

## Inoculum sources and infection pathways of pathogens causing stem-end rots of ‘Hass’ avocado (*Persea americana*)

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**Abstract** Species of *Colletotrichum*, *Botryosphaeria*, and *Phomopsis* causing postharvest rots in avocado (*Persea americana* Miller) fruits are present in the living and dead branches and twigs of avocado trees, and in the living pedicels. They dominate the fungal population within the extra-cambial tissues but are less common within the xylem elements. There is no evidence that invasion of these tissues is pathogenic. With the possible exception of *C. gloeosporioides* they appear to be discontinuously present and are more properly termed phellophytes rather than endophytes. There was a higher incidence of stem-end rots than of body rots in untreated (control) ‘Hass’ avocados in New Zealand experiments and most of these stem-end rots were associated with *B. parva* and *Phomopsis* spp. A high proportion of stem-end infections appeared to be initiated during harvesting. Picking the fruit by snapping the pedicels instead of clipping, as in commercial practice, resulted in an unusually high level of stem-end rots caused by *C. acutatum*. Frequently sterilising the clippers used to harvest the fruits reduced the incidence of stem-end infections, in particular those caused by *B. parva*, indicating that contamination of the clippers is an important source of infection. It is suggested that this contamination is probably present as fragments of infected extra-cambial tissue.

### INTRODUCTION

Previous descriptions of the development of postharvest rots of avocado (*Persea americana* Miller) fruits have been largely concerned with those caused by a single pathogen, *Colletotrichum gloeosporioides* (Penzig) Penzig & Saccardo. Hartill (1991) reported species of three fungal genera: *Colletotrichum*, *Botryosphaeria* (as the *Fusicoccum* anamorphs), and *Phomopsis* caused almost all of the postharvest rots of avocados in New Zealand. The *Fusicoccum* species are probably identical to the *Dothiorella* species reported elsewhere (Johnson 1994) but taxonomic revisions suggest the former name is correct (Pennycook & Samuels 1985). Most overseas reports are concerned with rots initiated by infections through the peel of the fruits. These rots will hereafter be termed “body rots”. There is good evidence that some, at least, of the body rot infections caused by *C. gloeosporioides* occur early in the development of the fruits (Binyamini & Shiffmann-Nadel 1972; Peterson 1978; Coates et al. 1993). The pathogen produces an appressorium and a short germ tube but then becomes inactive probably because of the presence of antifungal (presumably fungistatic) dienes in the fruit (Prusky et al. 1982, 1983, 1991). After the fruit is harvested the concentration of the dienes falls as a result of the action of lipoxigenase. Hyphal growth then resumes and a rot develops.

Some reports indicate that postharvest rots may be initiated by other pathogens and that infections starting at the pedicel end of the fruits (stem-end or neck rot infections) are also important (Zentmeyer 1951, 1953; Zauberman et al. 1975; Peterson 1978; Darvas & Kotze 1987; Darvas et al. 1987; Snowdon 1990; Johnson et al. 1991; Ohr et al. 1991).

A review of the pathogens causing rots in 1792 control fruits (i.e., those that had received no experimental or commercial fungicide treatments) collected in 13 experiments from various orchards in the Bay of Plenty, indicated that stem-end rots may be responsible for greater losses than body rots in New Zealand (Hartill unpubl. data). In these trials 25.6% of the fruits developed body rots whereas 37.9% had stem-end rots, and 6.4% had both types of rot. The pathogens most frequently isolated from these stem-end rots were *C. gloeosporioides* (in 5.5% of all fruits), *C. acutatum* (3.1%), *Botryosphaeria parva* (anamorph *Fusicoccum parvum*) (17.1%), and *Phomopsis* spp. (14.7%). The incidence of each pathogen varied considerably from trial to trial. Stem-end rots are also responsible for most of the defects in New Zealand avocados exported to Australia (Marie Piconne pers. comm.).

Two problems need to be solved before appropriate measures for the control of stem-end rots can be devised. First, the sources of inocula need to be identified and second, the pathways of infection leading to the development of the rots need to be established.

Fitzell (1988) found large numbers of *C. gloeosporioides* conidia were produced on dead leaves and infected and mummified fruits in avocado trees. A few were produced on dead twigs but none on the branches and green leaves. Darvas et al. (1987) found *C. gloeosporioides* conidia and perithecia in margins of leaf and twig lesions and in bark from the trunks of trees.

There are several reports that *Botryosphaeria* spp. are frequently found in various structures in the avocado tree canopy. Zentmyer (1953) records the presence of *B. ribis* Grossenbacher & Duggar and its anamorph *D. gregaria*, which should probably be known more correctly as *F. aesculi* Corda, teleomorph *B. dothidea* (Morgan-Jones & White 1987), on dead branches, twigs, and leaf margins. Darvas et al. (1987) found non-fruiting colonies of *D. aromatica* (Saccardo) Petrak & Sydow (= *F. luteum* (Pennycook & Samuels) (G. I. Johnson pers. comm.)) on avocado twigs and branches.

Darvas et al. (1987) also found *Phomopsis perseae* Zerova in all parts of the trees they sampled. Hartill (1991) did not identify the *Phomopsis* species isolated from avocado fruit rots but considered at least two distinct species were present.

In this paper a number of trials conducted to identify inoculum sources and preliminary trials aimed at elucidating the pathways of infection are described.

## METHODS

### Infections of necrotic tissues

Two types of necrotic tissues in avocado tree canopies were investigated, dead twigs and branches, and ring-neck necrotic lesions on pedicels (Hartill 1991, fig. 4).

