PATTERNS OF STEM-END ROT DEVELOPMENT IN COOLSTORAGE

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ABSTRACT

Stem-end rots are a major issue for the New Zealand avocado industry, especially as fruit age increases. The impact of stem-end rots in the eyes of the consumer is made worse because of their association with vascular browning. These experiments were designed to determine the effect of time in coolstorage on development of stem-end rots. After 35 days coolstorage at 5.5^{o} C, *Colletotrichum acutatum* had grown through the stalk and into the flesh of inoculated avocado fruit indicating that infection can occur before the fruit ripen. The rate of growth of *C. acutatum* in avocado fruit stems and flesh increased exponentially with time, indicating that as fruit age they become more susceptible to infection. Fruit inoculated with *C. acutatum* had a reduced storage life of 14 days relative to the uninoculated control. The pattern of stem-end rot development in coolstore was almost identical for inoculated and uninoculated fruit, but the actual amount of disease at any time during storage depends on the amount of disease at the start. This is likely to be related to the amount of inoculum (spores, fungal fragments) in the orchard when the fruit are picked. If the pattern of disease development is consistent for fruit from different orchards, there is potential to develop a predictive model for storability of avocado fruit based on inoculum load in the orchard.

Keywords: stem-end rots, coolstorage, *Colletotrichum acutatum*, disease progress curves.

INTRODUCTION

Stem-end rots have been identified by the Avocado Industry Council as the most serious rot problem for the New Zealand industry. Stem-end rots are strongly associated with vascular browning, which strongly decreases the acceptability to consumers. Although fruit can be only slightly affected (severity) by stem-end rots, the incidence of stem-end rots has been high in outturn monitoring data (Dixon, 2001). Prior to outturn monitoring, the results of the orchard rot survey had also shown that incidence of stem-end rots in a small sub-sample of orchards was also relatively high (Everett and Pak, 2001). Increasing our understanding of how stem-end rots develop during coolstorage of fruit can help to manage the risk of this disease limiting sale and distribution in the market.

Body rots are prevented from invading unripe fruit by antifungal chemicals present in the skin in fungitoxic concentrations. These chemicals break down when fruit ripen, and latently infecting fungi resume growth and penetrate into the fruit flesh to cause body rots. Although antifungal chemicals are present in fruit flesh, these chemicals are compartmentalised into oil cells and are not available in fungitoxic quantities to prevent fungal growth in unripe avocado flesh (Prusky *et al.*, 1991). Theoretically once fungi have grown down the cut stem there would be no barrier to infection of unripe fruit flesh.

Previous studies (Everett, 1999), in which a sample of fruit were cut and assessed every day after harvest, failed to provide any evidence of endophytic invasion of avocado fruit by naturally occurring stem-end rot pathogens. This tends to suggest that infection of the stem end is by fungal spores invading the wounded cut end of the stem as is supported by the results of Hartill *et al.* (2002). In fruit held at 20^{o} C, rots did not develop into the fruit from the wounded stem until the fruit were ripe.

Observational evidence of unripe coolstored fruit in South Africa suggested that stem-end rots are capable of invading and growing through unripe 'Fuerte' fruit in coolstorage. Because fruit stored at 20^{o} C showed no evidence of stem-end rots until ripe coolstorage may either predispose the fruit to invasion, or fungal growth continues in coolstorage to eventually penetrate into unripe fruit.

There is no published information on stem-end rot development in coolstorage. This would provide the industry with valuable information regarding the keeping quality of different lines of fruit. If avocado rots grow in coolstorage, then from the growth rate and historical disease data, the maximum storage life of individual lines of fruit should be able to be calculated. Further elucidation of the infection pathway by stem-end rot fungi is required in order to design better control strategies.

MATERIALS AND METHODS

Run of line export grade avocados in 20 count trays were collected from a packhouse in Katikati on 5th February 2002, a total of 1400 fruit. Of these fruit, 700 were inoculated with *Colletotrichum acutatum*. Six hundred inoculated and 600 uninoculated fruit, a total of 1200, were placed in coolstore at 5.5^{o} C at the Mount Albert Research Centre of HortResearch. The remaining 200 fruit were placed at 20^{o} C, and 20 fruit from each of the two treatments (inoculated and controls) were cut and assessed for stem-end rots, firmness by firmometer and by hand, and other disorders using the AIC assessment manual. A tray of inoculated and a tray of uninoculated fruit were cut on 5 occasions; 1, 3, 4, 6 and 7 days after 5th February, a total of 200 fruit.

