paull and Chen (1987) recorded a decline in TSS with storage. Again, Agravante and Lizada (cited in Lam and Kosiyachinda 1987) and Nanthachai et al. (1991) reported no significant change in TSS during storage. Whether these differences in observation may be related to cultivar or storage conditions is uncertain.

Titrateable acidity (TA) of the pulp has been reported to increase slightly with storage (Mendoza et al. 1972) although no significant changes have been reported (Agravante and Lizada, cited in Lam and Kosiyachinda 1987; Nanthachai et al. 1991). Paull and Chen (1987) reported an initial decline in TA followed by a subsequent increase to the original value. This appeared to be associated with a rapid decline in succinic acid and a gradual increase in citric acid. As with TSS, whether the differences are cultivar or trial related is uncertain. Where changes in TA and TSS occur, however, they appear to be very slight in comparison to climacteric fruit.

Few studies appear to have been made of the textural changes that occur in rambutans. Ahamad and Said (1983) observed pectin methylesterase and polygalacturonase activity to increase with increasing fruit maturity. Paull and Chen (1987) also reported breakdown of rambutan pulp after 11 days and 20 days storage at 22°C and 12°C, respectively, with the pulp having a water-soaked appearance.

## Physiological Disorders

Occasionally, premature seed germination is observed inside mature but nonsenescent fruit. This is usually associated with subsequent pulp softening and flavour loss (Kosiyachinda and Salma 1987). The cause of this phenomenon is unknown and its incidence is rare.

Skin splitting can also occur with thin-skinned cultivars (e.g. cv. Rongrien). Heavy rains or sudden

uptake of water during the last stages of fruit development can cause the pulp to expand at a faster rate than the skin, resulting in skin rupture (Lam and Tongumpai 1987).

Lam and Tongumpai have attributed poor filling of fruit at maturity as being due to low adaptability of fruit to dry conditions. Cultivars such as cvs. R4 and R9 appear to be more susceptible to this problem, whereas cvs. R3, R134, R156, R161 and R170 remain well-filled due to a reduction in fruit size. Poor filling of fruit has also been attributed to poor nutrition (Marshall 1988).

## Postharvest Pathology

*Botryodiplodia theobromae*, *Gliocephalotrichum bulbilium* and *Colletotrichum* sp. have been identified as the three major causes of postharvest disease in Thailand (Chayasombat and Sangchote 1983; Visarathanonth and Ilag 1987). Infection may occur either in the field or on lesions caused during harvesting or handling. *B. theobromae* infection usually occurs through the cut stem-end and results in stem-end rot of the fruit. A wound also appears to be necessary for rapid penetration and infection by *G. bulbilium*, although both *G. bulbilium* and *Colletotrichum* sp. can occur as latent, subcuticular field infections which develop during fruit senescence. *Phytophthora* sp. can also cause disease symptoms, but postharvest infection by this pathogen is uncommon. Farungsang et al. (1991) have also isolated *Pestalotopsis* sp., *Phomopsis* sp., *Colletotrichum gloeosporioides* and *Glomerella* sp. as causes of postharvest rots in Thailand. Growth of *Pestalotopsis* sp. was observed to be greater at 25°C than at 13°C, while growth of *Phomopsis* sp. was less.

In Australia, *Colletotrichum* sp., *Dothiorella dominicana*, *Fusarium* sp., *Penicillium* sp., *Pestalotopsis* sp., *Phoma* sp., and *Phomopsis* sp. have been isolated from fruit stored at 2.5–12.5°C (Johnson et al. 1989; O'Hare et al., unpublished). Preliminary trials also indicate that *Dothiorella* sp. growth is suppressed by low temperature. *G. bulbilium* has also been identified as a rambutan pathogen in the Philippines (Pordesimo and Luna-Ilag 1982). In Malaysia, Lam (1982) isolated *Candida* sp. (fruit stored at 20°C) as being the cause of pulp fermentation.

## Postharvest Entomology

Osman and Chettanachitara (1987) have listed seven insect pests of major postharvest importance in Southeast Asia: leaf minor (*Acrocercops cramerella*), armoured scale (*Phenacaspis* sp.), citrus mealybug (*Planococcus citri*), yellow peach moth (*Conogethes* [*Syn. Dichocrocis*] *punctiferalis*), oriental fruit fly (*Bactrocera* [*Syn. Dacus*] *dorsalis*) and driedfruit

beetles (*Carpophilus dimidiatus* and *C. marginellus*). All of these produce visible external damage, except *A. cramerella* whose larvae normally burrows into the fruit near the peduncle. Citrus mealybug is also commonly associated with sooty mould (*Meliola nephili*) which develops on its excretory products. Driedfruit beetles are reported to be a secondary pest as they usually enter through holes made by other insects. Watson (1984) claims that fruit flies (including *B. dorsalis*) are not a problem unless overripe fruit are left on the tree.

Watson (1988) has described banana spotting bug (*Amblypelta lutescens lutescens*), mealy bugs and mites as contributing to skin deterioration and discoloration in Australia. Preliminary observations have also indicated fruit piercing moth to be a problem with thin-skinned cultivars (e.g. cv. Rongrien).

## Conclusion

Rambutans must be harvested when they are at their visual and organoleptic optimum, as little improvement occurs once the fruit is removed from the tree. As the fruit gradually deteriorate with time, storage regimes that maintain the quality closest to that at harvest have been sought.

The major postharvest problem of rambutan is the rapid loss and deterioration of visual appearance. This can be partially alleviated by storing fruit under conditions which minimise moisture loss (e.g. 95% r.h.), but although this will greatly reduce desiccation and subsequent skin browning, it is insufficient on its own and must be accompanied by refrigeration. Depending on cultivar, temperatures between 7.5°C and 12.5°C have been found to extend storage life without inducing chilling injury. Under these conditions, rambutans currently have a marketable shelf life of approximately 10–16 days.

Other methods of prolonging shelf life have been investigated with varying success (e.g. CaCl<sub>2</sub>, CO<sub>2</sub>). Enhanced carbon dioxide atmospheres have been shown to increase storage life to approximately 18–20 days. The shelf life of rambutans is short, however, compared to temperate crops (such as apples) and they are much less robust under ambient conditions. If rambutan quality is to be maintained, fruit must be stored correctly. Shelf life is definitely associated with visual appearance, and future research should be directed into this area.

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# The Susceptibility of Thai and Australian Mango Cultivars to Sap Injury and Possible Means of Control

T.J. O'Hare\*

## Abstract

Thai mango cultivars (Nam Dok Mai, Nang Klang Wun, Tong Dum and Kaew Sawoey) were less susceptible to sap injury than 'Kensington' (an Australian cultivar). The degree of sap injury was regulated by several factors including total sap flow, oil content in sap, and lenticel distribution. Sap flow of Thai cultivars was considerably lower (0.17–0.47 mL/fruit) than 'Kensington' (1.67 mL/fruit). The proportion of oil in the initial sap fraction collected from Thai cultivars (2.2–15.8% oil) was also substantially lower than 'Kensington' (52% oil). Damage occurred where sap flowed over lenticels, and consequently fruit with higher lenticel densities were more prone to injury. Destemming fruit in a 1% Cold Power<sup>®</sup> (detergent) solution reduced sap injury to fruit, but was not as effective as 1% calcium hydroxide solution. Cold Power<sup>®</sup> appeared to increase the wettability of fruit, allowing penetration of sap into cuticle cracks, creating a finer, more evenly spread injury. Dipping fruit in Cold Power<sup>®</sup> immediately prior to destemming fruit in air was partially effective in reducing sap injury and may have acted as a physical barrier to sap entry.

MANGO skin injury caused by exuding sap is an important economic problem within the Australian mango industry (Brown et al. 1986). Damage is normally associated with the cultivar 'Kensington' which comprises more than 90% of total mango production. Mango sap removed from fruit will separate into two distinct fractions on standing: an oil and a protein-polysaccharide fraction, the former being responsible for skin injury (O'Hare and Prasad 1993). Injury occurs wherever the oil fraction makes contact with and enters the mango skin, which is normally via the lenticels.

The susceptibility of Thai cultivars to sap injury is unknown, although preliminary observations tend to indicate that damage is less severe than in cv. Kensington. Earlier studies have shown that sap injury in cv. Kensington can be largely negated by desapping fruit in alkaline solution (O'Hare and Prasad 1993), however, field studies indicate that commercial detergents also may be effective. The aim of this investigation was to determine how susceptible several important Thai cultivars are to sap injury, and to evaluate the use of detergent as a means of sap injury control.

## Materials and Methods

### Susceptibility of Thai cultivars

Four important Thai cultivars (cvs. Nam Dok Mai, Nang Klang Wun, Tong Dum and Kaew Sawoey) and one Australian cultivar (cv. Kensington) were evaluated for their susceptibility to sap injury following destemming. All cultivars except cv. Kaew Sawoey are normally eaten as ripe fruit. Fruit were sampled at commercial maturities (i.e. Thai cultivars — 20% dry matter; cv. Kensington — 14% dry matter).

Twenty fruit of each cultivar were harvested with stems attached and immediately taken to the laboratory. Ten fruit from each cultivar were destemmed and the sap collected over four time intervals (0–10, 10–30, 30–90, 90–300 seconds). Sap was collected into vials and allowed to settle into oil and protein fractions. After 3 hours, the volume of each fraction was recorded.

The remaining ten fruit from each cultivar were destemmed and placed pedicel upwards so that all sap exuded ran down the surface of the fruit. After 48 hours, fruit were measured for sap injury (nil/slight/moderate/severe) and lenticel distribution.

### Sap injury control

A commercial detergent (Cold Power<sup>®</sup>) was tested for its efficacy in controlling sap injury in cv. Kensington. A field technique of harvesting mangoes by

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pulling fruit directly off the tree and plunging into a Cold Power<sup>®</sup> dip is currently being used by some orchardists. Field concentrations of Cold Power<sup>®</sup> have been reported as 1% (10 g/L) and were duplicated in this trial.

Fruit were harvested with stems attached and taken directly to the laboratory. The efficacy of Cold Power<sup>®</sup> was tested by destemming fruit and allowing the sap to flow down the fruit surface for increasing increments of time (0–300 seconds). At the end of each increment, fruit (10 fruit per treatment) were then plunged into the Cold Power<sup>®</sup> solution for 5 minutes. Fruit were then removed and assessed visually for sap injury (1 = no damage; 5 = severe damage) after 48 hours.

The efficacy of Cold Power<sup>®</sup> was further tested against lime (1% Ca(OH)<sub>2</sub>) which has been previously reported to be effective in controlling sap injury when fruit were destemmed beneath this solution (O'Hare and Prasad 1993). Fruit were destemmed under 1% lime, 1% Cold Power<sup>®</sup>, or 1% lime + 1% Cold Power<sup>®</sup> for 5 minutes, after which fruit were removed and allowed to dry. Ten fruit were also destemmed in air as a control. An additional treatment of dipping fruit in Cold Power<sup>®</sup> for 5 seconds and then destemming was also tested. This method has been recommended by field staff as a possible means of coating the fruit to protect it from injury. After 48 hours, all fruit were assessed for sap injury.

## Results

### Susceptibility of Thai cultivars

All Thai cultivars suffered considerably less sap injury than cv. Kensington (Table 1). Of these cultivars, Tong Dum was the most damaged, although this was very mild in comparison with cv. Kensington. Sap injury occurred at lenticels and was similar to large, dark pin-pricks (< 1 mm diameter). Symptoms were similar with cv. Nang Klang Wun, although these were smaller. No injury was visible with cvs. Nam Dok Mai and Kaew Sawoey, apart from some slight damage to a single fruit of the latter.

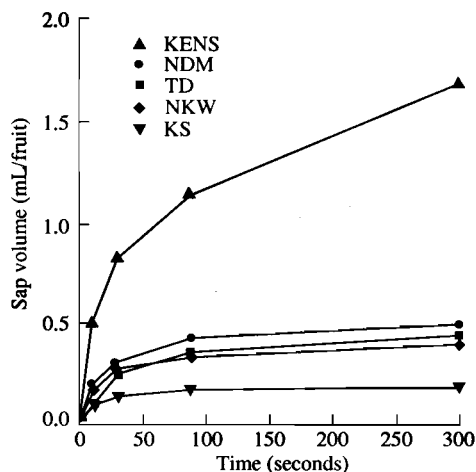
Sap volumes exuded from the Thai cultivars varied from 0.16 to 0.48 mL/fruit with at least 90% of sap exuding over the first 90 seconds (Fig. 1). Cv. Kaew Sawoey, which is normally eaten green, had the lowest sap flow, while cv. Kensington had a very high flow (1.67 mL/fruit) and actually squirted after destemming. Although fruit size differed between cultivars, the ratio of sap exuded to the weight of the fruit followed a similar trend to total sap volume per fruit (Table 1).

The oil content of sap exuded in the first ten seconds was greatest in cv. Kensington, and considerably lower in all Thai cultivars (Table 1). Of these, cv. Tong Dum had the highest oil content (15.8%), and cv. Nam Dok Mai the lowest (2.2%). The degree of

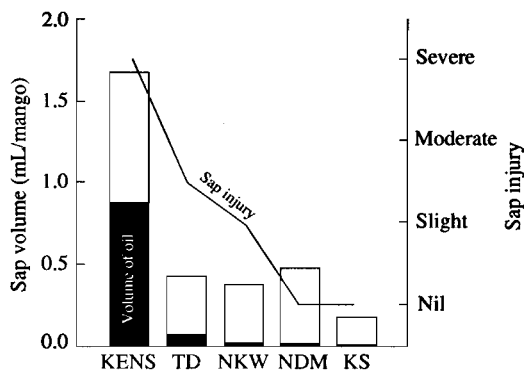
sap injury sustained was closely related to the oil content of the sap (Fig. 2). Lenticel distribution also varied amongst cultivars (Table 1) and appeared to modify the degree of sap injury as damage was localised at these areas.

### Sap injury control

The level of sap injury of fruit destemmed and then plunged into Cold Power<sup>®</sup> increased rapidly with the amount of time that sap was allowed to flow onto the



**Figure 1.** Sap exudation from mango cultivars during 0–5 minutes after destemming (KENS = Kensington; NDM = Nam Dok Mai; NKW = Nan Klang Wun; TD = Tong Dum; KS = Kaew Sawoey).



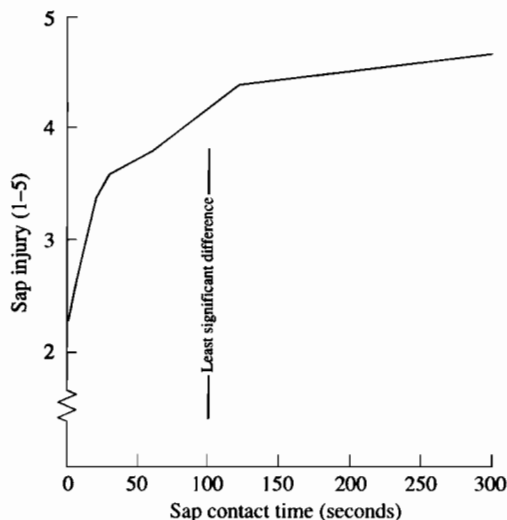
**Figure 2.** Sap volume (first 10 seconds) and skin injury of different cultivars of mango. Skin injury was closely related to the volume of oil (shown in black) in the sap (KENS = Kensington; NDM = Nam Dok Mai; NKW = Nan Klang Wun; TD = Tong Dum; KS = Kaew Sawoey).

fruit surface (Fig. 3). Two types of sap injury were observed. The first was similar to a spray and consisted of numerous red-brown spots ( $< 0.1$  mm). This was visible on all treatments (including fruit destemmed under solution), but decreased with increasing time increment. The second type of injury occurred where sap had run over the surface of the fruit and had the appearance of a drip stain. This injury was similar to that observed on fruit destemmed in air, however, browning was not confined to lenticels but covered the area between them. Consequently, injury was accentuated over 'normal' sap injury. In contrast to spray damage, this form of injury increased with time increment.

Destemming fruit under a 1% Cold Power<sup>®</sup> solution was not as effective as 1% lime, although it did reduce the incidence of injury in comparison to control fruit (Fig. 4). Combining Cold Power<sup>®</sup> with lime did not have any benefit over lime alone. Dipping fruit in Cold Power<sup>®</sup> for 5 seconds followed by destemming reduced sap injury (injury = 2.6), although this effect was only minor in comparison to control fruit (injury = 3.6).

## Discussion

Mango sap injury varied between cultivars, although the problem was considerably more severe with cv. Kensington. Only slight to moderate damage was observed in cv. Tong Dum and slight damage in cv.

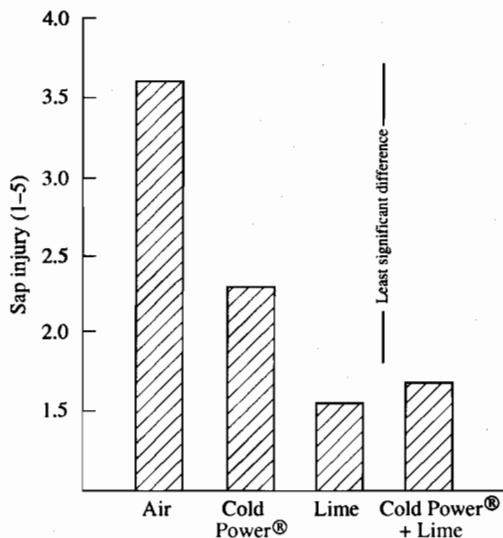


**Figure 3.** Sap injury of cv. Kensington following different sap contact times between stem removal and immersion into 1% Cold Power<sup>®</sup> for five minutes. Fruit were assessed for damage after 48 hours. Least significant difference is indicated by a vertical bar.

Nang Klang Wun (no damage in cvs. Nam Dok Mai or Kaew Sawoey). The degree of sap injury observed was related to at least three factors: total sap flow, oil content and lenticel density. In the present study, all Thai cultivars had a much lower sap flow and oil content than cv. Kensington and a much lower incidence of injury. Of the Thai cultivars, cv. Tong Dum had the greatest oil content which was consistent with the amount of injury observed. Lenticel density also appeared to have a modifying effect on injury, as damage only occurred where sap flowed over a lenticel. This was consistent with previous observations with cv. Kensington (O'Hare and Prasad 1993).

It is uncertain whether the chemical consistency of the oil fraction may have influenced the degree of sap injury in this trial, although it is possible that the oil fraction in the Thai cultivars may not be as injurious to skin as in cv. Kensington. Recent study (Loveys et al. 1993) has provided evidence that different terpenoids may cause varying amounts of damage to mango skin and that the chemical consistency of sap can vary between cultivars. In the present trial, the oil fraction was not separately applied to fruit, and consequently its potency was not assessed.

The use of Cold Power<sup>®</sup> as a means of reducing sap injury appeared doubtful from the results obtained in the present trial. Sap injury appeared in two forms (spray and drip) and was different from that observed previously (O'Hare and Prasad 1993). Spray damage appeared to be caused by sap exuded



**Figure 4.** Sap injury of cv. Kensington after destemming under 1% Cold Power<sup>®</sup> and 1% lime solutions for 5 minutes. Fruit were assessed for damage after 48 hours. Least significant difference is indicated by a vertical bar.

**Table 1.** Mango cultivar specifications and sap injury characteristics. Standard errors for dry matter and fruit weight are shown in parentheses (KENS = Kensington; NDM = Nam Dok Mai; NKW = Nan Klang Wun; TD = Tong Dum; KS = Kaew Sawoey).

	KENS	NDM	NKW	TD	KS
Dry matter (%)	14.9 (0.4)	21.2 (0.3)	20.6 (0.2)	20.3 (0.5)	20.8 (0.5)
Fruit wt (g)	436 (7)	351 (14)	288 (10)	323 (14)	232 (11)
Sap injury	Severe	Nil	Slight	Slight/moderate	Nil
Total sap (mL/mango)	1.67	0.47	0.38	0.42	0.17
Oil fraction (%)	52	2.2	4.3	15.8	4.2
Lentical density	Moderate	Moderate	Low	High	High
Sap/wt ratio (mL/g)	0.0038	0.0013	0.0013	0.0013	0.0007

directly into the dip solution which subsequently made contact with other mango fruit. This can also occur when desapping fruit in water, although the sap tends to remain in larger droplets and cause spotting (1–2 mm) only at lenticels (O'Hare and Prasad 1993). In this regard, it is possible that Cold Power<sup>®</sup> may be acting as a wetting agent on the fruit surface, allowing sap to gain entry into smaller skin openings (e.g. cracks in cuticle). Spray injury tended to lessen with time and this may have been directly related to the reduction in sap exudation over time. As less sap would have been flowing at the time of immersion, less sap would have been available to enter into the solution.

In contrast to spray damage, drip damage increased with time, and appeared to be directly related to the amount of sap flowing over the skin after destemming. This injury was not alleviated by dipping fruit in Cold Power<sup>®</sup>, but appeared to be accentuated due to browning between lenticels. Again, this may have been due to the wetting activity of Cold Power<sup>®</sup>. In this case, the browning was more intense than a spray, and covered the entire drip surface. This difference in intensity was probably related to a higher oil concentration in contact with the skin.

Desapping fruit under a solution of Cold Power<sup>®</sup> was not as effective as using lime, although it was better than desapping in air alone (Fig. 3). Lime provided good control, although small spots at lenticels were occasionally observed. The combination of Cold Power<sup>®</sup> with lime offered no improvement over lime alone and is not recommended.

The practise of dipping fruit quickly in Cold Power<sup>®</sup> before desapping was partially effective in reducing sap injury, although whether it should be recommended as a means of injury control on its own is questionable. How Cold Power<sup>®</sup> reduced injury is uncertain, although it is possible that it provided a physical coating that prevented entry of at least some

sap. It is suggested that a further modification (such as destemming fruit upside down after dipping) should be included before using this technique.

## Conclusion

The low incidence of sapburn in Thai cultivars, together with the tradition of marketing fruit with stems attached, would tend to preclude further research into minimising sap injury with Thai cultivars. Sap injury was largely confined to the Australian cultivar 'Kensington' to which the use of destemming techniques is most pertinent.

The use of Cold Power<sup>®</sup> as an agent for reducing sap injury is questionable at the present, as other forms of damage (e.g. spray) can be induced. In the field, fruit are broken off the tree and dropped onto a plastic sheet with Cold Power<sup>®</sup> running down and into a bath. From the present trial, some damage would be expected if sap dripped onto the fruit before it reached the sheet, and also in the bath if fruit were still exuding sap at this stage. Possibly, once the fruit are coated in Cold Power<sup>®</sup>, some protection from sap injury would be achieved. Certainly, this means of destemming should be tested before commercialisation.

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# Storage and Ripening Temperatures for Kensington Mangoes

R.L. McLauchlan\* and I.A. Wells†

## Abstract

Kensington mangoes were stored at 3, 7, 10 or 13°C for 0, 1, 2 or 3 weeks and ripened at 18, 22, 26 or 30°C. Fruit stored at 13°C coloured significantly during the first week of storage; the 10°C fruit coloured slowly in store; colour of the 7°C fruit remained static; and that of the 3°C fruit was severely affected after 2 weeks storage. Ripening temperatures had no effect on skin colour once it was temperature-affected. External chilling injuries (CI) were not significant in fruit stored at 7 to 13°C. After 2 weeks at 3°C, mangoes showed minor CI which increased in severity on ripening. After 3 weeks at 3°C, fruit were severely injured before and after ripening. Acidity levels in ripe fruit were increased by decreasing both storage and ripening temperatures. Eating quality of 13°C fruit was unacceptable after 1 weeks storage, indicating that fruit approaching its climacteric should be removed from store to a more appropriate ripening temperature. The 30°C ripening temperature was unsuitable for fruit stored for 1 week, while 26°C was also unsuitable after 3 weeks storage.

COOL storage is routinely used to extend the marketing life of many fruits and vegetables. As production increases, the desire for longer storage periods also increases, and recommended storage temperatures are reduced to their lowest limits. While some fruits (e.g. apples, pears, grapes) may be stored for several months at quite low (0–1°C) temperatures, tropical fruits are generally sensitive to low temperature storage and may suffer chilling injury (CI), even at moderate storage temperatures (10–13°C). Refrigerated storage under atmospheric conditions is limited by the sensitivity of a given commodity, usually in a time–temperature relationship.

Sadasivam et al. (1971) report that Bangalora and Neelam mangoes developed chilling injury (CI) after 15 days at 6 or 9°C. The injury increased after removal to 24°C, with fruit failing to ripen. CI was not affected by polyethylene overwrap or 4–10% wax dips. Saucedo Veloz et al. (1977) stored Kent mangoes at 8, 10 and 13°C for 10–22 days followed by ripening at 25°C. CI was evident at all temperatures and increased on ripening, with inhibited sugar, carotenoid and flavour development.

Alphonso mangoes stored at 10°C for 30 days developed CI as pitting (Thomas and Oke 1983). After ripening, fruit had poor flavour and carotenoid development. Cold adaption of 1 day each at 20, 15 and 10°C gave longer storage life and better out-turn quality. Nonstored, ripe fruit exhibited skin bronzing when subsequently stored for 3–4 days at 7–10°C. Storage at 10°C followed by ripening at 27–32°C and storage for 3 weeks at 4–7°C produced no CI. Thomas and Joshi (1988) report that Alphonso mangoes ripened at 27–34°C developed CI when subsequently stored at 5, 10 or 15°C. Fruit ripened at 20°C showed no CI after 14 days at 5 or 10°C. Unripe fruit stored for 30 days at 10°C then ripened at 27–34°C could also be cool stored for 7 days with no CI.

