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Market Survey of Stem-end Rot and Anthracnose on Fuerte Avocados and Comparison of Colletotrichum gloeosporioides *Isolates from Different Avocado Producing areas*

G M Sanders • L Korsten
Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria 0002

ABSTRACT

A market survey was carried out over a three year period to determine the incidence of stem-end rot and anthracnose in commercially available Fuerte fruit from different avocado producing areas. Two hundred and eighty seven isolates of C. gloeosporioides *isolated from avocados from the different areas were compared using fruit inoculation studies, colony and spore morphology, growth rate and benomyl resistance. Differences were observed, and isolates could be grouped based on criteria under evaluation. Data must be further analysed to determine whether groupings obtained for the different criteria correspond. Molecular techniques will be used to determine relationships as well as differences or similarities between isolates.*

INTRODUCTION

Avocados (*Persea Americana* Mill) are the sixth most important subtropical crop in the world, with South Africa being the tenth largest producer with an average annual production of 38 000 metric tons (Ploetz, 1994). Post-harvest diseases such as anthracnose caused by *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. and stem-end rot mostly caused by *Thyronectria pseudotrichia* (Schw.) Seeler, *C. gloeosporioides, Dothiorella aromatica* (Sacc.) Petrak & Sydow and *Phomopsis perseae* Zerova are responsible for a considerable loss of export revenue with incidences of 29 % and 15 % reported respectively on the overseas markets (Bezuidenhout & Kuschke, 1983).

Pre and post-harvest anthracnose on avocados caused by *C. gloeosporioides* has been reported in several countries including Australia (Fitzell, 1987), Israel (Binyamini & Schiffmann-Nagel, 1972), South Africa (Darvas & Kotzé, 1987) and Sri Lanka (Sivanathan & Adikaram, 1989). *C. gloeosporioides* is especially important as a quiescent pathogen (Jefferies *et al,* 1990) with symptoms developing post harvest, but serious losses also occur due to premature fruit ripening and abscission resulting from fungal infection (Fitzell, 1987). Disease incidence and severity varies throughout the growing season with some export consignments being virtually disease free and others, completely unmarketable (Kotzé, 1978). In order to better understand the epidemiology and thus reduce the financial impact of *C. gloeosporioides* on the avocado industry, it is important to know as much as possible about the pathogen itself.

Differences between isolates of C. *gloeosporioides* from various sources have been widely reported. Techniques ranging from inoculation studies in mango (Quimio & Quimio, 1975) and citrus (Agostini *et al,* 1992) to molecular techniques such as electrophoretic patterns from protein extracts and dsRNA patterns (Dale *et al,* 1988), restriction fragment length polymorphisms (RFLP's) (Sreenivasaprasad *et al,* 1993; Bernstein *et al,* 1995), random amplified polymorphic DNA (RAPDS) (Alahakoon *et al,* 1994) and isozyme analyses (Kaufmann & Weidemann, 1996) were used for this purpose.

The purpose of this study was to carry out a market survey of the incidence of stem-end rot and anthracnose in market fruit from different production areas. C. *gloeosporioides* isolates obtained during the course of these surveys were compared using a variety of criteria.

MATERIALSAND METHODS

Market survey and collection of isolates

Fuerte avocado fruit were collected from the Pretoria fresh produce market at weekly intervals for four weeks from 7 to 28 July 1994, seven weeks from 25 April to 22 June 1995 and bi-weekly from 26 March to 8 June 1996. Ten trays per area with fruit ranging from 12 to 24 fruit per tray were collected from the following areas: Tzaneen (including Duivelskloof and Politsi), Nelspruit (including the Kiepersol area), Louis Trichardt, Levubu and Kwa-Zulu-Natal (including the midlands and northern Natal). Fruit were collected from these areas subject to availability. On arrival from the market, fruit were evaluated, enumerated and general condition of the fruit noted. Fruit were left to ripen at ambient temperature and were evaluated at three stages of ripeness viz. eating ripe, slightly overripe and overripe. At each stage, the number of lesioned fruits per tray were noted and isolations made from all lesions, plated onto oatmeal agar (OA) and incubated at ambient temperature under mixed irradiation from near ultraviolet and daylight type fluorescent tubes (Phillips TL 40W/08RS, F40 B43 and TL 40W/33RS respectively) until spore formation was observed. Pure cultures of *C. gloeosporioides* isolates were prepared for further study and all isolates were preserved by freezing in 50% glycerol at 78°C as well as plating onto potato dextrose agar (PDA) slants and in sterile water. Two hundred and sixty seven isolates in total were selected randomly from all cultures obtained for further study.