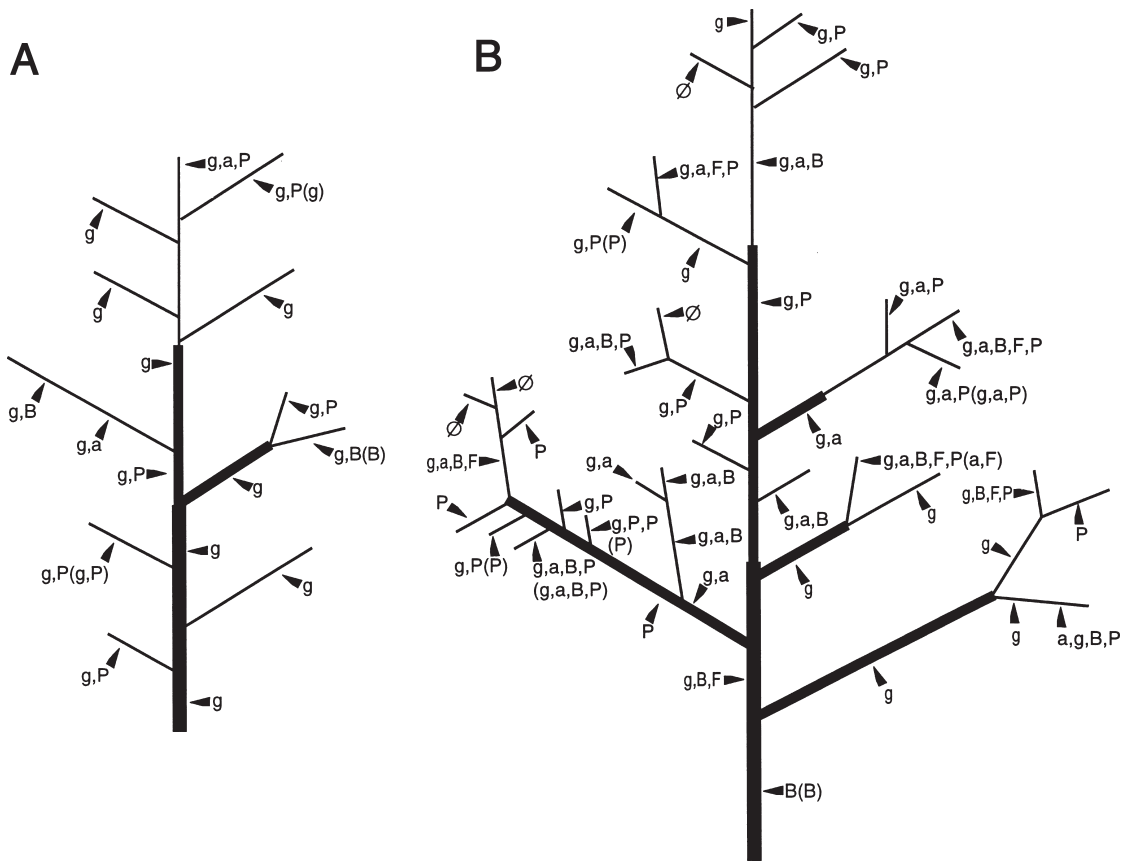
Dead twigs and branches were collected from various orchards during the course of these investigations. Several (3–10) small pieces of tissue were excised from the unsterilised surface of each twig and branch and plated onto Difco® potato dextrose agar containing 50 ppm streptomycin (PDSA). Fungi growing out of the tissues were then identified.

A total of 88 samples of avocado fruits with ring necrosis of the pedicels were collected on four occasions from two Bay of Plenty, New Zealand, sites in 1989 and individually identified. The surface tissue of the necrotic scars was scraped away with a sterile scalpel and pieces of the underlying necrotic tissue excised onto PDSA. The pedicels were then clipped off with flame-sterilised secateurs as near as possible to the fruit and the fruits left to ripen at ambient temperature. When ripe the fruits were halved and examined for internal rots. Tissue samples were isolated from the rots and plated onto PDSA. Fungi growing out of the ring-neck necrotic tissue fragments and from the fruit rot samples were identified and the species found in the fruit rots compared with those found in the ring-neck scars of the corresponding pedicels.

### Infections of living twigs and branches

Samples were taken on two occasions, November 1991 and March 1992, from 14-year-old trees in an unsprayed orchard at Te Puke, and on two further occasions, December 1992 and March 1993, from 6-year-old trees in an orchard at Ruahihi that had received three applications of copper hydroxide during the previous season.

Branches were cut from avocado trees at the point where they were 2.0–2.5 cm diameter (i.e., the samples included wood  $\leq 3$ ,  $\leq 2$ , and  $\leq 1$  years old). All leaves were removed and the samples returned to the Mt Albert Research Centre laboratories of The Horticulture and Food Research Institute of New Zealand Ltd (HortResearch). The branches were stored at 4°C for 1–4 days after collection while awaiting isolation procedures. Before isolations were made a complete map of each branch was prepared (e.g., Fig. 1). Isolations were made from segments, 5–6 cm long, cut from representative parts of the



**Fig. 1** A, B, Distribution of fruit-rot pathogens in two terminal shoots of avocado trees (*Persea americana*) collected from a Te Puke, New Zealand, orchard. Thicker lines indicate 3-year-old wood, thinner lines indicate 1-year-old wood. Open letters indicate pathogens found in whole cross-sections, letters in parentheses indicate pathogens found in the xylem elements only. (Key to pathogens: a, *Colletotrichum acutatum*; g, *C. gloeosporioides*; B, *Botryosphaeria parva*; F, *Fusicoccum luteum*; P = *Phomopsis* spp.; Ø indicates a sterile site.)

branches and twigs, and the locations from which the segments were taken were marked on the maps.

The segments were surface sterilised using the 3-step method of Petrini (1986) i.e., 1 min submersion in 90% alcohol, followed by 5 min in 2.5% sodium hypochlorite, then 1 min in 95% alcohol. The segments were held upright in a flame-sterilised vice, the distal 5 mm was removed, and transverse sections c. 1 mm thick sliced off from one half of each segment with flame-sterilised secateurs and the sections plated onto PDSA. The bark and extra-cambial tissues on the remaining half of each segment were sliced off with a flame-sterilised scalpel and further c. 1 mm thick sections of the xylem and pith were cut and plated onto PDSA. The number of sections plated depended on the diameter

of the segments; usually 3–4 sections were plated from 3-year old-segments and up to 12 sections from 1-year-old segments. Where possible, the fungi subsequently growing out from the sections were identified.

