STEM-END ROTS: THE INFECTION PORTAL

W.F.T. HARTILL[1], K.R. EVERETT[1] and H.A. PAK[2].

1) The Horticulture and Food Research Institute of New Zealand Ltd
Private Bag 92 169, Mt Albert, Auckland
2) Avocado Industry Council, PO Box 16004, Bethlehem, Tauranga

ABSTRACT

To design and implement an effective control strategy for stem-end rots, the pathway for infection needs to be determined. Three possible pathways for infection (1. aerial borne spores infect the picking wound, 2. fungi growing inside the stem tissue are activated by picking, 3. fungi growing just under the cuticle are smeared over the wound by secateurs) were tested using a variety of harvesting techniques. These included plucking, clipping pedicels to a button as in commercial practice, clipping pedicels at 2, 4 and 6 cm long, removing the extra-cambial layers before cutting the stem, and treating the picking wounds with various fungicides. The fruits were then stored and ripened at 20°C in a controlled temperature room. Avocados were cut when ripe, assessed for rots and fungi from stem-end rots isolated and identified. Results showed that entry via the picking wound is a major portal for pathogens initiating post-harvest rots because there was an inverse relationship between the incidence of post-harvest rots and the length of the pedicel remaining after harvest. Disease incidence and severity was highest in fruits harvested by plucking. This was mainly due to an increase in the incidence of rots associated with *Colletotrichum* spp. and to a lesser extent with *Phomopsis* sp. Applications of fungicides to the picking wounds had variable effects. Dipping the wounds of plucked fruit in a Sportak/Benlate mixture significantly reduced rot incidence and severity. The mixture had no effect when applied to the cut end of pedicels clipped to a button. Dipping secateurs in the mixture before clipping each pedicel was also ineffective in reducing rots. However, dipping the button in Benlate alone was more effective. Slicing off the extra-cambial layers before clipping the pedicel through the xylem elements was ineffective, probably because it proved impossible to remove the extra-cambial layers without damaging the xylem elements. This study shows that control of stem-end rots can be achieved by applying a systemic fungicide to the cut end of clipped fruit at harvest.

Keywords: infection pathways, inoculum sources, endophytes, phellophytes, stem-end rot control, avocado.

INTRODUCTION

Johnson *et al.* (1991) suggested that the stem-end rot pathogens are endophytes (fungi growing inside the vascular system), living in the stems, branches and pedicels of the avocado canopy, which grow into the fruits to initiate the stem-end rots. However, Hartill (1993) found that most of the fruit rot pathogens present in the canopy were symptomless phellophytes (fungi growing in tissues around the vascular system, just under the cuticle) confined to the extra-cortical layers, including those of the fruit pedicels. The distinction is important as true endophytes could grow unobstructed down the xylem elements into the fruit whereas phellophytes must breach a number of cell walls before entering the fruit either directly or via an initial breakout into the xylem. Extensive development of neck rots within the fruits is frequently via xylem elements (Hartill, 1991).

In a previous trial preliminary results indicated that picking methods can have a significant effect on the incidence of stem-end rots in ripened avocados (Hartill & Everett, 1997). Fruits harvested by plucking had the highest disease incidence and those harvested by clipping with secauteurs dipped in fungicides had the lowest. We propose that the normal clipping process, or plucking, may provide the means of entry for the pathogens, by exposing the xylem elements, and these pathogens then grow down the pedicel and infect the fruit. The fungi are transferred to the xylem elements during clipping and the pathogens then grow down the elements into the fruit. Hartill and Everett (1997) further postulated that infected fragments from the extra-cortical layers might be transferred from pedicel to pedicel during clipping.

This work investigates the method of entry of fungi into avocado fruit to cause stem-end rots with the aim of developing effective control strategies. Three possible ways that fungi can gain access into the fruit were tested; air-borne spores infecting the picking wound, fungi growing inside the stem tissue (endophytes) are activated by picking and thirdly fungi growing just under the cuticle (phellophytes) are smeared over the wound by secateurs.

