

Comparison of *Colletotrichum gloeosporioides* Isolates from Various Avocado Producing Areas

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ABSTRACT

Eighty six isolates of *C. gloeosporioides* isolated from avocados from various production areas were compared using fruit inoculation studies. Differences were observed, and isolates could be grouped into three groups based on lesion size. These results are preliminary and further investigation must be carried out to confirm these findings.

INTRODUCTION

Pre and postharvest anthracnose on avocados caused by *Colletotrichum gloeosporioides* (Penz). Penz & Sacc. in Penz has been reported in several countries including Australia (Fitzell, 1987), Israel (Binyamini & Schiffmann Nagel, 1972), South Africa (Dar vas & Kotzé, 1987) and Sri Lanka (Sivanathan & Adikaram, 1989). *C. gloeosporioides* is especially important as a quiescent pathogen (Jefferies *et al.*, 1990) with symptoms developing postharvest, but serious losses also occur due to premature fruit ripening and abscission resulting from fungal infection (Fitzell, 1987). 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) and restriction fragment length polymorphisms (Bernstein *et al.*, 1995) were used for this purpose.

The purpose of this study was to compare *C. gloeosporioides* isolates from various avocado producing areas using inoculation studies on avocado.

MATERIALS AND METHODS Collection of isolates

Fuente avocados were obtained from the Pretoria fresh produce market at weekly intervals for seven weeks from 25 April to 22 June 1995. Ten trays per area, with quantities ranging from 12 to 24 fruits per tray, were collected from the following areas: Tzaneen (including Duivelskloof and Politsi), Nelspruit (including the Kiepersol area)

and KwaZuluNatal (including the midlands and northern Natal). On arrival from the market, fruit was evaluated, counted and its general condition noted. Fruit was left to ripen at ambient temperature and evaluated at three stages of ripeness, viz. eating ripe, slightly overripe and overripe. At each stage, the number of lesioned fruits per tray was noted, and isolations made from all lesions, were plated onto oatmeal agar and incubated at ambient temperature under ultraviolet light 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 slants and in sterile water. Eighty six isolates in total were selected for further study.

Fruit inoculation studies: plug inoculation into Fuerte fruit

Untreated, unwaxed Fuerte fruit from Westfalia Estate was 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 stain less steel cork borer. Plugs were cut from actively sporulating areas from each culture on oatmeal agar which had been incubated at ambient temperature under ultraviolet light for five days. Three replicates from each culture were placed into holes in three different fruits and the fruit plugs were replaced and covered with parafilm. Fruits 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.

Fruit inoculation studies: spore inoculation into Fuerte fruit

Isolates were cultured as described, spores harvested and concentrations adjusted to 10^7 spores/ml. Two millimetre deep prick wounds were made using a 26gauge needle and 10 µl of each spore suspension was placed onto each wound. Replicates and incubation conditions were the same as for the plug trial, except that fruit were not incubated upright. Lesions were measured as described.

Fruit inoculation studies: plug inoculation into Hass fruit

Untreated, unwaxed Hass fruit from Westfalia Estate was used for the plug inoculation trials. The procedure was identical to that for Fuerte, except that fruits were evaluated by being cut open to measure the diameter and depth of the lesion.

RESULTS

Collection of isolates

The general condition of the fruit obtained from the market was good, with only a little mechanical damage, thrips damage and sunburn observed. Many *Colletotrichum gloeosporioides* isolates were obtained from the various areas from mid to late season. Most were isolated from the Duivelskloof area (330 isolates), followed by Politsi (252 isolates), Tzaneen (167 isolates), Kiepersol (89 isolates) and KwaZuluNatal (45

isolates). Eighty six isolates were selected randomly from these and used for further study.

Fruit inoculation studies: plug inoculation into Fuerte fruit

Nine subgroups could be distinguished within the eighty six isolates according to the size of lesion formation on Fuerte fruit. These groups ranged from 4 mm to 36 mm, and the distribution of lesion sizes in between, were close to normal (figure 1). Typical anthracnose lesions were produced by these isolates, with the exception of two isolates that did not produce any symptoms. Most isolates produced lesions with a diameter of 32 mm. These were then grouped into three main groups, avirulent, moderately virulent and highly virulent.

Fruit inoculation studies: spore inoculation into Fuerte fruit

Lesions obtained with spores were much smaller than those observed with plugs. The smallest lesions produced were only 1 mm wide, and the largest 11 mm (figure 2). The distribution of the lesion sizes amongst the isolates was considerably different from that observed when using plugs as inoculum.