### Infections of the pedicels

Fruits were collected on three occasions (18 October 1994, 5 December 1994, and 27 January 1995) from a different Te Puke orchard from that used in the previous trials. The same group of trees were used for each harvest. Copper sprays had been applied to the orchard at regular intervals earlier in the season. On 18 October 1994, 100 fruits were harvested at random from a row of eight trees in groups of 25 fruits each. The fruits were harvested complete with

their pedicels using secateurs that were flame-sterilised before harvesting every 4–5 fruits from the trees and were processed immediately before harvesting the next group. Processing consisted of removing the fruits to a laminar flow cabinet at the HortResearch Te Puke Research Centre laboratories, re-clipping the pedicels to discard the distal 5–10 mm, then surface sterilising the rest of the pedicel and the neck of the fruits by dipping in c. 90% ethanol and flaming. A further short section was clipped from the distal end of the pedicel and discarded, then three c. 1-mm-thick sections were cut off and plated onto PDSA. Most of the remainder of the pedicel was then discarded leaving a short button. Three further sections were removed from this button and plated onto PDSA. A transverse slice, 3–4 mm thick, was then taken from the neck of the fruit at the termination of the pedicel bundle sheath. Small parts of the xylem elements were excised from this slice with a sterile scalpel and plated onto PDSA. All sectioning was done with flame-sterilised secateurs and scalpels.

On the same day two further collections of 100 fruits were made. One collection was stored for 2 days in the Mt Albert laboratories at c. 23°C and then isolations made from the pedicels and fruits as above. The other collection was harvested by clipping the pedicels to a button as in commercial practice, ripened in the laboratory and fungi isolated as before from any rots that developed.

In each of the 5 December 1994 and 27 January 1995 harvests three collections of 75 fruits and one of 100 fruits were made. One collection, in three groups each of 25 fruits, was processed immediately, as described above, in the Te Puke laboratories. Another collection was similarly processed after storing for 2 days in the Mt Albert laboratories. On each of these two subsequent occasions a third collection of 75 fruits with complete pedicels was made and isolations made, as above, after the fruits had ripened, at which stage the pedicels had usually dehisced. The collection of 100 fruits was harvested by clipping the pedicels to a button and isolations made from any rots that developed after the fruits had ripened.

### Harvesting trials

In a 1990–91 trial the effect of plucking (i.e., harvesting the fruits by breaking the fruit from the tree by hand, preferably at the point where the fruit joins the pedicel) was compared with conventional harvesting, where the fruit is harvested with clippers so that a small piece of pedicel (the “button”) is left

attached to the fruit. Fifty fruits were harvested by each technique on three occasions, November, January, and February, from a copper-sprayed Tauranga orchard, then ripened and checked for rots.

In a later trial (1997) avocados from a Te Puke orchard that had also received regular copper sprays were harvested in different ways to test an hypothesis that many stem-end rots are initiated at harvesting. The treatments used were as follows—

Group A: Normal packing in moulded cardboard trays (“Plix”). (1) Fruits picked by the grower (“Grower pick”), clipped with unsterilised clippers and collected in a bucket before packing. (2) Fruits picked by the experimenter (“Normal pick”), clipped with unsterilised clippers and collected in a picking bag before packing. (3) Fruits clipped with sterilised clippers, which were also dipped in fungicide between each clipping (“Fungicide pick”), and collected in a picking bag before packing.

Group B: Fruits collected and packed individually. Fruits wrapped in tissues and separated by cardboard partitions in trays. (4) Fruits collected with unsterilised clippers (“Unsterile pick”). (5) Fruits collected with sterilised clippers (“Sterile pick”). (6) Fruits collected by plucking (“Plucked”).

The unsterilised clippers were commercial clippers used by the grower over a number of seasons (Grower pick), or secateurs (Normal pick). The sterilised clippers were the same secateurs after the blades had been dipped in 95% alcohol and flamed over a gas flame before harvesting the individual fruits. The fungicide dip used in the Fungicide pick was a mixture of 500 ppm benomyl (Benlate®) and 500 ppm prochloraz (Sportak®), fungicides shown to be effective in controlling avocado rots (Hartill 1992). A few of the fruits collected by plucking had remnants of the pedicel remaining attached, but in most instances the pedicel broke away cleanly at the point of attachment to the fruit. Each treatment consisted of 23–25 fruits and was replicated 4 times.

### Fruit ripening

Where appropriate the fruits were ripened at ambient temperature (c. 23°C) in the Mt Albert laboratories. The fruits were examined every 1–2 days for postharvest rots and those with external rots removed and the pathogen(s) isolated. The remaining fruits were halved when ripe and assessed for internal rots. An estimate was made of the extent of rotting in each fruit where: trace = rot symptoms barely visible; moderate = rots extending up to 5 mm into the flesh; and severe = rots extending greater than 5 mm into the flesh.

The data were used to generate a rot severity index using the following parameters:

$$\frac{[(\text{no. trace}) + (4 \times \text{no. moderate}) + (10 \times \text{no. rotten})] \times 100}{10 \times \text{total fruits in sample}}$$

Fruits with no rot, or only a trace of rot, were considered to be commercially acceptable.

The data obtained were subjected to one-way analysis of variance using MINITAB.

## RESULTS

### Infections of necrotic tissues

All the major postharvest rot pathogens were present in the dead twigs and branches collected from avocado trees (Table 1). Almost all the twigs were colonised by at least one pathogen and in some instances up to four pathogens were isolated. *Colletotrichum* spp. were isolated most frequently.

Most of the ring-neck necrotic scars yielded at least one fungal pathogen known to be associated

with postharvest rots (Table 2). A smaller number of the fruits attached to these pedicels developed rots and most of the fungi isolated from the stem-end rots differed from the fungi isolated from the adjacent pedicel. In only 11 instances (29%) was the same fungus isolated from a diseased fruit and its adjacent pedicel.