MATERIAL AND METHODS

Fruits were picked from the lower half of avocado trees at the orchard of Mr. Ron Bailey Rangiuru Road on 30 January 2002. Treatments were comprised of twelve different picking techniques as follows:

1 Fruits plucked.

2 Pedicels clipped to a *c.* 0.5 cm button as in commercial practice.

3 Pedicels clipped to a *c.* 2 cm length.

4 Pedicels clipped to a *c.* 4 cm length.

5 Pedicels clipped to a *c.* 6 cm length.

6 Pedicels clipped to a *c.* 0.5 cm button with secateurs dipped in Benlate + Sportak.

7 Fruits plucked and the picking wound immediately plunged onto a sponge pad soaked in Benlate + Sportak.

8 Pedicels clipped to a *c.* 0.5 cm button and the button immediately plunged onto a sponge pad soaked in Benlate + Sportak.

9 Pedicels clipped to a *c.* 0.5 cm button and the button immediately plunged onto a sponge pad soaked in Benlate.

10 Pedicels clipped to a *c.* 0.5 cm button and the button immediately plunged onto a sponge pad soaked in Captan.

11 Pedicels cut to a 8-10 cm length with secateurs sterilised in 70% alcohol, then girdled by removing the extra-cortical tissues approximately between the 4-6 cm point; then the pedicel re-cut in the middle of the girdled area with sterile secateurs and the wound sealed with a Vaseline/medicinal paraffin mixture.

12 Pedicels cut to a *c.* 2 cm length with secateurs sterilised in 70% alcohol and the wound sealed with a Vaseline/medicinal paraffin mixture.

Benlate, and Sportak were chosen because of their known activity against the post-harvest rot pathogens (Hartill, 1992). Captan was selected because of its efficacy in inhibiting spore germination *in vitro* (Everett, 1999). For the dip treatments an XLO® sponge was placed in a plastic tray and flooded with the required fungicide.

Each treatment was replicated five times. Each replicate treatment consisted of one tray of 20 fruits picked at random from one or more trees. The trays were then stored and ripened at 20^{o} C in a controlled temperature room. Avocados were cut when ripe, assessed for neck rots using the methods of the AIC assessment manual and fungi from rots isolated onto Potato Dextrose Agar containing 50 ppm streptomycin and identified.

The data obtained were subjected to one way analysis of variance using MINITAB and SAS. Data was normalised by arc-sine transformation when appropriate.

RESULTS

There was only a moderate level of post-harvest rots in this trial but sufficient to indicate that the level can be significantly influenced by the harvesting technique used. The incidence of post-harvest rots ranged from a high of 32% in the plucked fruits to a low of 4% in the fruits harvested with the pedicel clipped 6 cm long (Table 1).

The incidence of stem-end rots in fruits harvested with the pedicel clipped to a button (i.e. *c*. 0.5 cm long), as in commercial practice was 13%. The relative values recorded for stem-end rot severity followed a similar pattern to that recorded for rot incidence. Pedicel length was related to incidence of disease (Figure 1). The longer the pedicel left attached to the fruit, the lower the rot incidence and severity.

The incidence of *Colletotrichum* spp. was highest in fruits harvested by plucking (Table 2). These fruits also had the highest incidence of *Phomopsis* sp. Fruits harvested by cutting the pedicel with sterile secateurs, girdling, recutting and sealing the wound had the highest incidence of *Botryosphaeria* spp.

Of the fungicide treatments, Benlate® applied to the button by dipping gave best control. Captan was ineffective, as were the combination of Benlate® + Sportak® applied with secateurs or by button dipping. Rots in fruit plucked then dipped in Benlate® + Sportak® were reduced by half. This difference was statistically significant ($p<0.05$).

Table 1. The incidence and severity of post-harvest stem-end rots in avocado fruits harvested using different techniques.

Treatment	Stem-end rot incidence %	Stem-end rot incidence *Arcsine transformed*		Stem-end rot severity %	
Plucked	32	0.59	a	2.27	a
Button	13	0.34	b c d	0.76	b c d
2 cm button	11	0.29	c d e	0.45	c d
4 cm button	9	0.24	d e	0.38	c d
6 cm button	4	0.12	e	0.05	d
Secateurs dipped in Benlate + Sportak	11	0.30	b c d e	0.90	b c d
Plucked – end dipped in Benlate + Sportak	12	0.31	b c d e	0.43	c d
Button dipped in Benlate + Sportak	14	0.38	b c d	0.68	b c d
Button dipped in Benlate	6	0.22	d e	0.17	d
Button dipped in Captan	22	0.48	a b c	1.49	a b c
Pedicel cut with sterile secateurs, girdled, recut and sealed	24	0.50	a b	1.83	a b
Pedicel cut with sterile secateurs, and sealed	8	0.25	d e	0.54	b c d
L.s.d.		0.214		1.33	

Values in columns with no letters in common differ significantly at the $P < 0.05$ level.