Inoculation studies into Fuerte avocado fruit

Untreated, unwaxed, physiologically mature, but unripe Fuerte fruit from Westfalia Estate were used for the plug inoculation trials. Prior to inoculation, all fruit were swabbed with 70% ethanol and left to dry. Ten millimetre deep plugs were cut from fruit using a four millimetre diameter stainless steel cork borer. Plugs were cut from actively sporulating areas from each culture on OA which had been incubated as described for five days. Three replicates from each culture were placed into holes in three different fruit. Fruit plugs were replaced and covered with parafilm. Fruit were incubated upright at ambient temperature. After five days, lesions were evaluated by measuring the length

and breadth including the hole made by the cork borer. Data was statistically analysed with the SAS system using analysis of variance. Comparisons were made using Duncan's multiple range test and Pearson's correlation coefficient was used to correlate factors under investigation. All statistical analyses with probability values equal to or less than 0,05 were regarded as an indication of significant differences between variables.

Inoculation studies into Sensation mango fruit

Untreated, unwaxed Sensation fruit from Moria Mango were used for the plug inoculation trials. Preparation of inoculum, inoculation evaluation and data analyses were carried out in the same manner as the avocado trial.

Comparison of spore and culture morphology

Plugs were cut from actively sporulating areas from each culture prepared as described. For each culture, three replicate OA plates were incubated under the same conditions as described. After seven days, colony size, colour, texture, colour of conidia! masses and zonation was described using categories from Baxter *et al.* (1993). Plates were flooded with sterile distilled water and conidia harvested. Conidial concentration was adjusted to 1×10^7 conidia/m/ and examined microscopically. Lengths and widths of 30 conidia were measured using a Zeiss phase contrast microscope and stage micrometer. Mean conidium width and length were analysed using analysis of variance.

Comparison of growth rates and benomyl resistance

Inoculum prepared as previously described, was plated onto three replicate PDA plates as well as three replicate PDA plates amended with benomyl (Benlate, Du Pont) (Bernstein *et al,* 1995). Fungal colony diameter on both groups of media were measured daily for seven days. Growth rates were calculated from growth obtained on unamended media.

RESULTS

Market survey and collection of isolates

The general condition of fruit obtained from the market was good, with little mechanical, wind and thrips damage and sunburn. During the three years of surveys, more anthracnose was generally observed than stem-end rot (figure 1). Levels of stem end rot remained relatively constant except in 1995 where a peak was observed in the first week of May. Levels of anthracnose fluctuated throughout the study (figure 1).

