

## Calcium, fungicide sprays and canopy density influence postharvest rots of avocado

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**Abstract.** A study was carried out in eight orchards in 1999, 2000 and 2001 to examine the effect of preharvest factors on postharvest rots of 'Hass' avocados in New Zealand. A further 14 orchards were sampled in 1999 and, of these, 13 were sampled in 2000, depending on fruit availability. Orchards were selected following consultation with industry personnel to obtain a sample representative of fruit quality in New Zealand. Fruit were harvested in early January at a dry matter content of ~33%, then stored and ripened at 20°C. Fruit were assessed for stem-end and body rots when ripe, and fungi from rots were isolated and identified. For the 3 years' data collected from the same eight orchards, the incidence of stem-end rots was linearly related to the number of fungicide applications, mainly of copper and a few of benomyl. In 1999 and 2000, an index describing canopy density and the amount of dead branches in the canopy was significantly related to the incidence of body rots, but not to the incidence of stem-end rots. In 2000, a significant relationship was found between body rot incidence and severity and fruit calcium levels. Body rots were found to decrease with increasing temperature and rainfall in 2000 when these data were collected for seven orchards. In contrast, calcium content in fruit was positively related to these factors. The most common pathogen from isolations was *Botryosphaeria parva*, followed by *Colletotrichum acutatum*. To improve fruit quality of avocados in New Zealand, it is recommended that growers apply fungicides more than eight times and calcium to increase the (calcium + magnesium)/potassium ratio above 0.065 in fruit.

**Additional keywords:** *Botryosphaeria dothidea*, *Colletotrichum gloeosporioides*, *Phomopsis* sp.

### Introduction

The New Zealand avocado industry began in the 1980s. Recently, plantings have been increasing at an exponential rate (plantings 5 years or older were 946 ha in 1996–97, 2138 ha in 2003–04), but production has been at similar levels for the last 4 years (0.6 million trays [each of 5.5 kg] in 1996–97, 2.3 million trays in 2000–01, and 1.94 million trays in 2003–04; Anonymous 2004). Currently, over 98% of avocados grown in New Zealand are *Persea americana* 'Hass', and 64% of total production is based in the Bay of Plenty (Kaitikati and Te Puke), 23% in the mid-North (Whangarei and Mangawhai), 10% in the Far North (Kaitiaki), and 4% in the rest of New Zealand (Fig. 1; Anonymous 2004). Avocados in New Zealand set fruit mainly during October, and most are harvested 11–17 months later from September until March. New Zealand fruit are sold in the domestic market and exported to Australia, USA, Japan and other Asian countries. Fruit are cool-stored at 4–7°C for varying times up to ~30 days. Coolstorage can change fungal populations and affect the amount of rots (Everett 2003). Ripening temperatures can improve fruit quality by reducing rots (Hopkirk *et al.* 1994). Fruit in this study were stored at 20°C, a temperature that allowed sufficient rots to express in order to find differences among fruit from different orchards.

The fungi that infect avocados and cause postharvest rots are primarily water dispersed (Fitzell and Peak 1984; Fitzell 1987;

Ahimera *et al.* 2004). Avocado fruit are infected by *Colletotrichum gloeosporioides* as latent infections (Binyamini and Schiffmann-Nadel 1972). The mechanism of infection by other pathogens has not been reported for avocados, but in apples, Kim *et al.* (1999) showed both latent and direct infection by spores of *Botryosphaeria dothidea*, a fungus that also infects avocados. There are two forms of disease: body rots, infecting fruit through the sides, or stem-end rots, beginning at the wounded pedicel then growing into the flesh. In New Zealand, Hartill (1991) reported that *C. acutatum*, *C. gloeosporioides*, *B. parva*, *B. dothidea*, *Fusicoccum luteum* and *Phomopsis* spp. cause body rots and stem-end rots of avocados. Postharvest rots of avocados in New Zealand are thus best described as a disease complex, and infections by different pathogens are difficult to distinguish except by isolations. In all other avocado-growing countries, body rots are caused most commonly by *C. gloeosporioides*, and stem-end rots by *Dothiorella* spp. (Snowden 1990). The genera *Dothiorella* and *Fusicoccum* are synonymous with the anamorphs of *Botryosphaeria* (Pennycook and Samuels 1985).

Infections by *C. gloeosporioides* that lead to postharvest rot infections in avocados are latent (symptomless) while fruit are still attached to the tree and express only as fruit ripen (Prusky *et al.* 1991a, 1991b; Coates *et al.* 1993a). Infection

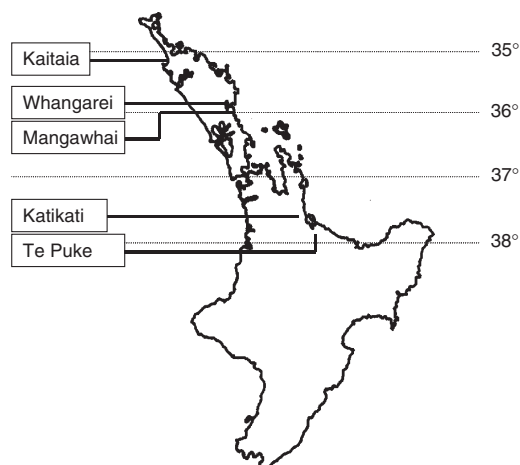


Fig. 1. Geographic location and latitude of the regions studied.

takes place in the field during the season (Binyamini and Schiffmann-Nadel 1972; Peterson 1978; Coates *et al.* 1993b) and there is very little evidence for postharvest infection. Usually the postharvest rot complex is controlled in the field by application of copper fungicides to reduce inoculum levels (Peterson and Inch 1980; Darvas *et al.* 1987; Hartill 1992). In New Zealand, only benomyl and copper were registered for use in avocado orchards even though recently, benomyl has been removed from sale. Benomyl was applied infrequently, not more than three applications per season, and copper can be applied up to 18 times in a season. All growers that spray apply copper but only a few apply benomyl as well. Efficacy trials in orchards have shown that both fungicides are equally effective (Hartill 1992). Additional control is achieved by applying postharvest fungicide applications of prochloraz, and by storing, transporting and selling fruit at optimal temperatures (Hartill 1992; Hopkirk *et al.* 1994; Everett and Korsten 1998). Despite this knowledge, postharvest rots of avocados can still cause economically significant losses in the marketplace (Olphert 2000).