After coolstorage for 14, 21, 28, 35, 42 and 49 days, 100 fruit from each treatment were removed and placed at 20^{o} C. A 40 fruit sub-sample (20 fruit from each of the 2 treatments) was cut on five occasions. These times were variable, and were chosen to enable fruit from all stages of the ripening curve to be assessed. The experimental design is described graphically in Figure 1.

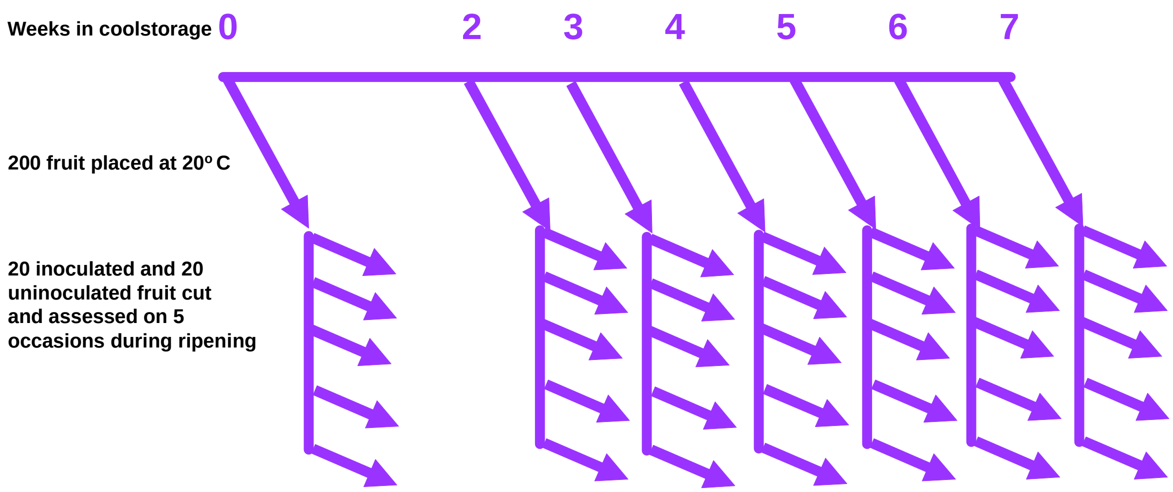

Figure 1. Experimental design showing exits points from storage (arrows).

RESULTS

Development of stem-end rots in coolstored fruit

The effect of firmness on rots

When mean hand firmness values are plotted against time in coolstorage there is no clear relationship (Figure 2). There was a strong linear relationship between fruit firmness and rot incidence (R^2 = 93.4%) for the uninoculated control (Figure 3a) with softer fruit developing more stem-end rots. Severity of stem-end rots increased with as firmness decreased (Figure 3b). Inoculated fruit developed a high incidence of stem-end rots, regardless of the firmness when assessed, other than at a firmness rating of 1(Figure 3a).

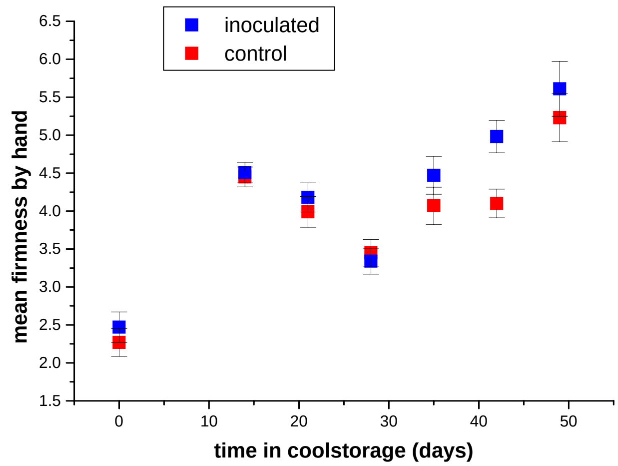

Figure 2. The effect of time in coolstorage on fruit firmness as determined by hand.

