PREDICTING AVOCADO FRUIT ROTS BY QUANTIFYING INOCULUM POTENTIAL IN THE ORCHARD BEFORE HARVEST

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SUMMARY

The fruit rot pathogens *Colletotrichum gloeosporioides* and *C. acutatum* infect avocados following germination of rain dispersed spores to produce quiescent appressoria. Stimulation of appressorial formation is by contact with a hard surface such as leaves and fruit. In a preliminary study a number of different methods to quantify inoculum potential pre-harvest without sacrificing valuable fruit were compared with historic fruit rot data. Fruit from several orchards with a consistent history of low and high disease were used. Previous survey work of 23 orchards over three seasons had shown that unless growers changed practice the relative amount of rots in their fruit generally remained constant. Four methods were compared; a. spores were washed from leaf discs, b. appressoria were counted, c. leaf tissue was surface sterilised and placed on fungal growth media, d. isolations were made from dead branches. Following quantification it was shown that appressoria and washed spore numbers were unrelated to historic fruit rots, but amount of leaf tissue infected with *Colletotrichum* spp. and *Botryosphaeria* sp. showed potential as a predictor of rots.

Key Words: *Colletotrichum acutatum*, *C. gloeosporioides*, *Glomerella cingulata*, *Botryosphaeria* spp.

INTRODUCTION

The most common avocado variety grown in New Zealand is 'Hass'. It is difficult to detect rots in ripe 'Hass' because the skin changes from green to 'black' upon ripening. In order to find rots the fruit has to be cut, and the best method of detection requires removal of the skin. Consumers are deterred from repeat purchasing this variety because of rots that only become obvious after preparation for consumption. Previous to 1999, New Zealand fruit was either sold locally, or to Aus-

tralia. Shipping to Australia took 3-4 days, and fruit was usually sold within 7 days of harvest. However, since that time the New Zealand crop has increased in volume requiring diversification of supply to include more distant markets such as California, USA. Shipping to USA requires fruit to be stored for a longer period of time. Industry out-turn monitoring (Dixon and Pak 2002) and research outcomes (Everett and Pak 2002) have shown that rots are the most important quality problem in New Zealand fruit. In experimental conditions, 74% of some lines of New Zealand fruit were shown to have rots (Everett and Pak 2001).s Rots can be controlled using a range of management practices, however, there is a lot of variation between growers with respect to implementation of such practices. In addition, even when no control measures are used there is large variation between orchards in the amount of rots. Given this large inherent variability in fruit quality the availability of a system for predicting the quality of fruit to enable export of the lines with the least rot potential would be very useful.

In New Zealand, six fungal pathogens (*Colletotrichum acutatum*, *Gloeosporioide*, *Botryosphaeria parva*, *B. dothidea*, *Fusicoccum luteum* and *Phomopsis* sp.) cause rots of avocados (Hartill 1991). These pathogens have been placed in four categories for the purposes of this study, viz., *C. acutatum*, *C. gloeosporioides*, *Botryosphaeria* spp. (*B. parva*, *B. dothidea* and *F. luteum*) and *Phomopsis sp.*. The process by which avocados are infected by *Botryosphaeria* spp. and *Phomopsis* sp. is largely unknown. Two pathogens of New Zealand avocados, viz. *Colletotrichum gloeosporioides* and *C. acutatum*, infect avocado fruit latently. Spores germinate in response to a hard surface to produce appressoria from which an infection peg penetrates a short distance into the skin of the avocado and then becomes quiescent (Binyamini and Schiffmann-Nadel 1972). Leaves are usually infected symptomlessly, and the purpose of this preliminary study was to investigate if leaves or dead branches could be used as an indicator of the inoculum potential in an avocado orchard. Fruit from several orchards where historic disease incidence information was available were used. Orchards with consistent high or low disease incidence were selected. Previous survey work of 23 orchards over three seasons had shown that unless growers changed practice the relative amount of rots in their fruit generally remained constant.