The time taken for fruits to ripen and the severity of body rots were not significantly affected by differences in the harvesting techniques (data not shown).

Table 2. The incidence (%) of post-harvest fruit rot pathogen species in avocado fruits harvested using different techniques (not analysed) by isolation from rot lesions.

Treatment	C.g.[1.]	C.a.	B.d.	B.p.	Phom.	Other
Plucked	6	13	1	5	5	1
Button	4	1	4	3	1	-
2 cm button	2	0	1	6	0	-
4 cm button	0	1	1	4	3	-
6 cm button	2	0	0	2	0	-
Secateurs dipped in Benlate + Sportak	1	2	3	5	1	-
Plucked – end dipped in Benlate + Sportak	1	7	1	1	2	-
Button dipped in Benlate + Sportak	3	0	5	5	1	-
Button dipped in Benlate	0	4	2	0	0	-
Button dipped in Captan	4	7	4	4	2	-
Pedicel cut with sterile secateurs, girdled, re-cut and sealed	1	1	8	9	2	1
Pedicel cut with sterile secateurs, and sealed	0	0	3	4	0	-

[1.] C.g.= *Colletotrichum gloeosporioides*, C.a.= *Colletotrichum acutatum*, B.d.= *Botryosphaeria dothidea*, B.p.=*Botryosphaeria parva*, Phom.= *Phomopsis* sp.

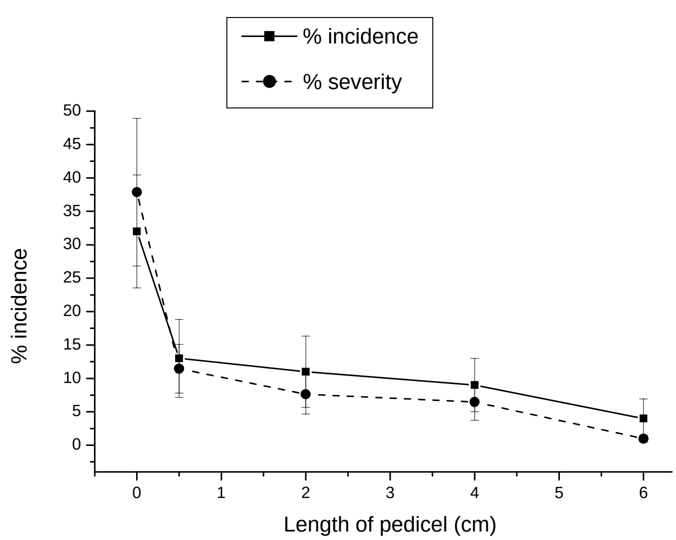

Figure 1. Relationship between pedicel length and incidence and severity of stem-end rots.

DISCUSSION

The incidence and severity of post-harvest rots were markedly affected by the different harvesting methods used. The results obtained support an hypothesis that fragments of infected tissue present as phellophytes within the extra-cambial layers of the pedicels (Hartill, 1993) and/or spores of the pathogens are deposited onto the open ends of the xylem elements when the fruits are harvested by clipping. Similarly in plucked fruits spore deposition probably initiates the major infection pathway. Picking by plucking is a more violent procedure than picking by clipping and could lead to the release of spores that then settle on the wounds. The pathogens then infect fruit by growing down the xylem elements. This hypothesis is supported by our finding that the incidence and severity of stem-end rots was negatively related to the length of the pedicel left after clipping (Figure 1). Plucked fruits, i.e. those that had little or no pedicel attached to each fruit after harvest, had the highest, and fruits with pedicels cut approximately 6 cm long had the lowest rot incidence and severity. It therefore seems obvious that any measure that slows or prevents this growth of the pathogen down the pedicels will result in a reduced incidence of stem-end rots. It may therefore be significant that the higher incidence of rots in the plucked fruits is associated with an increased incidence in those pathogens that produce the most spores on agar, *Colletotrichum* and *Phomopsis* spp. (Table 2, Figure 2). The increased incidence of rots in plucked fruit is associated with a relatively high incidence of *Colletotrichum acutatum* when compared with clipped fruit has been recorded in previous trials (Hartill & Everett, 1997; Hartill & Sale, 1991).