A) **B)**

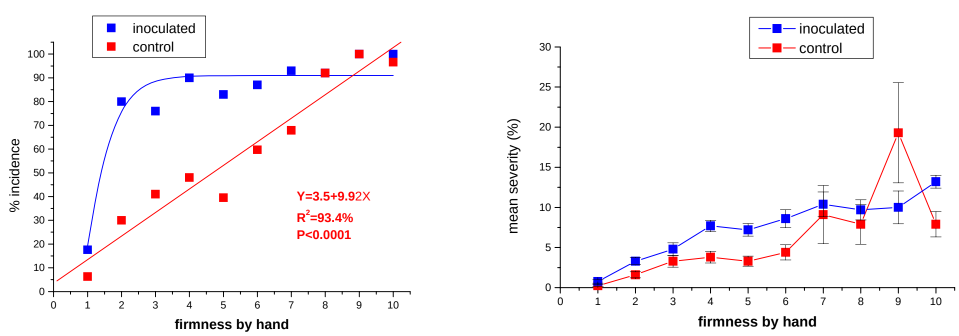

Figure 3. The relationship between fruit firmness as determined by hand and incidence and severity of stem-end rot.

Assessment of rots in unripe fruit immediately after removal from coolstorage

To investigate if stem-end rots develop in coolstored fruit, the amount of rot in fruit cut immediately after removal from coolstorage at each of the seven times tested was examined. Results show (Figure 4a) that after 35 days for inoculated fruit, and after 49 days for control fruit, more than 70% of fruit had stem-end rots immediately after removal from coolstorage. This result demonstrates clearly that rots do in fact develop in coolstored fruit. The higher the inoculum load (disease pressure), the more rots that will develop, leading to a reduction in storage life. The inoculum load affects incidence, but not severity (Figure 4b). In this experiment a high inoculum load decreased storage life from 42 days to 28 days.

A) B)

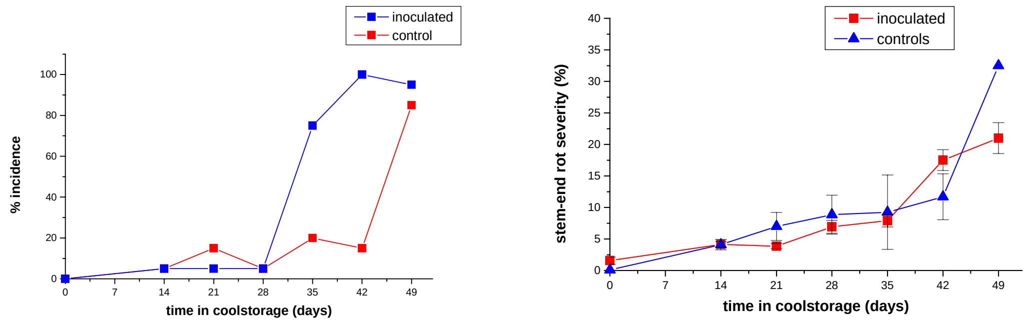

Figure 4. Development of stem-end rots in coolstorage. These fruit were assessed firm immediately upon removal from coolstorage.

Total number of stem-end rots developing in fruit after increasing time in coolstorage

If the total number of stem-end rots developing in fruit after varying times in coolstorage is graphically presented, it is apparent that rots increased in an 'S' shaped (sigmoidal) pattern (Figure 5a). This increase can be transformed to a straight line using a logistic transformation (Figure 5b). In this experiment the amount of stem-end rots in a sub-sample of fruit stored and ripened at 20^{o} C could be used to estimate the amount of rots developing at any time during extended coolstorage with a high level of accuracy. The slope of the transformed lines is the same for inoculated and control fruit (Figure 5b) indicating that rots are increasing at the same rate. The real difference between inoculated and uninoculated fruit in Figure 5a is that inoculated fruit will reach any given level of disease 14 days earlier than the uninoculated fruit.

A) B)

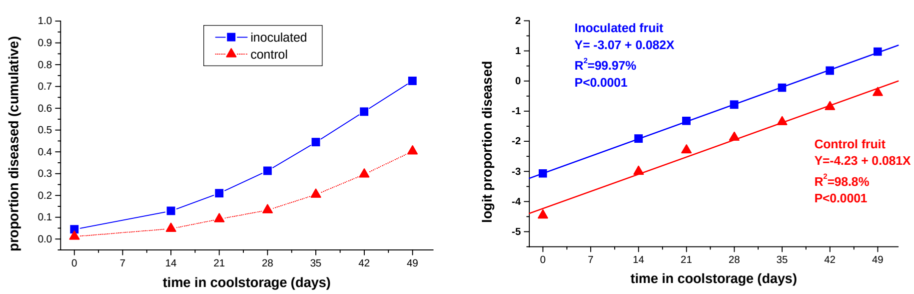

Figure 5. Total number of stem-end rots developing in inoculated and uninoculated fruit after increasing time in coolstorage. a) cumulative proportion diseased, b) logit transformation of proportion data.