MATERIAL AND METHODS

Three orchards were selected for study from the Bay of Plenty avocado growing region of New Zealand (37.49ºE, 176.2ºS). These orchards were selected on the basis of similarity of tree size and form, and differences in levels of rots in fruit. Fruit from these orchards had been sampled and assessed for rots under standardised conditions in early January for the previous two seasons (Table 1). Leaves and branches used in this current study were collected on 6th and 7th September 2000.

Six different methods for quantifying inoculum were tested:

1. leaf disc isolations: 12 leaves were taken from around each of 4 trees from each orchard. Twelve leaves were taken in this manner from four different tissue types; top and bottom (position), and old and young (age). Two leaf discs (5 mm diam.) from each leaf were surface sterilised (Petrini 1986) and placed on Difco® Potato Dextrose Agar (PDA) in Petri plates. Isolations were scored as total number of leaf discs from which fungi of interest (*C. acutatum*, *C. gloeosporioides*, *Botryosphaeria* spp. and *Phomopsis* sp.) grew, a total of 384 samples per orchard.

2. leaf piece isolations: Following excision with a scalpel, two leaf pieces (c. 5 x 5 mm) from the midrib and lamella near the base of each leaf, and two from the midrib only, were surface sterilised (Petrini 1986), and placed on Nobles' media (Nobles 1965)(NM) in Petri plates. Twenty-four leaves were collected from each tree, 12 from 1m above ground and 12 from 3m above ground. Lea-

ves were collected from the same four sectors as in method 1. Pieces were scored as number of leaf pieces from which colonies of interest grew, a total of 192 samples per orchard.

3. dilutions of leaf washings: 12 leaves were taken as in method 1. Dilutions were made from 5 ml of 0.5% (w/v) peptone containing 12 x 5 mm leaf discs (one disc from each of 12 leaves per tree; 48 samples per orchard) rotary shaken for 90 mins. Aliquots of 100ml of undiluted, 1:10 and 1:100 dilutions in sterile deionised water were spread on PDA. Numbers of fungal colonies of interest were counted.

4. appressoria on leaf discs: Appressoria were counted with the aid of the light microscope following clearing leaf discs with 50:50 acetic acid:ethanol (v/v) followed by autoclaving for 15 min. Four 1.5 cm diam. leaf discs were sampled from each tree (top, bottom, young, old), a total of 16 samples per orchard.

5. appressoria on leaf pieces: appressoria were counted with the aid of a dissecting microscope from 2.0 x 0.5 mm pieces of tissue from the midrib near the base of the leaf, 40 leaves per tree (top, bottom, young, old), a total of 160 samples per orchard.

6. isolations from fruit bodies on dead branches: A total of 20 branches (10 from within the canopy and 10 from the litter beneath the canopy) were taken from each of two trees from each of two orchards and examined with the aid of the dissecting microscope. A sample of four fruiting bodies was taken from each branch and placed onto NM (a total sample of 160).

The number of discs, pieces or fruiting bodies from which fungi of interest grew and the numbers of appressoria were recorded and analysed using the general linear model programme of MINI-TAB®. Leaf disc isolations and number of appressoria were plotted against fruit rot data collected the previous season using Microcal® Origin.

RESULTS AND DISCUSSION

Results of analysis of variance show that some measures of inoculum could be used to identify differences between orchards (Table 2). These methods were; counting appressoria on leaf pieces, isolations of *C. acutatum* from leaf discs and leaf pieces, and isolation of *C. gloeosporioides* from leaf pieces. Different amounts of inoculum were present on tissue sampled from different positions in the tree viz. 1 m or 3 m above the ground for leaf samples, and within or beneath the canopy for branch samples. However, only some methods were able to detect these differences (leaf pieces and branches). Leaf pieces were sampled from specific parts of the leaf, whereas selection of tissue for leaf discs was more random. This suggests that inoculum load is variable over the leaf surface and needs to be investigated further.

In this study only two orchards were compared by sampling leaf pieces and branches. For this reason only leaf disc isolations, which compared three orchards, were plotted against historic rot data (Fig. 1). Numbers of isolations of both *Colletotrichum* species and *Botryosphaeria* spp. were related to historic rot data, but neither appressorial counts nor isolations of *Phomopsis* sp. were related (Fig. 1). The relationship between mean numbers of isolations from leaf discs of *C. gloeosporioides* and *Botryosphaeria* spp. and percent fruit affected by body rots the previous season was very good (Fig. 2). No other relationships were statistically significant by linear regression.