### Infections of living twigs and branches

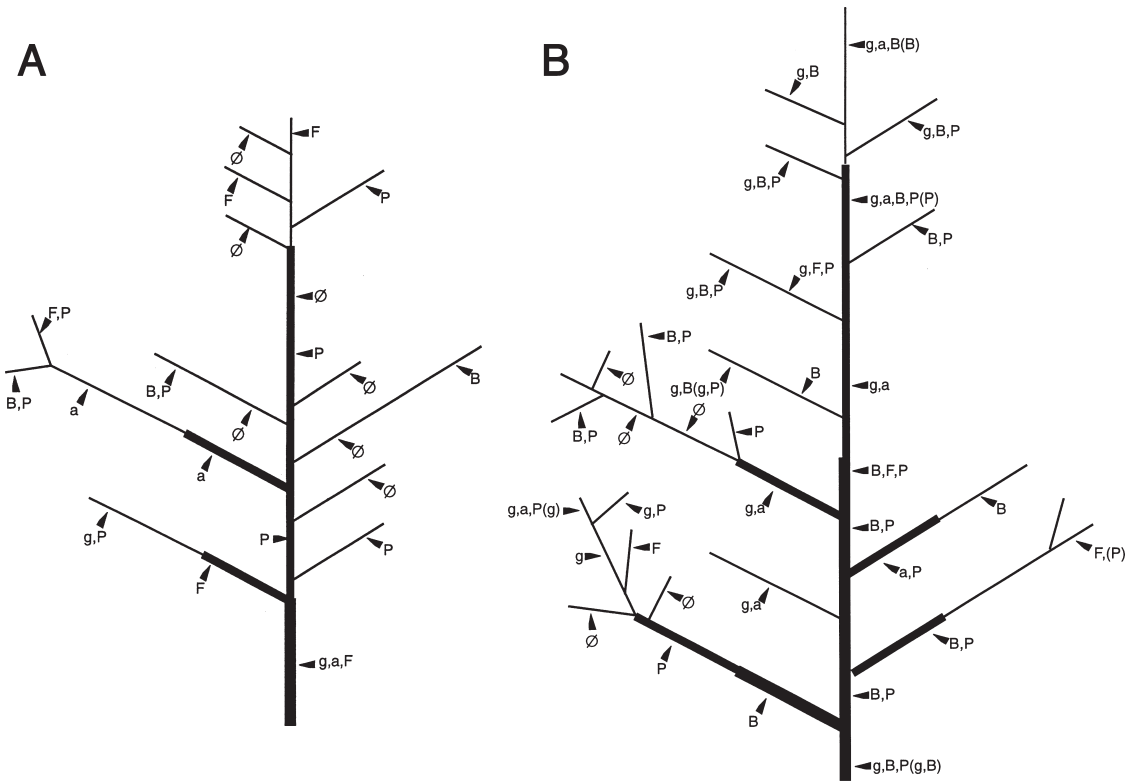
Most of the intact samples of twigs and branches sectioned (i.e., before removal of the extra-cambial tissues) were colonised by one or more fruit-rot pathogens (Table 3). The pathogens were isolated from 95 and 100% of the segments from the two Te Puke samples, and from 73 and 74% of the segments from the Ruahihi samples. There was a lower frequency of colonisation of the xylem and pith, pathogens being isolated from 17 and 49% of the Te Puke segments and from 4 and 15% of the Ruahihi segments. It was observed that colonisation of the xylem elements was sometimes associated with wound sites.

**Table 1** Frequency of isolations of postharvest rot pathogens from dead twigs and branches collected from three Bay of Plenty, New Zealand orchards.

Pathogen	Pyes Pa (20 samples)	Apata (18 samples)	Te Puke (20 samples)
<i>Colletotrichum acutatum</i>	12	4	13
<i>C. gloeosporioides</i>	19	15	14
<i>Botryosphaeria parva</i>	2	2	3
<i>B. dothidea</i>	1	0	0
<i>Fusicoccum luteum</i>	6	0	2
<i>Phomopsis</i> spp.	5	5	4
Uncolonised twigs	0	0	3

**Table 2** Isolations of postharvest rot pathogens from 88 ring-neck necrosis scars and their associated fruits.

Pathogen	Isolations (%)		
	From ring-neck scars	From fruit	Same pathogen from both
<i>Colletotrichum acutatum</i>	16	5	2
<i>C. gloeosporioides</i>	58	9	7
<i>Botryosphaeria parva</i>	2	6	0
<i>B. dothidea</i>	1	10	1
<i>Fusicoccum luteum</i>	3	0	0
<i>Phomopsis</i> spp.	31	13	3
<i>Fusarium</i> spp.	5	0	0
None isolated	13	55	0



**Fig. 2** A, B, Distribution of fruit-rot pathogens in two terminal shoots of avocado (*Persea americana*) trees collected from a Ruahihi, New Zealand orchard. For details see Fig. 1.

**Table 3** Frequency of isolations of postharvest rot pathogens from living twigs and branches collected from two Bay of Plenty, New Zealand, orchards.

Pathogen	Twig sample:	Te Puke Nov 1991 132 samples		Te Puke Mar 1992 41 samples		Ruahihi Dec 1992 82 samples		Ruahihi Mar 1993 86 samples	
		Intact	Xylem + pith	Intact	Xylem + pith	Intact	Xylem + pith	Intact	Xylem + pith
<i>Colletotrichum acutatum</i>		32	3	15	3	9	0	9	0
<i>C. gloeosporioides</i>		82	9	23	3	29	1	22	3
<i>Botryosphaeria parva</i>		26	4	12	2	15	2	26	3
<i>B. dothidea</i>		3	0	0	0	1	0	0	0
<i>Fusicoccum luteum</i>		22	1	11	0	14	1	14	0
<i>Phomopsis</i> spp.		56	11	33	14	24	0	42	4
<i>Fusarium</i> spp.		2	0	0	0	5	0	2	0
No pathogens present		6	109	0	21	22	79	22	73

Overall *C. gloeosporioides* was the fruit-rot pathogen most frequently isolated but most of the other pathogens were regularly isolated. However, *B. dothidea* was found in only four segments. When the distribution of the fruit-rot pathogens was plotted on the maps of the branches drawn before the samples were sectioned only *C. gloeosporioides* showed evidence of continuity of colonisation from section to section (Fig. 1A,B). There was no apparent continuity of colonisation by any of the pathogens in the Ruahihi samples (Fig. 2A,B). Some of the other pathogens were isolated from a few adjacent segments on the branches but showed no evidence of more widespread colonisation. It was further observed that pathogens were rarely present in all the sections plated from a single segment.