Preharvest factors other than inoculum levels are known to be important in the susceptibility of fruit to fungal pathogens. For instance, rot diseases in fruits other than avocados have been reduced by preharvest application of calcium (Ca) (Vang-Petersen 1980; Smith and Gupton 1993)

and nitrogen fertilisation (Davenport and Provost 1994), and *Botrytis* spp. in grapes can be managed by reducing canopy density (Gubler *et al.* 1987).

Calcium is frequently described as a ratio with magnesium (Mg) and potassium (K) because these other minerals interact with and affect Ca uptake (Martin-Prevel *et al.* 1987). In avocados, mineral nutrition, particularly fruit Ca status, has previously been implicated in a variety of storage problems including chilling injury, poor shelf-life and vascular browning (Tingwa and Young 1974; Chaplin and Scott 1980; Eaks 1985; Thorp *et al.* 1997). A significant inverse relationship between the incidence of body rots and fruit Ca and Mg status and the (Ca + Mg)/K ratio in 'Hass' avocados has been found by other workers (Hofman *et al.* 2002). Relationships have also been found between fruit storage disorders and the (Ca + Mg)/K ratio in the soil and leaves of 'Fuerte' avocados (Koen *et al.* 1990). Willingham *et al.* (2004) hypothesised that improved quality of 'Hass' avocado fruit from non-vigorous trees was a result of a higher concentration of Ca compared with levels in fruit from vigorous trees. The following study was instigated to investigate the effect of selected preharvest factors on postharvest rots of avocados.

## Materials and methods

### Fruit harvesting, maturity and nutrition

Fruit were sampled from eight avocado orchards in early January 1999, 2000 and 2001, when fruit dry matter averaged 33.9, 33 and 33.6%, respectively. January is late-season for New Zealand avocados, a time when rots are known to be prevalent. Orchards were selected on the basis of advice from industry personnel to include both good and bad orchards from each of the main avocado growing regions of New Zealand. Selected grower practices and environmental parameters were recorded and analysed to determine their influence on the incidence and severity of postharvest rots.

The orchard locations ranged from Kaitaia in Northland to Te Puke in the Bay of Plenty (Fig. 1). The same eight orchards were sampled each year, but in 1999 and 2000, fruit were sampled from additional orchards, a total of 22 and 21 orchards, respectively (Table 1). Fruit were harvested (11 fruit from each of 10 trees per orchard selected randomly), and immediately packed into avocado boxes containing fibreboard trays (Plix), with 11 fruit per box. Fruit were transported to a 20°C controlled temperature room at Mount Albert Research Centre (MARC)

Table 1. Number of orchards sampled in each region and rainfall and temperature data

Rainfall and temperature data are from long-term weather stations (New Zealand Meteorological Service 1980). Mangawhai and Katikati data are from weather stations about 30 km distant (Leigh and Tauranga). The eight orchards selected in 2001 were the four orchards with the fewest rots and the four orchards with the most rots in 2000

Region	No. of orchards sampled			Annual rainfall (mm)	Average daily temperature (°C)		No. of years data recorded
	1999	2000	2001		Maximum	Minimum	
Kaitaia	4	4	0	2234	19.5	11.7	32
Whangarei	6	6	2	2018	19.9	10.9	31
Mangawhai	2	2	2	1574	18.7	12.7	24
Katikati	6	5	1	1759	18.7	11.3	10
Te Puke	4	4	3	2608	18.8	9.3	7
Total	22	21	8				

Auckland, New Zealand, within 58 h of harvest. Each year, 5–10 fruit per orchard, selected randomly, were tested for maturity immediately after arrival at MARC by calculating percentage dry matter. The flesh of half of each fruit was grated after the peel and stone were removed, and percentage dry weight determined after drying at 80°C for 24 h and then dividing by fresh weight and multiplying by 100. A further 5–10 fruit were retained for mineral analysis. The flesh from these fruit was dried and ground before being analysed for Ca, Mg and K using nitric acid digestion and atomic absorption spectroscopy (Thorp *et al.* 1997). Nitrogen was reduced to ammonium using the Kjeldahl method by sulfuric acid and selenium digestion (Allen 1974). Ammonium concentration was determined by a salicylate–isocyanurate colourimetric assay (Adamsen *et al.* 1985).

#### *Storage and rot assessment*

Each year, avocados were stored at 20°C after harvest. When ripe, as determined by hand firmness (Everett *et al.* 1999), fruit were cut in quarters and assessed for rots similar to the method of Hartill (1991). In 2001, fruit were also peeled and rots recorded as described previously (Everett 2003) and elsewhere (Korsten *et al.* 1995; Willingham *et al.* 2004). Quartering was adopted as a timesaving method when sample numbers were large in 1999 and 2000, but peeling ensured that all rots were counted and was more suitable for the smaller sample size in 2001. In both methods, fruit were assessed for rot by estimating the percentage of the surface area of the fruit flesh affected (severity) and by counting the number of fruit with rots (incidence). A sample of rotten flesh was placed on potato dextrose agar (PDA) without sterilisation if from the peeled surface, and if exposed by cutting, was re-cut with a sterilised scalpel before placing on PDA. Fungi growing out from diseased tissue after 2–3 weeks in diffuse light at ambient temperatures (about 20°C) were identified based on diagnostic morphological characteristics. Rots were described separately as body rots and stem-end rots. These categories are similar to anthracnose and stem-end rots described elsewhere (Willingham *et al.* 2004). Anthracnose is specifically used to describe rots caused by *C. gloeosporioides* on avocados (Ohr *et al.* 2006). Because rots that infect through the sides of fruit are also caused by other fungi in New Zealand, the term body rots has been adopted (Everett 2003).