In comparing Sensation and Samar Bahisht mangoes stored for 0–16 days at 6–12°C and ripened at 25–28°C, Farooqi et al. (1985) found no CI from 4 days storage at any temperature. After 4 days, there was a rapid increase in CI at temperatures below 12°C, with Sensation being the more sensitive.

Chaplin et al. (1986a) determined that CI in Kensington mangoes stored for 18 days at 1–5°C could be reduced by regular warming (1 day at 20°C) and initial stepwise temperature reduction. Kensington, Zill, Carrie and Common mangoes stored at 1°C in air or polyethylene bags (PE: 0.04 mm) showed no CI after 3 days. After 5 days, CI developed, with Kensington and Common being more sensitive than Carrie and Zill (Chaplin et al. 1986b). CI was less severe in PE-stored fruit and did not develop until 7 days. Chaplin et al. (1991a) report that for Kensington

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mangoes, external appearance, flesh colour, aroma, sweetness and general acceptability all declined to unacceptable levels in fruit stored for more than 2–3 weeks at 5°C, and for as little as 1 week at 1°C. All fruit stored at 1 and 5°C showed CI on ripening, with increases in acidity. In a further study on Kensington mangoes, Chaplin et al. (1991b) reported CI after 1 week at 1–5°C becoming worse after ripening at 20°C and increasing with storage time. No CI was evident after 3 weeks at 10–15°C, but the lower temperature produced higher acid levels.

Smillie et al. (1987) correlated severe CI with a complete loss of chlorophyll fluorescence in Kensington mangoes stored at 0°C for 4 weeks then at 23°C for 4 days. Using fluorometry, they categorised several varieties for resistance to CI. Indian cultivars were less chill tolerant than Florida or Australian cultivars. Zauberman et al. (1988) related CI in Keitt mangoes stored at 5°C for 14 days to peroxidase and cellulase activity.

Medlicott et al. (1990) stored Amelie, Tommy Atkins and Keitt mangoes for 21 days at 8–12°C. Immature fruit performed better in storage, but subsequently failed to develop full ripe character. More mature fruit underwent less ripening at 8 and 10°C, but CI and inhibited ripening were evident at 8°C. A similar result was obtained with early-harvested fruit stored at 10°C. Fruit stored at low temperatures had less flavour after ripening compared to nonstored ripe fruit.

CI in mangoes is characterised by abnormal ripening processes; dark brown discoloration and pitting of the peel; uneven flesh softening; atypical aroma, flavour and carotenoid development; high acid retention; and increased susceptibility to decay. The injury is generally proportional to storage time and inversely to temperature. The critical time–temperature required for CI development can vary with cultivar, maturity and ripeness. This present study was aimed at determining how long Kensington mangoes could be stored at temperatures below the currently recommended 13°C (Hubbert and Ledger 1988) and if any attendant CI could be alleviated or reduced by removal from a given storage to an appropriate ripening temperature.

## Methods

First grade mangoes (48 trays, 340–400 g) were obtained from a commercial orchard in Childers (south Queensland). Fruit were harvested in mid-January 1990, treated with an approved fungicide, size graded, packed into trays and forwarded to Hamilton by road. Maturity was assessed by dry matter content on 24 mangoes. Fruit were randomly allocated to 64 samples of 12 mangoes.

Each fruit was labelled and its colour was measured (Hunter L, a, b: HunterLab Labscan 6000). External appearance based on surface marks/injuries was assessed on a 1 (severe blemish or chilling injury) to 9 (clean skin, nil injury) scale. Fruit were then stored at 3, 7, 10 or 13°C for 0, 1, 2 or 3 weeks. After storage, four trays were removed from each storage temperature and fruit colour and appearance were again measured. Fruit were held overnight under ethylene at 22°C to initiate ripening except where significant softening had occurred during storage (e.g. after 2 weeks at 13°C). Fruit were then transferred to 18, 22, 26 or 30°C rooms until fully ripe.

External ripe colour was assessed by three staff on a 1 (full green, unripe) to 9 (typical full yellow ripe colour) scale and Hunter L, a, b were again measured. Fruit were submitted to a taste panel for assessment of eating quality (EQ) on a 1 (dislike) to 9 (like) scale, and analysed for soluble solids (Abbe refractometer) and titratable acidity (MetrOhm autotitrator).

All data were subjected to factorial analyses of variance (anova). Where the anova returned a significant F test, least significant differences were calculated for pairwise comparison of treatment means (protected least squares test).

## Results

Mangoes averaged 15.6% dry matter, well above the current market standard of 14.0%. Ripening temperature significantly affected Hunter L and a values, acidity and colour of nonstored ripened mangoes (0 weeks storage). Other parameters (Hunter b, appearance, soluble solids and eating quality) were unaffected. At out-turn from storage after 1, 2 or 3 weeks, storage temperature and time significantly affected L, a, b and appearance of all fruit. After ripening, these parameters, as well as acidity and ripe colour, were significantly affected by both storage and ripening temperatures. Eating quality was affected by storage temperature only after 3 weeks storage. The more interesting results are presented in Figures 1 and 2.

Mangoes stored at 13°C coloured significantly during the first week of storage with out-turn a and b values typical of ripe fruit (Fig. 1). Fruit stored at 10°C also exhibited a and b data increasing with time but to a lesser extent. Colour of mangoes stored at 7°C remained relatively static during storage, while that of the 3°C fruit decreased in b (yellowness) indicating potential CI. This was confirmed when ripening of this fruit failed to produce typical yellow–red colours, irrespective of ripening temperature (Fig. 1).

Ripe colour ratings were generally commensurate with the Hunter a, b data (Fig. 2). Mangoes stored at 13°C produced typical ripe colours throughout. Ripe

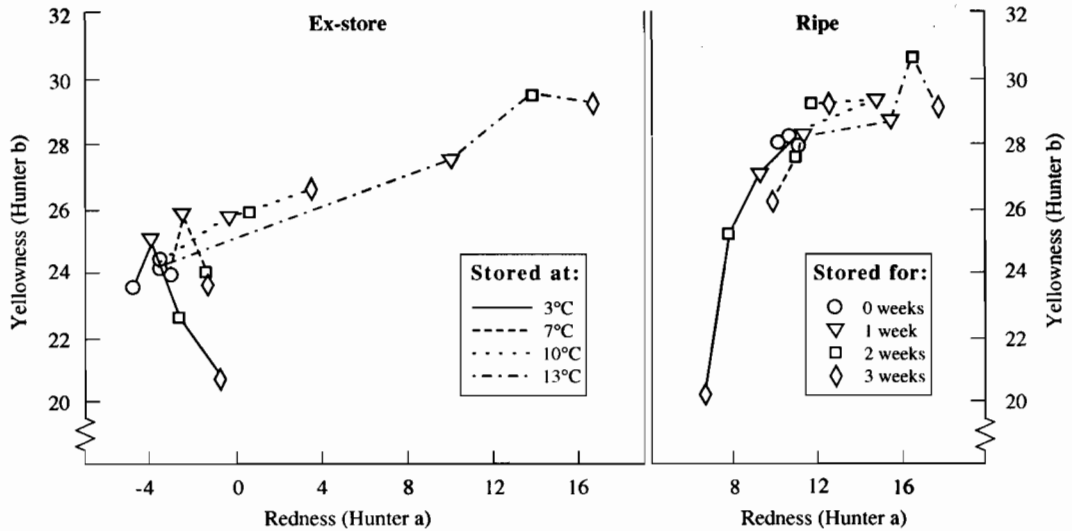


Figure 1. Effect of storage temperature and time on the skin colour of mangoes.

colour of 7 and 10°C fruit remained acceptable, although the 7°C ratings began to decline after 2 weeks storage. Fruit stored at 3°C had the poorest ripe colour at all removals. A ripening temperature of 22°C produced consistently good ripe colour.

All fruit stored at 7–13°C out-turned with good external appearance even after 3 weeks storage (Fig. 2). Appearance ratings of fruit stored at 3°C began to decline after 2 weeks and were significantly reduced after 3 weeks. Ripening tended to increase the level of surface marks and blemishes, generally reducing appearance ratings by 0.5 units for mangoes stored at 7–10°C, but by up to 2 units for fruit stored at 3°C for 2 weeks or longer. Ripening temperature significantly affected appearance ratings after 2 weeks storage with the higher temperatures (26 and 30°C) producing lower ratings.

Acidity levels in ripe fruit were inversely related to storage temperature (Fig. 2). The ability of Kensington mangoes to metabolise acid during ripening appears to be significantly reduced in a time-temperature relationship, i.e. by a combination of increasing storage time and decreasing storage temperature. The higher ripening temperatures produced lower acid levels after ripening. Fruit ripened at 18°C was consistently higher in acidity so that 18°C may be a little too low for ripening cool-stored fruit.

Eating quality of ripe fruit was somewhat variable. It was only after 3 weeks at 13°C that an effect of storage temperature became significant even though the 13°C fruit scored the lowest eating quality throughout storage. Differences between 3, 7 and 10°C storage temperatures were only minor. After 1

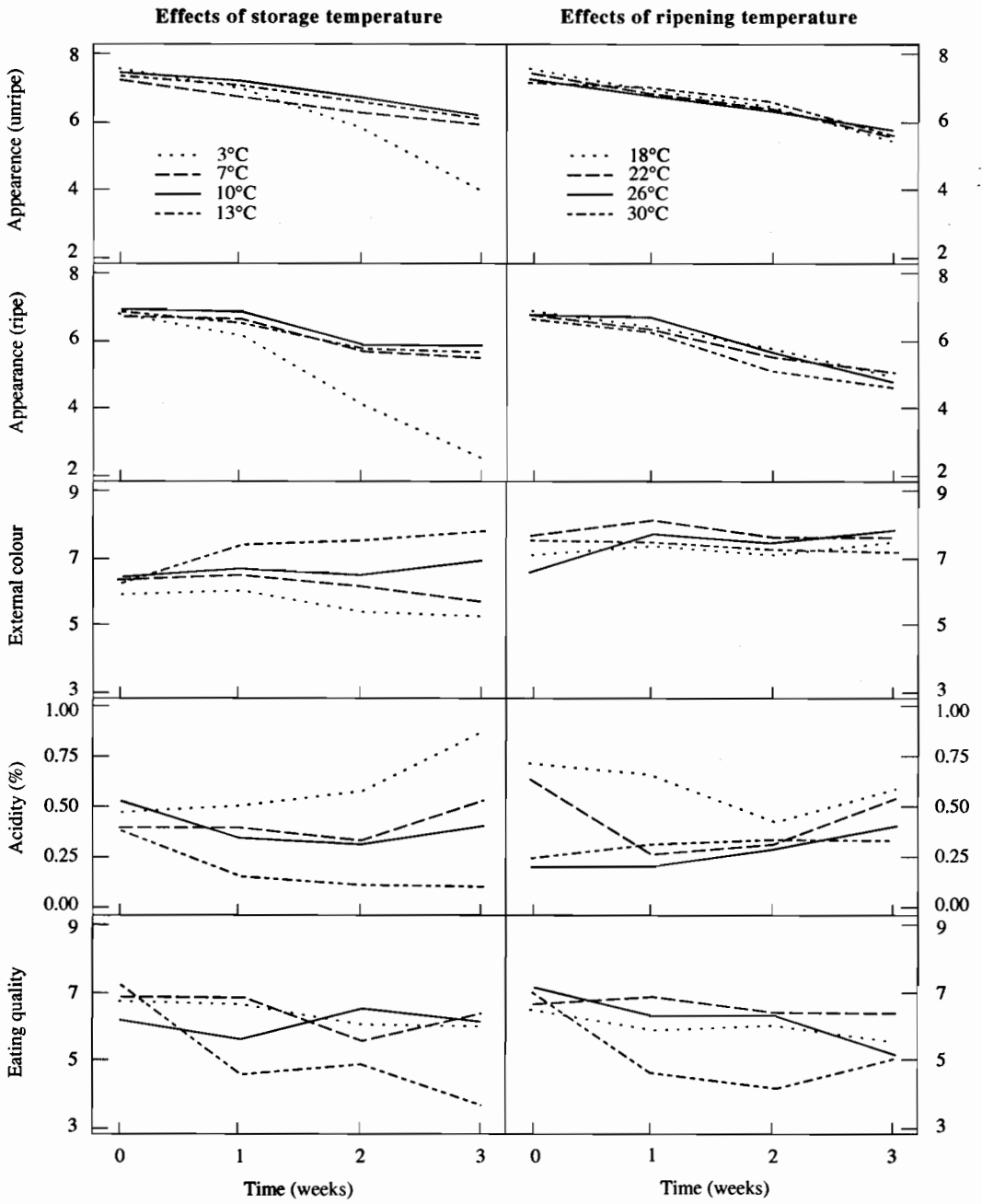
week of storage, fruit ripened at 30°C developed off-flavours (also at 3 weeks and ripened at 26°C) which significantly reduced eating quality. Best ripening temperature (based on eating quality) was 22°C.

## Conclusions

Kensington mangoes stored at 13°C ripened significantly during the first week of storage, indicating that 13°C is a short-term holding temperature only. The poor eating quality of ripe mangoes stored (and essentially ripened) at 13°C has been recorded previously at Hamilton where taste panels consistently report poor and/or lack of flavour in these fruit. Mangoes approaching their climacteric should be removed from cool store to a more appropriate ripening temperature.

The 10°C fruit coloured slowly in store while colour of the 7°C fruit remained static. External appearance and eating quality of these fruit were acceptable, even after 3 weeks storage. A temperature of 10°C can be used for storage of Kensington mangoes for up to 3 weeks; 7°C is suitable for 2 weeks after which ripe colour development may be retarded.

Colour and appearance of the 3°C fruit were severely affected after 2 weeks storage and were indicative of CI. Acid metabolism was severely retarded during ripening of these fruit. Mangoes can be stored for 1 week at 3°C but this should be attempted only if minimal change in fruit characteristics is essential. CI is highly likely after 1–2 weeks at 3°C.



**Figure 2.** Effects of storage and ripening temperatures on external appearance (injury), external colour, acidity and eating quality of mangoes.

Ripening temperature had no effect on skin colour once it was (storage) temperature-affected. CI from low temperature storage cannot be reversed by elevated ripening temperatures. The 30°C ripening temperature was unsuitable for fruit stored for 1 week, while 26°C was also unsuitable after 3 weeks storage, these temperatures generally producing off-flavours. Best overall ripening temperature was 22°C.

### Acknowledgments

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# The Use of Sulfur Dioxide and Low pH Treatment to Control Lychee (*Litchi chinensis* Sonn.) Pericarp Browning

S.J.R. Underhill, S. Gardiner and A. Prasad\*

## Abstract

Browning has been attributed to the by-products of phenolic degradation by polyphenol oxidase activity (PPO, EC 1.10.3.2). Fumigation of lychee fruit with 1.2% sulfur dioxide has previously been reported to inhibit PPO activity. This treatment also causes a rapid bleaching of the pericarp, due to the formation of a colourless anthocyanin-SO<sub>2</sub>H complex. Our results show that acidification of the fruit with 1N HCl results in an immediate improvement in pericarp colour of sulfur dioxide-treated fruit. Acidification is suggested to reverse the bleaching effect by promoting sulfur dioxide disassociation from the pigment complex. Increased pigment stability caused by acid-induced structural changes and possible Cl-ion inhibition of PPO activity may also contribute to colour improvement. Acidification did not reduce eating quality of the aril. Total titratable acid and pH levels were consistent with little penetration of HCl into the aril. Pericarp colour recovery was very stable, with no deterioration observed after 12 weeks storage.

SKIN (pericarp) browning is the most important post-harvest problem associated with marketing of fresh lychee fruit (Snowdon 1990). The brilliant red pericarp colour quickly deteriorates once fruit are harvested, in many cases during handling or shortly after transportation to markets. As eating quality of the aril is somewhat delayed, the browning process initially results in only a loss of aesthetic quality.

Moisture loss, specifically from the pericarp (Kuhn 1962) is thought to induce pericarp deterioration by promoting PPO activity. The use of sulfur dioxide (SO<sub>2</sub>) to inhibit lychee pericarp oxidation was first reported by Swarts (1985). Recent work by Zauber- man et al. (1991) demonstrates inhibition of PPO activity following SO<sub>2</sub> application. Sulfur dioxide fumigation for the control of pericarp browning and disease development is currently used commercially in Israel (Zauber- man et al. 1990), South Africa (Swarts 1985; 1989), Mauritius and Reunion (Menzel 1990).

Immediately after fumigation the pericarp develops a bright yellow appearance. Although partial colour recovery does occur, at best the pericarp becomes a dull orange, characteristic of SO<sub>2</sub>-treated fruit. This inability to return to the brilliant red colour is consid-

ered a major limitation to SO<sub>2</sub> technology. Recent work by Zauber- man et al (1991) reports that the application of HCl following SO<sub>2</sub> treatment could improve pericarp colour. This study was undertaken to investigate the technique of HCl treatment to improve pericarp colour of SO<sub>2</sub>-treated fruit.

## Materials and Methods

*Litchi chinensis* Sonn. cv. Tai So (synonym, Mauri- tius, Hong Huey) fruit were harvested from a com- mercial orchard in north Queensland (Lat. 17.1°S), and air freighted to the laboratory the next day. Cultivar Bengal (synonym, Madras) fruit were obtained from northern New South Wales (Lat. 28.6°S) and transported the same day to the laboratory. Cultivar Kwai May Pink (synonym, Gui Wei) fruit came from a commercial orchard in south east Queensland (Lat. 27.04°S).

Fruit were sealed in a 1 m<sup>3</sup> plastic container and fumigated with 1.2% gaseous SO<sub>2</sub> for 10 minutes. They were then either dipped into 1N HCl for 1 minute, or held as a SO<sub>2</sub> control. All fruit were stored for 6 days at 23°C, 60% r.h., and assessed daily.

Pericarp colour was determined visually using a 1 to 5 scale of redness— 1 = no red colour; 2 = 1/4 of the pericarp surface red; 3 = 1/2 of the pericarp surface red; 4 = 3/4 of the pericarp surface red; and 5 = fully red— by a five-member panel. Pericarp redness (CIE 'a' values) was further assessed using a Hunter

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labscan 6000 Spectrocolourimeter fitted with a 13 mm orifice, D65 illuminant and a 10 degree observer.

The pH was determined using either 2 g of pericarp or 3 g aril, obtained from three fruit. Tissue was thoroughly washed in  $\text{dH}_2\text{O}$  to remove juice, homogenised in 30 mL  $\text{dH}_2\text{O}$ , and the pH of the homogenate measured. Acidity was determined by titration of 10 g of homogenised aril against standardised 0.1 N NaOH to pH 8.2, using a Mettler DL20 automatic titrator.

A 50 g sample from the whole fruit, aril, or pericarp was obtained from a minimum of 30 fruit and stored overnight at  $-70^\circ\text{C}$ . Samples were then examined in duplicate for sulfite according to de Vries et al. (1986).

Mean eating quality of control and  $\text{SO}_2$ -HCl-treated fruit (cv. Bengal) was determined progressively during storage at  $23^\circ\text{C}$  60% r.h. Peeled fruit were rated on a 1 to 9 hedonic scale for general acceptability (1 = dislike extremely, 9 = like extremely) using a 12-member taste panel.

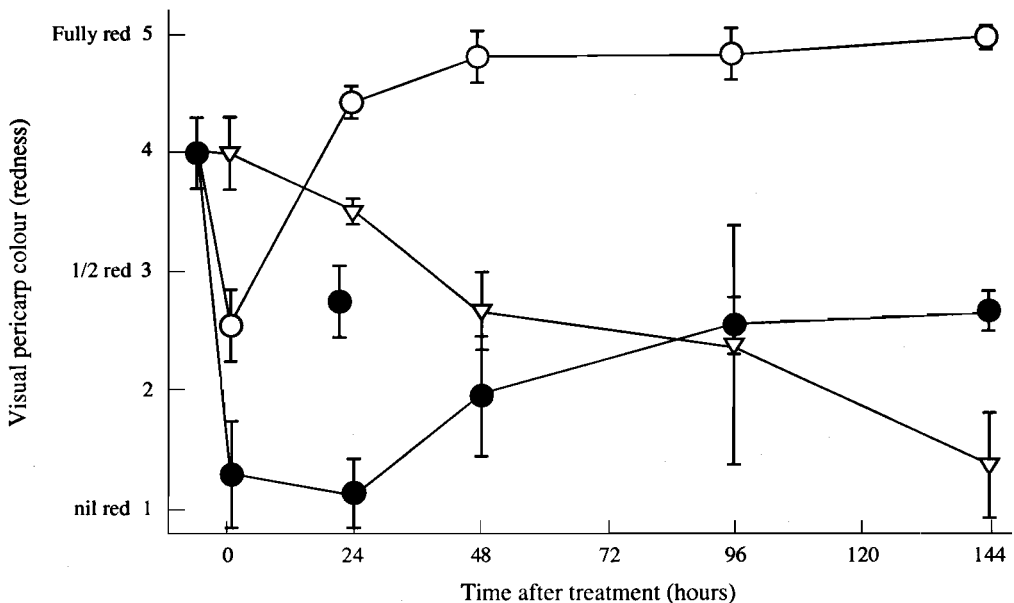
## Results and Discussion

Sulfur dioxide fumigation of the fruit resulted in instantaneous, uniform bleaching of the pericarp (Fig. 1a). Bleaching is a common response to sulfur application in many fruit, and has been attributed to the formation of a colourless anthocyanin- $\text{SO}_3\text{H}$  com-

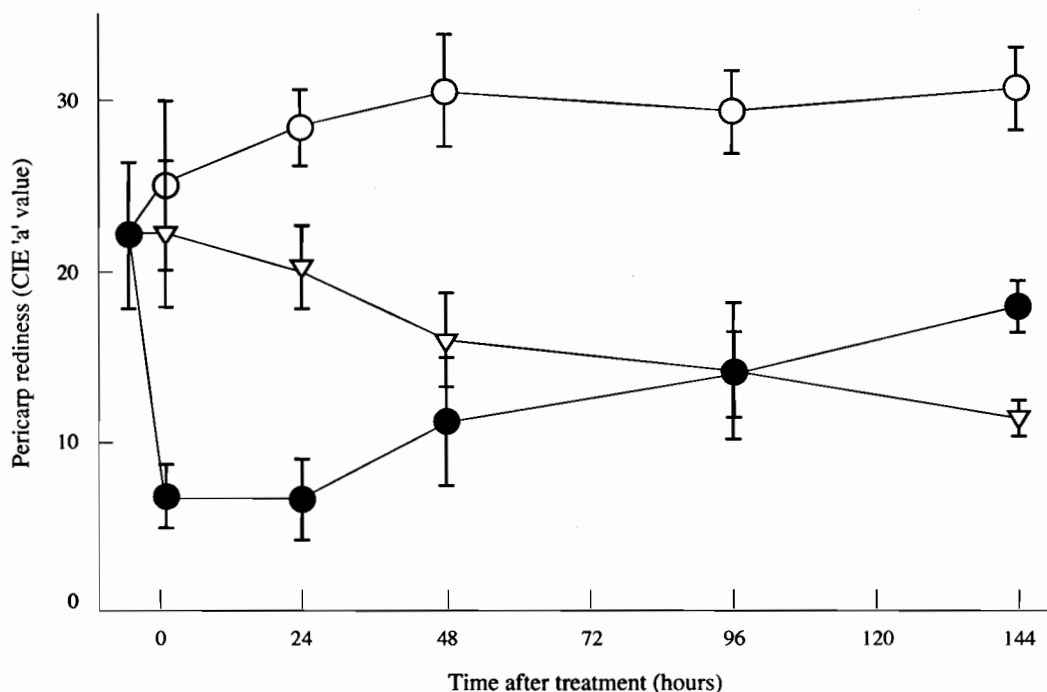
plex (Jurd 1964; Markakis 1982). Lychee pericarp colour improved during storage, but never achieved its initial redness (Fig. 1b). Colour recovery following  $\text{SO}_2$  application has also been reported in other fruit. Markakis (1982) attributed colour recovery to a dissociation of the pigment- $\text{SO}_3\text{H}$  complex, resulting in an increase of unbound sulphite in the tissue. We believe that partial colour recovery of the lychee pericarp following fumigation is due to incomplete  $\text{SO}_2$  dissociation, whereby enough  $\text{SO}_2$  is retained to maintain a degree of pigment decolorisation.

Acidification of the pericarp immediately after fumigation, using 1N HCl for 1 minute, increased pericarp colour (Fig. 1a). Colour development was initially localised, with the pericarp surface still partially bleached. Colour recovery occurred progressively over time with the pericarp fully red at 48 hours. No subsequent deterioration of colour was observed after 12 weeks storage. Recovery was restricted to direct contact with HCl (Fig. 2), indicating a highly localised effect. It is possible that progressive colour development, may be due to continued absorption of HCl into the pericarp.

Pericarp pH was significantly lowered by HCl treatment and remained below 3.5 for 6 days (Table 1). Markakis (1982) suggested that acidification of tissue could increase disassociation of the  $\text{SO}_2$  molecule from the anthocyanin- $\text{SO}_3\text{H}$  complex, thereby increasing the level of unbound sulphite in the tissue.