The relationship between inoculum and stem-end rots was not as good as the relationship with body rots (cf. Fig. 1a and 1b). There is evidence that the fungi that cause stem-end rots infect fruit predominantly at harvest from contaminated stem tissue (Hartill and Everett 2002). This suggests

that a measure of inoculum availability at harvest is required for better prediction of the amount of stem-end rots, or that inoculum on branches or stems may be more strongly correlated with this disease symptom.

Despite the good relationship between isolations of *C. gloeosporioides* or *Botryosphaeria* spp. from leaf discs and body rots, analysis of variance did not indicate that there was a significant difference between orchards. Presumably there was too much variation in the data for this analysis to be significant. For future work, variation needs to be reduced by such means as increasing number of trees sampled. Further work is required to ascertain if this relationship can be used to predict the amount of rots in fruit harvested in the same season, and if the relationship remains strong following the inclusion of more orchards.

The orchard with the lowest number of appressoria on leaves as determined by both methods used was the orchard on which samples were collected in the rain. This suggests that appressoria did not produce infection pegs on leaves before harvest and were washed off by heavy rain, although further experimentation is required.

The greatest number of isolations from leaves were *C. gloeosporioides*, and from branches *Botryosphaeria* spp. (Table 3). *C. gloeosporioides* was also isolated in large numbers from branches, and *C. acutatum* from leaves. *Phomopsis* sp. was isolated in low numbers from all tissue types. Isolations from branches may also be a good predictor of final fruit rots and requires further testing.

CONCLUSIONS

Of the methods tested in this study, isolating from surface sterilised leaf tissue showed the best relationship with historic rot data and shows the most potential for developing a rot prediction system.

Acknowledgements

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Table 1: Amount of rots in 100 fruit collected and ripened under standardised conditions from each of the sampled orchards in January 1999 and January 2000.

	Body rots (%)		Stem end rots (%)	
	1999	**2000**	**1999**	**2000**
Orchard 1	23	24	43	24
Orchard 2	19	12	46	23
Orchard 3	5	7	7	8

Table 2: Factors showing significant relationships with measures of inoculum following analysis by the general linear model of MINITAB.

Factors	appressoria on leaves		isolations from leaves							isolations from branches	
			discs			pieces			dilution		
	discs	pieces	B.	C.a.	C.g.	B.	C.a.	C.g.	plating	B.	C.g.
Orchard	n.s.	0.0011.	n.s.	0.0001	n.s.	n.s.	0.001	0.001	n.s.	n.s.	n.s.
Age	n.s.	n.t.	0.03	n.s.	n.s.	n.t.	n.t.	n.t.	n.s.	n.t.	n.t.
Position	n.s.	0.03	n.s.	n.s.	n.s.	0.05	0.003	0.0001	n.s.	n.s.	0.0001
Tree	n.s.	0.04	n.s.	n.s.	n.s.	0.007	n.s.	n.s.	n.s.	0.002	0.014
Leaf/branch	n.s.	n.s.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.s.	n.s.

[1.] P value from ANOVA table. n.t. = not tested; n.s. = not significant; B. = *Botryosphaeria spp.*; C.a.= *Colletotrichum acutatum* ; C.g.= *C. gloeosporioides*.Age = young or old leaves; Position = 1m or 3m above the ground for leaves, or from within the canopy or under the canopy for branches; Tree = 2 trees were sampled for leaf pieces and for branches, 4 trees were sampled for leaf discs and for dilution plating.

Table 3: Total number of isolations from branches and leaves.