#### *In-orchard data collection*

Every year, the following data were collected for each orchard: number of applications and type of fungicide from fruit set (October) to harvest 15 months later (January); shelter belt species and height; type of adjacent crop; the orchard latitude, longitude and altitude; slope (gradient of orchard); the type of irrigation; type and depth of mulch; canopy height (m); leaf height inside the canopy (m); a rating (0–3) of the amount of dead branches in the canopy; a rating (0–3) of canopy density; tree spacing (m); tree height (m) and age; and trees that were showing symptoms of *Phytophthora* infection. Symptoms of *Phytophthora* infection are yellowing and wilting followed by loss of leaves, first from the ends of branches and twigs, then progressively down the branch (Darvas *et al.* 1984). Leaf height inside the canopy was determined by standing inside the canopy and estimating the vertical height at which leaves were first

apparent. Canopy density was estimated by standing inside the canopy and taking a photograph by pointing a Nikon F601 camera skyward using a Nikon/Nikkor 80-mm lens focused at infinity. Resultant print photographs were separated into three groups, where 1 was the least dense canopy and 3 the most dense. The score of zero (no canopy) was not given. A canopy index was derived from these data using the formula: canopy index = dead branches + [(canopy density × leaf height inside canopy)/tree spacing]. This index was derived following analysis of canopy-related factors using multiple regression, stepwise regression and best subsets regression (Minitab Release 12, Minitab Inc., State College, PA) to ascertain the best fit to body rots. No fit was found to stem-end rots. Removal of dead branches has been reported to reduce rots (Hartill 1992), presumably by reducing inoculum sources (Hartill and Everett 2002), and inclusion of this factor improved the relationship with body rots.

Additional information was collected for each of the 21 orchards in 2000: fungicide rate, method of application and volume per hectare; mean weight of fruit; and leaf weight and area. Rainfall and temperature were monitored for 40 days before harvest on or near (~1 km away for one site) seven sites. Forty days before harvest was the time period selected for monitoring because fungi that infect avocados are water-dispersed and more infections may take place in periods of high rainfall immediately before harvest (Pak *et al.* 2002). Temperature was also monitored because mean daily temperatures above a threshold are required for infection to occur (Everett and Pak 2002) and these temperatures are sometimes not exceeded during January in New Zealand (Table 1).

#### *Data analysis*

Each of the factors described above was examined separately and, for nutrition, canopy index and fungicides, in combination using scatter plots, linear model analysis of variance (ANOVA) and regression analysis (best subsets and stepwise) to ascertain any influence on body rots and stem-end rots. Scatter plots showed if data required transformation to meet the assumptions of ANOVA and linear regression. When linearisation was thus required, data was transformed using the logistic [ $\log(p/1-p) = a + bx$ ] or logarithmic ( $\log_{10}$ ) functions. All factors were compared with each other for each of the 3 years. Mean fungicide data were compared with mean body and stem-end rots for the 3 years studied for the eight orchards sampled every year. The statistical and graphics packages, Minitab Release 12, Microcal Origin (OriginLab, Northampton, MA), SAS (SAS Institute Inc., Cary, NC) and MathSoft S-plus 4.5 (MathSoft International, Bagshot, UK) were used.

## **Results**

### *Number of rots*

The mean total rot incidence for the eight individual orchards sampled every year decreased over the 3 years of the survey (Table 2). There was a higher incidence of body rots in 2000 than in 1999 and 2001. The mean number of stem-end rots was higher in 1999 than in 2000 and 2001.

### *Isolations*

The most common fungus isolated from postharvest rots of avocado in New Zealand was *B. parva* (Fig. 6). The second most

**Table 2. Rot levels (mean % incidence of 100 fruit per orchard  $\pm$  s.e.) for each year of the study**

Fruit were stored and ripened at 20°C, and rots were assessed when ripe			
Factor	1999	2000	2001
Body rots	14.5 $\pm$ 8.8	21.3 $\pm$ 11.4	6.5 $\pm$ 4.6
Stem end rots	41.0 $\pm$ 19.1	16.3 $\pm$ 8.4	22.5 $\pm$ 17.1
Total rots	48.2 $\pm$ 19.8	33.9 $\pm$ 14.3	27.4 $\pm$ 16.7
No. of fungicide applications	3.9 $\pm$ 4.0	8.1 $\pm$ 6.1	9.9 $\pm$ 7.0

**Table 3. Correlations (linear correlation coefficient,  $r$ ) between rots and calcium (Ca), magnesium (Mg), potassium (K) and nitrogen (N) in 2000**

Incidence values were logit-transformed ( $\log(p/1-p)$ ) before linear regression analysis. Severity values were  $\log_{10}$  transformed before linear regression analysis. \*\* $P \leq 0.0001$ , \* $P \leq 0.02$

	(Ca + Mg)/K	Ca	Mg	K	N
Body rot incidence	-0.72**	-0.37	-0.28	0.14	-0.30
Stem end rot incidence	0.10	-0.17	0.14	0.04	0.19
Body rot severity	-0.76**	-0.51*	-0.12	0.50*	0.35
Stem end rot severity	-0.22	-0.05	0.12	-0.11	-0.04

common fungus was *C. acutatum*. *Phomopsis* sp. was seldom isolated from body rots. Analyses of regional and seasonal differences for 1999 and 2000 showed that *C. acutatum* was significantly more frequently isolated from body rots in fruit from Kaitaia than in fruit from Whangarei and Te Puke (Tukey's test,  $P < 0.05$ ), and from stem-end rots in Te Puke compared with all other regions (Fig. 7 and Table 4). All fungi apart from *B. parva* were more frequently isolated from stem-end rots in 2000 than in 1999 ( $P < 0.05$ ). *Botryosphaeria parva* was more frequently isolated from body rots in 1999 than in 2000 ( $P < 0.05$ ).