**Figure 1a.** Visual pericarp colour (cv. Tai So) during storage at  $23^\circ\text{C}$ , 60% r.h. Control (▼); fruit fumigated with 1.2%  $\text{SO}_2$  (●); fruit fumigated with  $\text{SO}_2$  and dipped into 1N HCl (○). Values are the mean of 20 fruit rated on a 1 to 5 scale  $\pm$ SE. Variation is indicated by the length of the vertical bars.



**Figure 1b.** External pericarp redness, (CIE 'a' value) during storage at 23°C, 60% r.h.. Control (▼); fruit fumigated with 1.2% SO<sub>2</sub> (●); and fruit fumigated with SO<sub>2</sub> and dipped into 1N HCl (○). Values are the mean of 20 fruit ±SE. Variation is indicated by the length of the vertical bars.

Jurd (1972) also indicated complete regeneration of anthocyanins following acidification. Acidification may therefore decrease the level of pigment-bound sulfite in the pericarp to a concentration too low to induce bleaching, while retaining sufficient concentration to inhibit PPO activity. Goodman and Markakis (1965) demonstrated that an SO<sub>2</sub> concen-

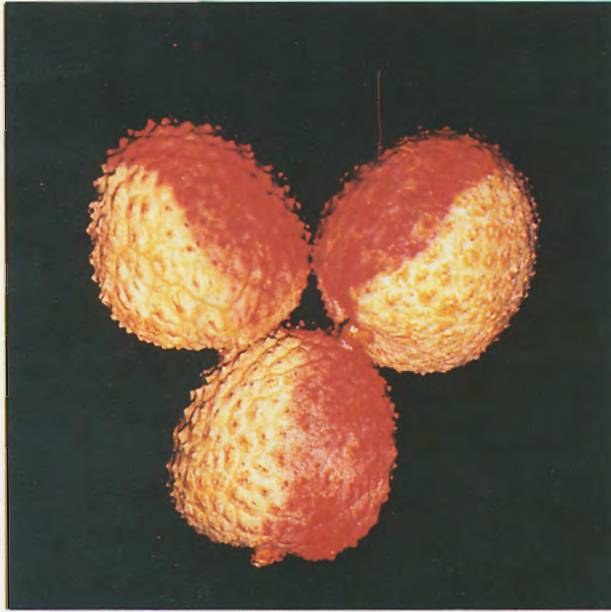
tration of 30 ppm in cherries was sufficient to inhibit enzymatic activity, without causing pigment bleaching. Attempts to reduce initial SO<sub>2</sub> application concentration or exposure time directly, however, did not show any benefit. Instead, fruit developed uneven colour recovery and localised browning, particularly at the stalk base.

**Table 1.** Pericarp and aril pH of lychee fruit cv. Tai So fumigated with sulfur dioxide, emersed in 1N HCl for 1 minute, and stored at 23°C, 60% r.h.

Tissue	Treatment	Storage time (hours)				
		0	24	48	96	144
Pericarp	control	4.57 a	4.48 a	4.54 a	4.93 a	4.94 a
	SO <sub>2</sub>	3.92 b	4.17 b	3.93 b	4.37 b	4.35 b
	SO <sub>2</sub> + HCl	2.84 c	2.88 c	2.51 c	2.79 c	3.46 c
Aril	control	4.35 a	4.31 a	4.90 a	4.64 a	4.82 a
	SO <sub>2</sub>	4.20 b	4.27 a	4.81 a	4.51 a	4.84 a
	SO <sub>2</sub> + HCl	4.41 a	4.13 b	4.48 c	4.24 c	4.58 b

Means (in columns) followed by the same letter are not significantly different ( $P < 0.05$ ) ±SE. Values are the mean of five independent extractions.





**Figure 2.** Sulfur dioxide-fumigated lychee fruit. One half of the fruit has been immersed in 1N HCl for 1 minute to illustrate the extent of pericarp colour recovery following acidification. (Photograph courtesy of Dr G. Zauberman, Volcani Centre Israel.)

Lowering the pericarp pH may also increase anthocyanin structural stability. Anthocyanins are very unstable even within intact plant tissue, due to an electron deficiency. Under alkaline conditions they are readily converted into an anhydro base form, which rapidly hydrates to a colourless chromenol, and ultimately results in pigment decomposition (Markakis 1982). Acidic conditions have been widely reported to increase anthocyanin stability, inhibit decomposition, and enhance red colour (Jurd 1972; Asen et al. 1973). Denny et al. (1986) reported a similar improvement in peach skin coloration following acidification, attributing it to anthocyanin structural and composition changes.

Increased pericarp colour following acidification (Fig. 1b) may also involve regeneration of anthocyanin. The conversion of anthocyanins to chromenols is reversible upon acidification (Markakis 1982), and would be expected to enhance pericarp colour. It is difficult, however, to determine chromenol concentration and therefore the importance of this response. Anthocyanins are extracted in the presence of HCl, allowing chromenol conversion to occur during extraction. Conversion of leucoanthocyanidins to flavylum may also have an additional role in increasing pericarp coloration. Leucoanthocyanidins are colour-

less flavanoids which are readily converted to anthocyanidins in the presence of mineral acids. Although leucoanthocyanidins have been isolated from lychee seeds (Chadha and Seshadri 1962), they may also occur in the woody pericarp. Prior to fumigation, anthocyanin was observed in the mid to upper mesocarp and epicarp regions of the pericarp. Following acidification this distribution was extended to include the endocarp. Acidification of isolated endocarp tissue also resulted in tissue coloration, and would discount the possibility of pigment leakage from the mesocarp. This disparity between pigment distribution after acidification may provide circumstantial evidence for the conversion of leucoanthocyanidins to coloured anthocyanidins in the endocarp.

Changes in cellular pH may inhibit enzymatic activity. Since the pH optimum for PPO has been reported to be between pH 5.0 and 7.0 (Mayer and Harel 1979), a reduction in the pericarp homogenate below this would be expected to limit activity. Chloride ions have also been reported by Mathew and Parpia (1971) to inhibit PPO activity nonselectively. The possible inhibition of PPO activity by Cl<sup>-</sup> ions may also explain why acidification using other inorganic acids proved less effective than HCl (unpublished data).

**Table 2.** Mean eating quality and total titratable acidity of the aril (% citric acid) of lychee fruit cv. Bengal (control) compared to fruit fumigated with sulfur dioxide, emersed in 1N HCl for 1 minute, and stored at 23°C, 60% r.h.

Treatment	Time (hours)	Mean eating quality <sup>a</sup>	Titratable acidity (% citric acid) <sup>b</sup>
Control	0	7.54 a	0.34 a,b
	24	7.54 a	0.32 a,c
	48	nd	0.22 d
	96	7.08 a	0.27 c
SO <sub>2</sub> + HCl	0	7.25 a	0.32 a,c
	24	7.13 a	0.39 b
	48	nd.	0.30 c
	96	6.70 a	0.33 a

Means (in columns) followed by the same letter are not significantly different at (P<0.05).

<sup>a</sup> Means of twelve tasters ±SE.

<sup>b</sup> Mean of five replicates ±SE.

No data available = nd.

**Table 3.** Mean total sulfite residue of three lychee cultivars after fumigation with 1.2% SO<sub>2</sub> and the application of 1N HCl. Values are individual samples expressed as ppm.

Cultivar	Time after fumigation (hours)	Sulfite residue (ppm)	
		Pericarp	Aril
Bengal	untreated	6	7
	0	1101	42
	24	323	115
	48	311	135
	96	101	64
Tai So	0	685	21
	24	212	16
	48	139	12
	96	169	6
Kwai May Pink	0	232	61
	24	105	40
	48	67	12
	96	92	nd

No data available = nd.

Acidification did not reduce eating quality of the aril, although total titratable acid levels were increased slightly (Table 2). Aril pH decreased immediately after SO<sub>2</sub> application, possibly due to unbound SO<sub>2</sub> in the tissue-forming sulfurous acid (Table 1). Although subsequent acidification did not initially reduce pH, aril pH decreased at 24 hours and remained significantly lower than the control. This would suggest some HCl penetration into the aril 24 hours after treatment.

Most of the SO<sub>2</sub> residue was located in the non-edible pericarp, with little initial penetration into the aril (Table 3). Pericarp residue levels decreased during storage, with most of the SO<sub>2</sub> lost in the first 24 hours. Residue levels in both the aril and pericarp varied considerably between cultivars. Highest residue levels were obtained in cv. Bengal, with least residue in cv. Tai So. Sulfur dioxide levels in the aril (cv. Bengal) increased during the first 48 hours after treatment, and would imply some transfer of free SO<sub>2</sub> between the pericarp and the aril.

The effect of acid treatment on SO<sub>2</sub> residue levels is not clear. Although acidification is thought to release free SO<sub>2</sub> from the anthocyanin-SO<sub>3</sub>H complex, it is not known whether this disassociation equates to increased loss of SO<sub>2</sub> from the tissue. Swarts (1985) reported SO<sub>2</sub> residue levels in the aril of cv. Tai So fruit at 21 ppm after 72 hours. This was higher than for the same cultivar, following acid treatment and similar storage (Table 3). Pericarp residue levels of acidified fruit were also lower than those reported by Tongdee (1990) using just SO<sub>2</sub> fumigation. Acid treatment may decrease total SO<sub>2</sub> residue in aril, and possibly the pericarp. Sulfur dioxide application, however, can result in considerable residue variability (Schutte et al. 1990), and further work is required to verify a possible relationship between acidification and reduced residue levels.

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# An Overview of Lychee Postharvest Technology

S.J.R. Underhill\*

## Abstract

Lychee fruit are prone to a uniform browning of the pericarp surface. Injury is commonly associated with rapid pericarp desiccation. When moisture loss is reduced, skin colour can be retained for up to 5 weeks. A combination of low temperature (2–5°C), high humidity and plastic packaging is currently used commercially to control browning. Alternative treatments to inhibit enzymatic activity, using sulfur dioxide (SO<sub>2</sub>) fumigation have been reported recently. Additional acidification of the fruit has proved beneficial in removing the problem of anthocyanin bleaching.

LYCHEE, like most arillate fruit, deteriorates rapidly once harvested. The first and most significant symptom is a browning of the pericarp or skin. Once brown, consumer demand decreases dramatically, resulting in significant postharvest loss. Skin browning initially represents a simple loss of aesthetic quality, as deterioration of the edible pulp is somewhat delayed. Skin colour is an intrinsic component of fruit quality and freshness, and as a consequence browning is the most important postharvest problem associated with the fruit. This review outlines the historical progression of lychee postharvest technology with emphasis on skin colour retention. Aspects of fruit maturity also have been included to complete the focus on fruit quality.

## Early Postharvest Handling Techniques

Attempts to control browning are evident in some of the earliest Chinese literature (Groff 1921). Fruit destined for the Emperor highlights the extremes. Offerings were transported using a relay of couriers on horse back. Timing and speed were essential, and in many cases the displeasure of receiving brown fruit resulted in grave consequences (Groff 1921).

The importance of fruit desiccation in the control of browning has long been recognised. Early attempts

to control moisture loss were based on various forms of packaging, using woven grass bags, matting or bamboo baskets lined with green leaves or moss (Singh 1957). Large bamboo and reed baskets are still used in parts of India and Southeast Asia, especially for transportation. Water was often splashed or sprayed over the fruit during transit to reduce fruit desiccation. The incidence of disease was usually promoted as a result. However, overall colour retention appears to have been extended (Nip 1988).

Although lychees were first introduced into western culture in the late 17th century, no effort was made to retain lychee colour. The substantial distances involved and time taken in shipping consignments from say China to Europe made this an impossible task. Instead, fruit were dried and sold as 'lychee nuts', establishing a significant demand amongst Chinese expatriates. This practice still continues today, especially in southern China and Vietnam where storage of fresh fruit is particularly difficult, and postharvest losses are very high. It was not until plantations were established in Florida, South Africa and Australia in the late 1800s, that storage and colour retention again became an issue.

The application of cool storage technology in the 1930s was critical in the development of lychee industries. For the first time fruit could be stored, and commercial expansion, especially in South Africa and Florida, soon resulted. Temperature management still remains the most effective means of facilitating long-term storage, in terms of controlling both pathogens and reducing fruit desiccation. Numerous handling techniques have been developed depending on the availability of cold room facilities. These include

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hydro-cooling, ice-slurry packaging, and forced air cooling.

## Packaging

Initial attempts at storage involved the development of more effective forms of packaging, incorporating various types of paper and cloth bags (Mukerjee 1957); perforated plastic bags (Gaur and Bajpai 1978); plastic films and PVC bags (Singh 1957; Campbell 1959; Hatton et al. 1966); and in one interesting report even individually wrapping fruit in aluminium foil (Macfie 1955). Results were highly variable, with either heavy condensation promoting disease, or inadequate moisture control. Attempts to develop moisture absorbents in packaged lychees were reported (Mukerjee 1957; Singh 1957); however, it was not until the advent of effective chemical disease control (Hargreaves 1983), that browning could be controlled. Plastic packaging and cool storage at 5°C combined with a postharvest fungicide treatment increased pericarp colour retention for up to 5 weeks (Scott et al. 1982; Huang and Scott 1985; Wong et al. 1991) (Fig. 1).

## Surface Coatings

Considering the success of surface coatings in other fruit, they would appear particularly suited to the underlying problem of rapid fruit desiccation. Although wax emulsions have been investigated (Datta et al. 1963; Prasad and Bilgrami 1973; Gaur and Bajpai 1978; Bhullar et al. 1983), little benefit has been demonstrated, due to either continued dehydration or skin discoloration (i.e. a darkening of the pericarp surface, as distinct from pericarp browning). Recent work by Underhill and Simons (unpublished data) reports the development of pericarp micro-cracking shortly after harvest. Similar cracking was also observed in wax-treated fruit after 24 hours, and is thought to enhance desiccation. This may partly explain the inability of current commercial coatings to restrict water loss and therefore inhibit resultant pericarp browning. The development of 'skin darkening' has not been reported previously in the literature. Discoloration usually occurs soon after the wax is applied and appears to be restricted to high pH wax coatings (greater than pH 7). Polysaccharide and similar sucrose ester-based coatings show no detrimental



**Figure 1.** Lychee fruit stored at ambient temperature (25°C) for 1 week, compared to the fruit which have been stored in punnets overwrapped with a PVC film to control fruit desiccation. (Photograph; source unknown)

affect. Discoloration is reversible upon acidification of the fruit, and may simply reflect an adverse pigment-pH response.

## Sulfur Dioxide Technology

Sulfur dioxide has been used recently in South Africa (Swarts 1985, 1989), Reunion (Menzel 1990), Thailand (Tongdee pers com.), and Israel (Zauberman et al. 1991) as an alternative treatment for pericarp colour retention. Fruit are usually treated on farm, by either burning sulfur powder (Zauberman et al. 1989), or fumigating directly with SO<sub>2</sub> gas. Sulfur dioxide is thought to work by inhibiting polyphenol oxidase activity (Goodman and Markakis 1965; Mayer and Harel 1979); however, it also has fungistatic properties. One of the main problems with SO<sub>2</sub> treatment is that it rapidly bleaches the pericarp surface due to the formation of a colourless anthocyanin-SO<sub>3</sub>H complex. Although colour will return after 24 to 48 hours, fruit are a characteristic dull orange. The degree of colour recovery depends on the rate of subsequent SO<sub>2</sub> release, which itself is a function of storage temperature and air flow around the fruit.

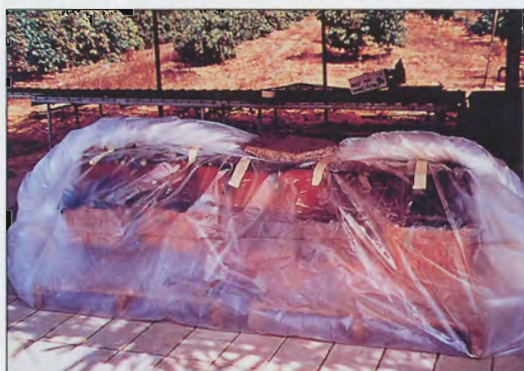
The method of SO<sub>2</sub> application is critical to treatment success. Numerous techniques, from metabisulfite dipping, slow release 'grape-guard'-type pads, burning sulfur powder, and direct SO<sub>2</sub> fumigation have been used, with variable results reported. Burning sulfur powder is possibly the most common method used (Fig. 2); however, problems regulating dosage, leading to tainting of the pulp, are regularly reported (Lonsdale and Kremer-Kohne 1990). Low

residue levels are possible (Swarts 1985), but the current practice of burning sulfur is too inaccurate. Fumigation using gaseous SO<sub>2</sub> tends to be far more precise (Fig. 3). The extra cost involved, however, has tended to limit its commercial use. The application of SO<sub>2</sub> in gaseous form is faster (10 minutes total treatment time), yields more reproducible dosage rates, and should be considered as a commercial alternative. Other forms of application such as slow release pads have been used, but problems with contact damage and dosage regulation still exist.

Recent work by Zauberman et al. (1989, 1991) and Underhill and Critchley (1990) demonstrate the additional value of acid dips, where original pericarp coloration can be restored after SO<sub>2</sub> treatment. Fruit are immersed in 1N HCl for 2 minutes with complete colour recovery within 24 hours. Eating quality is not affected, with little evidence of acid penetration into the pulp. Acidification can be applied immediately after fumigation; however, this sometimes causes fruit splitting. Delaying treatment until 8 hours after fumigation is commonly used in Israel to avoid this problem (Avshalom, pers. comm.).

Sulfur dioxide-low pH technology is used commercially in Israel for export to Europe, particularly the United Kingdom, France and Holland. Treatment use is likely to expand in the future, especially in those countries currently using SO<sub>2</sub>, such as South Africa. Increasing concern over chemical usage on horticultural produce, however, may limit its long-term value.

It is important to appreciate that browning is a general injury symptom, which will result from a wide range of preharvest and postharvest stresses (Nip



**Figure 2.** Sulfur dioxide application by burning sulfur powder. This technique is widely used in South Africa and Israel. Left: fruit are fumigated on farm by wrapping the picking crates with a high density plastic. Crates are stacked in an open manner to allow free movement of gas around the fruit. Sulfur powder is placed on a small tray and heated using a small burner. Following treatment plastic is removed and the fruit left to stand for several hours. Right: fumigation using large plastic tents is also common. Again, fumigated is by heating sulfur powder to release SO<sub>2</sub> gas. Treatment is on farm, usually in half to one tonne loads. Little attempt is made to completely seal the chamber and leakage in both cases is an obvious limitation.



**Figure 3.** Sulfur dioxide application using a gas fumigation cabinet. This technique is used in Thailand, for both lychee and longan. Gaseous  $\text{SO}_2$  is trickle fed into the sealed chamber to achieve a set internal concentration. Once treatment is complete,  $\text{SO}_2$  is rapidly removed using a large scrubbing chamber, whereby the gas is passed through a fine water mist.

1988). This has led to considerable confusion, both in the literature and the industry. In many cases it is difficult to distinguish the initial cause of browning. A good example is the common confusion over pathological and desiccation browning. Both forms of browning develop during storage, and although symptoms are quite similar, the appropriate control is quite distinct. In many cases, postharvest browning is a combination of both, as shown by Scott et al. (1982).

### Fruit Maturity

Emphasis on skin colour retention assumes fruit are mature and fully developed. In many cases the physiological maturity of the fruit is highly variable. Lychee maturity is of major concern in those countries where industry development is relatively new, and there is poor consumer awareness. In the absence of maturity regulations, immature fruit are being sold on the commercial markets. Although returns are initially high, poor fruit quality and reduced product reputation dramatically lowers subsequent prices.

When this is combined with the high incidence of 'first buyers' and product competition, the damage caused by retailing immature fruit is severe.

A wide variety of parameters are currently used to predict fruit maturity, including skin colour; pulp acidity (Batten 1989); brix:acid ratio (Underhill and Wong 1990); and days from anthesis (Gaur and Bajpai 1977). Although these parameters are all correlated with fruit eating quality, their relative value will depend on cultivar and region variability (Tongdee et al. 1982).

### Conclusions

Over the last 10 years there has been a resurgence in lychee postharvest research, similar to that of the late 1950s. Technology aimed at either inhibition of enzymatic degradation, or control of initial desiccation has made significant progress. Advances in acidification technology, surface coatings and the availability of new active plastic films, may have a major impact on postharvest storage quality in the future. For the first time the physiology of pericarp browning is

being investigated in detail, and much of the past confusion over uniformity of symptomatology is being clarified.

## Acknowledgments

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# Controlled Atmospheres for Kensington Mango Storage: Classical Atmospheres

R.L. McLauchlan and L.R. Barker\*

## Abstract

Kensington mangoes were stored under 36 different atmospheres in a 6 oxygen (2, 4, 5, 6, 8, 10% O<sub>2</sub>) × 6 carbon dioxide (0, 2, 4, 6, 8, 10% CO<sub>2</sub>) matrix while control fruit were held in air at 13°C for 33 days. Colour development was linearly retarded by decreasing O<sub>2</sub> (10 to 2%) and increasing CO<sub>2</sub> (0 to 4% only, no further effect from 4 to 10%) concentrations. Fruit from the lower O<sub>2</sub> atmospheres also had high titratable acidity at out-turn. After 5 days at ambient conditions, these fruit continued to develop typical external colour and metabolise acid, thereby increasing palatability. Fruit from all atmospheres were soft at out-turn. Within the concentrations studied, optimum atmosphere appears to be around 4% CO<sub>2</sub> and 2–4% O<sub>2</sub> but the data does suggest that further research is required below the 2% O<sub>2</sub> and above the 10% CO<sub>2</sub> levels of this trial, as well as lower storage temperatures to control softening. Disease control also needs improvement for long-term storage of mango.

MANGO production in Australia has increased steadily in recent years, the Kensington variety accounting for 90% of the crop. Virtually the whole crop has been sold on the domestic market with occasional cool storage during the peak of the season. Cool storage alone does not provide the storage life needed for export by sea, so that only token quantities are exported by air to high-priced markets. With substantial plantings over the past decade about to come into full production in Queensland and the Northern Territory, the export market will assume an increasing importance.

Controlled atmosphere storage is used to substantially increase the market life of many fruits, notably apples and pears. However, tropical fruits are susceptible to chilling injury at the temperatures commonly used for pome fruit storage. Controlled atmosphere has been shown to provide slight to moderate increases in the storage life of many mango varieties compared to cool storage alone.

Hatton and Reeder (1966) stored Keitt mangoes in controlled atmospheres of 1/5, 3/5, 5/5, 1/8 and 4/9

%O<sub>2</sub>/CO<sub>2</sub> at 12.8°C. After 20 days storage, they determined that 5/5 was the best of the atmospheres used. At 1% O<sub>2</sub>, off-flavours, green colour and skin discoloration were evident. Pantastico et al. (1970) determined that refrigerated controlled atmosphere storage (14.4°C, 5/5) of Carabao mango produced a storage life of 16.5 days compared to 10 days for controls (air) and 15.5 days for modified atmosphere storage in polyethylene bags.

Spalding and Reeder (1974) report that in Israel, Haden mangoes stored in controlled atmospheres of 2/1 and 2/5 suffered heavy fungal losses after 6 weeks. More mature fruit could be stored at 10–11°C while less mature fruit required 13°C to avoid chilling. Peacock and Jobin (1985) measured the time to first softening in Kensington mangoes stored at 13°C in several controlled atmospheres of 1–20% O<sub>2</sub>/0–6% CO<sub>2</sub>. While the results from three trials were variable, they suggested that the optimum controlled atmosphere of 5/1 did increase storage life by around 30%.

In simulated export trials, Peacock (1987) stored Kensington mangoes in a shipping container at 13°C in a 5/1 using lime to absorb evolved CO<sub>2</sub>. After 3 weeks, the lime was exhausted and CO<sub>2</sub> levels rose. Stem-end rots also caused heavy losses. Peacock and Kosiyachinda (1987) similarly stored five Thai mango varieties. Storage in 8% O<sub>2</sub> gave best results

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after 3 weeks, 5–5.5% enabled 4 weeks storage, and 4.5% was suitable for more backward fruit. Best overall was a controlled atmosphere of 5/1.

Noomhorm and Tiasuwan (1988) stored Rad mango at 13°C in 9 controlled atmospheres (4, 6 and 8% O<sub>2</sub>×CO<sub>2</sub>). They experienced some difficulty in maintaining the atmospheres but reported that a controlled atmosphere of 6/4 gave 25 days storage to 50% yellow colour and 32 days to full ripe, compared to 20 days for controls.

To date, most research on controlled atmosphere storage of mango has suffered from an inability to produce a wide range of accurately controlled atmospheres to fully test the effects of O<sub>2</sub>, CO<sub>2</sub> and temperature. The recent development of relatively inexpensive gas generating, mixing, control and monitoring devices has enabled a more rigorous study of the effects of controlled atmospheres on the storage of mango to be undertaken.

## Methods

First grade mangoes (38 trays) were purchased from Mareeba (north Queensland). Fruit were harvested in mid-December 1989, treated with an approved fungicide, size graded, packed into trays and forwarded to Brisbane by air. One fruit per carton was randomly sampled for maturity testing by % dry matter. Mangoes (17) were allocated to each controlled atmosphere treatment by randomly selecting from each size grade (3×20 count + 12×18 + 2×16), with an average total treatment weight of 5.9 kg.

