CHAPTER 3

PATHOGENICITY OF *BOTRYOSPHAERIA* SPECIES ON TWO MANGO CULTIVARS IN SOUTH AFRICA

ABSTRACT

Botryosphaeria spp. cause a wide range of disease symptoms on mango trees and fruit in South Africa. There are limited options available for control of these diseases and this is partly attributed to a lack of understanding of the etiology of these fungi. The purpose of this study was, therefore, to evaluate the pathogenicity of four *Botryosphaeria* spp. from mango in South Africa and to evaluate the susceptibility of two commercial cultivars, Keitt and Tommy Atkins. Isolates obtained from mango in South Africa were grown on potato dextrose agar, and screened for pathogenicity using an apple-based screening procedure. Most pathogenic isolates were then inoculated into the stems of potted mango trees representing both cultivars. All isolates were pathogenic to mango trees, but they varied in the extent of lesion development. The most pathogenic species on both cultivars were *B. parva*, *B. rhodina* and *Fusicoccum indigoticum*. *Fusicoccum bacilliforme* isolates were least pathogenic on both mango cultivars. Results of this study represent the first inoculations with the newly described *Botryosphaeria* spp., *F. indigoticum* and *F. bacilliforme*. They also provide the first clear indication of the relative importance of the four *Botryosphaeria* spp. now known to occur on mango in South Africa.

INTRODUCTION

The South African mango (*Mangiferae indica* Linn.) industry is relatively small in export value (59 000 tons for the 2000 season) compared to other fruit exports, but it remains an important source of foreign exchange for the country (Finnemore, 2000). Locally, this is one of the most important sub-tropical crops, earning in excess of R 98 million annually. The area planted to mango in South Africa has increased rapidly during the last decade (1990 – 2000), however, fruit production did not increase by the same factor during this period (Finnemore, 2000). This is partly due to increased levels of tree and fruit diseases, and in particular fungal diseases such as those caused by *Botryosphaeria* spp. Similarly, these fungal pathogens are threatening mango industries world-wide (Donkin & Oosthuyse, 1996; Finnemore, 2000).

Botryosphaeria spp. cause various disease symptoms on mango throughout the tropical and sub-tropical regions of the world (Ramos *et al.*, 1991; Johnson, 1992). These fungi infect through natural or mechanical wounds and directly through natural plant openings such as stomata (Britton & Hendrix, 1986; Johnson, 1992; Lonsdale, 1993). In South Africa, die-back, stem cankers, blossom blight, stem end rot (SER) and soft brown rot (SBR) are all associated with *Botryosphaeria* spp. infecting mango (Lonsdale, 1993). Stress weakens host defence responses and predisposes trees to infection by *Botryosphaeria* spp. (McPartland & Schoeneweiss, 1984). Infection, disease incidence and symptom expression may, therefore, vary due to seasonal and environmental factors (Singh, 1960; Britton & Hendrix, 1986; Darvas, 1991; Johnson, 1992; Nakasone & Paul, 1998).

Botryosphaeria spp. are recognised as endophytes and latent pathogens of mango (Johnson, 1992). When trees are weakened by stress, the quiescent state of these endophytic fungi ends, and disease symptoms develop (Schoeneweiss, 1979; Wene & Schoeneweiss, 1980; Johnson, 1992). Predisposition of mango trees, is mainly due to mineral deficiencies (iron, zinc, manganese) and environmental factors such as sunscald and hail damage (Schaffer *et al.*, 1988; Ramos *et al.*, 1991). Water stress is known to predispose young vascular tissue near the cambium to attack by *Botryosphaeria* spp. (Schoeneweiss, 1979; Pusey, 1989; Ramos *et al.*, 1991). Trees can, however, outgrow infection during periods of vigorous growth, which in turn reduces disease impact (Brown & Britton, 1986; Britton & Hendrix, 1986; Johnson, 1992).

Botryosphaeria spp. systemically colonise the vascular system of their hosts (Maas & Uecker, 1984; McPartland & Schoeneweiss, 1984). Infection of young mango trees results in a light reddish brown discoloration of the xylem vessels, while light to dark brown discoloration of the phloem becomes visible later (Herbert & Grech, 1987; Shearer *et al.*, 1987). Xylem and phloem vessels become clogged with tyloses and mycelium and later become necrotic, as the pathogen spreads through the trees. This necrosis may lead to branch and stem dieback, canker formation and eventually tree death (Maas & Uecker, 1984; Shearer *et al.*, 1987; Ramos *et al.*, 1991).

Controlling *Botryosphaeria* diseases on various economically important fruit crops has had limited success (Johnson *et al.*, 1991; Peterson *et al.*, 1991; Sanchote, 1991; Johnson, 1992; Johnson & Sanchote, 1994). In mango, fruit infections by *Botryosphaeria* spp. from external sources can be minimised by spraying with copper oxychloride, but this fails to control endophytic infections (Peterson *et al.*, 1991; Johnson, 1992; Johnson & Sanchote,

1994; Sanchote, 1993). Therefore, in order to manage *Botryosphaeria* diseases, an integrated strategy that combines orchard management, reduction of inoculum through pre- and postharvest practices and the use of timely field spray or postharvest application of chemicals, is used. There is, however, a growing demand for new cultivars with improved resistance to *Botryosphaeria* and other mango pathogens (Johnson & Sanchote, 1994; Finnemore, 2000).