### Fungicide application

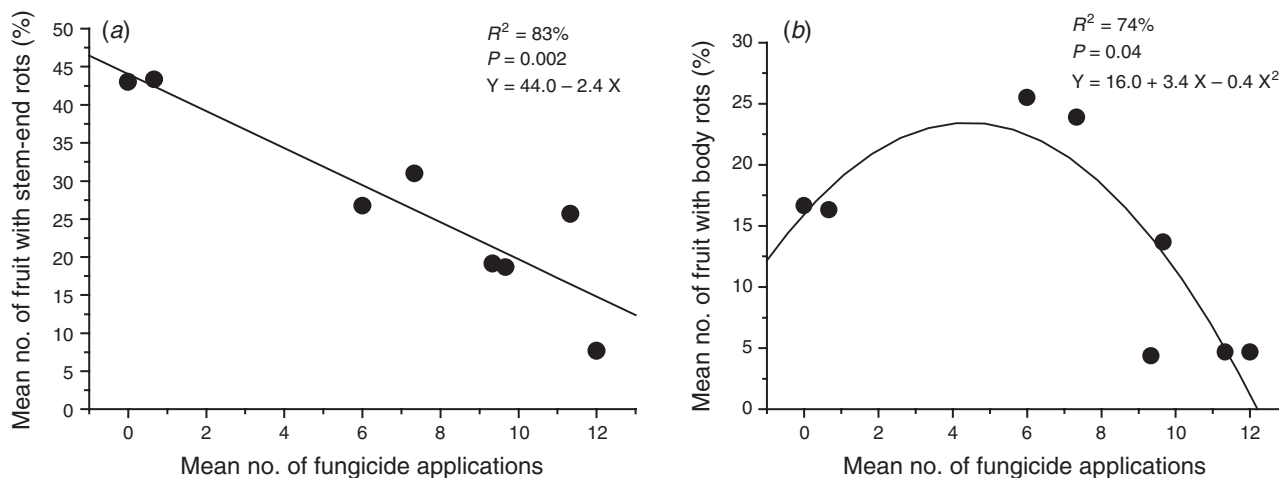
The relationship between fungicide application and rots improved when the number of months that fungicides were applied was analysed, rather than the number of times fungicides were applied. Both benomyl and copper were counted as a fungicide application because of their similar efficacy. There was no obvious relationship between spray volume, timing and rates and rots. There was a steady increase in the number of fungicide applications over the three seasons surveyed, and this was linearly related ( $P = 0.009$ ,  $R^2 = 99\%$ ) with a decrease in the numbers of rots in fruit (Table 2).

Further analysis of rot incidence in relation to fungicide application using linear and non-linear curve fitting and the mean of 3 years' data for eight orchards sampled every year showed that stem-end rot incidence was inversely linearly related to fungicide use ( $P = 0.002$ ,  $R^2 = 83\%$ ; Fig. 2a). In contrast, there was, overall, a quadratic relationship between body rot incidence and fungicide application ( $P = 0.04$ ,  $R^2 = 74\%$ ; Fig. 2b). However, in the third year, the incidence of body rots was linearly related to the number of fungicide applications ( $P = 0.01$ ,  $R^2 = 69\%$ ; Fig. 3).

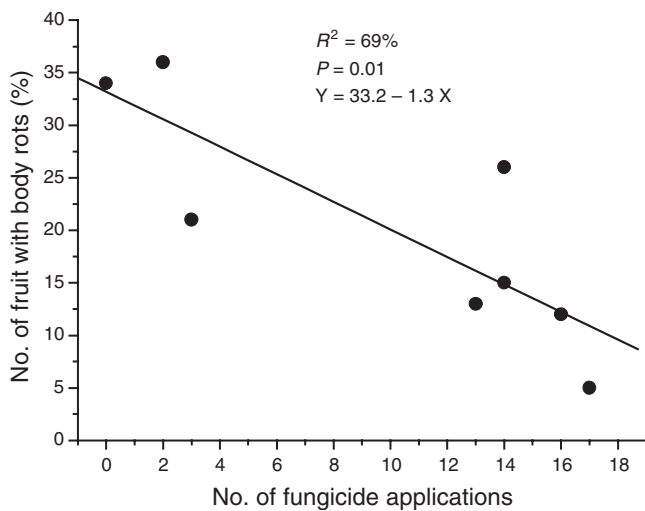
### Relationship between rots and nutrition

Mineral analysis of fruit from 22 orchards in 2000 showed there was a significant relationship between Ca and K and body rot severity ( $P = 0.02$ ,  $R^2 = 26\%$  for Ca; and  $P = 0.01$ ,  $R^2 = 28\%$  for K). Body rot incidence decreased as the amount of Ca in fruit increased, and increased with increasing K concentration. A stronger statistical relationship ( $P = 0.0001$ ,  $R^2 = 58\%$ ) was found when the incidence was compared with the ratio of (Ca + Mg)/K and this relationship was improved ( $P = 0.0001$ ,  $R^2 = 59\%$ ) when body rot severity was compared with the ratio. There were no significant relationships between body rot or stem-end incidence or severity and nitrogen or Mg (Table 3).

Statistical analysis of the 2001 data found no significant relationships between body rot incidence and the severity and the fruit content of any of the nutrients measured, or the ratio



**Fig. 2.** Relationship between the mean number of fungicide spray applications per season (14 months from November to January) and mean incidence of body rots and stem-end rots of avocado fruit ( $n = 100$  fruit per orchard). The same eight orchards were sampled in January 1999, 2000 and 2001.



**Fig. 3.** Relationship between number of peeled avocado fruit with body rots and number of fungicide applications to eight orchards in 2001.