*Fusarium* spp. were also found occasionally but these were isolated infrequently from fruit rots (Hartill 1991). Other fungi, not known to cause fruit rots, were frequently isolated from the samples, an unidentified white sterile fungus and a species of *Sporormiella* being the most common.

**Table 4** Twig and branch age and percentage frequency of infection (%).

Age	Te Puke	Te Puke	Ruahihi	Ruahihi
	Nov 1991	Mar 1992	Dec 1992	March 1993
3rd-year wood	100	100	91	100
2nd-year wood	88	100	80	72
1st-year wood	97	100	65	71

The most frequently colonised sections were those taken from 3rd-year wood (Table 4). However, the number of 3rd-year wood sections investigated was much fewer than the numbers of 1st-year and 2nd-year samples. There was no statistically significant relationship between the frequency of isolation of the fruit-rot pathogens and the age of the sections.

### Infections of the pedicels

Fruit-rot pathogens were present in almost all the pedicels of the harvested fruits throughout the 1994–95 trial. *Phomopsis* and *Colletotrichum* spp. were the fungi most frequently isolated from the pedicels immediately after harvest (Table 5). They were frequently isolated from both ends of the same pedicel. There was only a small variation in the incidence of these fungi between the October 1994 and December 1994 samples but there was a marked decrease in the incidence of *Phomopsis* spp. and an increase in the incidence of *C. acutatum* in the January 1995 sample.

The *Botryosphaeria* (*Fusicoccum*) species were found in a smaller proportion of the pedicels than were the other fruit-rot pathogens throughout the trial and only *B. parva* was sometimes found in both ends of the same pedicel and then only on a few occasions. Similar isolation frequencies were obtained from the pedicels sampled after storage for 2 days (unpubl. data).

There was a greater incidence of *C. gloeosporioides* and a smaller incidence of *C. acutatum* and *Fusicoccum luteum* in pedicels left attached to the fruits and sampled at fruit ripening

**Table 5** Fungal species isolated from avocado (*Persea americana*) pedicels immediately after harvest, October and December 1994, and January 1995. (NB. October data are based on 100 pedicels; December and January data on 75 pedicels.)

Fungus	Isolates from pedicel (%)						Identical fungus at both ends (%)		
	Tree end			Fruit end			Oct	Dec	Jan
	Oct	Dec	Jan	Oct	Dec	Jan			
<i>Phomopsis</i> spp.	74	65	23	66	65	28	55	44	11
<i>Colletotrichum gloeosporioides</i>	16	20	36	26	23	26	8	16	15
<i>C. acutatum</i>	16	24	33	19	24	45	5	8	21
<i>Botryosphaeria parva</i>	3	6	4	11	14	12	0	4	3
<i>B. dothidea</i>	0	1	0	1	1	0	0	0	0
<i>Fusicoccum luteum</i>	2	6	3	9	6	8	0	0	0
Saprophytes	15	5	12	9	13	10	0	0	0
Sterile sites	5	19	15	5	6	4	1	4	7

(Table 6) than in pedicels sampled immediately after harvest (Table 5). The incidence of *B. parva* was similar in both sets of samples. Storing until the fruits had ripened had little effect on the incidences of the other fungi.

Fungi other than known fruit-rot pathogens were also isolated from a few pedicels, including a few unidentified, sterile colonies, not included in the tables. However, none of these occurred as frequently as the fruit-rot pathogens.

There was a marked tendency, especially with the *Botryosphaeria* species, for the pathogens to be isolated more frequently from the fruit-end of the pedicel, than from the tree-end. However, there was little evidence of endophytic growth of pathogens

through the pedicels and into the fruits in the October 1994 and December 1994 samples (Table 7). A few of the fruits harvested in January 1995 and stored with the pedicels attached developed stem-end rots but the pathogens isolated were at a lower incidence than in the corresponding fruit-ends of the pedicels (Tables 6 and 7). Only two possible non-pathogens, an actinomycete and a white sterile fungus, were isolated from fruit rots.

**Harvesting trials**

In the 1990–91 trial the severity of postharvest rots in fruits harvested by clipping the pedicels increased as the season progressed (Table 8). In plucked fruits the severity was greatest in the November 1990

**Table 6** Fungal species isolated from avocado (*Persea americana*) pedicels after the attached fruits had ripened, December 1994, and January 1995. (NB. Data are based on 75 pedicels sampled on both occasions.)

Fungus	Tree end		Fruit end		Identical fungus at both ends (%)	
	Dec	Jan	Dec	Jan	Dec	Jan
<i>Phomopsis</i> spp.	38	20	49	29	22	8
<i>Colletotrichum gloeosporioides</i>	42	59	61	56	35	36
<i>C. acutatum</i>	15	11	34	19	5	0
<i>Botryosphaeria parva</i>	3	0	14	16	3	0
<i>B. dothidea</i>	0	0	3	0	0	0
<i>Fusicoccum luteum</i>	3	1	0	3	0	0
Saprophytes	6	17	19	17	0	2
Sterile sites	15	8	4	1	–	–

**Table 7** Fungal species isolated from the stem-end of avocado (*Persea americana*) fruits immediately after harvest, after ripening with attached pedicels, and from commercially picked fruits, October and December 1994, and January 1995. (NB. October and commercial crop data are based on 100 fruits sampled; other December and January data are based on 75 fruits.)