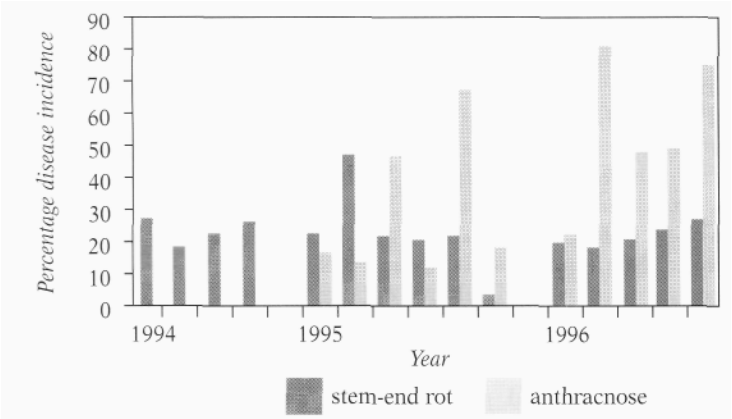

Figure 1
Total incidence of stem-end rot and anthracnose from market fruit

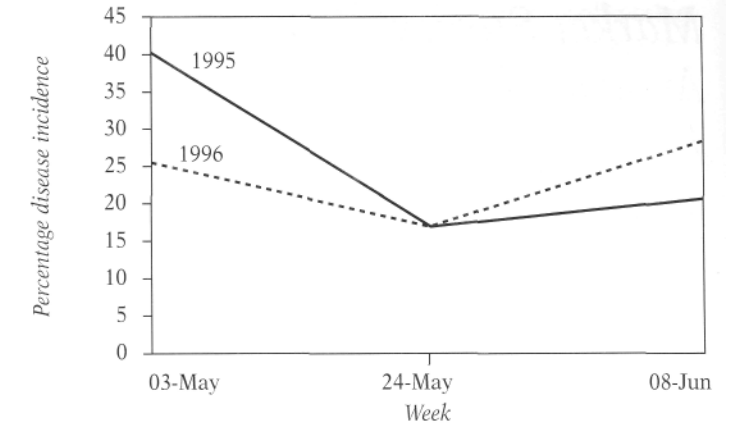

Figure 2
Incidence of stem-end rot from market fruit from Tzaneen as observed over a two year period.

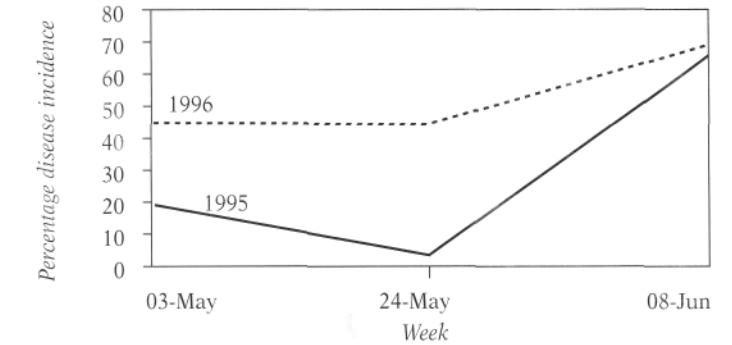

Figure 3
Incidence of anthracnose from market fruit from Tzaneen as observed over a two year period

Fruit from the Tzaneen area was used as a model to compare the incidence of anthracnose and stem-end rot in 1995 and 1996. In order to compare disease incidence on fruit collected over the two years, only corresponding collection dales were selected to represent trends observed in this study. The incidence of stem end rot was lower in 1995 except in the first week of May, when the incidence was higher than the corresponding period in 1996, although similar patterns were observed during the two year period under investigation (figure 2). The incidence of anthracnose was much higher in 1996, although the first week in June showed very similar levels for the two years surveyed (figure 3)

In 1994, a total of 2 303 fruit were evaluated only for stem end rot where the total incidence in all market fruit was 22,40%. The most stem-end rot was observed in Levubu (38,57%) followed by Tzaneen (28,53%), Nelspruit (19,36%) and Louis Trichardt (17,13%). In 1995, a total of 1 634 fruit were evaluated for anthracnose and stem-end rot. The total incidence of stem end rot was 25,09% with the highest incidence recorded in Nelspruit (table 1). The total incidence of anthracnose was 23,62% with the most observed in KwaZulu-Natal (table 1). In 1996, a total of 2390 fruit were evaluated for anthracnose and stem-end rot. The total incidence of stem-end rot was 15,94% with the most observed in Nelspruit (table 1). The total incidence of anthracnose was 48.20% with the most observed in KwaZulu-Natal (table 1) Many *C. gloeosporioides* isolates were obtained from the various areas from early to late season. In 1996, the most *C. gloeosporioides* were isolated from Tzaneen, followed by Louis Trichardt, Levubu, Nelspruit and KwaZulu-Natal (table 2). In 1996, more *C. gloeosporioides* was isolated from anthracnose than stem-end lesions in all areas except Nelspruit and Louis Trichardt (table 2).

Isolates were selected randomly from all positively identified as *C. gloeosporioides* and used for further study.