**Table 4.** Significance of the effect of region and season on the incidence of body rots and stem-end rots  
n.a., not applicable as only one isolation was made

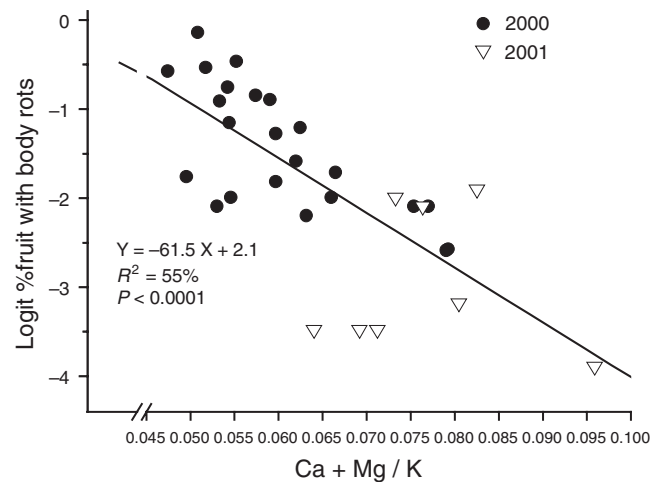
Fungus	Body rots		Stem-end rots	
	Region	Year	Region	Year
<i>C. acutatum</i>	0.011	0.811	0.027	0.002
<i>C. gloeosporioides</i>	0.295	0.597	0.217	0.0001
<i>B. parva</i>	0.345	0.002	0.741	0.443
<i>B. dothidea</i>	0.698	0.483	0.514	0.0001
<i>Phomopsis</i>	n.a.	n.a.	0.655	0.017

(Ca + Mg)/K, but when included with the 2000 data, the fit with rot incidence was improved (Fig. 4). The lack of significant relationship for the 2001 data may have been for several reasons including the smaller number of orchards sampled in 2001, the lower overall incidence of body rots (7.5–47% in 2000 and 2.5–12% in 2001), or the higher (Ca + Mg)/K ratios (0.048–0.080 in 2000 compared with 0.065–0.085 in 2001).

#### Climate factors

In 2000, body rot incidence and severity decreased as the mean temperature over the 40 days before harvest increased for seven orchards. When incidence of body rots were logit-transformed, the linear relationship was significant ( $P = 0.01$ ,  $R^2 = 74.3\%$ ; Fig. 5).

The relationship between rot incidence and severity and rainfall was examined by analysing total rainfall over 24 and 48 h, and 10-, 20-, 30- and 40-day periods before picking. Body rots decreased as rainfall increased for 20- ( $P = 0.03$ ) and 30-day periods ( $P = 0.04$ ) (Fig. 5). There was no significant relationship with stem-end rots. Temperature and rainfall were not interrelated. There were strong relationships between the (Ca + Mg)/K ratio, logit body rots, temperature and rainfall (Fig. 5). The (Ca + Mg)/K ratio was positively linearly related with temperature ( $P = 0.02$ ,  $R^2 = 67.2\%$ ) and rainfall ( $P = 0.03$ ,  $R^2 = 66.2\%$ ; Fig. 5).



**Fig. 4.** Relationship between calcium (Ca), magnesium (Mg) and potassium (K) expressed as (Ca + Mg)/K and frequency of body rots in avocado fruit. Data from 2000 and 2001 are combined.

The incidence of body rots in fruit from the same orchards sampled the following year (2001) was not related with the previous year's rainfall. There was a positive linear relationship between temperature in orchards for 40 days before harvest in 2000 and the (Ca + Mg)/K ratio of avocado fruit sampled the following season (2001) ( $P = 0.03$ ,  $R^2 = 72\%$ ).

#### Canopy index

There was a weak relationship between body rot incidence and severity and canopy index for each of the 2 years that this factor was investigated (1999:  $P = 0.04$ ,  $R^2 = 18.5\%$ ; and 2000:  $P = 0.02$ ,  $R^2 = 23.3\%$ ). The higher the canopy index, the greater the incidence × severity score for body rots.

#### Nutrition, body rots, canopy index and fungicides

In 2000, the three main factors that might be linked to incidence of body rots [number of fungicide spray applications, the (Ca + Mg)/K ratio and the canopy index] were each examined after adjusting for the effects of the remaining two factors. Following this analysis, only the (Ca + Mg)/K ratio remained significant ( $P < 0.001$ ,  $R^2 = 64.8\%$ ). When these three factors were used in a three-way ANOVA, the relationship with body rot severity was highly significant ( $P < 0.001$ ,  $R^2 = 67.4\%$ ).

#### Other factors

For 1 year only, several factors were related to rots. Stem-end rot incidence decreased as altitude ( $P = 0.05$ ,  $R^2 = 18\%$ ), latitude ( $P = 0.02$ ,  $R^2 = 23\%$ ), shelf life ( $P = 0.01$ ,  $R^2 = 25\%$ ) and depth of avocado mulch ( $P = 0.03$ ,  $R^2 = 36.8\%$ ) increased, and increased as depth of other types of mulch than avocado increased ( $P = 0.01$ ,  $R^2 = 54.8\%$ ). Incidence × severity of stem-end rots was positively related to depth of other types of mulch than avocado ( $P = 0.01$ ,  $R^2 = 55\%$ ). Body rot incidence decreased as latitude and maturity increased ( $P = 0.03$ ,  $R^2 = 21\%$  and  $P = 0.02$ ,  $R^2 = 23\%$ , respectively), and