Fungus	Isolated immediately after picking (%)			Isolated after storage for 2 days (%)			Isolated after ripening with complete pedicel attached (%)			Isolated from ripened commercial crop (%)		
	Oct	Dec	Jan	Oct	Dec	Jan	Oct	Dec	Jan	Oct	Dec	Jan
<i>Phomopsis</i> spp.	2	3	0	0	1	0	–	0	7	0	7	7
<i>Colletotrichum gloeosporioides</i>	0	0	0	0	0	0	–	1	3	0	3	3
<i>C. acutatum</i>	0	0	0	0	0	0	–	0	0	0	0	0
<i>Botryosphaeria parva</i>	0	0	0	0	0	0	–	0	2	0	13	3
<i>B. dothidea</i>	0	0	0	0	0	0	–	0	0	1	0	1
<i>Fusicoccum luteum</i>	0	0	0	0	0	0	–	0	0	0	0	0
Saprophytes	0	0	0	0	0	0	–	0	0	0	1	1
Unidentified	0	1	3	0	0	0	–	0	2	0	1	0
Sterile fruits	98	96	97	100	99	100	–	99	86	99	85	85



sample, almost entirely because of a high incidence of stem-end rots caused by *C. acutatum*. The fruits were dry when harvested but earlier rainfall was still present on some leaves. In the January 1991 sample the severity of postharvest rots was greater in the clipped fruit mainly because of a relatively high incidence of *C. gloeosporioides*.

In the February 1991 sample the plucked fruits again had a high proportion of rots caused by *C. acutatum*. The fruits were picked following a heavy dew but appeared to be dry. The incidence of *B. parva* was much higher than in the previous samples but was similar in both plucked and clipped fruits. Frequently more than one pathogen was isolated from a single fruit.

In the 1997 trial, fruits harvested with sterilised clippers, with or without further dipping in fungicide, had statistically significantly fewer diseased fruits ( $P < 0.01$ ), and more acceptable fruits ( $P < 0.05$ ) than fruits harvested by plucking (Table 9). The

differences in the rot severity index between these treatments was more variable but approached significance at the  $P = 0.05$  level.

These differences were almost entirely because of differences in the incidence of stem-end rot infections (Table 9). The differences in the incidence of body rots were relatively small although the incidence in the Grower pick treatment was somewhat higher than in the other treatments almost entirely because of a high incidence of *C. gloeosporioides* in a single replicate.

There was no overall significant difference between treatments in the incidence of *Botryosphaeria* spp. present in the stem-end rots although there was a strong indication that the incidence was reduced by sterilising the clippers (Table 10). Plucked fruits had an intermediate incidence of stem-end rots caused by *Botryosphaeria* spp. The incidence of *C. acutatum* was lower than that of *Botryosphaeria* spp. In isolations from stem-end rots

**Table 8** Severity of stem-end postharvest rots in avocado (*Persea americana*) fruits harvested by clipping or by plucking and the pathogens isolated from those rots. (50 fruits were sampled on each occasion.) (*C. a.*, *Colletotrichum acutatum*; *C. g.*, *C. gloeosporioides*; *B. p.*, *Botryosphaeria parva*; *B. d.*, *B. dothidea*; *Phom.*, *Phomopsis* spp.)

Harvested month and method	% fruits		Rot index (%)		% of fruits with pathogens				
	Healthy	Acceptable	All rots	Stem-end rots	<i>C. a.</i>	<i>C. g.</i>	<i>B. p.</i>	<i>B. d.</i>	<i>Phom.</i>
<b>Nov</b>									
Clipped	78	86	13.6	7.4	10	4	4	4	0
Plucked	20	31	56.9	55.7	71	4	4	0	0
<b>Jan</b>									
Clipped	52	62	26.2	22.2	8	32	6	2	2
Plucked	78	79	12.3	7.7	15	13	0	2	0
<b>Feb</b>									
Clipped	46	60	34.2	18.0	14	4	52	0	0
Plucked	20	36	47.6	38.4	58	10	46	0	2

**Table 9** Proportion of healthy and acceptable fruits, rot severity index, stem-end rot, and body rots in samples of fruits harvested using different methods.

	Healthy fruits (%)	Acceptable fruits (%)	Rot severity index (%)	Stem-end rots (%)	Body rots (%)
Grower pick	69.5 ± 13.9	76.3 ± 10.4	20.0 ± 13.3	20 ± 12	14 ± 11
Normal pick	76.5 ± 7.5	86.0 ± 9.1	15.4 ± 8.5	23 ± 12	4 ± 4
Fungicide pick	89.3 ± 7.4	91.3 ± 6.1	5.8 ± 5.4	8 ± 6	4 ± 4
Unsterile pick	76.0 ± 8.6	79.0 ± 8.3	17.4 ± 5.2	22 ± 11	5 ± 2
Sterile pick	86.0 ± 5.2	89.0 ± 6.0	8.3 ± 4.9	7 ± 4	8 ± 3
Plucked	61.0 ± 8.7	69.0 ± 10.5	21.6 ± 6.3	31 ± 7	6 ± 2

**Table 10** Incidence of *Botryosphaeria* (*Fusicoccum*) species and *Colletotrichum acutatum* isolated from stem-end rots in samples of fruits harvested using different methods.

	<i>Botryosphaeria</i> spp. (%)	<i>Colletotrichum</i> <i>acutatum</i> (%)
Grower pick	18 ± 10	3 ± 2
Normal pick	17 ± 9	1 ± 2
Fungicide pick	6 ± 4	2 ± 2
Unsterile pick	17 ± 4	4 ± 6
Sterile pick	5 ± 2	1 ± 2
Plucked	12 ± 4	16 ± 7

except in the plucked treatment where it was significantly greater ( $P < 0.001$ ) than in all other treatments.

Scrapings of old plant material, taken from the grower's clippers before they were used in this trial, were plated onto PDSA. *C. gloeosporioides*, *C. acutatum*, *B. parva*, and a number of saprophytes were present in the surface debris.