Johnson *et al.* (1991) suggested that the stem-end rot pathogens are endophytes. The present results do not support this suggestion. If the pathogens are endophytes there is no need to propose a special relationship between the method of harvesting and the incidence of post harvest rots. The pathogens would be present throughout the pedicels at harvest and the subsequent development of post-harvest rots would not be affected by the length at which the pedicels are clipped.

However, the finding that there were more rots in fruit treated with Captan, a contact fungicide, than those treated with Benlate, which has systemic action may give some support to the suggestion that the infections are endophytic. Otherwise the time between picking and applying the fungicide must have been sufficient for infective propagules to have reached the fruit or alternatively the break in the water column occurring immediately after clipping the fruits must have prevented the fungicide being drawn into the xylem elements. However, M. Manning (pers.comm.) stated that in kiwifruit, if Captan is applied immediately after picking as was done in this trial, then stem-end rots were controlled. It should be noted that although Captan has been shown to be very effective against the avocado pathogens *in vitro* it has not been fully field-tested under New Zealand conditions.

The results from treatment 11 where the pedicels were cut with sterile secateurs, girdled, re-cut and sealed do not support the phellophytic hypothesis, probably because the exposed vasculature was susceptible to infection throughout its length not just at the cut end. The effect of the treatment was to increase the numbers of infected fruit compared to fruit clipped to a 4 cm stalk then sealed with Vaseline/paraffin oil. This was probably because the inoculation site in the fruit that were girdled then clipped and sealed had a greater surface area, increasing the chances of infection taking place. It was not clear whether the vasculature had been damaged apart from at the end. Microscopical examination would be required to confirm or otherwise if damage had occurred. If the exposed vasculature had been sterilised before cutting and sealing, this may have reduced infections.

The fungicide treatments did not provide statistically significant control which is in contrast to previous findings (Hartill and Everett, 1997). None of the treatments entirely eliminated stem-end rots. Other, minor, infection pathways may also exist. Fruits harvested with secateurs dipped in Benlate® and Sportak® had a similar level of rots to those harvested with untreated clippers. Least disease occurred in fruits where the button was dipped in Benlate® immediately after harvest, a result which was due to the total control of *C. gloeosporioides* and *B. parva*. However, when Sportak® was added to the Benlate® with the aim of also controlling *C. acutatum*, disease incidence and severity was not affected. In contrast dipping the wounds of plucked fruits in a Sportak®/Benlate® mixture immediately after picking significantly reduced the incidence and severity of stem-end rots when compared with undipped fruits (Table 1). This treatment reduced the incidence of all pathogens present. The failure of this mixture to control rots in clipped fruits is difficult to explain.

The best fungicide treatment (Benlate® applied by dipping to the button) halved the rot incidence compared with fruit with untreated buttons although these did not differ significantly. This treatment shows promise as an effective control method for stem-end rots, but unfortunately Benlate® was recently withdrawn from sale. Related fungicides, e.g. thiabendazole, methyl thiophanate, carbendazim, need to be tested on a number of different orchards to see if they are suitable replacements.

Fungicide treatments appear to hold some promise in situations such as picking from a hydraladder or when re-clipping pedicels of fruits picked with long-arm clippers or picking poles where the person clipping the fruit has the dipping suspension held in a fixed position before them. There is also no need to use a conventional field fungicide as persistence is not required here so that disinfectants, food preservatives, biological agents, and similar materials may also be effective. Residue tests may be required before commercialisation.

Since the introduction of long-arm clippers many fruits are now initially picked with long pedicels. We have shown that this treatment reduces the incidence of rots. Delaying the re-clipping of the pedicels, in a manner analogous to the air curing of kiwifruit should also be investigated.

SUMMARY

This work investigates the method of entry of fungi into avocado fruit to cause stem-end rots with the aim of developing effective control strategies. The results indicate that either fragments of infected tissue present as phellophytes within the extra-cambial layers of the pedicels (Hartill, 1993) and/or spores of the pathogens are deposited onto the open ends of the xylem elements when the fruits are harvested. The pathogens then infect fruit

by growing down the xylem elements. The longer the pedicel of the fruit when harvested, the lower the incidence of stem-end rots. This is most likely due to the length of time taken for fruit to grow down the pedicel, before entering the fruit. The best fungicide treatment (Benlate® applied by dipping to the button) halved the rot incidence compared with fruit with untreated buttons although these did not differ significantly. This treatment shows promise as an effective control method for stem-end rots.

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