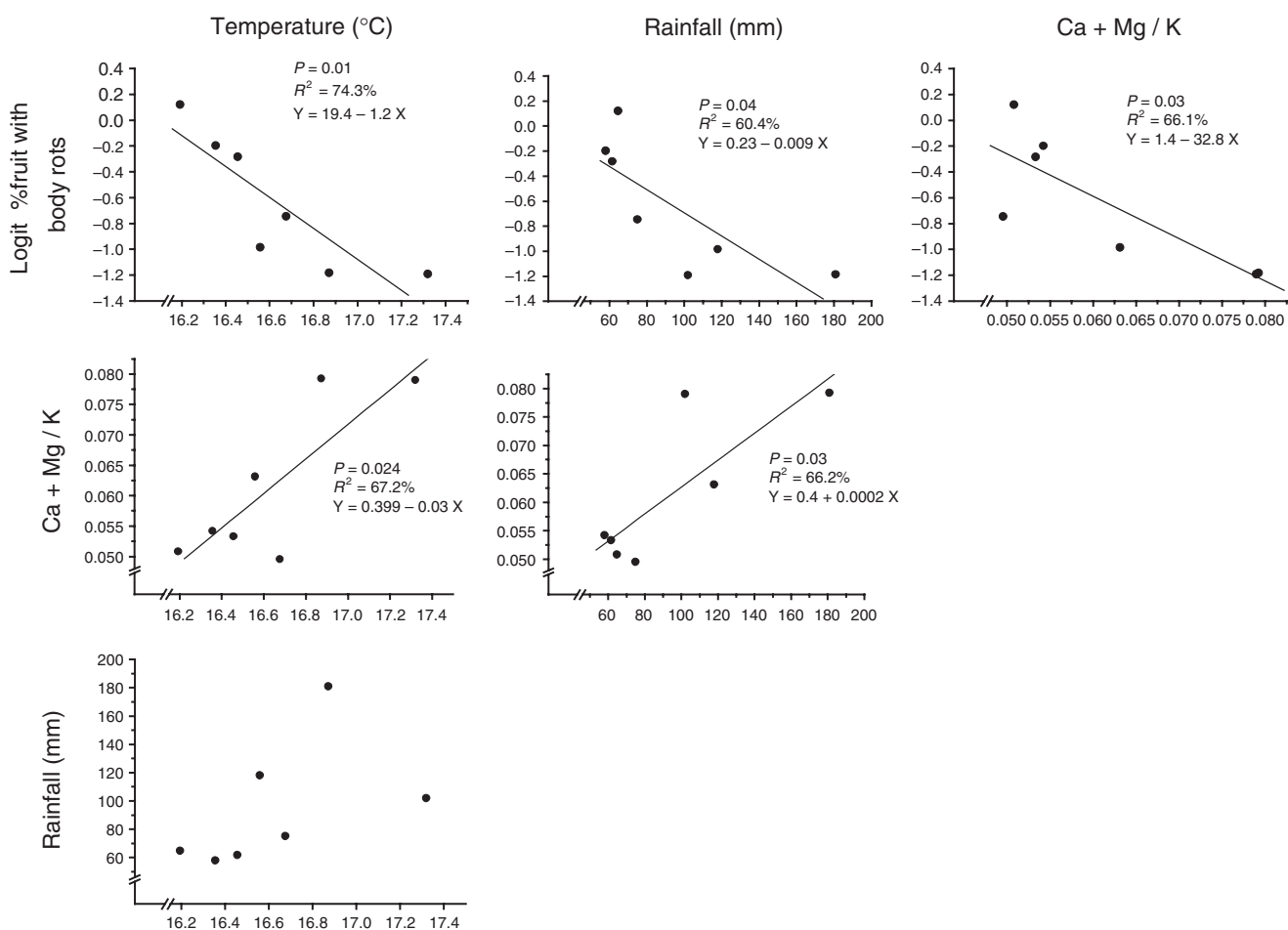


Fig. 5. Relationships between body rots of avocado fruit and calcium (Ca), magnesium (Mg) and potassium (K) contents in fruit expressed as (Ca + Mg)/K, total rainfall for 30 days before harvest and mean daily temperature for 40 days before harvest in 2000. Seven orchards were sampled.

incidence  $\times$  severity of body rots decreased as depth of other types of mulch than avocado increased ( $P = 0.04$ ,  $R^2 = 19\%$ ). There were no significant relationships between rots and any of the other factors.

### Discussion

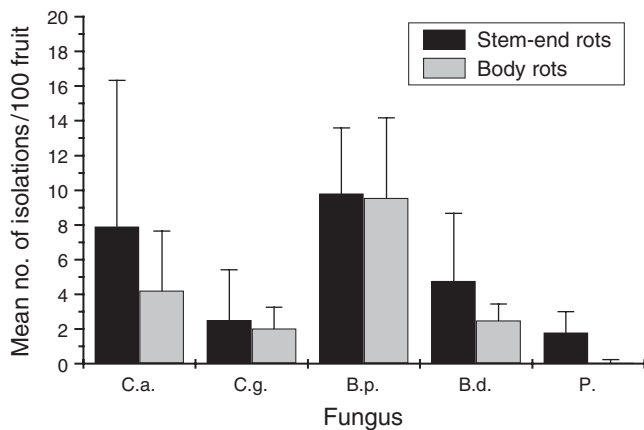
Fruit quality increased over the 3 years of this study and there was a related increase in the number of fungicide applications per season, and an increase in the Ca content in fruit. Every year, a report was sent to participating growers, results were reported in the New Zealand avocado industry journal, and in 2000, growers were instructed on applying Ca supplements at the annual research conference. Fungicide and Ca usage may have increased during the course of this study due to this feedback to growers.

*C. gloeosporioides* was less frequently isolated from avocado body rots than *B. parva*, *B. dothidea* and *C. acutatum*. Researchers in other countries report that *C. gloeosporioides* is the most common fungus isolated from rots that infect through the sides of the fruit (Snowden 1990). The reason for this difference may be climatic; New Zealand's mean

daily temperatures are lower than most other avocado growing countries (World Avocado Data, [www.avocadosource.com](http://www.avocadosource.com), verified 10 October 2006). Everett and Pak (2002) showed that the mean daily temperatures required for spore germination of *C. gloeosporioides* are only sometimes exceeded in avocado growing districts in New Zealand. In contrast, *B. parva* spore germination was not inhibited. Spores of *C. acutatum* are able to germinate at cooler temperatures ( $>16.5^\circ\text{C}$ ) than are spores of *C. gloeosporioides* ( $>20^\circ\text{C}$ ) (Everett and Pak 2002; Everett 2003).

It is possible that in the warmer far north region of Kaitaia, *C. acutatum* is able to compete with *B. parva* more effectively to cause more body rots than elsewhere. The reason for the prevalence of *C. acutatum* in stem-end rots from Te Puke in 2000 is not clear. The effect of different factors on the relative importance of each of these fungi requires further investigation.