## DISCUSSION

Avocado fruit-rot pathogens were found throughout the branches and twigs in the avocado canopy samples examined, as has been reported in avocados and mangoes (Johnson et al. 1991; Johnson et al. 1992). A high proportion of the isolations made from the living twigs and branches of the trees came from the extra-cambial tissues (Fig. 1A,B; Fig. 2A,B; Table 3). Such infections should more properly be termed "phellyphytes" rather than endophytes (Kowalski & Kehr 1996). Endophytic growth of fungi generally occurs via the xylem elements and relatively few isolations were made from these tissues in the work reported here (Table 3). Although the pathogens were isolated from dead twigs and branches (Table 1) they appeared to be present as saprophytes and there was no evidence that they had killed the tissues. A similar pattern of infection has been found for *B. dothidea* in peaches (Pusey 1993). The fruit-rot pathogens dominated the fungal population present in the twigs and branches; no other fungi were isolated as frequently as the fruit-rot pathogens, apart from *B. dothidea*.

There are at least four possible pathways of infection that could lead to the initiation of stem-end rots: (1) the site may be infected at flowering and

the infection then remain dormant until the fruit starts to ripen; (2) the site may be less resistant to, or have a more favourable microclimate for, infection throughout the development of the fruit; (3) the pathogens may be endophytic within the branches and twigs of the tree and from there grow into the fruits (Johnson et al. 1991); or (4) airborne spores or fragments of pathogens present in or on the outer cell layers of the pedicels may be transferred to the exposed xylem elements at harvest and from there grow into the ripening fruits. The trials reported here were designed to give some indication of the extent to which these pathways occur in practice with special emphasis on pathways 3 and 4.

Stem-end rots do not become apparent until the fruit has started to ripen. In the case of infection via pathways 1, 2, and 3, this might be explained by the presence of antifungal dienes in the unripe fruits (Prusky et al. 1982, 1983, 1991). However, there is a report that while the dienes within the skin are active the dienes within the flesh of the fruit are compartmentalised and have no effect on fungal invasion (Kobiler et al. 1993). Activity at the stem-end of the fruit does not appear to have been investigated. Pathway 4, which is not initiated until the fruits are harvested, would be less affected by the presence of antifungal dienes since these break down once the fruit starts to ripen after harvest.

The incidence of stem-end rots in New Zealand 'Hass' avocados tends to be low until January when the fruits are c. 13 months old (Hartill unpubl. data). A similar relationship between fruit maturity and stem-end rot incidence has been reported from Queensland (Peterson 1978). This discounts the importance of an early latent infection, e.g., at flowering (pathway 1), in the initiation of stem-end rots since the expression of such an early infection should not be affected by the time of harvest unless some other, as yet unreported, maturity factor is involved. In ripening fruits, stem-end rots frequently spread through the fruits via the xylem elements (see Fig. 1, Hartill 1991) unlike the body rots which develop more or less evenly through all the tissues. This, in turn, tends to discount the possible importance of a peculiarly favourable microclimate site (pathway 2) which is equivalent to a special form of body rot.

The evidence supporting endophytic growth through the tree as the source of infection (pathway 3) is also not strong. Pathogens were infrequently isolated continuously throughout each twig or branch sample examined, and except for *C. gloeosporioides*, were rarely found in more than a few one or two

contiguous sections (Fig. 1). The pathogens were found at much greater frequency in the extra-cambial tissues than in the xylem elements indicating they are more likely to be phellophytes than endophytes. Data from the trial where the infection of the pedicels was studied is, at best, only weakly supportive of endophytic growth (Table 6). Pathogens were isolated from less than 1% of the fruits examined immediately after picking or after 2 days of storage, and only in the fruits harvested in January and ripened with intact pedicels did disease incidence approach that in ripened commercially harvested fruits. It is probable that the pathogens were able to grow down the intact pedicels from the picking wound and into the fruits before the pedicels dehisced (pathway 4). In a small observational trial where fruits were harvested with intact pedicels it was noted that the incidence of stem-end rots appeared to be related to the length of the pedicel (unpubl. data) as has been reported to occur in mangoes (Sangchote 1988). In contrast Johnson et al. (1991, 1992) provide strong evidence that stem-end rot in mangoes is related to the incidence of pathogens present endophytically in the pedicels.

There is stronger evidence in favour of infection occurring during the harvesting procedures (pathway 4), especially that obtained in trials where plucking the fruits was compared to other harvesting methods (Tables 8 and 9). Plucking results in a larger and more jagged wounding than clipping thus exposing a greater area of the xylem elements to infection. On three of the four occasions where stem-end rots in plucked fruits were compared to those in fruits conventionally harvested by clipping there was an exceptionally high incidence of *C. acutatum* in the plucked fruits (Tables 8 and 10), possibly because *C. acutatum* is a prolific spore producer. The incidence of *C. acutatum* was low in all the clipped treatments (Table 11) and not affected by sterilisation of the clippers. However, sterilising the clippers, with or without further dipping them in fungicide, did appear to reduce the incidence of *Botryosphaeria* spp. The most frequently isolated of these species, *B. parva* is also a prolific producer of spores (Hartill unpubl. data) but the incidence of *B. parva* in plucked fruits was intermediate between that in fruits harvested with sterilised clippers and that in fruits harvested with unsterilised clippers. It therefore seems probable that some of the rots are initiated by contamination from the unsterilised clippers, possibly when fragments of infected extra-cambial tissues are transferred to the open ends of the xylem elements.

Although the data presented here do not entirely discount the possibility that some stem-end rots may be initiated at flowering, or early in fruit development, or through the endophytic development of the pathogens through the pedicels into the fruits, they do indicate that infections initiated during harvesting are important. If this is confirmed then ways of reducing the incidence of stem-end rots by modification of harvesting techniques should be investigated.