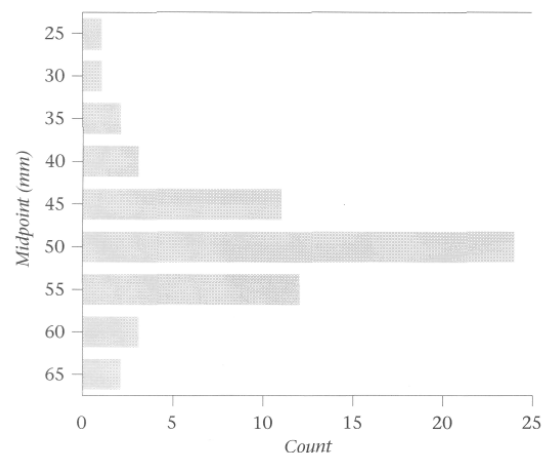
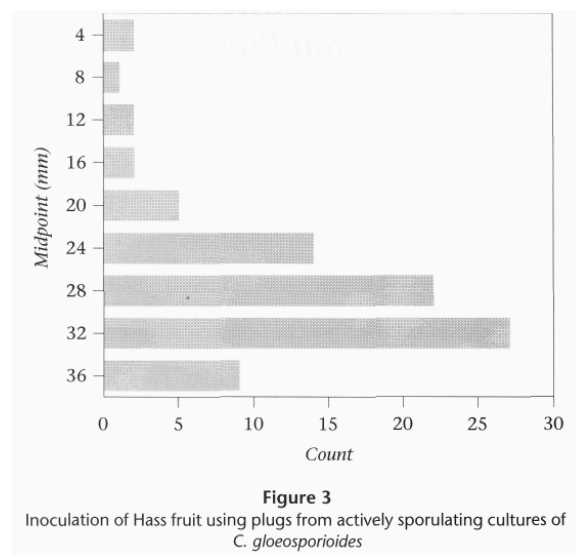
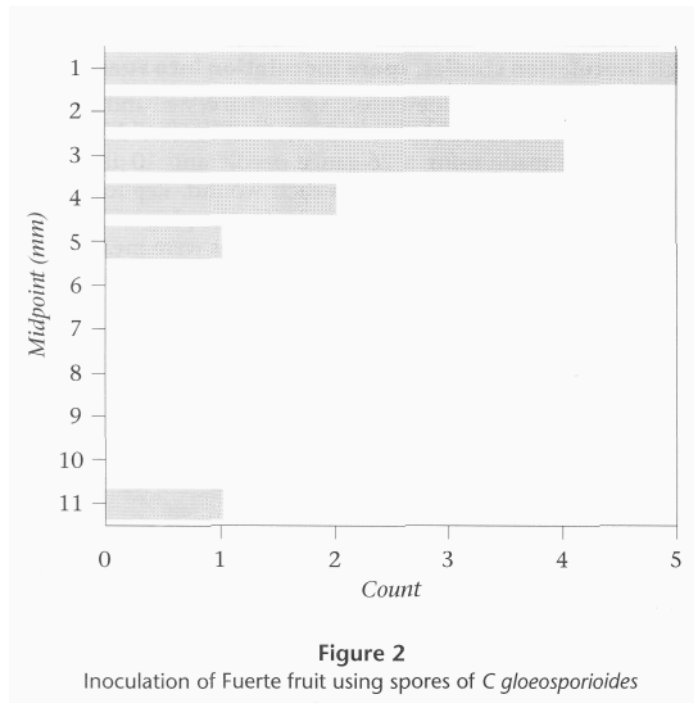


Figure 1
Inoculation of Fuerte fruit using plugs from actively sporulating cultures
of *C. gloeosporioides*



Fruit inoculation studies: plug inoculation into Hass fruit

Since the depth of all the lesions was found to be the same for all the isolates, it was not taken into account when evaluating differences between isolates. Lesions observed were larger than those obtained when inoculating Fuerite fruit with plugs. The smallest lesion diameter was 25 mm and the largest 65 mm, with most isolates producing lesions of 50 mm (figure 3). The distribution pattern was similar to that observed in the Fuerite plug trial, being close to normal.

DISCUSSION

The data obtained in these experiments, although preliminary, gives a clear indication that there are indeed differences between isolates of *C. gloeosporioides* from avocado. Similar findings have been reported for *C. gloeosporioides* isolates from various sources, such as citrus (Agostini *et al.*, 1992; Liyanage *et al.*, 1992), straw berry (Denoyes & Baudry, 1995) and mango (Hayden *et al.*, 1994; Quimio & Quimio, 1975). Most of the isolates tested were found to be highly virulent, with the greatest distribution of isolates producing large lesions. The isolates could also be grouped into three groups based on lesion formation, viz. avirulent, moderately virulent and highly virulent. Similar groupings were found 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). Significantly larger lesions were obtained when plugs from actively sporulating cultures of *C. gloeosporioides* were inoculated into Hass fruit than when inoculated into Fuerte. This was unexpected, since Fuerte is more susceptible to infection by *C. gloeosporioides* than Hass (Fitzell, 1987), but the Hass fruit was slightly ripe when studies were carried out, which may explain these findings.

Hayden *et al.*, (1994) successfully used spores in cross infection studies. However, in these trials more reproducible results were obtained when using plugs as inoculum, since spore inoculations did not yield satisfactory results when compared to findings obtained with plugs.

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 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.

This work forms part of an ongoing project, in which molecular techniques will be combined with morphological and physiological comparisons to determine whether or not there are differences between isolates of *C. gloeosporioides* and what the significance of these will be to the avocado industry.

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