Controlled atmospheres were generated by mixing N<sub>2</sub> (from a membrane generator), air (compressor) and CO<sub>2</sub> (bottled) in appropriate proportions to achieve all 36 combinations of O<sub>2</sub> (2, 4, 5, 6, 8, 10%) × CO<sub>2</sub> (0, 2, 4, 6, 8, 10%) plus an air control. A second (nonstored) control was ripened by exposing to ethylene for 1 day and holding for 1 week at 20°C. Controlled atmosphere concentrations were monitored by gas chromatography. Dew point was maintained at 12–12.5°C (94–97% r.h.) by bubbling through water. Fruit were stored in gas-tight barrels (25 L) at 13°C with controlled atmosphere flow rates of 200 mL/minute. Exhaust streams were directed via a sampling valve to a gas analyser (Bishop 701) for determination of O<sub>2</sub> and CO<sub>2</sub> which were logged (DataTaker DT100) every 3 hours.

Ten fruit (18 count size) per treatment were labelled, their colours measured (L, a, b: HunterLab Labscan 6000) and rated for ripe colour (1=green, 9=full ripe). After 33 days storage, the air control fruit was inspected and found to be soft and highly coloured so that storage was terminated. The labelled fruit were again used for L,a,b and ripe colour measurement. Firmness was determined on an Instron 1122 (force required to indent fruit 2 mm by a 12 mm

hemispherical plunger at 20 mm/minute). Disease severity was assessed as the percent of surface area affected. Fruit were submitted to a five member taste panel for assessment of eating quality on a 1 (dislike) to 9 (like) scale, and analysed for soluble solids (Abbe refractometer) and acidity (MetrOhm autotitrator).

It was noted that fruit from the low O<sub>2</sub> atmospheres (2–5%) had high acidity and low colour scores at out-turn. After 2 days at 20°C, these fruit developed substantial ripe colour so that three disease-free fruit from each of these treatments were maintained at 20°C for a further 5 days. They were again assessed for colour (L,a,b), acidity and eating quality.

All data were subjected to factorial analyses of variance (anova). Where the anova returned a significant F test, least significant differences were calculated for pairwise comparison of treatment means.

## Results

Maturity testing showed no significant differences between size grades, although the larger fruit had higher dry matter content (20 count 12.6%, 18 count 13.0% and 16 count 13.6%). The fruit was considered mature for the district even though the overall average of 13.1% was below the current market standard (14.0%). Nonstored controls were colour-rated at 2.0 (1=green, 9=ripe) at the start and ripened to a very acceptable 7.4. Input controlled atmospheres were generally as required. All O<sub>2</sub> levels were an average 7–8% low and the 8% CO<sub>2</sub> level was slightly high (Table 1). All data was analysed against the actual controlled atmospheres.

Concentration of CO<sub>2</sub> in the effluent streams was about 1% higher than in the inlet atmospheres. Mangoes stored in air produced a broad climacteric peak centred on day 17 (±8 days), CO<sub>2</sub> rising to 1.7%. Respiratory climacterics were not evident in most controlled atmospheres, especially those with >3% input CO<sub>2</sub> or <6% input O<sub>2</sub>. Of the parameters measured, external (ripe) colour rating, firmness, acidity and eating quality were considered the most important in assessing out-turn quality (Table 2).

At out-turn, mangoes stored in air were highly coloured (8.3), soft (3.7 N), low in acid (0.210%) with poor eating quality (2.8). The low eating quality was due to overripe fruit with a bland flavour.

**Table 1.** Actual vs desired O<sub>2</sub> and CO<sub>2</sub> concentrations (%).

Desired %	0.0	2.0	4.0	5.0	6.0	8.0	10.0
Actual	O <sub>2</sub>	-	1.9	3.7	4.7	5.5	7.4
	CO <sub>2</sub>	0.0	2.0	4.0	-	6.0	8.7

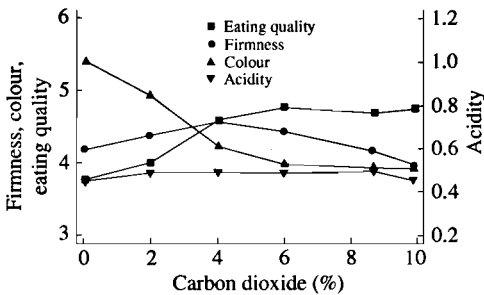
Colour, firmness and eating quality were all significantly affected by CO<sub>2</sub>, but acidity was not (Table 2; Fig. 1). While the effect of CO<sub>2</sub> on firmness is significant, it is of minor importance, since all fruit were at an eating-ripe (3.8–4.8 N) firmness which was maximal (4.6 N) at 4% CO<sub>2</sub>. Ripe colour was linearly retarded by the addition of up to 4% CO<sub>2</sub>; above this level CO<sub>2</sub> had no effect on colour. Eating quality was higher in fruit stored in 4–10% CO<sub>2</sub>, although all taste panel scores are rather poor (3.8–4.8). Low eating quality was generally the result of lack of flavour, aroma and volatiles.

The effects of O<sub>2</sub> on mangoes were stronger than those of CO<sub>2</sub>, with colour, firmness, acidity and eating quality all significantly affected (Table 2, Fig. 2). Firmness and acidity were relatively unaffected above 5% O<sub>2</sub>; below 5% they were significantly retained. Colour and eating quality increased continuously with increasing O<sub>2</sub> but were generally poor (colour: 3.4–5.4; eating quality: 3.2–5.4).

Acidity in mangoes stored under low O<sub>2</sub> (1.9–4.7%) decreased substantially during the 7 days at 20°C after controlled atmosphere storage (Fig. 3). This metabolism of acid is typical of ripening mango but occurred at a faster rate than in nonstored fruit.

**Table 2.** Correlations (r) between major mango quality parameters and controlled atmospheres.

	O <sub>2</sub>	CO <sub>2</sub>	Acid	Colour	Eating quality
CO <sub>2</sub>	0.00				
Acidity	-0.81	0.01			
Colour	0.63	-0.58	-0.57		
Eating quality	0.47	0.37	-0.51	0.06	
Firmness	-0.61	-0.11	0.80	-0.50	-0.36



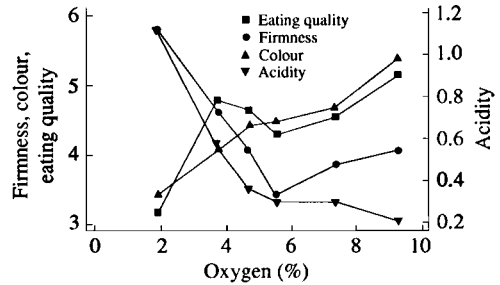
**Figure 1.** Effect of CO<sub>2</sub> concentration on firmness, colour, eating quality and acidity of controlled atmosphere-stored mango.

The decrease in acidity was similar in all CO<sub>2</sub> treatments. Although ripe colour also developed, final eating quality was poor, with disease increasing during this period and causing off-flavours.

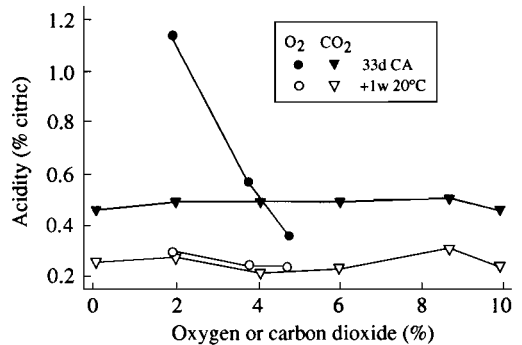
An average 2.2% of surface area was affected by disease, which ranged from 0–65%, with no controlled atmosphere treatment. Assuming fruit with >5% surface area showing disease are unmarketable, then the 3.3% unmarketable in the low (1.9–3.7%) O<sub>2</sub> treatments was significantly lower than the 5.8–16.7% recorded in the higher (4.7–9.3%) O<sub>2</sub> levels. Increased disease levels are likely to be directly related to increased ripeness and hence indirectly to the storage controlled atmosphere. Disease will be a major problem in long-term storage of mango.

## Conclusions

On out-turn from controlled atmosphere storage, fruit should be either close to full ripe (coloured, soft, low acid) and ready for immediate consumption; or semi-



**Figure 2.** Effect of O<sub>2</sub> concentration on firmness, colour, eating quality and acidity of controlled atmosphere-stored mango.



**Figure 3.** Effect of CO<sub>2</sub> and O<sub>2</sub> on the acidity of mangoes after 33 days controlled atmosphere and after a further 1 week storage at ambient conditions.

ripe (firm, half-coloured, moderately high acid content) with sufficient shelf life to permit typical wholesale and retail marketing. Also, the latter should ripen subsequently to typical full colour, softness, low acidity and high eating quality.

If ready-to-eat fruit are desired, then a controlled atmosphere of 4/4% O<sub>2</sub>/CO<sub>2</sub> should give 20–30 days storage compared to 14–20 days in air at 13°C. If fruit with some days of remaining shelf life are preferred, then lower O<sub>2</sub> levels (2–3%) should be used.

The effects of low O<sub>2</sub> levels (1.9–3.7%) are quite strong, suggesting that even greater storage life may be obtained in controlled atmospheres containing lower O<sub>2</sub> levels (say 0.5–2% O<sub>2</sub>) than was used in this trial. Our controlled atmosphere system has since been modified to generate these O<sub>2</sub> levels. Given the minor effects from the CO<sub>2</sub> levels used (0–10%), higher CO<sub>2</sub> controlled atmospheres (10–20%) may be also worth investigating.

Controlled atmosphere storage was at 13°C, the currently recommended storage temperature for Kensington mangoes. Other trials in this laboratory suggest that 13°C is really only suitable as a short-term holding temperature and is too high for long-term mango storage. Mangoes that ripen at 13°C almost invariably have a poor to bland flavour as occurred in this trial. Storage at lower temperatures, 7–10°C, should be investigated in conjunction with controlled atmosphere.

The use of hot fungicide dips on mangoes has considerably improved disease control in recent times. However, disease remains a major problem in long-term mango storage and further work is required for controlled atmosphere storage. Fruit may need fungicide application before and after storage to ensure that disease is controlled during ripening after storage.

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# Benomyl Resistance of *Colletotrichum* Species Associated with Mango and Rambutan Fruit Rots in Thailand

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## Abstract

Mature fruits of rambutan and mango were collected randomly from various locations in Thailand: rambutan from the east (Rayong, Chanthaburi and Trat) and south (Chumphon and Nakhon Si Thammarat); and mango from the central (Ratchaburi, Nakhon Pathom, Chon Buri and Nakhon Ratchasima). The fruits were incubated to assess the incidence of fruit decay, and *Colletotrichum* spp. were isolated from the disease lesions. The benomyl tolerance of these isolates was determined by examining their growth on potato dextrose agar (PDA) supplemented with benomyl (PDA+B), at concentrations of 0, 0.1, 1.0, 5.0 and 10.0 ppm. Thirty-one isolates from mango and 36 (east) and 42 (south) isolates from rambutan were tested. Resistance to benomyl was rated at three levels: strongly resistant, moderately resistant and sensitive. The differences in colonial characteristics were noted in isolates that grew on PDA+B, but the abnormalities were absent in the strongly resistant isolates.

Of the rambutan isolates from the east, three isolates were strongly resistant, and one isolate moderately resistant to benomyl at 10.0 ppm. Of the rambutan isolates from the south, two isolates were strongly resistant and one isolate was moderately resistant to benomyl at 1.0 ppm. Similarly, four isolates from mango were moderately resistant to benomyl at 1.0 ppm. This was the first report of resistance to benomyl of field-collected isolates of *Colletotrichum* spp. in Thailand. Their occurrence in relation to the field spraying of benomyl will be discussed.

CHEMICAL fungicides have been used widely in orchards. Continuous use of systemic fungicide over long periods may result in the development of chemical resistance in fungi (Griffiee 1973; Dekker 1977; Dekker and Georgopoulos 1982; Ogawa et al. 1983). Benomyl was recommended for control of anthracnose disease both in the field and after harvest.

Resistance against benomyl of *Colletotrichum* spp. has been reported in United States, Japan, Kenya and West Indies (Ramos and Kamidi 1982; Ogawa et al 1983; Sommartaya 1985). In Thailand benomyl has been used widely since 1967. Naturally-selected resistant isolates of *Colletotrichum* spp. may have developed. This work was done to determine the benomyl-resistance of *Colletotrichum* spp. isolated from mango and rambutan.

## Materials and Methods

### Isolation and collection of *Colletotrichum* spp.

Mature green fruits of mango and rambutan were collected from major planting locations. Naturally infected *Colletotrichum* spp. were isolated from diseased fruits after a period of incubation. The obtained isolates were allowed to grow on potato carrot agar (PCA) supplemented with streptomycin sulfate to avoid bacterial contamination, then preserved for further study.

### Mycelium growth on potato dextrose agar (PDA) supplemented with benomyl

Inoculum of each *Colletotrichum* isolate was prepared on PDA plate. Mycelial discs (5 mm) were transferred to PDA supplemented with 0, 0.1, 1.5 and 10 ppm of benomyl concentrations (PDA+B). Ten replications for each isolate were made. Replicates were stored under 12 hours/day fluorescent lighting and incubated at room temperature and near ultraviolet light conditions. Radial growth (RG) of each isolate was recorded every 24 hours for 7 days. Benomyl

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resistance was classified as follows: A = highly resistant (RG on PDA+B plates was more than 2/3 of that on PDA plates); B = moderately resistant (RG on PDA+B plates was more than 1/3 of that on PDA plates); and C = sensitive (RG on PDA+B plates was less than 1/3 of that on PDA plates).

#### Colonial characteristics of *Colletotrichum* isolates on PDA+B plates

Development of *Colletotrichum* isolates was determined on PDA+B plates and then the plates were sampled randomly from the three levels of benomyl resistance. The fungi were mounted on slides and observed microscopically.

#### The frequency of chemical application and fungal resistance

Chemical use and frequency of application in the orchard were determined from interviews with growers.

## Results and Discussion

#### Isolation and collection of *Colletotrichum* spp.

Seventy-eight isolates of *Colletotrichum* spp. were obtained from rambutan, 36 isolates from the eastern part and 42 from the southern part of Thailand. Thirty-one isolates of *Colletotrichum* spp. were obtained from mangoes.

Two different types of *Colletotrichum* isolates were obtained. Type I, colony cottony with delicate aerial mycelia which appeared grey in colour with orange concentric rings of conidial masses. It was isolated from both mango and rambutan. Type II, col-

ony submerged with white mycelia, numerous conidia formed in masses which appeared brown or greenish-brown. It was isolated only from rambutan.

#### Mycelium growth on potato dextrose agar supplemented with benomyl

The fungal isolates obtained from rambutan in the east showed high and moderate resistance to 10 ppm. benomyl concentration, with the percentages of 5.33 and 2.78 respectively. High and moderate resistance to 5 ppm was also present — percentages of 11.11 and 5.56 respectively (Table 1). Rambutan isolates of *Colletotrichum* spp. obtained from the south were moderately resistant to 5 ppm of benomyl concentration (Table 2). *Colletotrichum* isolates obtained from mango showed moderate resistance to 1 ppm of benomyl concentration. None showed resistance to 5 or 10 ppm (Table 3).

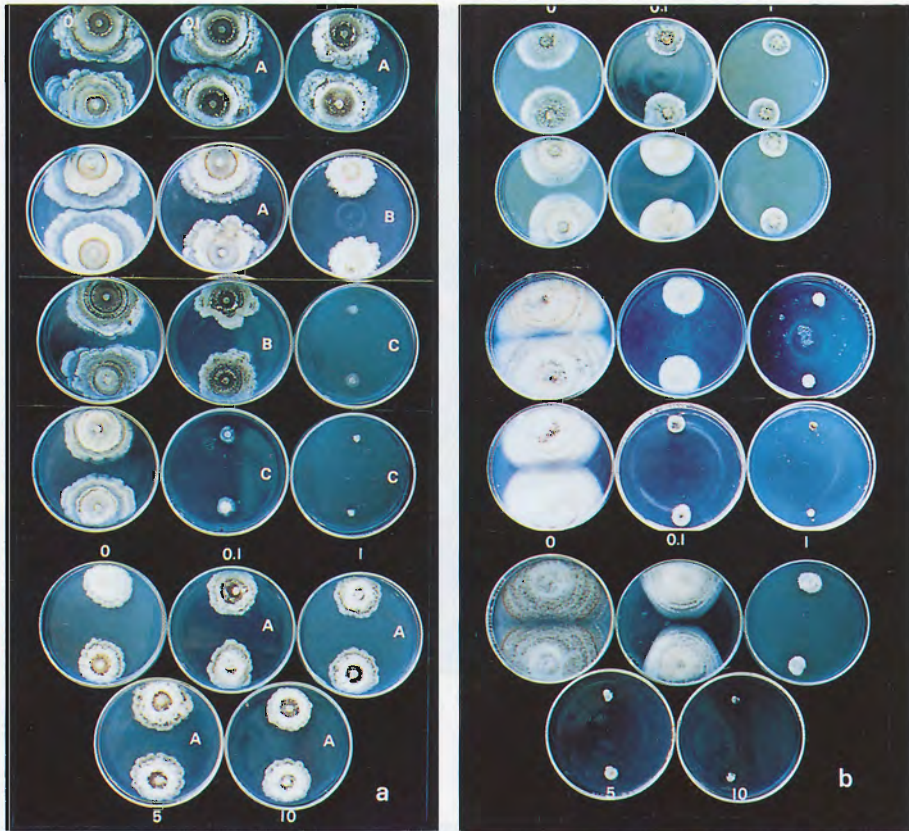
#### Colonial characteristics of *Colletotrichum* isolates on potato dextrose agar supplemented with benomyl

Normal or slightly abnormal colony formation with reduced growth rate was found in highly resistant isolates. The abnormality in colony and growth rate was increased in moderately resistant isolates (Fig. 1). Abnormal colony formation was very distinct in the sensitive isolates, which were grown on PDA+B, and sometimes no growth was obtained. The abnormalities were presented as rugged colonies consisting of fruiting bodies and conidial masses with or without white-rough-cottony mycelia (Fig. 2). Microscopic observation showed normal spore formation of resistant isolates; abnormal conidia together with abnormal fruiting bodies, and some-

Table 1. Benomyl resistance of *Colletotrichum* isolates obtained from rambutan collected from the east of Thailand.

Location	No. of tested isolates	Resistance level <sup>a</sup>	No. of fungal isolates at benomyl concentrations of (ppm)			
			0.1	1.0	5	10
Chanthaburi	24	A	6	3	2	1
		B	9	1	1	1
		C	9	20	21	22
Trat	12	A	4	3	2	2
		B	3	1	1	0
		C	5	8	9	10
Total	36	A	10	6	4	3
		B	12	2	2	1
		C	14	28	30	32

<sup>a</sup> A = highly resistant; B = moderately resistant; C = sensitive.

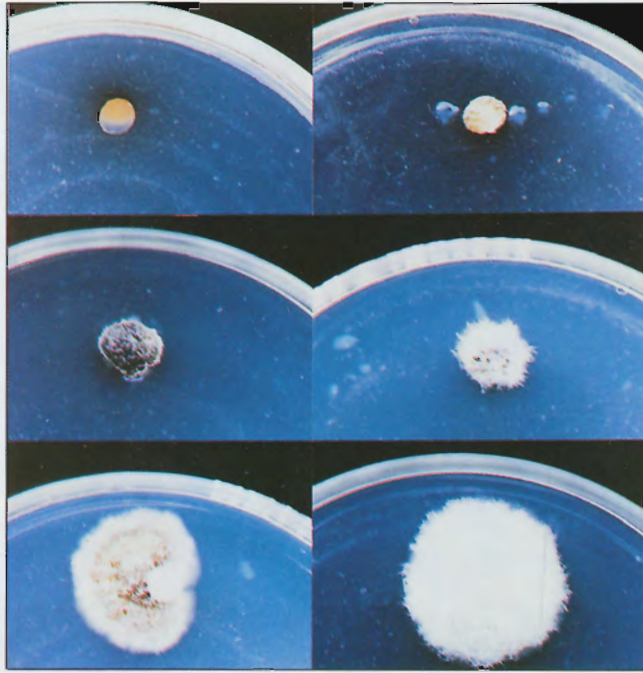


**Figure 1.** Benomyl resistance of *Colletotrichum* spp. isolated from rambutan (left) and mango (right). Resistance level: A= highly; B= moderately; and C= sensitive.

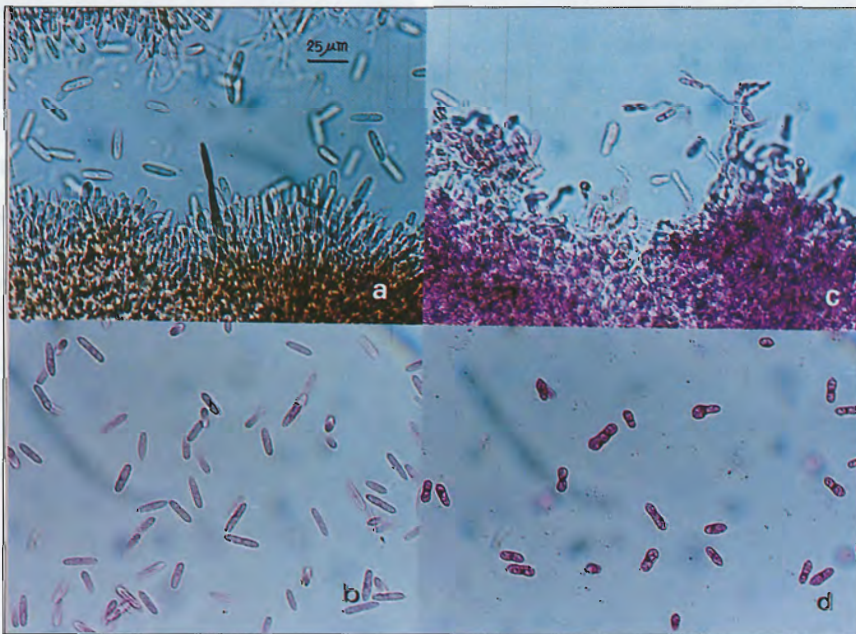
**Table 2.** Benomyl resistance of *Colletotrichum* isolates obtained from rambutan collected from the south of Thailand.

Location	No. of tested isolates	Resistance level <sup>a</sup>	No. of fungal isolates at benomyl concentrations of (ppm)			
			0.1	1.0	5	10
Chumphon	27	A	2	1	0	0
		B	4	1	1	0
		C	21	25	26	27
Nakhon Si Thammarat	15	A	1	1	0	0
		B	1	0	0	0
		C	13	14	15	15
Total	42	A	3	2	0	0
		B	5	1	1	0
		C	34	39	41	42

<sup>a</sup> A = highly resistant; B = moderately resistant; C = sensitive.



**Figure 2.** Abnormal colonies of *Colletotrichum* isolates formed on PDA+B.



**Figure 3.** Acervuli and conidia of *Colletotrichum* spp. Type I, isolated from mango, formed on (a,b) PDA and (c,d) PDA+B.



**Table 3.** Benomyl resistance of *Colletotrichum* isolates obtained from mango collected from central Thailand.

Location	No. of tested isolates	Resistance level <sup>a</sup>	No. of fungal isolates at benomyl concentrations of (ppm)			
			0.1	1.0	5	10
Chon Buri	6	B	4	2	0	0
Nakhon Pathom	6	B	1	1	0	0
Nakhon Ratchasima	13	B	3	0	0	0
Ratchaburi	6	B	3	1	0	0
Total	31	B	11	4	0	0
		C	20	27	31	31

<sup>a</sup> B = moderately resistant; C = sensitive.

times no spore formation of sensitive isolates on benomyl-supplemented PDA (Figs 3 and 4). The frequency of chemical application on fungal resistance

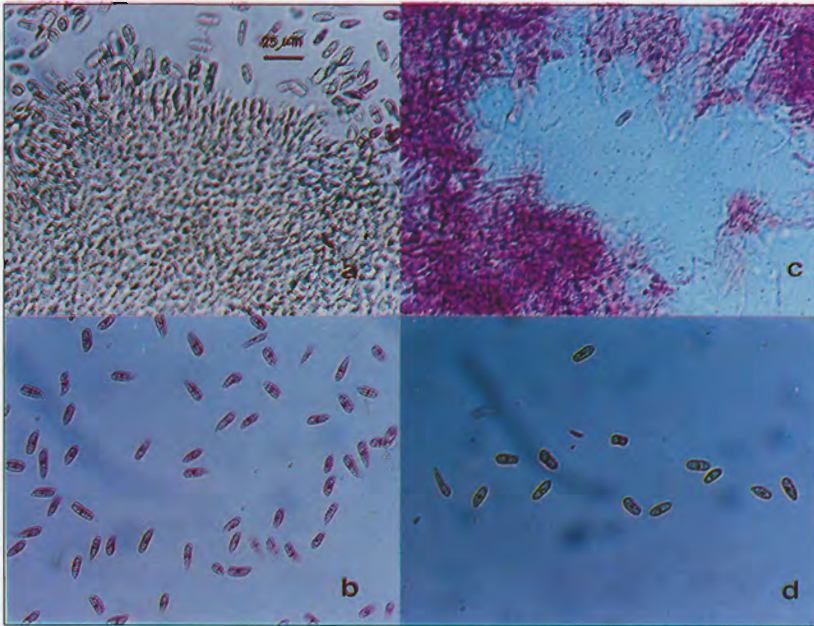
Resistant isolates of *Colletotrichum* spp. were developed naturally under conditions of continuously applied chemical. Resistance percentages were 58.89, 77.78, 66.67 and 44.45 to 0.1, 1, 5 and 10 ppm benomyl concentrations, respectively. The percentages of resistance isolates developed under seldomly applied chemical were 51.85 and 3.63 to 0.1 and 1 ppm, respectively but zero to 5 and 10 ppm benomyl concentrations (Table 4). Populations of *Colletotrichum*

spp. obtained from diseased rambutan fruits from the orchards with continuous fungicide application were less than those from the orchards where chemical was seldomly applied. However, higher percentages of chemical resistance of *Colletotrichum* isolates were obtained from the continuously applied orchards than from the others. It showed that continuous application enhanced fungal pathogen development against chemical fungicides as reported by many researchers (Griffiee 1973; Ogawa et al. 1983). Resistance was frequently found to be associated with isolates with white strong mycelia and brown or greenish-brown conidial masses (Type II). This is the

**Table 4.** Effects of application frequency on benomyl resistance of *Colletotrichum* isolates obtained from rambutan from eastern Thailand.