Inoculation studies into Fuerte avocado fruit

Sub-groups could be distinguished within the isolates according to the size of lesion formation on Fuerte fruit. Typical anthracnose lesions (up to 36 mm in diameter) were produced by these isolates, with the exception of seven isolates, which did not produce any symptoms. Isolates from certain areas were found to be more virulent than others. Isolates from KwaZulu-Natal were found to be less virulent than isolates obtained from Louis Trichardt, Levubu and Hazyview (table 3).

Table 1

A comparison of the incidence of stem-end-rot and anthracnose from different avocado production areas for 1995 and 1996

Year	*Area*	*Incidence SE (% of total fruit evaluated*	*Incidence anthracnose (% of total fruit evaluated)*
1995	Nelspruit	43,00	17,66
	KwaZulu-Natal	29,00	31,25
	Tzaneen	20,90	28,79
	Louis Trichardt	19,50	3,00
1996	Nelspruit	38,30	28,71
	KwaZulu-Natal	28,47	71,52
	Louis Trichardt	19,50	43.06
	Levubu	11,14	48,12
	Tzaneen	11,20	55,80

Table 2

A comparison of the incidence of *Colletotrichum gloeosporioides* isolated from anthracnose and stem-end rot lesions from different avocado producing areas in 1996

Area	*Total incidence*[a]	*Total numbers of isolations*	*Incidence from anthracnose*[b]	*Incidence from SE*[c]
Tzaneen	48,61	1 008	342	148
Nelspruit	44,57	525	98	136
Louis Trichardt	33,80	1 053	159	197
KwaZulu-Natal	24,73	186	40	6
Levubu	24,31	1 131	161	114

a Percentage *C. gloeosporioides* obtained from all isolations made
b Number of *C. gloeosporioides* isolated from anthracnose lesions
c Number of *C. gloeosporioides* isolated from stem-end rot lesions

Table 3

Comparison of lesion size on Fuerte avocado obtained from inoculation of *Colletotrichum gloeosporioides* isolates from different production areas

Area	*Mean lesion diameter (mm)*
Hazyview	24,33a
Levubu	21,15a
Louis Trichardt	19,95a
Tzaneen	17,31ab
Nelspruit	16,43ab
KwaZulu-Natal	1,58b
Pr > F	0,0019

Means followed by the same letter do not differ significantly according to Duncan's multiple Range Test at the 95% confidence level.

The stage of ripeness at which isolations were made, also had an effect on the virulence of isolates tested. Isolates obtained from slightly overripe fruit were significantly less virulent than isolates obtained from eating ripe and very overripe fruit (P = 0,019). No significant differences were observed in virulence between isolates obtained from stem-end rot or anthracnose lesions. A similar tendency was found for all production areas tested, except Nelspruit where isolates obtained from anthracnose lesions were significantly less virulent than isolates obtained from stem-end rot lesions (table 4).

Table 4
Comparison of virulence of different isolates obtained from stem-end rot and anthracnose lesions from different production areas on avocado fruit

	Average lesion diameter			
Symptom	*Tzaneen*	*Levubu*	*Louis Trichardt*	*Nelspruit*
Anthracnose	17,28a	21,31a	18,28a	14,37b
Stem-end rot	17,37a	20,66a	21,023a	19,58a
Pr >F	0,9433	0,8076	0,1186	0,0212

A correlation was observed between the stage of ripeness at which the isolations were made and virulence (P = 0,0238). Isolates obtained from less ripe fruit produced larger lesions, while isolates obtained from ripe fruit produced smaller lesions.

Inoculation studies into Sensation mango fruit

Sub-groups could also be distinguished within the isolates according to the size of lesion formation on Sensation mango fruit. Typical anthracnose lesions up to 45 mm in diameter were produced by these isolates. Origin and stage of ripeness from which isolations were made, did not have any significant effects on lesion size produced in mango. A correlation exists with respect to lesion size in avocado when compared to mango (P = 0,036). Isolates which appear to be highly virulent in avocado only produce small lesions in mangos. However, the avocado isolates can infect mangos equally well, since lesions in avocados are not significantly larger than those formed by the same isolates in mango.

Comparison of spore and culture morphology

Culture morphology and conidial shapes and sizes varied between isolates. These differences can be used to further characterise the isolates.