Factors	leaf discs[1.]			leaf pieces[2.]		branches[3.]	
Orchard	**1**	**2**	**3**	**1**	**2**	**1**	**2**
C. acutatum	24	5	3	22	3	6	1
C. gloeosporioides	65	48	41	104	65	44	28
Botryosphaeria spp.	10	5	5	11	12	44	42
Phomopsis sp.	9	1	7	6	3	7	7
total	108	59	56	143	83	101	78

[1.] out of 384 per orchard; [2.] out of 192 per orchard; [3.] out of 160 per orchard
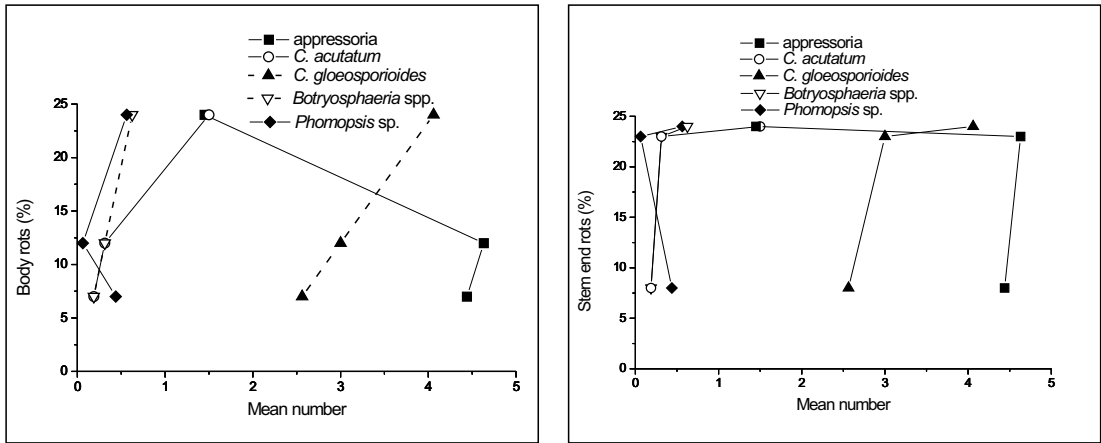
Figure 1: Mean number of isolations of four fungal pathogens (*C.acutatum*, *C. gloeosporioides*, *Botryosphaeria* spp. and *Phomopsis* sp.) and mean numbers of appressoria from leaf discs from three orchards in the Bay of Plenty, New Zealand, versus percentage of fruit with rots tested the previous season from these same orchards. Dashed lines indicate best fit.
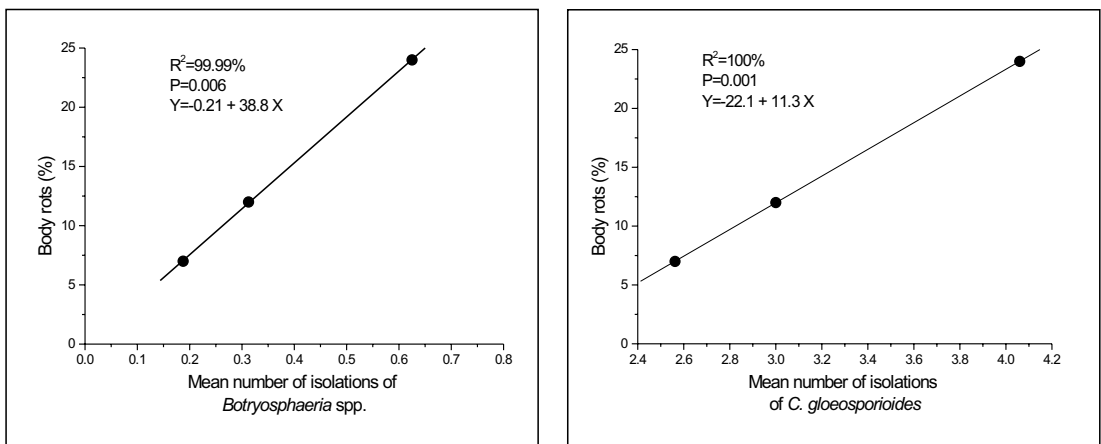

Figure 2: Linear regression of mean number of isolations of *Botryosphaeria* spp. and *C. gloeosporioides* from three orchards in the Bay of Plenty, New Zealand, plotted against percentage of fruit with body rots tested the previous season from these same orchards.