Location	Application frequency	No. of isolates tested	Resistance level <sup>a</sup>	No. of fungal isolates at benomyl concentrations of (ppm)			
				0.1	1.0	5	10
Chanthaburi	Continuous	5	A	3	3	2	1
			B	1	0	1	1
	Seldom	19	A	3	0	0	0
			B	8	1	0	0
Trat	Continuous	4	A	3	3	2	2
			B	1	1	1	0
	Seldom	8	A	1	0	0	0
			B	2	0	0	0
			A+B	8	7	6	4
			A+B	14	1	0	0

<sup>a</sup> A = highly resistant; B = moderately resistant; C = sensitive.



**Figure 4.** Acervuli and conidia of *Colletotrichum* spp. Type II, isolated from rambutan, formed on (a,b) PDA and (c,d) PDA+B.

first report in Thailand on the resistant development of *Colletotrichum* spp. against chemical fungicide. This indicates that chemical fungicides must be used with caution.

### Acknowledgment

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# Rambutan Postharvest Diseases in Thailand

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

## Abstract

Mature rambutan fruits were collected from growing areas in the east and south of Thailand and stored at 13°C or 25°C for 17 days. Percentage of disease incidence at 13°C was reduced and shelflife (as 50% of fruit remaining disease-free) was doubled as compared with 25°C storage.

The pathogenic fungi isolated from infected fruits were *Colletotrichum gloeosporioides* (two types), *Gliocephalotrichum bulbilium*, *Lasiodiplodia theobromae*, *Pestalotiopsis* sp., *Phomopsis* sp. and *Glomerella cingulata*. The spectrum of the fungi varied with storage temperature. *C. gloeosporioides* and *L. theobromae* could severely infect other kinds of fruits.

Chemical postharvest treatment gave unsatisfactory results in controlling disease. Further studies of biological control techniques for postharvest treatments are therefore warranted.

RAMBUTAN (*Nephelium lappaceum* Linn.) is a native tree fruit of Southeast Asia; Thailand is the leading producer. Major producing areas are located in the east and south. Fresh fruit is a high value export. Fruit rot affects shelf life of rambutan. It is caused by various fungal pathogens (Chayasombat and Sangchote 1983; Visarathanonth 1988). The aim of this research is to determine: the loss due to disease; the effect of low-temperature storage on species and development of fungal pathogens; and postharvest handling treatments.

## Materials and Methods

### Pathogen biology

Experiments were conducted during 1990. Areas examined were the rambutan growing areas in the east and south of Thailand.

### Fruit rot pathogens and disease incidence, and storage temperature

Mature rambutan fruits were collected, making sure they did not touch the ground, packed in carton boxes (50 fruit/box), and stored at different temperatures, 13 and 25°C (eight boxes each). After 3 days

storage, the numbers of decayed fruits were recorded every 2 days and fruit rot pathogens were isolated. The isolated fungi were identified based on culture and microscopic characteristics and on literature reports (Sangchote et al. 1983).

### Pathogenicity of rambutan fruit rot fungi

To determine pathogenicity of the species obtained, wound inoculations on detached mature rambutan and five other kinds of tropical fruit (banana cv. Hom-Thong [*Musa sapientum* L.], banana cv. Nam-Wah [*M. sapientum* L.], guava [*Psidium guajava* L.], papaya [*Carica papaya* L.], and rose apple [*Eugenia* sp.]) were conducted and symptoms allowed to develop at room temperature.

### Control measure

#### Chemical control

For postharvest control of fruit rot disease, chemical control was determined. Carbendazim, imazalil, iprodione and thiabendazole at a concentration of 500 ppm were applied as preharvest sprays to attached fruits 1 week before harvest. A postharvest dip was also tested, using chemicals including benomyl, imazalil, iprodione, and thiabendazole at a concentration of 500 ppm with 2 minutes dipping. Later, the concentration was increased to 750 ppm and dipping to 5 minutes.

#### Biological control

Flushing leaves, fruit at various maturity stages, and dew were collected. The plant parts were shaken

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for 15 minutes in a normal saline solution to obtain surface microorganisms in suspension. The suspensions were diluted and spread on NYDA-plates (nutrient–yeast–dextrose agar) (Wilson and Chalutz 1989; Janisiewicz 1986). The colonies developed on NYDA-plates were 80% randomly collected and primary tested for their antagonistic activities on dual-culture against two types of *C. gloeosporioides* on PDA-plates (potato dextrose agar).

## Results and Discussion

### Pathogen biology

#### *Fruit rot pathogens and disease incidence was affected by storage temperature*

Various fungal species were isolated, including two types of *Colletotrichum gloeosporioides*, *Glioccephalotrichum bulbilium*, *Lasiodiplodia theobromae*, *Pestalotiopsis* sp., *Phomopsis* sp. and *Glomerella cingulata* (Figs 1–7). The spectrum of the fungi varied with storage temperature. The incidence of *C. gloeosporioides* (42.1 and 43.4%), *G. bulbilium* (2.9 and 3.4%) and *L. theobromae* (10.3 and 13.5%) were similar in both storage temperatures. However, the incidence of *Pestalotiopsis* sp. was increased (11.9 to 21.9%) while that of *Phomopsis* sp. decreased (30.4 to 22.7%) with decreasing storage temperature (Table 1). Percentage of disease incidence at 13°C was approximately two times lower than at 25°C over the same period (Fig. 8).

#### *Pathogenicity of rambutan fruit rot fungi*

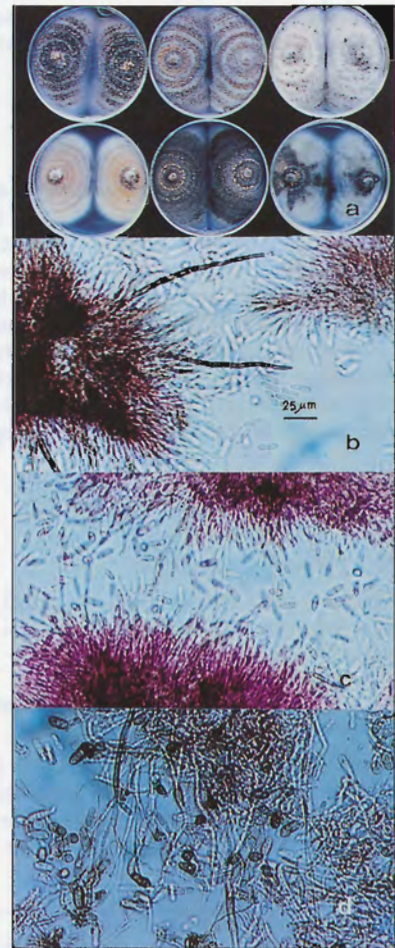
Wounded rambutan fruits were infected by all of the fungal isolates. Severe infection was caused by *C. gloeosporioides*, and *L. theobromae*, while *G. bulbilium*, *Pestalotiopsis* sp., and *Phomopsis* sp. caused moderate infection (Table 2). Banana cv. Hom-Thong, Banana cv. Nam-Wah, guava, papaya, and rose apple were infected by *C. gloeosporioides* Type I, *L. theobromae*, *Pestalotiopsis* sp. and *Phomopsis* sp. with various severities. *C. gloeosporioides* Type II caused moderate infection on banana cv. Hom-Thong and rose apple but did not infect guava and papaya. *G. bulbilium* caused moderate infection on banana cv. Hom-Thong and rose apple, slight infection on guava, but no infection on banana cv. Nam-Wah and papaya (Table 3). Most of the fungal species caused similar disease symptoms on rambutan — dark-brown to black lesions. Distinct symptoms were produced by *L. theobromae* and *G. bulbilium* as — grey and light-yellowish cottony mycelium on the infected fruits, respectively (Fig. 9). On the other five kinds of fruit, distinct symptoms were produced by *C. gloeosporioides* Type I as concentric orange conidial masses; *L. theobromae* as light to dark grey cottony

mycelia and/or pycnidia; and *Phomopsis* sp. as white mycelia with dark-grey pycnidia (Figs 10–12).

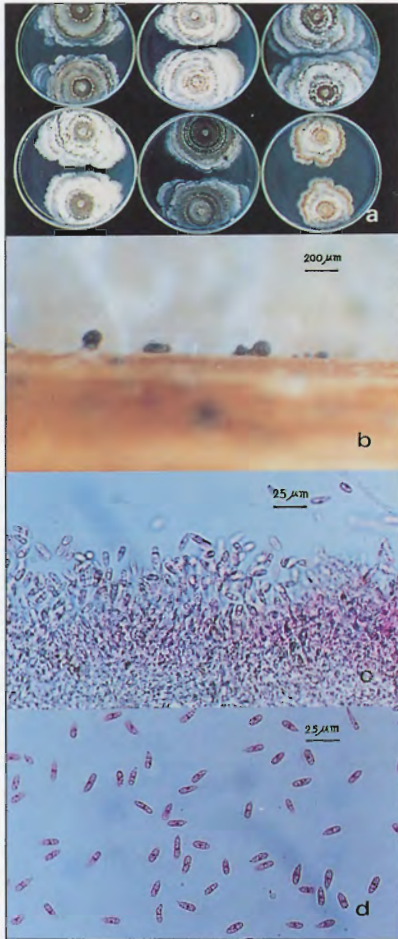
### Control measure

#### *Chemical control*

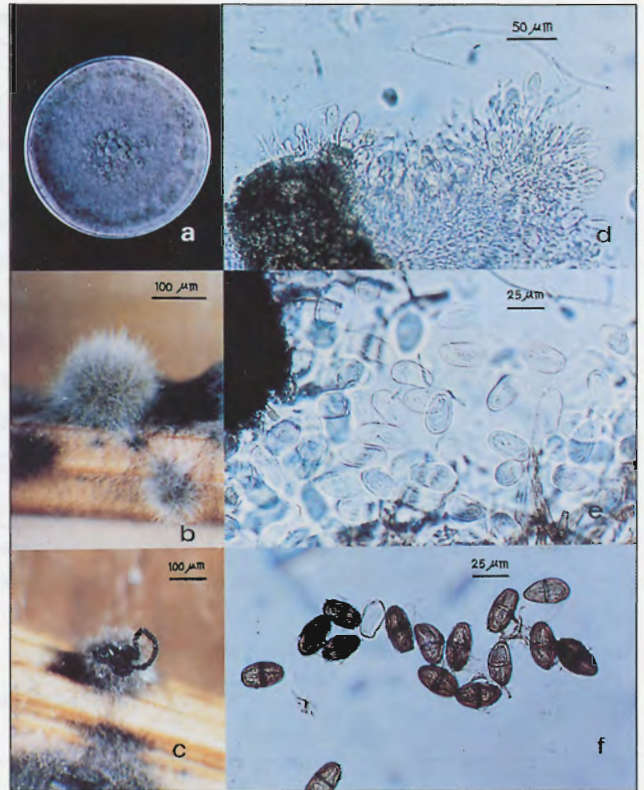
Preharvest chemical application could slightly reduce postharvest disease incidence. Carbendazim was the most effective chemical. The disease incidence was reduced to 32.63% as compared to 52.89% in unsprayed fruits (Table 4). Unsatisfactory control was obtained from postharvest chemical treatments.



**Figure 1.** *Colletotrichum gloeosporioides* Type I: (a) colonies on PDA; (b) acervuli with setae; (c) acervuli without setae; (d) appressoria.



**Figure 2.** *Colletotrichum gloeosporioides* Type II: (a) colonies on PDA; (b), (c) acervuli without setae; (d) conidia.



**Figure 3.** *Lasiodiplodia theobromae*: (a) colony on PDA; (b) young pycnidia; (c) conidia-releasing pycnidium; (d) conidia forming in pycnidia; (e) young conidia; (f) mature conidia.

### Biological control

Various colony types of bacteria were observed on NYDA-plates. The dominant types were: a) small orange convex colonies, approximately 5 mm diameter with limited growth; b) extended polish-bright yellow colonies with very fast growth; c) small white-milky colonies approximately 2 mm diameter, slow growth; d) big polish-creamy colonies approximately 10 mm diameter; and e) extended matted-creamy coloured colonies. Most antagonistic activity shown in this experiment was of the antibiotic type.

Fungal species was unaffected either by storage temperature or producing area. The development of some pathogens, including *Pestalotiopsis* sp. and *Phomopsis* sp., was influenced by storage tempera-

ture. Disease development was delayed by 13°C storage due to the retardation of fungal development (Johnson and Cooke 1990; Johnson et al. 1990, 1991). However, low temperature storage may be expensive (Wilson and Pusey 1985). Several fungal species detected in this experiment have been reported previously (Chayasombat and Sangchote 1983; Visarathanonth 1988), but *Glomerella cingulata* was a new species detected on rambutan. The classification of *C. gloeosporioides* Type II needs to be clarified on its morphology, pathogenicity, and other biological properties compared to Type I (Farungsang and Farungsang 1991). *C. gloeosporioides* and *L. theobromae* were the most virulent pathogens on rambutan.

**Table 1.** Population of rambutan fruit rot fungi affected by storage temperature

Location <sup>a</sup>	Storage temperature (°C)	Population (%) <sup>b</sup>				
		C	G	L	Pe	Po
Chanthaburi	13	38.4	4.4	10.8	19.2	22.4
	25	42.8	3.6	11.2	12.0	28.0
Trat	13	56.7	1.3	8.7	26.0	20.0
	25	47.3	1.3	18.0	12.7	36.7
Chumphon	13	41.5	4.9	8.8	19.1	26.2
	25	35.6	3.2	6.0	9.6	40.3
Nakhon Si Thammarat	13	38.6	0.0	9.0	18.9	30.1
	25	45.1	0.0	18.3	7.9	25.6
Mean	13	42.1	2.9	10.3	21.9a	22.7x
	25	43.4	3.4	13.5	11.9b	30.4y

<sup>a</sup> Mean of five sublocations in Chanthaburi; three sublocations in Trat; and two sublocations in Chumphon and Nakhon Si Thammarat.

<sup>b</sup> C = *C. gloeosporioides* (I+II); G = *G. bulbilium*; L = *L. theobromae*; Pe = *Pestalotiopsis* sp.; and Po = *Phomopsis* sp.

**Table 2.** Pathogenicity of isolated fungi on rambutan

Fungal pathogens	Infection diameter (mm) <sup>a</sup> after		
	3 days	5 days	7 days
<i>C. gloeosporioides</i> <sup>b</sup>	17.2	53.9	81.5
<i>G. bulbilium</i>	8.4	18.3	51.8
<i>L. theobromae</i>	87.5	90.0	90.0
<i>Pestalotiopsis</i> sp.	4.8	15.3	45.4
<i>Phomopsis</i> sp.	4.0	25.8	57.4

<sup>a</sup> Average of four isolates (40 fruits/isolate).

<sup>b</sup> *C. gloeosporioides* (two types).

**Table 3.** Pathogenicity of rambutan fruit rot fungi on some kinds of tropical fruit

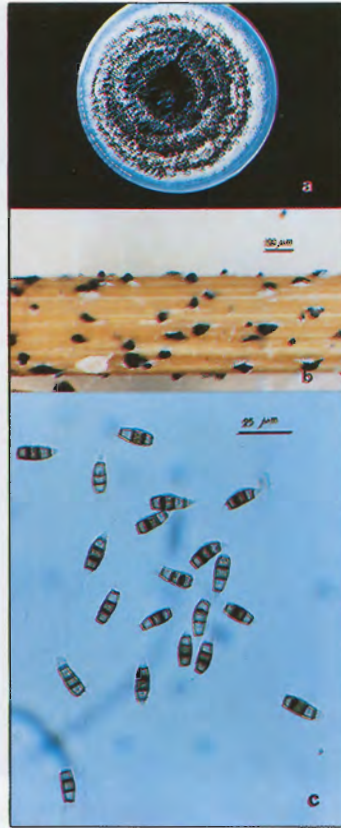
Fungal pathogens	Infection diameter (mm) <sup>a</sup> on				
	Rose apple	Banana (Hom-Thong)	Banana (Nam-Wah)	Guava	Papaya
<i>C. gloeosporioides</i> I	30.6b	38.2b	30.9b	16.6bc	15.8b
<i>C. gloeosporioides</i> II	15.3c	31.7b	0.0e	0.0d	0.0c
<i>G. bulbilium</i>	15.4c	48.3b	0.0e	10.9c	2.4c
<i>L. theobromae</i>	128.0a	137.7a	84.4a	85.9a	49.2a
<i>Pestalotiopsis</i> sp.	18.3c	37.5b	3.9d	21.0b	13.9b
<i>Phomopsis</i> sp.	31.8b	35.7b	16.7c	25.8b	17.4b

<sup>a</sup> Average of three isolates (10 fruits/isolate) at 5 days after inoculation.

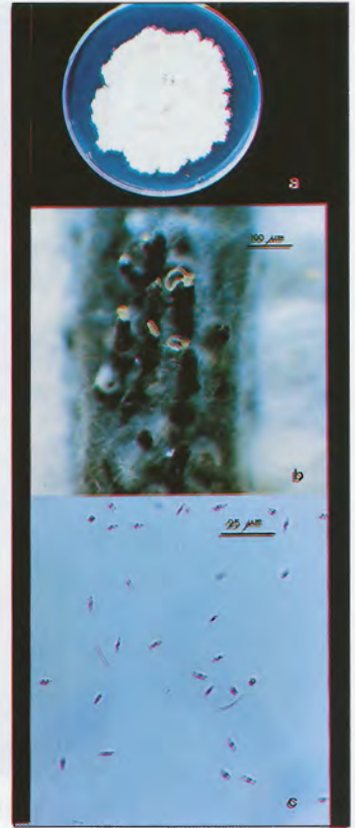
<sup>b</sup> Averages followed by the same letter are not significantly different.



**Figure 4.** *Glocephalotrichum bulbilium*: (a) colony on PDA; (b) conidial heads; (c) penicillate conidiophores and conidia.



**Figure 5.** *Pestalotiopsis* sp.: (a) colony on PDA; (b) acervuli; (c) conidia.



**Figure 6.** *Phomopsis* sp.: (a) colony on PDA; (b) conidia-releasing pycnidia; (c) alpha and beta conidia.

**Table 4.** Postharvest disease incidence affected by preharvest chemical treatment.

Treatment	Time interval before harvest	Fruit rot (%)
Untreated	—	52.89
Carbendazim (500 ppm)	1 week	32.63
Imazalil (500 ppm)	1 week	54.06
Iprodione (500 ppm)	1 week	51.27
Thiabendazole (500 ppm)	1 week	41.91

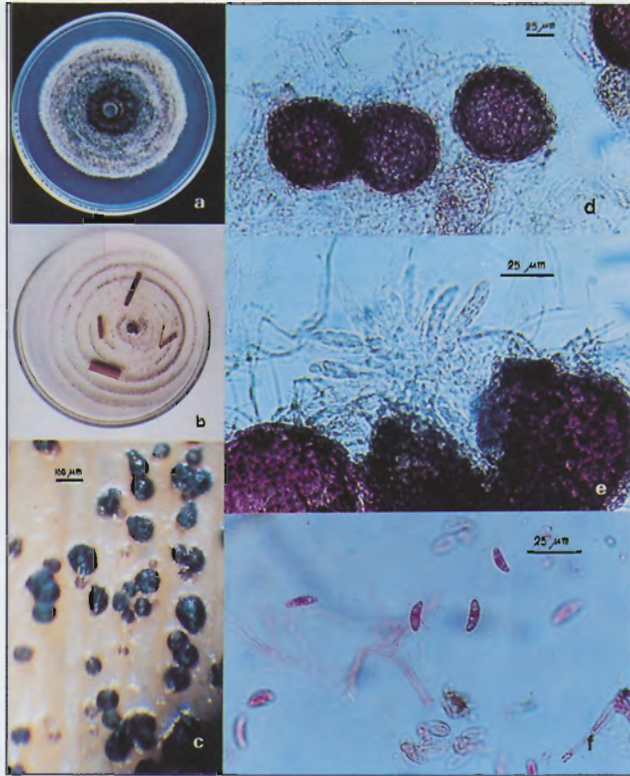
All of the rambutan pathogens could infect five kinds of tropical fruit — except *C. gloeosporioides*

Type II, which was rather host-specific. It could infect banana cv. Hom-Thong and rose apple.

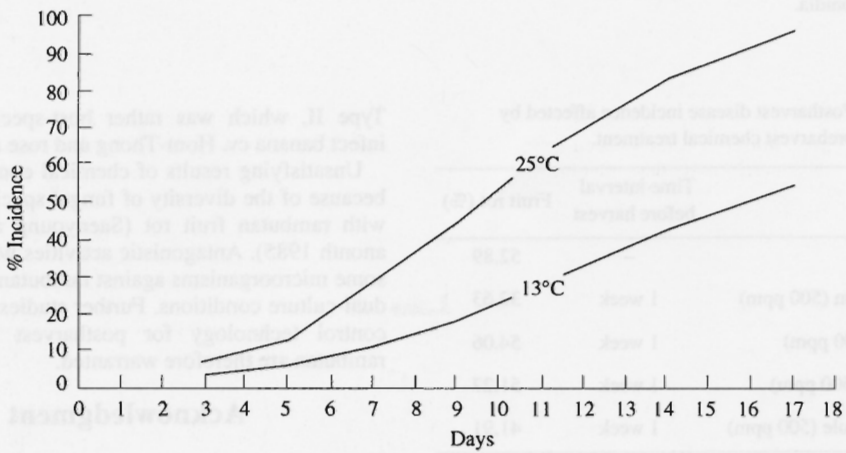
Unsatisfying results of chemical control might be because of the diversity of fungal species associated with rambutan fruit rot (Saenyong and Visarathanonth 1985). Antagonistic activities were shown by some microorganisms against rambutan pathogens in dual-culture conditions. Further studies of biological control technology for postharvest treatment of rambutan are therefore warranted.

### Acknowledgment

This work was conducted as part of a cooperative research program being sponsored by the Australian Centre for International Agricultural Research (ACIAR project 8844).

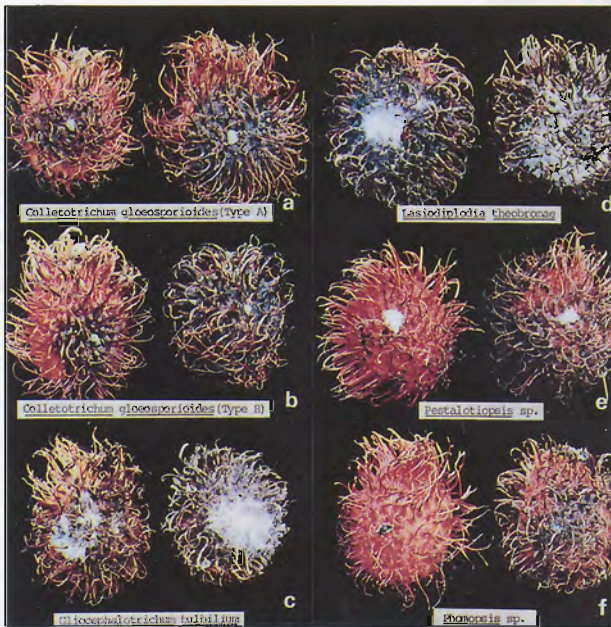


**Figure 7.** *Glomerella cingulata*: (a) colony on PDA; (b) colony on PCA; (c,d) perithecia; (e) asci; (f) ascospores.



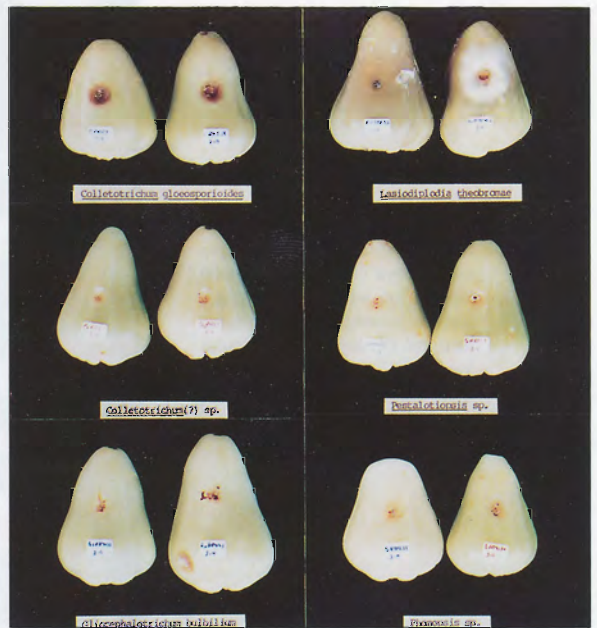
**Figure 8.** Effect of storage temperatures on disease incidence in rambutan.