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#### REFERENCES

- Binyamini, N.; Schiffmann-Nadel, M. 1972: Latent infection in avocado fruit due to *Colletotrichum gloeosporioides*. *Phytopathology* 62: 592–594.
- Coates, L. M.; Muirhead, I. F.; Irwin, J. A. G.; Gowanlock, D. H. 1993: Initial infection processes by *Colletotrichum gloeosporioides* on avocado fruit. *Mycological Research* 97: 1363–1370.
- Darvas, J. M.; Kotze, J. M. 1987: Fungi associated with pre- and post-harvest diseases of avocado fruit at Westfalia Estate, South Africa. *Phytophylactica* 19: 83–85.
- Darvas, J. M.; Kotze, J. M.; Wehner, F. C. 1987: Field occurrence and control of fungi causing postharvest decay of avocados. *Phytophylactica* 19: 453–455.
- Fitzell, R. 1988: Pre-harvest disease control. Proceedings of the Australian Avocado Growers Federation, Bicentennial Conference, Caloundra. Pp. 68–69.
- Hartill, W. F. T. 1991: Post-harvest diseases of avocado fruits in New Zealand. *New Zealand Journal of Crop and Horticultural Science* 19: 297–304.
- Hartill, W. F. T. 1992: Post-harvest rots of avocados in New Zealand and their control. *Brighton Crop Protection Conference Pests and Diseases—1992(3)*: 1157–1162.

- Johnson, G. I. 1994: Stem-end rot. *In*: Ploetz, R. C.; Zentmyer, G. A.; Nishijima, W. T.; Rohrbach, K. G.; Ohr, H. D. *ed.* Compendium of tropical fruit diseases. St Paul, Minnesota, APS Press. Pp. 39–40.
- Johnson, G. I.; Cooke, A. W.; Mead, A. J.; Wells, I. A. 1991a: Stem rot pathogens of fruit are endophytes. Australasian Plant Pathology Society 8th Conference, Sydney. P. 34 (Abstract).
- Johnson, G. I.; Mead, A. J.; Cooke, A. W.; Dean, J. R. 1991b: Mango stem end rot pathogens—infection levels between flowering and harvest. *Annals of Applied Biology* 119: 465–473.
- Johnson, G. I.; Mead, A. J.; Cooke, A. W.; Dean, J. R. 1992: Mango stem end rot pathogens—fruit infection by endophytic colonization of the inflorescence and pedicel. *Annals of Applied Biology* 120: 225–234.
- Kobiler, I.; Prusky, D.; Midland, S.; Sims, J. J.; Keen, N. T. 1993: Compartmentation of antifungal compounds in oil cells of avocado fruit mesocarp and its effect on susceptibility to *Colletotrichum gloeosporioides*. *Physiological and Molecular Plant Pathology* 43: 319–328.
- Kowalski, T.; Kehr, R. D. 1996: Fungal endophytes of living branch bases in several European tree species. *In*: Redlin, S. C.; Carris, L. M. *ed.* Endophytic fungi in grasses and woody plants. Minnesota, APS Press. Pp. 67–86.
- Morgan-Jones, G.; White, J. F. 1987: Notes on Coelomycetes. II. Concerning the *Fusicoccum* anamorph of *Botryosphaeria ribis*. *Mycotaxon* 30: 117–125.
- Ohr, H. D.; Coffee, M. D.; McMillan, R. D. 1991: Avocado (*Persea americana* Miller). *In*: Common names for plant diseases. *Plant Disease* 75: 225–226.
- Pennycook S. R.; Samuels, G. J. 1985: *Botryosphaeria* and *Fusicoccum* species associated with ripe fruit rot of *Actinidia deliciosa* (kiwifruit) in New Zealand. *Mycotaxon* 24: 445–458.
- Peterson, R. A. 1978: Susceptibility of Fuerte avocado fruit at various stages of growth, to infection by anthracnose and stem end rot fungi. *Australian Journal of Experimental Agriculture and Animal Husbandry* 18: 158–160.
- Petrini, O. 1986: Taxonomy of endophytic fungi in aerial plant tissues. *In*: Fokkema, N. J.; van den Heuvel, J. *ed.* Microbiology of the phyllosphere. Cambridge, Cambridge University Press. Pp. 175–187.
- Prusky, D.; Keen, N. T.; Eaks, I. L. 1983: Further evidence for the involvement of a preformed antifungal compound in the latency of *Colletotrichum gloeosporioides* in unripe avocado fruits. *Physiological Plant Pathology* 22: 189–198.
- Prusky, D.; Keen, N. T.; Sims, J. J.; Midland, S. 1982: Possible involvement of a preformed antifungal compound in the latency of *Colletotrichum gloeosporioides* in unripe avocado fruits. *Phytopathology* 72: 1578–1582.
- Prusky, D.; Plumbley, R. A.; Kobiler, I. 1991: The relationship between antifungal diene levels and fungal inhibition during quiescent infection of unripe avocado fruits by *Colletotrichum gloeosporioides*. *Plant Pathology* 40: 45–52.
- Pusey, P. L. 1993: Role of *Botryosphaeria* species in peach tree gummosis on the basis of differential isolation from outer and inner bark. *Plant Disease* 77: 170–174.
- Sangchote, S. 1988: Botryodiplodia stem end rot of mango and its control. *Kasetsart Journal, Natural Sciences* 22: 67–70.
- Snowdon, A. L. 1990: A colour atlas of post-harvest diseases and disorders of fruits and vegetables. Volume 1. London, Wolfe Scientific. Pp. 93–101.
- Zauberman, G.; Schiffmann-Nadel, M.; Fuchs, Y.; Yanko, U. 1975: La biologie et la pathogénicité des champignons causant les pourritures de l'avocat après la récolte. *Fruits* 30: 499–502.
- Zentmyer, G. A. 1951: Avocado diseases. California Avocado Society 1951 Yearbook. Pp. 97–100.
- Zentmyer, G. A. 1953: Diseases of the avocado. United States Department of Agriculture Yearbook. Washington, DC, United States Department of Agriculture. Pp. 875–880.