In the 2000 season, when 21 orchards were surveyed, body rot incidence and severity decreased as the (Ca + Mg)/K ratio in fruit increased, in agreement with other studies on avocado (Koen *et al.* 1990; Hofman *et al.* 2002; Willingham *et al.* 2004).



**Fig. 6.** Mean number of isolations from avocado fruit rots  $\pm$  s.e. Values are the means of orchards sampled in 1999, 2000 and 2001, a total of 51 orchard samples of 100 fruit each. C.a., *Colletotrichum acutatum*; C.g., *C. gloeosporioides*; B.p., *Botryosphaeria parva*; B.d., *B. dothidea*; P., *Phomopsis* sp.

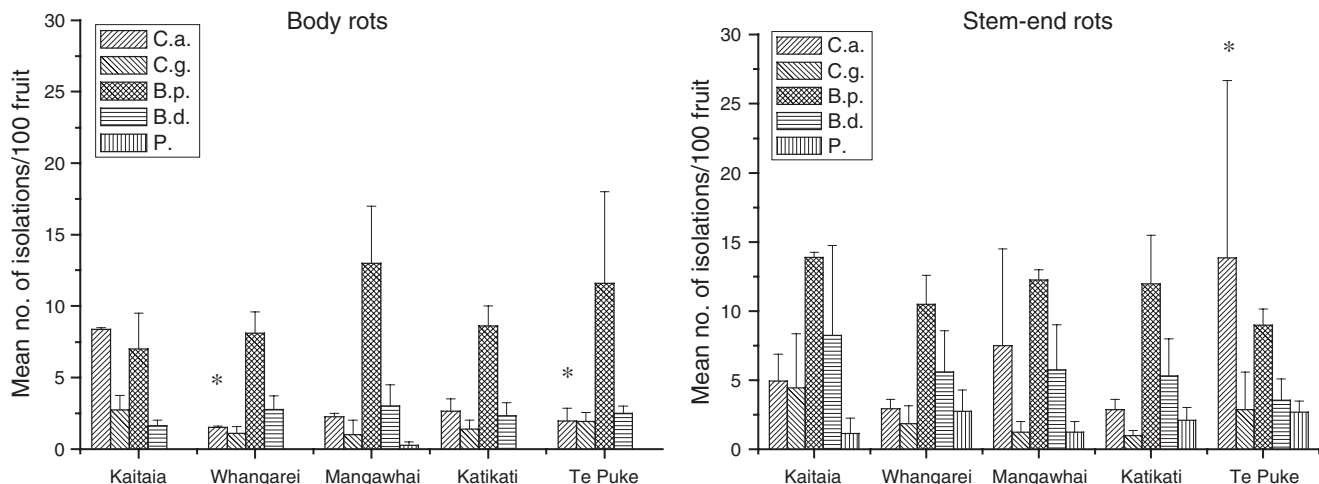
Body rot pathogens infect through the skin, either directly or after first forming an appressorium (Binyamini and Schiffmann-Nadel 1972; Kim *et al.* 1999). Increasing Ca levels in apple fruit by either preharvest sprays or postharvest dips can reduce the incidence of storage rots (Vang-Petersen 1980; Fallahi *et al.* 1997; Conway *et al.* 1999). Similar results have been found in other crops where reduced rot incidence has been associated with slower rates of softening (Ferguson and Boyd 2001). This suggests that Ca may inhibit cell wall breakdown, thus influencing both processes. A direct effect of Ca on cell wall integrity has also been implicated (Willingham *et al.* 2004).

In 2001, the relationship between body rot incidence and severity and the (Ca + Mg)/K ratio was not significant. This is a typical finding in many studies examining relationships between fruit quality and mineral nutrition (Hofman *et al.* 2002;

Ferguson *et al.* 2003). There are many reasons why a statistically significant relationship can be found in one season and not another. In the current study, this may be a result of the small sample numbers, a lower incidence of rots or less spread in the (Ca + Mg)/K ratio in 2001 (8 orchards compared with 21 in 2000). Another explanation is that the (Ca + Mg)/K ratio in the fruit harvested from all orchards in 2001 was  $\geq 0.065$ , and the growers in this study may have improved their nutrition program such that no further decrease in fruit susceptibility could be obtained by increasing Ca levels. In another study in New Zealand where the ratio of (Ca + Mg)/K in fruit from all orchards sampled was above 0.065, no relationship was found (Thorp *et al.* 1997). However, a possible Ca effect on the incidence of body rots was found when the ratio was above 0.065 for avocado fruit grown in the soil types and climate of Queensland, Australia (Willingham *et al.* 2004). It is possible that Ca levels are only related to rots when below a certain threshold in fruit in New Zealand.

There was no relationship between stem-end rot incidence and severity and the mineral levels in sampled fruit. This is not unexpected as stem-end rots infect through the picking wound (Hartill and Everett 2002). If penetration takes place through the open ends of the vascular system of the stem-end then cell wall strength, which is related to mineral nutrition, would be unlikely to influence the initial infection.

There was a strong relationship between body rot incidence and severity and both temperature and rainfall, even though results from more seasons would be useful to verify this relationship found for only seven orchards. Uptake and movement of Ca in the plant is essentially passive and, therefore, acropetal (Martin-Prevel *et al.* 1987). There are several studies that suggest that the transpiration stream is important in Ca uptake. Seedling 'Duke' and 'Topa Topa' avocados grown in soils with high moisture levels had greater total Ca levels than plants grown in soils with low moisture levels (Slowik *et al.* 1979). In apples, adequate evaporation from primary leaves is crucial to achieving high levels of fruit Ca (Jones and



**Fig. 7.** Mean number of isolations from avocado fruit rots  $\pm$  s.e. from each of the regions studied (Kaitaia, Whangarei, Mangawhai, Katikati, and Te Puke). Values are the means of orchards sampled in 1999 and 2000, a total of 43 orchard samples of 100 fruit each. C.a., *Colletotrichum acutatum*; C.g., *C. gloeosporioides*; B.p., *Botryosphaeria parva*; B.d., *B. dothidea*; P., *Phomopsis* sp. Bars marked with asterisks indicate significant differences following analysis of variance and Tukey's test ( $P < 0.05$ ).