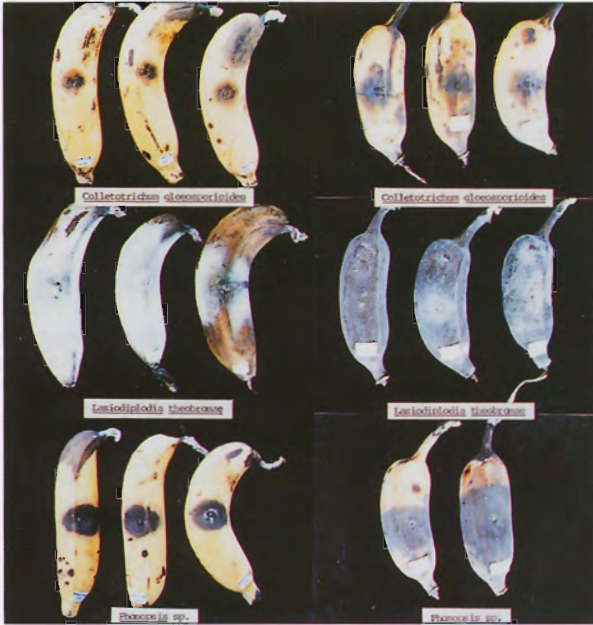




**Figure 9.** Disease symptoms on rambutan caused by artificial inoculation with various fungal species isolated from fruit rot (5 days after inoculation).

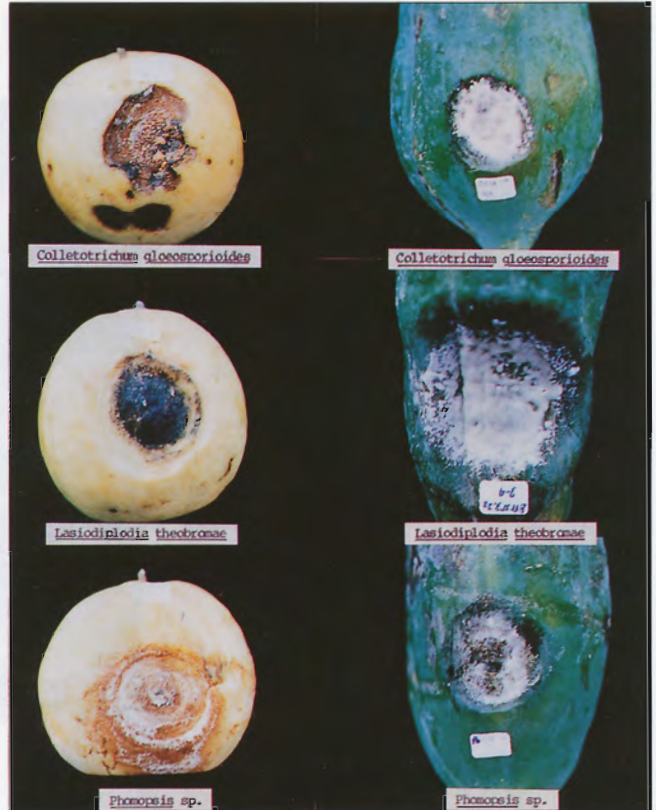
**Figure 10.** Disease symptoms on rose apple caused by artificial inoculation with various fungal species isolated from rambutan fruit rot (3 days after inoculation).





**Figure 11.** Disease symptoms on banana cv. Hom-Thong and banana cv. Nam-Wah, caused by artificial inoculation with various fungal species isolated from rambutan fruit rot (7 days after inoculation).

**Figure 12.** Disease symptoms on guava and papaya caused by artificial inoculation with various fungal species isolated from rambutan fruit rot (7 days after inoculation).



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# Effects of Plant Extracts on the In Vitro and In Vivo Development of Fruit Pathogens

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## Abstract

The antifungal activity of several plants occurring in Thailand were assessed: *Acorus calamus* L.; *Allium sativum* L.; *Centella asiatica* Urban.; *Cyperus rotundus* L.; *Languas galanga* (L.) Stuntz.; and *Rhinacanthus nasutus* Kurz. Twenty grams of each plant were ground and suspended in 100 mL of ethanol for 5 days before filtering to remove plant tissue. Using a rotary evaporator, the extracts were reduced to 10 mL and retained as test concentrates. Potato dextrose agar (PDA) was amended with the test extracts at 0.001, 0.01, 0.1 and 1.0% prior to pouring the plates. The plates were then inoculated with *Lasiodiplodia theobromae*, and incubated at 25–35°C, and mycelial growth was measured daily. Only *Acorus calamus* extract at 1.0% completely inhibited the growth of *L. theobromae*.

The antifungal activity of *A. calamus* was tested in vivo, by immersing longan fruit in 0.5 and 1.0% dilutions. The treated fruit were air-dried and plastic-wrapped in punnets, with fruit dipped in distilled water and undipped fruit serving as control treatments. The treated fruit were then stored at either 25, 10, 5°C and observed every second day. The extract showed no antifungal activity in vivo, with a similar spectrum of fungi to that reported previously associated with surface discoloured areas.

THE skin of longan turn brown and many fungal infections occur during storage. Fumigation of the fruit with SO<sub>2</sub> provides a promising disease control measure. However, there are some alternative methods which can be used to inhibit or delay symptom development, such as benomyl treatment (Chaiwangsri 1992) and cool storage (O'Hare et al. 1990). Treating the fruit with plant extract before storage might be one way to prolong the life of longan. *Lasiodiplodia theobromae* is one of the most prominent pathogens isolated from infected longan during storage (Chaiwangsri 1992). In this experiment the effect of plant extract on this pathogen is examined.

## Materials and Methods

### Effect of plant extracts in vitro

Five species of plant occurring in Thailand were assessed: *Acorus calamus* L.; *Allium sativum* L.; *Centella asiatica* Urban.; *Rhinacanthus nasutus* Kurz.;

*Languas galanga* (L.) Stuntz.; and *Cyperus rotundus* L. Twenty grams of each plant were ground and suspended in 100 mL ethanol (95%) for 5 days, before being filtered through Whatman No. 1 filter paper to remove plant tissue. Using a rotary evaporator, the extracts were reduced to 10 mL and retained as test concentrates. Potato dextrose agar (PDA) was amended with the test extracts at 0.001, 0.01, 0.1 and 1.0% prior to pouring the plates. The plates were inoculated at the centre with *Lasiodiplodia theobromae* and incubated at 25–35°C. Mycelial growth was measured daily. There were five replications for each treatment.

### Effect of plant extracts in vivo

Longan fruits cv. Daw freshly harvested from an orchard, were divided into four groups, with 240 fruit per group. The first group was directly packed in plastic punnets, each punnet containing 16 fruit. The fruit in the second group were dipped in sterile distilled water, air dried and packed in plastic punnets. Fruits in the third and the fourth groups were dipped in the extract of *Acorus calamus* at 0.5 and 1.0% concentrations, respectively, before being air dried and packed into plastic punnets. The fruit were incubated at 25, 10 and 5°C. Five punnets of each group were incubated at each temperature. Skin discoloration was observed every second day. The fungal growth

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on the skin was isolated into pure cultures and identified. Ten fruit from each punnet were sampled to isolate and calculate the frequency of the fungus found.

**Table 1.** Average diameter of *Lasiodiplodia theobromae* colonies on PDA medium amended with different concentrations of various plant extracts.

Plants	Concentration of plant extract (%)			
	1.0	0.1	0.01	0.001
<i>Allium sativum</i> L.	1.46	1.88	1.97	2.58
<i>Acorus calamus</i> L.	0.00	1.77	1.88	2.62
<i>Centella asiatica</i> Urban.	0.88	1.30	1.60	2.22
<i>Rhinacanthus nasatus</i> Kurz.	1.19	1.80	3.35	2.46
<i>Cyperus rotundus</i> L.	1.60	2.38	2.35	2.24
<i>Languas galanga</i> Stuntz.	1.68	2.01	2.23	2.32
Control	1.98	2.40	2.40	1.97

LSD = 0.41.

**Table 2.** The frequency of fungi isolated from longan fruit treated with sterile distilled water or plant extract (*Acorus calamus*) at 0.5 and 1.0% concentrations. The fungi were isolated from four spots of each fruit and 120 fruit per treatment.

Fungal isolates	Before treating	Concentration of plant extract (%) / Storage temperatures (°C)												Total
		0.5			1.0			Distilled water			Control <sup>a</sup>			
		5	10	25	5	10	25	5	10	25	5	10	25	
<i>Pestalotiopsis</i> sp.	6	86	91	87	100	76	105	77	99	137	128	99	115	1206
<i>Lasiodiplodia</i> sp.	18	36	68	126	44	89	119	43	55	129	42	61	126	956
<i>Cladosporium</i> sp.	52	35	56	-	18	38	-	21	38	-	22	59	-	339
<i>Fusarium</i> sp. (1)	-	19	36	36	34	40	1	32	36	4	14	20	16	288
<i>Fusarium</i> sp. (2)	5	28	17	8	22	18	9	32	20	2	2	22	-	185
<i>Penicillium</i> sp.	-	-	-	2	-	-	10	-	-	4	2	-	-	18
<i>Aspergillus niger</i> .	-	-	-	2	-	-	-	-	-	-	2	-	-	4
Unidentified (1)	5	9	-	-	6	-	-	5	1	3	-	6	-	35
Unidentified (2)	-	16	-	-	-	-	-	6	-	-	8	-	-	30
Unidentified (3)	1	-	-	-	-	-	-	-	-	-	-	1	-	2
<b>Total</b>	<b>87</b>	<b>229</b>	<b>268</b>	<b>261</b>	<b>224</b>	<b>261</b>	<b>244</b>	<b>216</b>	<b>249</b>	<b>279</b>	<b>220</b>	<b>268</b>	<b>257</b>	<b>3063</b>

<sup>a</sup>The fruit without any treatment were packed in punnets before storage.

## Results

### Effect of plant extracts in vitro

The test fungus grew well on the agar medium mixed with each of the plant extracts at 0.001% concentration. Inhibition effect was found in the crude extract of *Allium*, *Acorus* and *Centella* at the concentration of 0.01% and higher, while *Rhinacanthus* began to show the effect at 0.1%. Only *Acorus calamus* at the concentration of 1.0% could completely inhibit mycelial growth of the fungus (Table 1).

### Effect of plant extract in vivo

The extract showed no antifungal activity in vivo, with a similar spectrum of fungi to that reported previously. The common ones are *Pestalotiopsis* sp., *Lasiodiplodia theobromae*, *Cladosporium* sp. and *Fusarium* spp. (Table 2). Browning of the skin in treated fruit was the same as in the control groups at both storage temperatures (Table 3).

## Discussion

The experiment showed that the extract of *Acorus calamus* could completely inhibit the growth of *Lasiodiplodia theobromae* in vitro. Korpraditskul et al. (1990) reported that among 61 samples of plant

**Table 3.** Skin browning of longan fruit cv. Daw which had been dipped in 0.5% and 1.0% solution of *Acorus calamus* extract before being packed in plastic punnets and overwrapped with PVC film. The punnets were stored at 5, 10 and 25°C.

Days after storage	Distilled water			0.5%			0.1%			Control		
	5°C	10°C	25°C	5°C	10°C	25°C	5°C	10°C	25°C	5°C	10°C	25°C
2	2.8	4.0	5.0	3.0	4.0	5.4	3.0	4.0	5.0	2.8	4.0	5.0
4	3.4	4.0	7.6	4.0	5.4	7.0	4.0	5.4	7.6	3.0	5.2	7.0
6	3.8	5.6	9.2	4.2	5.8	8.2	4.2	5.8	9.0	3.8	5.4	8.0
8	5.0	7.0	10	5.0	7.2	9.4	5.0	7.2	10	4.8	6.4	9.6
10	5.4	7.6		5.6	8.0	10	5.8	8.0		5.6	8.0	10
12	5.8	8.6		6.4	8.8		6.2	9.0		6.2	8.6	
14	6.2	9.0		7.2	9.2		7.0	9.4		6.4	9.0	
16	6.6	9.6		7.8	9.8		7.6	10		7.0	9.8	
18	7.0	10		8.4	10		8.2			7.4	10	
20	8.2			8.6			8.8			7.6		
22	8.4			9.0			9.2			8.2		
24	9.0			9.2			9.8			9.0		
26	9.4			9.8			10			9.4		
28	9.8			10						9.8		
30	10									10		

extracts, *Acorus calamus* was the most effective in inhibiting the growth of *Colletotrichum gloeosporioides*. However, the extract was ineffective in controlling the growth of fungi on the fruit surface. It is possible that interaction between the extract and chemical residues on the fruit surface may have reduced the efficiency of the extract. More research should be conducted to determine the appropriate method of applying the extract.

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# Mathematical Modelling of Modified Atmosphere Conditions

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## Abstract

In this report, modelling of MA conditions ( $O_2$  and  $CO_2$  concentrations) inside a film package of Nam Dok Mai mango was undertaken. Parameters for PVC, PE and PP films were included. A method of determining respiration function, from the time history of the gas concentrations inside the film package, instead of from direct measurements, was developed.

THE main objective of modified atmosphere (MA) modelling is to predict the atmospheric conditions inside a sealed film package of horticultural produce, as a function of time, given produce parameters such as respiration functions, and film parameters such as surface area and permeability to  $O_2$  and  $CO_2$ . These parameters can then be manipulated in the model to obtain prespecified atmospheric conditions inside the film package to allow for an optimal storage life of the produce. The modelling takes the form of two coupled ordinary differential equations, which can be solved numerically with standard schemes. The film parameters such as permeability to various gases can also be measured readily. It is the respiration rates of the produce, as functions of several variables such as produce maturity, gas concentrations inside the package, and temperature, which require extensive measurements.

## Literature Review

Henig and Gilbert (1975) described the rates of change of  $O_2$  and  $CO_2$  inside a film package of produce by the following two differential equations:

$$\frac{dV_o}{dt} = K_1 A \left( 0.21 - \frac{V_o}{V} \right) - f_1 \left( \frac{V_o}{V}, \frac{V_c}{V} \right) \quad (1)$$

where  $V_o$  and  $V_c$  are volumes of  $O_2$  and  $CO_2$  in the package,  $V$  is the free volume in the package,  $K_1$  and  $K_2$  are permeability ( $mL/m^2/s/Pa$ ) of the film for  $O_2$

$$\frac{dV_c}{dt} = f_2 \left( \frac{V_o}{V}, \frac{V_c}{V} \right) - K_2 A \frac{V_c}{V} \quad (2)$$

and  $CO_2$ , and  $A$  is the surface area of the film. Respiration of the produce is represented by the functions  $f_1$  and  $f_2$ , which describe the  $O_2$  consumption rate and  $CO_2$  production rate of the produce, respectively. These equations are ordinary differential equations which may be solved numerically by a standard scheme such as the Runge-Kutta method. Henig and Gilbert (1975) solved the equations and compared the time history of  $O_2$  and  $CO_2$  volume inside the film package with experiments on tomatoes. They obtained good agreement between prediction and experimental data.

Hayakawa et al. (1975) further developed the model by assuming linear functions of the  $O_2$  consumption and  $CO_2$  production rates of the form

$$R_o = C_1 \left( \frac{V_o}{V} \right) + C_2 \left( \frac{V_c}{V} \right) + C_3 \quad (3)$$

$$R_c = C_4 \left( \frac{V_o}{V} \right) + C_5 \left( \frac{V_c}{V} \right) + C_6 \quad (4)$$

where  $R_o$  and  $R_c$  are rates of  $O_2$  consumption ( $mL/h/kg$ ) and  $CO_2$  production, respectively, and  $C_1, C_2, C_3, C_4, C_5, C_6$  are constants. They then proceeded to solve the equations analytically with the Laplace transform method. Subsequently, they assumed  $C_2 = C_4 = 0$  (that is, the rate of  $O_2$  consumption is dependent on  $O_2$  concentration only, and the rate of  $CO_2$  production is dependent on  $CO_2$  concentration only), to simplify the solutions. Their experiments with field tomatoes supported these assumptions, particularly for the final equilibrium values of  $O_2$  and  $CO_2$  inside the package.

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Plasse and Raghaven (1985) added a leakage term  $L$ , to equations (1) and (2), to account for imperfect gas tightness of film package in actual practice. Deily and Rizvi (1981), assuming constant respiration rates, applied the equations to Crest Haven peach. They concluded that a polymeric film with low permeability to  $\text{CO}_2$  and high permeability to  $\text{O}_2$  was needed for optimum storage. Such a film was not commercially available.

Wade and Graham (1987) showed from basic properties of gas molecules that it would be impossible to obtain a polymeric film with higher permeability to  $\text{O}_2$  than  $\text{CO}_2$ , and that the permeability of the film would always be in the order  $\text{CO}_2 > \text{O}_2 > \text{N}_2$ . They then showed that at equilibrium conditions, the following relation between  $V_o$ ,  $V_c$ ,  $K_1$  and  $K_2$  must hold:

$$\frac{\left(0.21 - \frac{V_o}{V}\right)}{\frac{V_c}{V}} = \frac{K_2}{K_1} \frac{1}{R_Q} \quad (5)$$

where  $R_Q$  is the respiratory quotient.

Cameron et al. (1989) derived  $\text{O}_2$  uptake rate for tomato as a function of  $\text{O}_2$  concentration, using a closed chamber to monitor  $\text{O}_2$  depletion curve with time. They used an equation of the form

$$R_o = C_1 \left[1 - \exp(-C_2 p_o)\right]^3 \quad (6)$$

where  $p_o$  = partial pressure of  $\text{O}_2$  inside the package.

They gave an example of MA package design for tomato, and concluded that to have an equilibrium  $\text{O}_2$  level of 2–4%, the types of plastic film to be used should be poly (1, 3-butadiene), plasticised polyvinyl chloride, or polyethylene vinyl acetate, all of which have sufficiently high permeability to  $\text{O}_2$ . They also found that, for tomato, there was a degree of flexibility in terms of film area per fruit weight, which allowed for a nearly two-fold increase in fruit weight for a given film while still maintaining the  $\text{O}_2$  concentration inside the package between 2–4%.

## Materials and Methods

In order to be able to predict a modified atmosphere (MA) condition inside a film package of produce and thereby manipulate the film parameters to obtain the desired MA conditions, the respiration rate of the produce must be known. This respiration rate is a function of at least the MA conditions surrounding the produce, particularly the concentrations of  $\text{O}_2$  and  $\text{CO}_2$  gases, as well as the produce temperature.

The work reported here is therefore divided into two major parts:

a) development of a technique to estimate the respiration rate as a function of  $\text{O}_2$  and  $\text{CO}_2$  concentrations, based on the changes in  $\text{O}_2$  and  $\text{CO}_2$  levels inside the MA package with time; and

b) development of a computer program to solve equations (1) and (2) by numerical integration, and using the program to design sample MA packages for Num Dok Mai mango.

## Determination of Respiration Functions

### Mathematical analysis

To illustrate this method, consider the transient behaviour of  $\text{O}_2$  and  $\text{CO}_2$  concentrations inside a film package of produce as shown in Fig. 1. The initial values of  $\text{O}_2$  and  $\text{CO}_2$  concentrations are approximately 0.21 and 0.0003 atm respectively. As the storage time proceeds,  $\text{O}_2$  concentration will drop and  $\text{CO}_2$  concentration will increase, due to respiration activity of the produce. At some time further on, the equilibrium values of  $\text{O}_2$  and  $\text{CO}_2$  concentrations will be reached, when the respiration activity has been adjusted to match the inflow of  $\text{O}_2$  and the outflow of  $\text{CO}_2$  through the film. The shape of the curves will depend on the respiration functions of oxygen uptake ( $R_o$ ) and  $\text{CO}_2$  production ( $R_c$ ). If we measure the  $\text{O}_2$  and  $\text{CO}_2$  concentrations inside the film package at regular intervals, we will have a set of  $N$  data points from time  $t_1, \dots$  to  $t_N$ .

We then approximate the differential equations (1) and (2) by a numerical scheme, say, a simple finite difference:

$$\frac{dV_o}{dt} \approx \frac{V_{o,n+1} - V_{o,n}}{\Delta t} \quad (7)$$

$$\frac{dV_c}{dt} = \frac{V_{c,n+1} - V_{c,n}}{\Delta t} \quad (8)$$

where the subscripts  $n$  and  $n+1$  refer to values at time  $n$  and  $n+1$  respectively, and  $\Delta t$  is simply  $t_{n+1} - t_n$ .

We also have to assume forms of the respiration functions  $f_1$  and  $f_2$ , for example along the lines of equations (3) and (4),

$$f_1 = w \cdot R_o = w \{C_1(p_o) + C_2(p_c) + C_3\} \quad (9)$$

$$f_2 = w \cdot R_c = w \{C_4(p_o) + C_5(p_c) + C_6\} \quad (10)$$

where  $p_o$ ,  $p_c$  are partial pressures of  $\text{O}_2$  and  $\text{CO}_2$  respectively inside the package.

If we then substitute (6)–(9) into equations (1) and (2), we will get



$$\frac{V_{o,n+1} - V_{o,n}}{\Delta t} \approx K_1 A (0.21 - p_o) - w \{C_1(p_o) + C_2(p_c) + C_3\} \quad (11)$$

$$\frac{V_{c,n+1} - V_{c,n}}{\Delta t} \approx w \{C_4(p_o) + C_5(p_c) + C_6\} - K_2 A (p_c) \quad (12)$$

From  $N$  sets of experimental data points, we will have  $N - 1$  equations of the  $O_2$  uptake (11) and  $N - 1$  equations of the  $CO_2$  production (12). In each set of equations, all constants  $C_1$ ,  $C_2$  and  $C_3$  are for the  $O_2$  uptake equation, and  $C_4$ ,  $C_5$  and  $C_6$  for the  $CO_2$ . The respiration functions  $f_1$  and  $f_2$  will therefore be determined from equations (9) and (10).

The advantage of this method is that the measurements required are relatively straightforward, needing only  $O_2$  and  $CO_2$  gas analysers. In many cases these measurements are already a part of modified atmosphere (MA) studies. It does not need elaborate controlled atmosphere (CA) respiration rate equipment.

The forms of the respiration function as shown in equations (8) and (9) are only examples. There can be many other forms, for example:

a power function

$$R_o = C_1 (p_o)^{c_2} \quad (13)$$

an exponential function

$$R_o = C_1 \exp(p_o C_2) \quad (14)$$

and others. However, in concocting these various functional forms, two important points must be kept in mind:

a) the functions should yield normal respiration rates of the produce in air when  $p_o = 0.21$  and  $p_c = 0.0003$  are substituted into them; and

b) the respiration rate should approach a constant value (asymptotic) as time increases and equilibrium conditions are approached.

In this work, we have tested the following three forms of respiration functions for Nam Dok Mai mango:

$$(a) \quad R_o = C_1 p_o + C_2 p_c + C_3 \quad (15)$$

$$R_c = C_4 p_o + C_5 p_c + C_6 \quad (16)$$

$$(b) \quad R_o = \frac{C_1}{t\rho} \left( \frac{p_o}{p_c} \right)^{c_2} \quad (17)$$

$$R_o = \frac{C_1}{t\rho} \left( \frac{p_o}{p_c} \right)^{c_4} \quad (18)$$

$$(c) \quad R_o - R_{o,a} = \frac{C_1}{t\rho} \left( \frac{p_o}{p_c} \right)^{c_2} \quad (19)$$

$$R_c - R_{c,a} = \frac{C_3}{t\rho} \left( \frac{p_o}{p_c} \right)^{c_4} \quad (20)$$

where  $R_{o,a}$  and  $R_{c,a}$  are the  $O_2$  uptake and  $CO_2$  production rates in normal atmosphere, which change with time, and  $\rho$  is the density of the mango (a maturity index).

### Experimental verification

In the first experiment, Num Dok Mai mangoes were individually packaged in test chambers with three types of films (Table 1).

**Table 1.** Plastic films used in experiments.

Type	$O_2$ permeability (mL/h/m <sup>2</sup> /atm)	$CO_2$ permeability (mL/h/m <sup>2</sup> /atm)
PVC	1360	7359
PE	1274	2516
PP	354	562

The mangoes were pre-treated with 500 ppm benomyl solution at 52°C before being packaged, then stored at 13°C. Two sizes of film surface area and four replications were employed, giving a total of 24 experiments (24 fruits) and about 300 data points before the  $O_2$  level dropped below 5%, which was taken as the cut off point for aerobic respiration. Oxygen and  $CO_2$  concentrations inside the packages were sampled every 12 hours in the early stage, and every 24 hours in the later stage.

In the second experiment, three mangoes were packaged in each chamber, to average out the fruit-to-fruit variation. Only one type of film, PVC, was used, and a parallel experiment using a completely closed chamber (zero permeability) was conducted. Three replications were employed, giving a total of six experiments (18 fruits). Gas was sampled every 3 hours. Stirrers were used to completely mix the gas inside the chambers before each sampling. A total of about 100 data points were collected after 4 days.