Stem-end rots in optimally ripe fruit

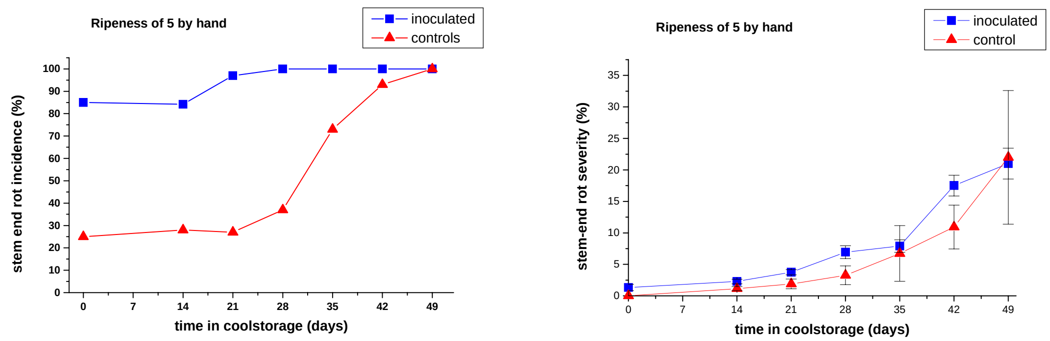

Figure 6. Incidence and severity of stem-end rots in fruit assessed at a hand ripeness of 5.

If rot incidence is recorded only for those fruit assessed at a firmness by hand of 5, then almost all inoculated fruit had rots irrespective of time in coolstorage (Figure 6a). For uninoculated controls, rot incidence was a constant 25% until an increase after 35 days to 100% in an 'S' shaped curve. Severity of stem-end rots increased at a constant rate throughout the experiment (Figure 6b).

Cumulative disease incidence (Figure 7a) could be transformed to a straight line by the logistic transformation (Figure 7b). The slope of the line for the fitted model (Figure 7b) was similar for inoculated compared to control fruit. Also the slopes for the logit transformations are similar in Figures 5 and 7. This indicates that the rate at which stem-end rots develop is not dependent on the firmness at which they are assessed. Following linearisation by logit transformation (Figure 7a and 7b), the relative growth rate of the stem- end rots can be calculated according to the formula in Appendix 1.

A) **B)**

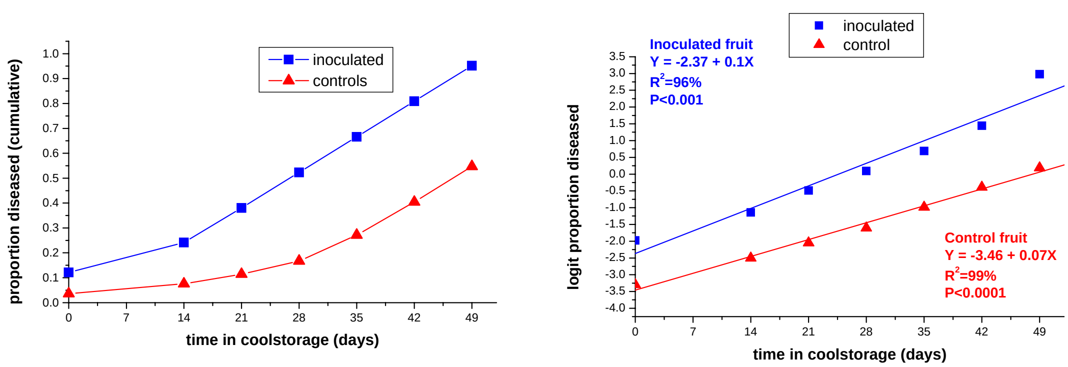

Figure 7. Number of stem-end rots in inoculated and uninoculated fruit assessed at optimal ripeness after increasing time in coolstorage. a) cumulative proportion disease b) logit transformation of proportion data.

Growth rate of avocado rot pathogens in coolstored fruit tissue

To estimate the growth rate of avocado rot pathogens in coolstored fruit tissue, the percent stem-end rot data generated from evaluating fruit according to the AIC assessment manual was converted to linear data (Table 1).