Comparison of growth rates and benomyl resistance

Differences in growth rates were observed between all isolates tested and were sufficient to enable grouping of isolates. Of the 287 isolates tested, 97 (33,79% of the isolates evaluated) were resistant at varying levels.

DISCUSSION

The results obtained in this study show that anthracnose and stem-end rot still play an important role in post-harvest losses in the South African avocado industry. The incidence of stem-end rot recorded was lower than that of anthracnose, but still resulted in a 25% loss of marketable fruit. The relatively low incidence of mechanical and other damage to fruit shows the increased awareness of producers to cosmetic quality of locally marketed fruit.

The prevalence of anthracnose in Tzaneen was found to be much higher in 1996 than 1995, and this may be ascribed to the higher rainfall and slightly higher temperature for the corresponding periods in 1996 (127 mm compared to 25 mm in 1995). It is well known that *C. gloeosporioides* has minimum moisture requirements for successful infection and colonisation (Jeffries *et al,* 1990; Dodd *et al,* 1992). Kotzé (1978) also showed that at a temperature between 20 24 °C lesions caused by *C. gloeosporioides* develop most rapidly.

The incidence of stem-end rot and anthracnose varied considerably from area to area. Several factors could be involved, most specifically climate. These findings are in accordance with a study carried out in 1989 by Korsten *et al.* (1994) who found that isolation rates of *C. gloeosporioides* from fruit rots differed according to area at a given time.

It is interesting to note that isolates obtained from certain areas are more virulent than others while no significant differences in virulence were found between isolates obtained for anthracnose or stem-end rot.

A high percentage of the isolates tested were found to be resistant to benomyl. It has been reported that there is an increase in the number of isolates of *C*. *gloeosporioides* that are resistant to benomyl, and it is suspected that at a genetic level, the teleomorph stage, *Glomerella cingulata* (Stonem.) Spauld & v. Schrenk may play a role in how the pathogen adapts to its host and environment (Dodd *et al,* 1992).

Preliminary comparative data obtained in these experiments, gives a clear indication that there are differences between isolates of *C. gloeosporioides.* Similar findings have been reported for *C*. *gloeosporioides* isolates from various other sources, such as citrus (Agostini *et al.,* 1992; Liyanage *et al.,* 1992), strawberry (Denoyes & Baudry, 1995) and mango (Hayden *et ai,* 1994; Quimio & Quimio, 1975). Most of the isolates inoculated into avocados were found to be highly virulent, with the greatest distribution of isolates producing large lesions. The isolates could also be grouped based on lesion formation. Upon inoculation into mango fruit, it was found that lesions were smaller, though not significantly. Infection rates were equally high irrespective of host inoculated. Quimio & Quimio (1975) reported similar results with isolates from mango, citrus and papaya but with greater variability in pathogenicity. Alahakoon *et al.* (1994) also found that isolates

obtained from a specific host were more pathogenic on that specific crop than on others. It has been shown that isolates of *C*. *gloeosporioides* from different sources did not have the same ribosomal DNA (rDNA) or mitochondrial DNA (mtDNA) restriction patterns (Hodson *et al,* 1993) or random amplified polymorphic DNA banding patterns (Mills *et al,* 1992). A great deal of information may be obtained using cross infection studies and morphological and physiological comparisons. Molecular analysis at the DNA level has shown that genetically distinct fungi exist within the *C*. *gloeosporioides* complex (Hayden *et al.,* 1994). It is therefore important to characterise all isolates obtained at a molecular level in order to elucidate findings.

Grouping of isolates was also done by Agostini *et al* (1992), but grouping was made based on morphology, growth rate and colony characteristics. Currently, these avocado isolates are being compared using similar criteria, and findings thus far indicate that trends found by Agostini *et al.* (1992) may also be true for avocado isolates of *C. gloeosporioides* (unpublished data).

It is thus important to make use of a combination of techniques to determine whether differences occur between isolates. This has been successfully carried out by Hayden *et al* (1994) who used a combination of cross infection studies and RAPDS (Random amplified polymorphic DNA) to group isolates of *C. gloeosporioides* from mango. Braithwaite *et al.* (1990) could correlate pathogenicity groups of *C. gloeosporioides* from *Stylosanthes* spp. and with genetically distinct groups, also using RAPDS.

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