## Results

The resulting correlations for Nam Dok Mai mango were

$$(a) \quad R_o = -5.45 \times 10^{-7} p_o - 7.03 \times 10^{-7} p_c + 1.87 \times 10^{-5} \quad (21)$$

$$R_c = 6.32 \times 10^{-7} p_o + 5.63 \times 10^{-7} p_c + 1.83 \times 10^{-5} \quad (22)$$

$$(b) \quad R_o = \frac{0.323}{tp} \left( \frac{P_o}{P_c} \right)^{-0.611} \quad (23)$$

$$R_c = \frac{0.353}{tp} \left( \frac{P_o}{P_c} \right)^{-0.783} \quad (24)$$

$$(c) \quad R_o = R_{o,a} - \frac{2.42}{tp} \left( \frac{P_o}{P_c} \right)^{-0.75} \quad (25)$$

$$R_c = R_{c,a} - \frac{1.32}{tp} \left( \frac{P_o}{P_c} \right)^{-1.278} \quad (26)$$

The last set of equations (25, 26) showed the highest goodness of fit ( $r^2=0.89$  for  $R_c$ ). This is quite satisfactory considering the number of fruits used and the fruit-to-fruit variation involved.

In addition, other methods of correlation were tested in an attempt to improve the correlation. These included a change to a second-order Runge-Kutta method instead of equations (7) and (8) which are first-order approximations of the time derivatives. The resulting equations were more complex, consisting of two time values at  $n$  and  $n+1$ . However, the correlations were not improved. The maturity index,  $\rho$ , was also omitted from the correlating equations in one test; the resulting correlations did not differ significantly from the previous ones, signifying that the effect of  $\rho$  on the goodness of fit was small. Therefore, in the case of Nam Dok Mai mango, the density of the fruit could be excluded as a variable in the correlation.

## Prediction Model and Computer Program

The prediction model is a computer program written in Pascal to solve the governing equations (1) and (2) by the fourth-order Runge-Kutta method. The inputs needed by the program are: package characteristics — film permeability to  $O_2$  and  $CO_2$ , film area, free volume; fruit parameters — fruit weight, fruit respiration functions, maturity index (if applicable); surrounding atmosphere —  $O_2$  concentration (normally

0.21 atm),  $CO_2$  concentration (normally 0.0003 atm); other — simulation time limit.

The user has several choices of respiration functions, selected from a general function form. For example, if the general form of the function is,

$$R_o \text{ or } R_c = C_1 + C_2 p_o + C_3 p_c + C_4 t + C_5 t^2 + \frac{C_6}{t} \left( \frac{P_o}{P_c} \right)^{C_7} \quad (27)$$

By judicious choice of the constants  $C_1$  to  $C_7$ , many forms of respiration function can be selected. For example, setting  $C_4$ ,  $C_5$ ,  $C_6$  to zero would give the linear form (15 and 16), while setting  $C_2$  and  $C_3$  to zero would give the power-function form of equations 19 and 20, with  $R_{o,a} = C_1 + C_4 t + C_5 t^2$  (assuming a quadratic relationship of respiration rate in normal air with time), and  $\rho$  included in the constant  $C_6$ .

The output of the program is the changes in  $O_2$  and  $CO_2$  concentrations in the package with time, until the pre-set simulation time limit is reached. Both numerical and graphical outputs are provided.

To design an MA package for a specific fruit is to come up with an appropriate film area for a certain fruit weight, given a certain type of film. The process is an iterative one, involving the following steps:

a) Select film type ( $O_2$  and  $CO_2$  permeability), free volume, fruit weight per package.

b) Select respiration functions  $R_o$  and  $R_c$ , by setting the appropriate constants in equation (27) to desired values.

c) Decide on the final concentrations of  $O_2$  and  $CO_2$  required at the end of the simulation time, or after equilibrium is reached.

d) Choose a value of film area and begin the program execution. After the calculation is completed, check the resulting final (or equilibrium) values of  $O_2$  and  $CO_2$  concentrations, and adjust the film area. Repeat until a chosen film area gives the required final (or equilibrium) values of  $O_2$  or  $CO_2$  concentrations.

e) If the resulting film area is not practically feasible, change the film type (step a) and repeat step d).

It should be noted that generally it will not be possible to require both  $O_2$  and  $CO_2$  concentrations to reach certain values at equilibrium. By adjusting film area, the program can choose to achieve only one gas concentration (either  $O_2$  or  $CO_2$ ), and the other would follow naturally. This is because a given type of film will have fixed values of permeability to  $O_2$  and  $CO_2$ ; the two permeabilities are not independently variable.

## Conclusions

The work reported here should be considered only as groundwork on indirect respiration rate determination and MA modelling for tropical fruits. Much more work is needed, both experimentally and numerically, to determine the respiration functions and appropriate

MA packages for other tropical fruits, especially those which have been found to respond well to MA storage. The methodology will be basically the same; the main effort will be in finding a good-fitting respiration function form among the various possibilities.

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# Postharvest Diseases of Lychee in Australia, and Their Control

L.M. Coates\*, G.I. Johnson†, U. Sardud§ and A.W. Cooke\*

## Abstract

A range of fungi previously isolated from diseased lychee fruit were tested for pathogenicity in Kwai May Pink and Wai Chee lychee fruit. The pathogenicity of the following genera of fungi was confirmed in both wounded and unwounded fruit: *Phomopsis*, *Phoma*, *Colletotrichum*, *Alternaria*, *Dothiorella*, *Lasiodiplodia*, *Fusarium*, *Stemphylium* and *Curvularia*.

Postharvest sulfur dioxide (SO<sub>2</sub>)-treatment using either SO<sub>2</sub> slow-release pads or SO<sub>2</sub> fumigation was evaluated for disease control in lychee fruit. Sulfur dioxide slow release pads provided good disease control, but caused bleaching of the pericarp. Partial recovery of pericarp colour occurred after removal of the SO<sub>2</sub> slow-release pads. *Penicillium* sp. colonised incompletely-fumigated fruit. Sulfur dioxide fumigation (1% SO<sub>2</sub> for 20 minutes) also provided excellent control of diseases resulting from latent infections of *Colletotrichum* sp. and *Alternaria* sp., although treated fruit were highly susceptible to colonisation by *Penicillium* sp. The application of prochloraz to fruit following SO<sub>2</sub> treatment prevented the development of blue mould (*Penicillium* sp.). Benomyl imazalil and iprodione were all ineffective in controlling blue mould in SO<sub>2</sub>-treated fruit.

Hot benomyl (at 50 or 52°C) and prochloraz treatments were effective in controlling postharvest disease lesions (*Alternaria alternata*, *Phomopsis* sp. and other fungi) on lychees cv. Bengal during storage at 5°C for up to 30 days. Despite its demonstrated disease control efficacy, prochloraz caused tainting of the aril in the thin-skinned cultivar Kwai May Pink. The fungicides imazalil and iprodione did not give acceptable control of postharvest disease in cool-stored Bengal lychees.

THERE is only limited information available on the fungal pathogens which cause postharvest disease in lychee. Prasad and Bilgrami (1973) described 11 fungal pathogens of lychee fruit in India. These included nine virulent pathogens (*Aspergillus* spp., *Botryodiplodia theobromae*, *Colletotrichum gloeosporioides*, *Cylindrocarpon tonkinense* and *Pestalotiopsis* sp., and two mild pathogens (*Penicillium lilacinum* and *Fusarium* sp.). Subsequent studies reported that lychee fruit could also be infected by *Geotrichum candidum* (Tandon and Tandon 1975), *Stemphylium* and *Fusarium* spp. (Tongdee et al. 1982; Roth 1963).

The aim of the study reported here was to identify the pathogens causing postharvest diseases of lychee

in Australia, and to develop effective postharvest treatments for the control of these diseases.

## Pathogens of Lychee Fruit

The pathogenicity of a range of fungal isolates was tested on both unwounded and wounded lychee fruit. In Bengal lychee, the pathogenicity of *Colletotrichum gloeosporioides* (ex rambutan), *C. acutatum* (ex avocado), *Alternaria alternata* (ex lychee) and *Pestalotiopsis* sp. (ex mango) was confirmed. *Dothiorella dominicana* (ex mango), *D. mangiferae* (ex mango) and *Phomopsis* sp. (ex mango and lychee) were not re-isolated from fruit inoculated with these fungi.

In subsequent trials, the pathogenicity of the following genera of fungi, all previously isolated from lychee, was confirmed in both unwounded and wounded Kwai May Pink and Wai Chee lychee fruit: *Phomopsis*, *Phoma*, *Colletotrichum*, *Alternaria*, *Dothiorella*, *Lasiodiplodia*, *Fusarium*, *Stemphylium* and *Curvularia*. These results indicate that lychee fruit are susceptible to attack by a wide range of pathogens which commonly cause disease in a number of other tropical and subtropical fruit crops.

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## Sulfur Dioxide Treatment of Lychee Fruit for Disease Control

Postharvest sulfur dioxide (SO<sub>2</sub>) treatment using either SO<sub>2</sub> slow-release pads or fumigation with gaseous SO<sub>2</sub> was evaluated for disease control in lychee.

Sulfur dioxide slow-release pads provided good disease control, but caused bleaching of the pericarp. Partial recovery of pericarp colour occurred after removal of the SO<sub>2</sub> slow-release pads. *Penicillium* sp. colonised incompletely-fumigated fruit.

Fumigation with gaseous SO<sub>2</sub> (1.2% SO<sub>2</sub> for 20 minutes) also provided excellent control of diseases resulting from latent infections of *Colletotrichum* spp. and *Alternaria* sp., although treated fruit were highly susceptible to blue mould caused by *Penicillium* sp. Loss of pericarp colour caused by fumigation was reversed by dipping fruit in a low pH solution (IN HCl for 1 minute 30 seconds) following SO<sub>2</sub> treatment. The development of blue mould in SO<sub>2</sub>-fumigated lychees was prevented by either: (1) packing fruit in punnets overwrapped with polyethylene film; or (2) dipping fruit in prochloraz. Benomyl, imazalil and iprodione were all ineffective in controlling blue mould.

### Alternatives to Sulfur Dioxide Fumigation for Disease Control in Lychee

Hot benomyl gave good control of postharvest disease (*Alternaria alternata*, *Phomopsis* sp. and other fungi) in Bengal lychees stored at 5°C. Treatment at 50°C was equally as effective as treatment at 52°C. Hot benomyl appeared less effective on Kwai May Pink lychees.

Prochloraz was equally effective as hot benomyl in controlling postharvest disease in cool-stored lychees, although tainting of the aril was detected in Chai May Pink fruit. The fungicides iprodione and imazalil gave inadequate levels of disease control. Cool storage alone (50°C) provided good disease

control for 17 days. Disease incidence and severity increased with storage temperature.

## Conclusions

The results of this study confirm that lychee fruit are susceptible to attack by a wide range of postharvest pathogens, most of which are commonly encountered in a number of other tropical fruit. Sulfur dioxide treatment was found to give effective disease control in lychee, although treated fruit were highly susceptible to blue mould. Postharvest dipping in heated benomyl or unheated prochloraz were also found to give effective disease control.

Hot benomyl treatment (50°C for 2 minutes) is recommended for the control of postharvest disease in lychee fruit. Prochloraz is not recommended for postharvest treatment of lychees because of the risk of tainting.

It is recommended that further research be conducted into the application of postharvest SO<sub>2</sub> treatments for disease control in lychee. In particular, the problem of blue mould development following SO<sub>2</sub> treatment needs to be addressed. Further research should also be conducted into the development of nonchemical treatments for postharvest disease control in lychee, given the strong possibility that fungicides such as benomyl may not be available for postharvest use in the near future.

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# Postharvest Control of Mango Diseases

G.I. Johnson\*, L.M. Coates†, A.W. Cooke† and I.A. Wells§

## Abstract

In Australia, the current recommendation for postharvest control of anthracnose (*Colletotrichum gloeosporioides*) in mango is a 5-minute heated (52°C) benomyl dip or a 30-second unheated overhead spray of prochloraz on the packing line. This research demonstrated the diminishing efficacy of these fungicides during long-term (4–6 weeks) cool or controlled atmosphere storage. When applied alone, both fungicides gave unacceptable control of anthracnose under long-term storage conditions (26 days in 5% O<sub>2</sub>/2% CO<sub>2</sub> at 13°C, then 11 days in air at 20°C). A dual treatment consisting of hot benomyl followed by prochloraz was shown to give superior control of anthracnose under these storage conditions.

It was also shown that rain on fruit at harvest increased disease severity, resulting in reduced efficacy of hot benomyl treatment. Mangoes harvested during wet weather were also more susceptible to brush damage following hot benomyl treatment at 52°C for 5 minutes. Brush damage was reduced or eliminated by applying benomyl at 50°C for 4 minutes. Disease control, however, was not satisfactory. Mangoes harvested in wet weather require careful handling to ensure adequate disease control and to avoid brush damage.

THE two major postharvest diseases of mango are anthracnose (*Colletotrichum gloeosporioides*) and stem-end rot (*Dothiorella dominicana*, *Lasiodiplodia theobromae* and other fungi). In Australia, the current recommendation for the control of these diseases is a 5-minute dip in hot benomyl at 52°C. Where stem-end rot is not a problem, an alternative treatment of unheated prochloraz (applied as a 30-second overhead spray) can be used. Although these treatments have served the mango industry well, research conducted in ACIAR projects 8356 and 8844 have identified some limitations in the application of these treatments. This paper discusses these limitations.

## Disease Control During Long-term Storage

Diminishing efficacy during long-term storage is one of the major problems of the hot benomyl and prochloraz treatments.

When applied alone, both hot benomyl and prochloraz gave unacceptable control of anthracnose

and stem-end rot under long-term storage conditions (26 days in 5% O<sub>2</sub>/2% CO<sub>2</sub> at 13°C, then 11 days in air at 20°C). A dual treatment of hot benomyl followed by prochloraz was shown to give superior control of both anthracnose and stem-end rot under these storage conditions.

## Effect of Rain at Harvest and Benomyl Dip Temperature on Mango Diseases

Comparisons were made between our experimental tank (50 L) in which mangoes were immersed in hot benomyl at 52°C for 5 minutes, and a commercial tank (3600 L) in which mangoes were immersed in hot benomyl at 49.6°C for 4.15 minutes. The experimental tank achieved a higher level of disease control than the commercial tank.

Control of stem-end rot (*Dothiorella* spp.) was acceptable on fruit from both tanks, but control of stem-end lesions caused by *Colletotrichum gloeosporioides* was not acceptable using the commercial tank.

Normally, infections by *Colletotrichum* on the sides of fruit are less deep-seated than those of *Dothiorella* and are easier to control. However, *Colletotrichum* infections at the stem end must have been more deep-seated than those of *Dothiorella*, so that it was harder for the hot benomyl treatment to control them. None of the *Colletotrichum* isolates showed any sign of resistance to benomyl.

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**Table 1.** Effect of brushing, fungicide and dip temperature on quality of Kensington mangoes after cool storage for 24 days.

Treatment; Br - brushing, B - benomyl, P - prochloraz	Skin damage (0-3) <sup>a</sup>	Disease incidence (% fruit)
Experimental tank		
Control	0.30a	91.6g
Br + P	0.59ab	60.0e
B (52°C)	0.78b	37.5b
B (52.1°C) + Br + P	2.40d	21.6a
B (49.5°C) + Br + P	1.47c	45.0d
B (47.9°C) + Br + P	1.20c	45.0d
B (46.3°C) + Br + P	0.81b	40.0c
Commercial tank		
B (49.6°C) + Br + P	0.45a	78.3f

<sup>a</sup> 0 = no damage, 3 = severe damage

Figures followed by the same letter are not significantly different at 5% level.

Spraying fruit with prochloraz improved disease control on fruit dipped in either tank.

Rain on fruit at harvest increased disease severity, reinforcing the need for treating fruit in hot benomyl for the correct time and temperature.

Two benomyl formulations (WP and DF) performed equally well in controlling anthracnose (*Colletotrichum gloeosporioides*) and stem-end rot (*Dothiorella* spp.) during 4 weeks storage of mango.

### Effect of Brushing on Mango Quality

Mangoes harvested during wet weather were susceptible to brush damage following dipping in hot benomyl at 52°C for 5 minutes. Brush damage was reduced or eliminated at lower temperatures but disease control was not satisfactory.

Mangoes harvested in wet weather require careful handling to ensure adequate disease control and avoidance of brush damage. Some growers claim they can avoid brush damage by delaying brushing for 24 hours after dipping. If this is so, we need to know what change occurs in the fruit making it less susceptible to damage. This knowledge might enable us to speed the process up and/or avoid brushing if it is unnecessary on a particular fruit line.

Fruit harvested in wet weather need to be marketed as quickly as possible because of higher disease lev-

els. Cool storage of fruit for more than three days should be avoided.

### Conclusions

This research has highlighted some problems with the current recommendations for postharvest disease control in mango, particularly the hot benomyl treatment. Growers need to be aware of these problems so that they can modify their harvesting, handling and storage practices appropriately. Future research should aim to overcome these limitations in the hot benomyl and prochloraz treatments. In addition, alternative nonchemical treatments need to be developed for postharvest disease control in mango.

While the Australian mango industry currently relies heavily on benomyl and prochloraz for the control of anthracnose and other postharvest disease, such fungicides may not always be available for postharvest use. With increasing consumer concern over the use of postharvest chemicals in horticulture, future research should aim to develop nonchemical disease control measures which will reduce our reliance on fungicides. The development of such measures relies on a detailed knowledge of the pathogen and how it infects its host.

# Stem-end Rot Diseases of Tropical Fruit — Mode of Infection in Mango, and Prospects for Control

G.I. Johnson, A.J. Mead, A.W. Cooke and I.A. Wells\*

## Abstract

Stem-end rots caused by *Lasiodiplodia theobromae*, *Dothiorella* spp. *Phomopsis mangiferae* and other fungi are serious postharvest diseases of tropical and subtropical fruit including mango, avocado, citrus, mangosteen and carambola. In Australia, *Dothiorella* spp. are generally the main cause of stem-end rot of mango.

In mango, postharvest losses do not result from infection at flowering or fruit set — fruitlets infected at this time are aborted. Natural infections by *Dothiorella* spp. reappear in stem-end tissue (10% affected) of fruit 11 weeks after full flowering and are more prevalent in the peduncle (60% affected) at that time.

All fungi which cause stem-end rots of mango (*Dothiorella dominicana*, Petrak & Cif. *D. mangiferae* Syd. & But., D. 'long', *P. mangiferae*, *L. theobromae*, *Cytosphaeria mangiferae* Died and *Pestalotiopsis* sp.) were found as endophytes associated with healthy stem tissue prior to inflorescence emergence. Some of these fungi progressively colonised the inflorescence tissue, and were present in the pedicel tissue of some fruit 8 weeks after flowering.

Our results suggest that stem-end rot pathogens are endophytes occurring widely on mature branches of mango trees. Mycelia of the fungi colonise inflorescence tissue as it matures and in certain conditions, reach the stem end of fruit. Infections then remain latent until after harvest or until the unharvested fruit senesce.

In the future, control of this disease may be achieved by slowing down the colonisation process so that the fungi do not reach the stem end of fruit before harvest. Factors which may affect colonisation include watering regimes, defoliation and pruning practices.

POSTHARVEST diseases of tropical fruit are a major limiting factor to shelf-life extension. Anthracnose (*Colletotrichum gloeosporioides* Penz.) and stem-end rot (*Lasiodiplodia theobromae* (Pat.) Griff & Maubl., *Dothiorella* spp. etc.) cause serious losses in several commodities including mango, papaya and avocado. In Australia, *Dothiorella* spp. are the main cause of stem-end rot in mangoes (Johnson et al. 1991a).

Frequently, stem-end rot emerges as a problem when anthracnose is well controlled by pre and post-harvest management (Johnson et al. 1990). While the infection process and latency of *C. gloeosporioides* are well established, little information is available on infection and latency with stem-end rot pathogens.

Infection by *L. theobromae* (as *Diplodia natalensis* (Pat.)) Nowell has been reported to occur via colonisation of the pedicel scar after harvest (for mango)

(Pathak and Srivastava 1967) or by colonisation of remnant floral tissue during flowering and fruit set (for oranges) (Nadel 1944; Minz 1946; Hildebrand 1947).

While infection by colonisation of the cut surface of the stem end is plausible, it is unlikely to be responsible for postharvest losses in mangoes from Australian orchards where fruit are harvested with long stems (>2 cm), destemmed in the packing shed and immediately inverted on a conveyer that passes beneath overhead sprays for 20 minutes to remove sap. Removing the stem, and washing the fruit would eliminate any superficial inoculum deposited on the fruit or stem at harvest.

We followed colonisation of floral remnants and pedicel tissue of mango by *Dothiorella* spp. between flowering and harvest in one season, and in the next season followed colonisation of stem and inflorescence tissue from before flowering until harvest, to determine the location and time of infection (Johnson et al. 1991b; Johnson et al. 1992). The results of this work are discussed here.

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## Materials and Methods

### Flower and fruit sampling

Isolations to laboratory media were made from buds, flowers, senescent flowers, flowers with fruit forming, young fruit with spent floral parts, developing fruit and mature fruit collected from tagged trees at regular intervals between flowering and harvest. Tissue samples were sterilised in sodium hypochlorite (1.25%) for 30 seconds. The results of the isolations were used to follow sequential changes in the incidence of fungi associated with petal-scar tissue, pedicel-fruit connexion tissue and peduncle tissue, and to monitor the occurrence of *Dothiorella* spp. As the maturity of fruit at sampling increased, the incidence of stem-end rot following fruit ripening was also assessed.

### Stem and inflorescence sampling

Samples of recent vegetative growth flushes and inflorescences were collected from tagged trees at regular intervals from before inflorescence emergence (preflowering) until after harvest (Fig. 1). Additional samples were collected from other locations at less frequent intervals between preflowering and harvest. Tissue sections were cut from various parts of individual stem and inflorescence samples, and triple sterilised (90% ethanol, 60 seconds; 2.5% sodium hypochlorite, 3 minutes; 95% ethanol, 30 seconds) (Petrini 1986), to eliminate superficial colonisers. As harvest approached, the accompanying fruit

from each stem/inflorescence sample was ripened to determine whether infection of stem tissue by stem-end rot pathogens resulted in the development of stem-end rot in fruit.

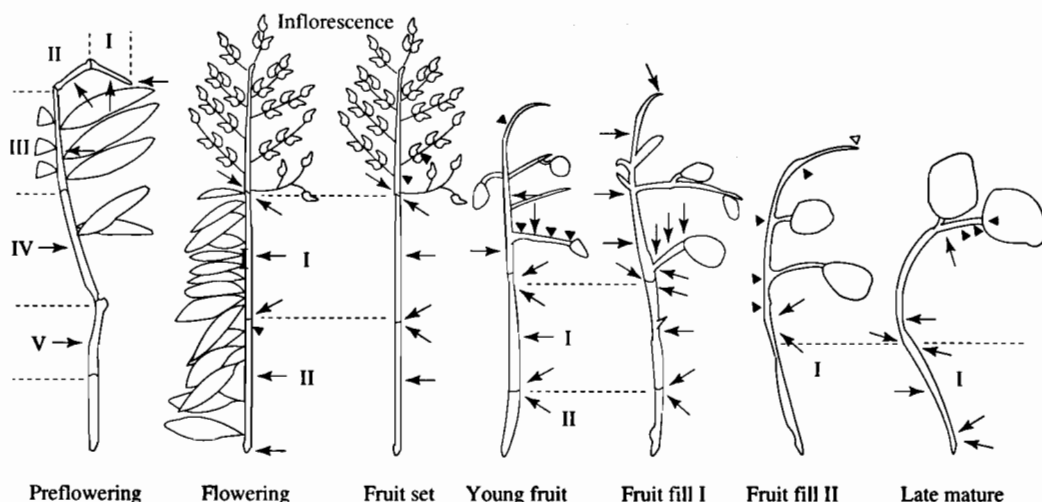
Where appropriate, fungi were identified to species level using cultures grown on Sach's wheat-straw agar (Hebert, 1971) under 12 hour U.V. illumination, and the pathogenicity of selected fungi was confirmed by fruit inoculation.

## Results

### Flower and fruit sampling

During flowering and fruit set, colonisation by *Alternaria alternata* (Fr.) Kiessler, *Cladosporium cladosporioides* (Fresen.) de Vres, *Dothiorella dominicana*, *Dothiorella mangiferae*, *Dothiorella* 'long', *Epicoccurn purpurascens* Ehren. ex Sch. and *Pestalotiopsis* sp. increased as the flowers senesced and young fruit formed.

The incidence of *Dothiorella* spp. associated with mango fruit-pedicel tissue declined in the third week after peak flowering, suggesting that initial infections by *Dothiorella* spp. caused fruit drop (Fig. 2). Levels of *Dothiorella* spp. in fruit-pedicel tissue remained low for the next 6 weeks, after which they increased. The appearance of *Dothiorella* spp. causing stem-end rot in incubated fruit was followed by the reappearance of *Dothiorella* spp. in fruit pedicel tissue. In this period, *Dothiorella* spp. were detected in the peduncle more frequently than in the fruit-pedicel tissue.



**Figure 1.** Sequential colonisation of the mango inflorescence and developing fruit by stem-end rot pathogens. Isolation points on growth flushes I to V (V = oldest growth sampled) yielding either (▲) no stem end rot pathogen, or (→) *Dothiorella* spp.