Table 1. Conversion of severity data to linear data

Severity category	Distance up stem-end (cm)
0	0
1	0.6
2	0.7
3	0.8
4	0.9
5	1.0
6	1.2
7	1.4
8	1.6
9	1.8
10	2.0
15	3.0
20	4.0
25	5.0
30	6.0
40	8.0

The mean distance of penetration for fruit assessed immediately after removal from coolstorage was plotted against duration of coolstorage (Figure 8). This data showed an exponential increase, but for modelling purposes were considered as the first phase of a logistic growth curve. This expoential increase indicates that as the furit get older they become more susceptible to invasion by stem-end rots fungi.

A) **B)**

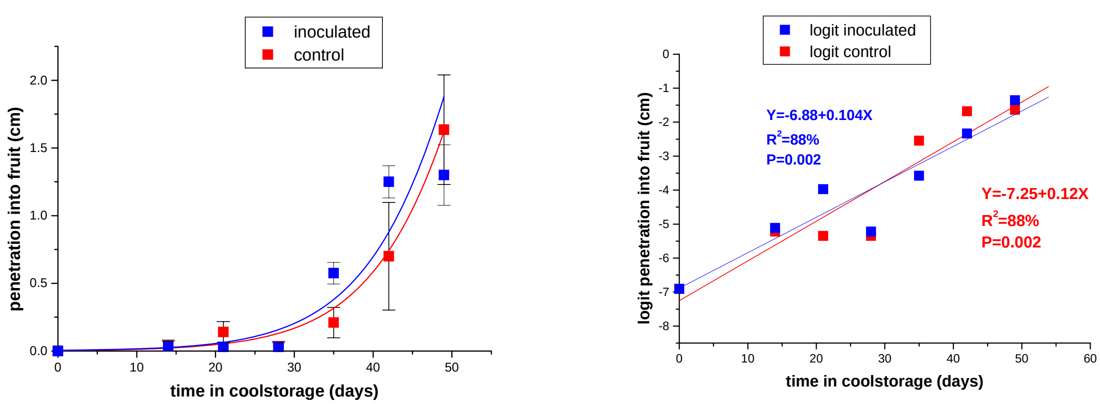

Figure 8. Penetration of fungi into fruit during coolstorage: a) means ± standard errors b) logit transformed data.

DISCUSSION

This experiment examined patterns of disease development in coolstorage. These showed that stem-end rots are able to grow in unripe fruit in coolstorage, and that the fungus *C. acutatum* reaches the flesh through the stem remnant in 32-37 days after picking. This agrees with patterns of development of stem-end rots in offshore monitoring, where after about 34 days there was a sharp increase in numbers of fruit with rots (Dixon 2001).

There appeared to be two separate infection pathways by stem-end rot pathogens. Until 35 days in coolstorage 25% of ripened fruit had rots. After this time, rots increased in an 'S' shaped pattern to 100% by 49 days. It may be that a number of spores penetrated into the stems further than others at picking, possibly into the vasculature, and caused 25% of fruit to rot. The remaining spores may have grown down the cut stem remnant by 32-37 days, and by 49 days had caused 100% of fruit to rot.

A higher inoculum load (rot potential) reduced storage life from 42 days to 28 days. Analysis of the available data to determine the rate of growth showed that the response was exponential. This pattern of increase is probably related to build up of fungal mass generating more inoculum and accelerating growth at an exponential rate. It is possible to accurately model this increase in rot incidence with increasing time in coolstorage. If this model is consistent for different lines of fruit, it has the potential for providing a robust predictor of storage life.

There is an urgent need to decide what criteria will be used to determine fruit acceptability to either use or extend the results of this experiment. Rot incidence in fruit assessed at optimal ripeness would probably generate the most meaningful acceptability criteria. If more lines of fruit are included in subsequent experiments, then these results suggest that rot incidence in fruit ripened at 20^{o} C will be able to be used to predict storage life. Inoculum levels in the stalks from which the avocados are removed at harvest may be able to provide this data more rapidly and without any fruit costs.

ACKNOWLEDGEMENTS

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Appendix 1. Formula for calculation of relative growth rate of *C. acutatum* in avocado fruit, assuming fruit is 12 cm in length.

$$y' = \frac{12\beta}{[1+\mathbf{e}^{-(\alpha+\beta x)}]^2} \cdot \mathbf{e}^{-(\alpha+\beta x)}$$

Where: α = y intercept on logistic transformed straight line
β = slope of logistic transformed straight line
e = exponential
x = time at which growth rate is required
y' = relative growth rate