Samuelson 1983). Irrigation increased fruit Ca uptake in apples (Nielsen and Stevenson 1986). Ca translocation to tomato fruit was reduced in plants grown in conditions of high humidity, compared with those of plants grown in conditions of low humidity (Gislerod *et al.* 1987; Ho 1989). Because both temperature and rainfall have an effect on the transpiration stream, these factors may also influence Ca uptake. Therefore, the effect of temperature and rainfall on body rots may be through its relation to Ca uptake rather than through a direct effect on the fungi that cause disease. This is further supported by the inverse nature of the linear relationship between rainfall and rots in this study. If rainfall was directly related to spore dissemination, germination and infection, the relationship would be expected to be positive rather than negative. The negative linear relationship between temperature and rots would also be unexpected if the principal effect of temperature was on spore germination. For the pathogens that cause rots in New Zealand, increase in temperature would be expected to enhance spore germination and thus infection (Everett and Pak 2002), rather than reduce numbers of fruit infected with body rots (Fig. 5). Additionally, if the temperature and rainfall relationships were directly affecting spore germination and infection, then a relationship with the number of fruit with stem-end rots would be expected, but there was none.

Generally the temperature of different sites relative to each other does not vary greatly from one year to another, and, therefore, it is not surprising that the Ca level of fruit harvested in 2001 was also related to temperature in 2000. It is also possible that Ca uptake occurs mainly when fruit is young as it does for apples (Wieneke and Fuhr 1973; Ferguson and Watkins 1981), but if this was so, then the temperature for 2000 should not have influenced Ca levels in mature fruit harvested 3 months later. Avocado fruit cells continue dividing until harvest (Schroeder 1953; Valmayor 1967), thus new cells are continually forming and continuous uptake of Ca is probably helpful in disease control throughout the season.

The linear relationship between the number of fungicide applications and stem-end rots suggests that the incidence of these rots is primarily influenced by the amount of inoculum. Frequency of fungicide sprays was the only factor consistently related with stem-end rots in this survey, providing further supporting evidence for this hypothesis. Inoculum sources have been identified as dead leaves, mummified fruit (Fitzell 1987), leaf margins, twig lesions, bark on trunks (Darvas *et al.* 1987) and the outer layers of the pedicel and twig cambium (Hartill and Everett 2002). Preliminary trials have suggested that removal of some of these inoculum sources, especially dead twigs and mummified fruit, can in some circumstances reduce incidence of rots (Hartill 1992). It would be useful to further investigate removal of inoculum sources as a method of rot control.

Unless more than eight fungicides were applied, body rots were no fewer than the numbers of body rots in unsprayed fruit. Fruit to which 6–7 fungicide applications were made had more body rots than fruit that were unsprayed, or sprayed once during the season. This is in agreement with previous results that showed that 3–4 fungicide applications increased the numbers of rots in fruit (W. F. T. Hartill, pers. comm.). The reason for the detrimental effect of a few fungicide applications is not clear,

but it could be related to reducing the population of non-target beneficial microorganisms.

The relationship of body rot incidence to fungicide applications was described using a quadratic equation. This suggests that factors other than the amount of inoculum are important in determining the numbers of fruit infected through the side by rot fungi. Indeed, results of this survey have shown that in one year, Ca was an important determinant of body rot incidence and severity. In 2001, when this factor was no longer important, the relationship between body rots and fungicides was inversely linear. Rots were assessed in peeled fruit for the data presented in this graph (Fig. 3). It is not clear if the linear nature of this relationship is because Ca was no longer an important factor, or if this is an anomaly of the different assessment techniques used (quartering compared with peeling the fruits). A sample of 1032 fruit was assessed using both methods. The quartering method under-reported body rots, but the amount of rots assessed was significantly related to the amount assessed using the peeling method ( $P < 0.0001$ ,  $R^2 = 55\%$ ). There was no difference between the two methods for assessing stem-end rots. Although assessing body rots following cutting in quarters may not be as accurate as assessing fruit following peeling, and the peeling method may have been a better indicator of actual rot levels, clear relationships were identified by the quartering method.

In this study, body rot incidence and severity were directly related with canopy index ( $R^2 = 18.5$  and  $23.3\%$  in 1999 and 2000, respectively). The canopy index included a factor that represented the amount of inoculum (amount of dead branches) as well as canopy density. A high canopy index value thus indicated a denser canopy with more inoculum. Although this relationship was relatively weak, the effect of canopy density warrants further investigation because of a similar relationship recorded in other crops (Gubler *et al.* 1987). Spores of the fungal pathogens that cause postharvest rots of avocados require conditions of high humidity for dissemination, germination and infection (Estrada *et al.* 2000; Ahimera *et al.* 2004). Humidity is reduced in a more open canopy, and this may minimise infections.

There were other factors that had a relationship to rots that were significant for 1 year only. This does not mean that they were not important, analysis and interpretation of data generated from an observational study such as the orchard rot survey needs to be careful. Because there are a large number of factors that can influence rots in the orchard, lack of reporting of an unknown influential factor can adversely affect conclusions that are made. Results of replicated and blocked trials are, therefore, more reliable because the experimental design removes the effect of unknown factors. However, those factors identified as important in this study, e.g. fungicides, Ca and canopy, have been identified by other workers as influencing rots from the results of replicated trials in avocado or in other crops. This study has identified some factors, such as the effect of mulch, that need to be investigated further in replicated blocked trials. Although the survey did not find any significant relationships between rots and the use of irrigation, this factor may also be important because of the relationship found between rainfall and body rots. From these results, it is recommended that Ca is applied to avocado orchards to achieve a ratio of (Ca + Mg)/K above 0.065 in fruit, and that



more than eight fungicides are applied each season to reduce rots. Further trials are required to investigate different strategies for managing the canopy of avocados and their effect on body rots before recommendations can be made to growers.

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