By 16 weeks, the incidence of *Dothiorella* spp. in fruit-pedicel connexion tissue was similar to the level of stem-end rot caused by *Dothiorella* spp. which developed in fruit harvested at the time (Fig. 2).

### Stem and inflorescence sampling

Symptomless infections by *Dothiorella* spp. and *P. mangiferae* occurred endophytically in the stem tissue of both older and most recent growth flushes prior to inflorescence emergence in mango trees with a history of high losses for stem-end rot. Colonisation did not extend into the youngest growth flush in samples from trees with a history of low levels of stem-end rot (Table 1).

At a site with a history of high levels of stem-end rot, sequential monitoring of inflorescence tissue between flowering and harvest revealed that at least some stem-end rot pathogens (*Dothiorella* spp., *P. mangiferae*, and *C. mangiferae*) gradually colonised the inflorescence, reaching the pedicel tissue of young fruit 57 days after flowering (Fig. 1). Subsequently, (at 74 days and later) detection frequency for the pathogen declined, possibly because of interference from copper residues (from field sprays) accumulating on tissue samples. Figure 1 shows the progression of colonisation by *Dothiorella* spp.

Endophytic colonisation by *Dothiorella* spp. and *P. mangiferae* was detected more frequently in stem, inflorescence and pedicel tissue of mature fruit speci-

mens from two other sites — one unsprayed (Indooroopilly) and the other regularly sprayed with copper (Childers). Colonisation was detected more frequently in the unsprayed tree samples (Table 2). Despite apparently less frequent colonisation of pedicel tissue, fruit from the sprayed orchard subsequently developed a high level of stem-end rot caused by *D. dominicana*, while a lower level of stem-end rot developed in unsprayed fruit (Table 1), possibly because the latter fruit were also extensively diseased by anthracnose (*Colletotrichum gloeosporioides*).

### Discussion

Our results suggest that early colonisation of floral remnants and fruit-pedicel tissue leads to premature fruit drop, and is therefore unlikely to be the primary infection phase for fungi causing stem-end rot in mature mangoes. The infections which initiate stem-end rot occur later in fruit development and at least some result from endophytic colonisation of the pedicel by fungi harboured in the stem.

The absence of *Dothiorella* spp. from the pedicel-fruit tissue in young mangoes (because endophytic colonisation doesn't reach there until later in fruit development) agrees with the results of Peterson (1978), who found that *Dothiorella aromatica* causing stem-end rot in avocado was not present in peduncle or stem tissues early in fruit development, but appeared as fruit matured.

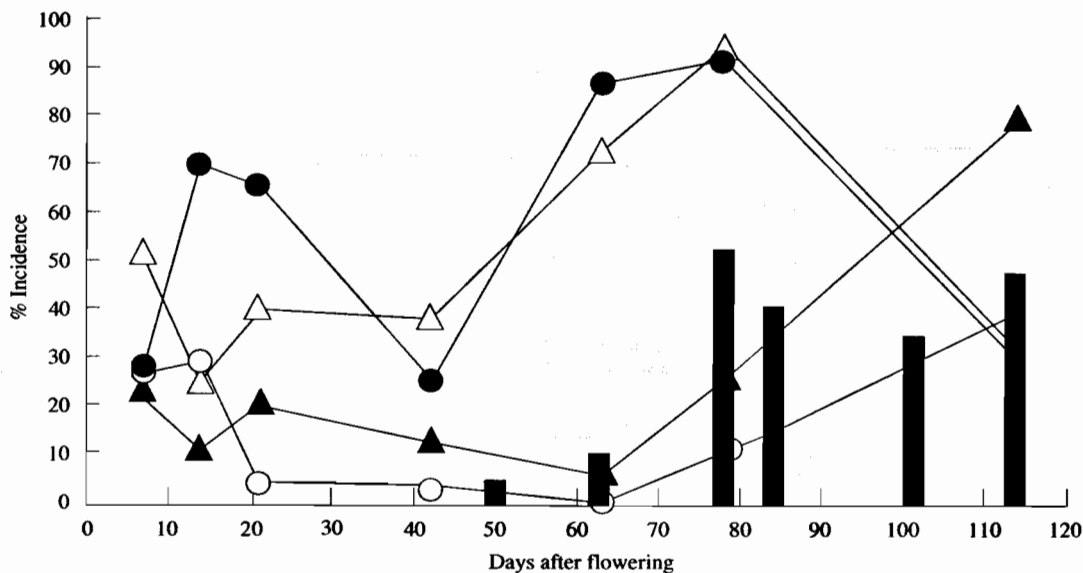


Figure 2. The incidence of fungi in fruit/pedicel connection tissue between flowering and harvest. *Dothiorella* spp. (O), *Alternaria alternata* (●), *Cladosporium cladosporioides* (Δ), *Pestalotiopsis* sp. (▲). Stem-end rot incidence (after 3 weeks at 23°C) shown as bar graphs.

**Table 1.** Incidence of endophytic (a) *Dothiorella* spp. and (b) *P. mangiferae* in mango stem tissue prior to inflorescence emergence.

Stem-end rot losses <sup>a</sup>	Incidence (%) of fungi in mango stem					
	Ayr		Mutchilba		Mareeba	
	a	b	a	b	a	b
	Moderate		Low		High	
V	40	0	20	20	0	40
IV	80	0	40	0	20	20
III	20	0	20	0	10	40
II	40	20	40	40	60	20
I	20	0	0	0	60	20
Tip	80	40	0	0	60	20
Total <sup>b</sup>	16	3	6	3	11	8

<sup>a</sup> Stem-end rot losses in 3 years of survey (Johnson et al. 1991a).

<sup>b</sup> Mean number of isolation points yielding the pathogen.

**Table 2.** Incidence of endophytic *Dothiorella* spp. in mango stem and inflorescence tissue in sprayed (Childers) and unsprayed (Indooroopilly) mango trees during harvest, as detected by isolations from tissue segments. Stem-end rot levels in fruit samples after 3 weeks at 23°C are also shown.

Growth flush (numbered I–III from youngest)	Incidence of <i>Dothiorella</i> spp. %	
	Childers	Indooroopilly
III – base	67	75
top	40	100
II – base	40	40
top	20	60
I – base	0	60
top	80	60
Inflorescence – base	20	60
mid	-	60
Side inflorescence	40	-
Peduncle	20	60
Pedicel	20	80
Stem-end rot	100	20

Peterson's (1978) observation that levels of *D. aromatica* rose more quickly in the peduncle than in the stem-end tissue of avocado fruit suggests that endophytic colonisation also occurs in avocado.

Future strategies for controlling stem-end rot could aim to slow down the process of endophytic colonisation so that the pathogens do not reach the stem-end tissue of fruit before harvest. Physiological factors such as water stress and defoliation may favour increased colonisation by *Dothiorella* spp. and other fungi as suggested by Crist and Schoeneweiss (1975) and Pusey (1989) for the anamorph of *Botryosphaeria dothidea* ( $\equiv$  *D. dominicana*, Johnson, unpublished) on temperate tree species. Irrigation scheduling, stored carbohydrate levels and pruning practices may have critical effects on the frequency of stem-end rot infections in harvested fruit.

The low level of stem-end rot observed in fruit from unsprayed trees, despite extensive colonisation of pedicel tissue by stem-end rot pathogens and other fungi, suggests that colonisation by other endophytes, or *C. gloeosporoides* could have delayed or prevented fruit colonisation by stem-end rot fungi. Competitive inhibition, antagonism or induced host resistance could be involved. Clarification of these effects may lead to further novel control methods for stem-end rot in tropical fruit.

We have shown that *Dothiorella* spp. and other stem-end rot fungi occur as endophytes in mango stems and colonise the pedicels of fruit during maturation. The induction of stem-end rot by endophytic *Dothiorella* spp. in mangoes after harvest could serve two purposes — first, it makes the fruit unpalatable to animals, and second it quickly destroys the fruit flesh, so the seed can germinate and grow.

Mummification of the fruit flesh would increase the likelihood of seedlings becoming infected as they emerged through the remains of the mango, ensuring perpetuation of the endophytic association. Carroll (1986) cited examples of other host benefits accruing from some endophytic associations (e.g. insect or herbivore resistance). Similar functions for associations between *Dothiorella* spp. or *P. mangiferae* and mango may also exist.

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# Effects of Postfumigation Washing Treatments and Storage Temperature on Disease Development in Fresh Longan

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## Abstract

Longan fruit with and without prior fumigation in sulfur dioxide (SO<sub>2</sub>) were subdivided into three groups. One group remained unwashed, and was packed in plastic punnets and overwrapped with PVC film, the second group was washed in sterile distilled water in the laboratory, while the third group was washed in water that had been collected from a longan fruit washing tank, before air drying and packing as described. The treated fruit were stored at either 28, 10 or 8°C and disease development was monitored daily. Skin browning began to appear in unfumigated fruit within 1 day of treatment, with development most rapid at 25°C. The spectrum of fungi associated with the browning was similar, regardless of washing treatment. *Lasiodiplodia theobromae*, *Pestalotiopsis* sp., *Cladosporium* sp. and *Fusarium* spp. were isolated frequently from the dark skin areas that became covered with mycelium, and *Aspergillus niger* and other fungi were detected occasionally. The fruit that had been fumigated with SO<sub>2</sub> showed no signs of skin browning or disease, even following storage at 10 or 5°C for up to 11 months (however, in this time the pulp shrank). At 25°C the fumigated fruit turned brown within 2 months and dark brown in 5 months.

SULPHUR dioxide for disease control is adopted by the Australian grape industry. The South Africa and Israel lychee industries have incorporated sulfur dioxide as a standard postharvest treatment for consignments to Europe (Underhill and Bangshaw 1990). Longan treated with a certain amount of sulfur dioxide gave evidence of rot control, and with a higher rate there was a complete inhibition of surface mould (Tongdee 1990). The fumigated longan fruit are chrome-yellow colour, whereas the untreated fruit are brown colour. In Thailand sulfur dioxide fumigation has become a practical method used to control disease development and skin browning of export longan. There are many kinds of fungi which cause rotting of longan (Chaiwangsri 1992). Therefore it would be possible that the treated fruit might be recontaminated during storage.

## Materials and Methods

Longan fruit with and without prior fumigation in sulfur dioxide (SO<sub>2</sub>) were divided into three groups. Each group had 64 fruit. One group remained

unwashed, and was packed in plastic punnets and overwrapped with PVC film. One punnet contained 16 fruit. The second group was washed in sterile distilled water in the laboratory, while the third group was washed in water that had been collected from a longan washing tank, before air drying and packing as described. The treated fruit were stored at either 23, 10 or 5°C. Two more sets of longan fruit treated with SO<sub>2</sub> and untreated were kept in a freezer or a refrigerator and disease development was monitored daily. Disease symptoms were evaluated at 5 months on the fruit stored at 25°C and at 11 months on those stored at 10 and 5°C and in the freezer.

## Results

Skin browning began to appear in unfumigated fruit within 1 day of treatment, with development most rapid at 25°C (Table 1). The whole skin of the unfumigated fruit stored at 25, 10, and 5°C became brown within 10, 16 and 22–26 days, respectively. The spectrum of fungi associated with the browning was similar, regardless of washing treatment (Table 2). *Lasiodiplodia theobromae*, *Pestalotiopsis* sp., *Cladosporium* sp. and *Fusarium* sp. (isolate 2) with reddish colony were isolated frequently on the dark skin areas that became covered with mycelium. *Aspergillus niger*, *Fusarium* sp. (isolate 1) with whitish colony, and three more unidentified fungi were occasionally detected. *Lasiodiplodia theobromae* and

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*Pestalotiopsis* sp. were more frequently isolated from the longan stored at 25 and 10°C than at 5°C.

There was no fungal growth on the SO<sub>2</sub>-fumigated fruit stored at 25°C for 5 months. At 25°C storage the skin of the fruit became brown within two months and dark brown in five months when they were discarded. At 5 and 10°C storage, the fumigated fruit maintained a chrome-yellow colour on the skin surface up to 11 months, which was the end of this experiment. By this time, the fruit was still a good shape and colour but the pulp had shrunk. The skin of the fruit turned brown after they were removed from 10 and 5°C and kept in a refrigerator. However, the fruit from 5°C were lighter in colour. After they were placed in the fridge for 3 weeks, one fruit from 10°C was infected by *Lasiodipledia theobromae*. For the extra sets of fruit kept continuously in the freezer, the skin of the unfumigated fruit became dark brown within 5 days while the fumigated fruit were still a chrome colour after 11 months storage. The pulp of the fruit kept in the freezer was always of good quality, up to the end of this experiment. When the fruit were removed from the freezer and kept in the refrigerator for 3 weeks, the skin colour remained unchanged while the pulp was soft and juicy.

## Discussion

Regardless of washing treatments, the fumigated fruit were still free from contaminants during 5 months storage at 25°C. It is possible that the residue of SO<sub>2</sub> on the fruit surface on day one when the fruit were washed with the contaminated water and wrapped in PVC film was high enough to kill all recontaminants, or SO<sub>2</sub> might have been released from the fruit and sterilised both punnet and fruit wrapped inside the PVC film. Tongdee (1990) reported that with a high amount of SO<sub>2</sub> fumigation (35 mL) a residue of more than 2500 ppm was maintained on the rind on day one. She also mentioned that fungal growth on the fruit fumigated with 25 mL of SO<sub>2</sub> or higher was significantly reduced or inhibited.

Drying is a good method for preservation of longan. Generally a modified oven heated with burning wood was used for drying the fruit. It took about 4–5 days to dry them. This method is rather costly and firewood is scarce. Therefore, in combination with SO<sub>2</sub> fumigation it could be possible to dry the fruit under the sun without worrying about rotting.

**Table 1.** Skin browning of unfumigated longan fruit which had been dipped in sterile distilled water and in water collected from a longan washing tank, air dried, packed in plastic punnets and overwrapped with PVC film before storing at three different temperatures (percent of skin brown).

Storage period (days)	Distilled Water			Longan washing water			Control		
	5°C	10°C	25°C	5°C	10°C	25°C	5°C	10°C	25°C
1	2.0	3.5	4.0	2.0	4.0	4.0	2.0	3.3	4.0
2	2.0	3.5	4.5	2.0	4.0	4.3	2.0	3.5	4.5
4	2.5	4.3	6.3	2.8	4.5	6.0	2.3	4.0	6.5
6	3.8	6.0	8.3	4.0	6.3	7.8	3.5	5.0	7.8
8	4.8	6.5	9.3	4.8	6.5	8.8	4.0	5.3	8.5
10	5.3	7.5	10.0	5.8	7.8	9.5	5.0	6.8	9.5
12	6.0	8.0		6.5	8.5	10.0	6.0	7.3	10.0
14	6.5	9.3		7.3	9.0		6.5	8.3	
16	7.3	10.0		7.8	10.0		7.0	9.3	
18	7.5			8.8			7.5	10.0	
20	8.0			9.8			8.5		
22	9.0			10.0			9.0		
24	9.5						9.8		
26	10.0						10.0		

**Table 2.** The frequency of fungi isolated from unfumigated longan fruit which had been dipped in sterile distilled water or in water collected from a longan washing tank, air dried, packed in plastic punnets and overwrapped with PVC film before storage at three different temperatures. The fungi were isolated from four spots of each fruit and 120 fruit per treatment.

Fungi isolated	Before treatment	Distilled water			Longan washing water			No dipping			Total
	25°C	5°C	10°C	25°C	5°C	10°C	25°C	5°C	10°C	25°C	
<i>Aspergillus niger</i>	19	-	-	-	-	-	-	-	-	-	19
<i>Lasiodiplodia theobromae</i>	14	77	104	112	82	92	95	76	126	103	881
<i>Cladosporium</i> sp.	-	48	15	-	35	25	-	40	24	-	187
<i>Fusarium</i> sp. (1)	7	-	-	5	6	-	14	4	4	5	45
<i>Fusarium</i> sp. (2)	-	22	40	24	38	36	5	19	20	21	225
<i>Pestalotiopsis</i> sp.	37	44	58	85	57	86	85	73	72	102	699
Unidentified (1)	2	-	-	-	-	-	-	-	-	3	5
Unidentified (2)	-	-	-	-	-	-	3	-	-	2	5
Unidentified (3)	-	13	6	-	9	-	-	8	-	-	36
<b>Total</b>	<b>79</b>	<b>204</b>	<b>223</b>	<b>226</b>	<b>227</b>	<b>239</b>	<b>202</b>	<b>220</b>	<b>246</b>	<b>236</b>	<b>2120</b>

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# Factors Affecting the Incidence of Stem-end Rot on Mango

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## Abstract

Pedicels near the stem end of mango fruits (1 cm from stem end) were infected with many microorganisms. Within this group of microorganisms, *Lasiodiplodia theobromae*, *Dothiorella dominicana* and *Phomopsis mangiferae* were the cause of stem-end rot. Mango fruits harvested with pedicels and detached before ripening at 25°C had less *L. theobromae* infection than the fruits harvested without pedicel and desapped on the ground in the orchard. This phenomenon was not found in the case of *D. dominicana* and *Phomopsis mangiferae*. Storage temperature (13°C, 25°C) had no effect on the incidence of *Dothiorella* and *Lasiodiplodia* rot but *Phomopsis* rot incidence was increased as the temperature was decreased.

Surface disinfection with 1% sodium hypochlorite before storage also had no effect on the stem-end rot incidence.

STEM-END rot of mango is a major postharvest disease in Thailand. Many organisms are involved in this disease, including *Lasiodiplodia theobromae*, *Dothiorella dominicana*, *D. mangiferae*, and *Phomopsis mangiferae* but the main pathogens are *L. theobromae* and *D. dominicana* (Sangchote 1987). They infect mango flower and young fruits in the field (Darvas 1991).

*D. dominicana* infects immature green fruits and produces dark spot lesions on the skin of mango in the field. *L. theobromae* and *D. dominicana* can survive and produce pycnidia on the dead twig (Sangchote and Chana 1990). They infect through the wounded part, stem end and pedicel of the fruits. *L. theobromae* infect the stem end of fruit with long pedicels slower than fruit with short pedicels (Sangchote 1989).

Fruits naturally infected with stem-end rot microorganisms from the orchard develop symptoms in the storage room and market after ripening (Sangchote 1987). Development of the symptoms can be delayed by cold storage (13°C) but development of other microorganisms in long-term storage occurs (Johnson et al. 1990). Postharvest treatment with hot benomyl 500 ppm at 52°C for 5 minutes followed by prochloraz at 750 ppm for 30 seconds is an effective control

measure for this disease. The combination of this treatment and controlled atmosphere storage extended shelf life of the fruits to 26 days (Johnson et al. 1990) but controlled atmosphere storage facilities in Thailand are expensive.

The work here was undertaken to determine the relationship between pedicel and stem-end infection, storage temperature, surface disinfection and modified atmosphere storage on the incidence of stem-end rot.

## Materials and Methods

### Pedicel infection

Mature mango fruits were harvested with pedicel. Pedicels were cut 1 cm from the stem end and cut longitudinally into two pieces, then surface sterilised with 1% sodium hypochlorite for 5 minutes. They were plated on potato dextrose agar (PDA). Percentages of the fungal colonies occurring on the agar were counted and microorganisms identified.

### Effect of pedicel and stem-end infection on the incidence of stem-end rot

Mature green fruits were harvested from the orchard; one group with pedicel and the other group by detaching pedicel and desapping on the ground. The two groups were brought to the ripening chamber where the pedicels of the first group were detached and the stem end covered with tape to prevent other infection. Fruit were ripened at 25°C. Percentages of disease incidence were recorded when the fruit were ripe.

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### Storage temperature, surface disinfection and stem-end rot incidence

Mango fruits were harvested from different locations including Nakom Pathom, Cha Choeng Sao and Rat Cha Buri and stored at 13°C and 25°C until ripe. Percentages of stem-end rot were recorded and the causal organisms isolated. Two groups of mango fruits from the same locations were harvested: one group was surface disinfected with 1% sodium hypochlorite for 5 minutes and the other was not surface disinfected. Both groups were stored at 13°C and 25°C until ripe. Percentages of stem-end rot were recorded and the causal organisms isolated.

## Results and Discussion

### Pedicle infection

Many microorganisms (*C. gloeosporioides*, *Lasiodiplodia theobromae*, *Dothiorella dominicana*, *Phomopsis mangiferae*, *Pestalotiopsis* sp., *Alternaria* sp. and *Fusarium* sp.) infected the pedicel near the stem end (Table 1).

**Table 1.** Percentages of fungi isolated from the pedicel near stem end (1 cm from stem end) of mango fruits.

Fungi	Percentages
<i>Alternaria</i> sp.	60.9
<i>Colletotrichum gloeosporioides</i>	5.4
<i>Dothiorella dominicana</i>	2.7
<i>Fusarium</i> sp.	16.1
<i>Lasiodiplodia theobromae</i>	10.7
<i>Pestalotiopsis</i> sp.	42.8
<i>Phomopsis mangiferae</i>	28.1

*L. theobromae*, *D. dominicana* and *P. mangiferae* were related to stem-end rot on the fruits (Sangchote 1987). Possibly, these fungi could pass through the pedicel to the stem end of the fruits (Sangchote 1984). Inoculum from the pedicel could be the other source of inoculums apart from flower and twig (Sangchote and Chana 1990).

### Effect of pedicel and stem-end infection on the incidence of stem-end rot

Different percentages of stem-end rot microorganisms were found at the stem end of the fruits, both harvested with and without pedicels (Table 2).

The percentages of *L. theobromae* on the fruits harvested with pedicel (1.1%) and detached before ripening showed no correspondence to pedicel infection (10.7%) in Table 1. The fungal mycelium generally were not reaching the stem end of the fruits. When the fruits were harvested as normal practice, the percentage of infection was higher due to contamination from the spore of fungus in the air and soil (Sangchote 1989). Percentage of pedicel infection in this orchard indicated the level of inoculum of this fungus was high. In contrast, percentage of *Dothiorella dominicana* was 2.7% (Table 1) and infection of the fruits with and without pedicel was 4.4% and 1.6%, respectively. The level of *D. dominicana* inoculum was not high but it could infect mature green fruit with a few symptoms (Sangchote 1989), and inoculum in the soil was less important for this fungus. *P. mangiferae* was a rather weak pathogen as compared to the others (Sangchote and Chana 1990). Even its rather high incidence on the pedicel (28.1%) only resulted in a low infection (Table 2).

### Storage temperature, surface disinfection and stem-end rot incidence

Storage temperatures of 13°C and 25°C had little effect on the incidence of *Lasiodiplodia* and *Dothiorella* rot but it had an effect on *Phomopsis* rot. *Phomopsis* rot increased (22.3 to 42.3%) as the temperature was decreased (25°C to 13°C) (Table 3).

**Table 2.** Disease incidence (%) on mango fruits harvested with and without pedicels from the orchard and stored at 25°C for 6 days.

Causal organisms	Disease incidence (%)	
	Without pedicel	With pedicel
<i>Dothiorella dominicana</i>	1.6a	4.4b
<i>Lasiodiplodia theobromae</i>	4.4a	1.1b
<i>Phomopsis mangiferae</i>	0.6a	1.1b

Means followed by the same letter in the column are not significantly different at the 5% level.

**Table 3.** Disease incidence (%) on mango fruits harvested from different locations and stored at 13°C and 25°C.

Locations	Storage temperature (°C)	Disease incidence	
		<i>Lasiodiplodia</i> and <i>Dothiorella</i> rot	<i>Phomopsis</i> rot
Nakorn Pathom	13	45	62
	25	60	37
Cha Choeng Sao	13	51	15
	25	50	9
Rat Cha Buri	13	21	50
	25	20	21
Average	13	39.0	42.3
	25	43.3	22.3

Temperature had an effect on the disease incidence but not on the kind of microorganisms as Johnson et al. (1990) found in short-term storage. In the long term other microorganisms apart from stem-end rot microorganisms, including *Alternaria alternata*, *Rhizopus stolonifer*, *Penicillium expansum*, *Botrytis cinerea*, *Stemphylium vesicarium* and *Mucor circinelloides* occurred. Surface disinfection with 1% sodium

hypochlorite also had little effect on stem-end rot incidence (Table 4). Treatment of the fruits with 1% sodium hypochlorite could partially reduce stem-end rot incidence because stem-end rot microorganisms mostly infect the fruits as latent infection (Sangchote 1987). Sodium hypochlorite could kill microorganisms on the surface (Eckert and Ogawa 1988).

**Table 4.** Disease incidence (%) on mango fruits harvested from different locations, treated with 1% sodium hypochlorite and stored at 13°C and 25°C.

Treatments	Storage temperature (°C)	Location	Disease incidence (%)		
			<i>Lasiodiplodia</i> and <i>Dothiorella</i> rot	<i>Phomopsis</i> rot	
NaOCL	13	Nakorn Pathom	42	60	
		Cha Choeng Sao	44	18	
		Rat Cha Buri	14	54	
	25	Nakorn Pathom	58	40	
		Cha Choeng Sao	54	40	
		Rat Cha Buri	20	24	
	Average		38.7	34.3	
	Untreated	13	Nakorn Pathom	48	64
			Cha Choeng Sao	58	12
Rat Cha Buri			28	46	
25		Nakorn Pathom	62	34	
		Cha Choeng Sao	46	8	
		Rat Cha Buri	20	18	
Average			43.7	30.3	

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