Until recently, little has been known regarding the taxonomy of *Botryosphaeria* spp. on mango in South Africa. Names of fungi belonging to this group have been used interchangeably and arbitrarily. In a recent study (Chapter 2), we have shown that four *Botryosphaeria* spp. occur on mango in South Africa. They include *F. parvum* Penycook & Sameuls, *B. rhodina* (Pat.) Griff. et Maubl., *F. indigoticum* Jacobs, Slippers & Wingf. and *F. bacilliforme* Jacobs, Slippers & Wingf. Virtually nothing is known regarding the pathogenicity of these fungi on mango. Although some pathogenicity tests have been done in the past (Ramos *et al.*, 1991; Johnson, 1992), the isolates were from other continents and the taxa of the fungi involved has received considerable attention since then. The aim of this study was to test the pathogenicity of the four species occurring on mango in South Africa. Two commercially important mango cultivars grown in South Africa were also evaluated for their resistance to the four *Botryosphaeria* spp. under glasshouse conditions.

Isolates used

Fourty eight monoconidial *Botryosphaeria* isolates, representing all four *Botryosphaeria* species that have been isolated from mango in South Africa (Chapter 2), were used in a preliminary apple-based screening trial. Based on the results of this preliminary screening, nine isolates representing the four *Botryosphaeria* spp. from mango in South Africa, were chosen for inoculation of mango trees (Table 1; p111). All isolates were grown on potato dextrose agar (PDA) (Biolab) for seven days prior to inoculations. Preservation and maintenance of all cultures was the same as that used in a previous study (Chapter 2).

Apple fruit assay

Granny Smith apples were used for an initial assessment of pathogenicity of all 48 isolates. This assay was chosen because it has been shown to provide an indication of pathogenicity in other fungi (Enebak *et al.*, 1994; De Lange *et al.*, 1996; Steenkamp *et al.*, 1996) and because mango fruit were not available. Healthy fruit were selected for uniform size and ripeness. Fruit were surface disinfested by dipping them for two minutes in a 2% sodium hypochlorite (NaHOCl) solution, followed by a distilled water rinse and 70% ethanol (EtOH) dip for two minutes. Fruit were left to air-dry for approximately five minutes.

Inoculation wounds were made in the apples by cutting a single three to four mm deep well in the apple body with a cork borer (5mm diam.). Mycelial plugs (5mm diam.) were cut from the edge of actively growing cultures, with a cork borer. Three fruit were inoculated once for each isolate to be screened. Six fruit were used as controls with half of these being either wounded and not inoculated or inoculated with a sterile PDA plug. All wounds were

covered with strips of parafilm for the duration of the trial, to prevent desiccation and secondary infection.

Fruit were incubated at 25°C for approximately eight days, until they were completely rotten. Lesion lengths and widths were measured every two-days from the second day after inoculation. Average sizes of lesions including the initial wound were computed and data were analysed.

Inoculation of mango trees

Isolates for the inoculation on mango trees were selected based on the apple screening assay. One of the most and least pathogenic isolates of all four species were selected to be inoculated in mango trees. For *B. parva*, an intermediately pathogenic isolate was also included. The isolates chosen for tree inoculations were *B. rhodina* [BOT2399, BOT2376], *B. parva* [BOT2413, BOT2302, BOT2353], *F. indigoticum* [BOT2351, BOT2355] and *F. bacilliforme* [BOT2421, BOT2417].

A total of 120 one-year-old trees (60 of each of the cultivars Keitt and Tommy Atkins) were obtained from Westvalia Estates, Tzaneen. These cultivars are respectively reported to be tolerant and susceptible to *Botryosphaeria* spp. in the orchard (Lonsdale, personal communication). Trees were maintained in a glasshouse at 20°C – 28°C for six weeks, prior to inoculation. Inoculations were conducted on two completely randomised blocks of trees, during July - September (mid-winter to early spring) 2001. In each trial, three trees of each cultivar were inoculated per isolate or sterile PDA plugs, which served as controls. Stems were surface disinfested by wiping them with 70% EtOH prior to inoculation. Wounds were made with a cork borer (5mm diam.) between two nodes, situated above the

graft union, but underneath the first branch. Mycelium plugs (5mm diam.) were cut with a cork borer from the edges of actively growing colonies and inserted into the wounds. The inoculation wounds were covered with Parafilm to prevent desiccation and contamination. Lesion length measurements were taken six weeks after inoculation. The lesions were measured by calculating the maximum length of vascular discoloration below the bark of the tree (Britton *et al.*, 1990). For re-isolation of the inoculated fruit, small tissue pieces were cut from the edges of discoloured tissue and incubated on PDA at 25°C.

Statistical analyses

In this study, pathogenicity was defined based on the extent of lesion development arising from inoculation. All statistical analyses were performed using SAS statistical software (Version 7, SAS Institution, Cary, NC). For the apple fruit assay and potted tree inoculation, analysis of variance (ANOVA) was by the General Linear Model (GLM) procedure. Data were not corrected and all actual values are reported. Means were grouped by Duncan's multiple range test with $P = 0.05$.

RESULTS

Apple fruit assay

All isolates inoculated on apples produced typical fruit rots (Fig. 1; p113). Inoculations of fruit with the four *Botryosphaeria* spp. mostly produced a soft rotten circular area with a light tan colour, up to the lesion edges. Inoculations with *Fusicoccum indigoticum,* however, produced a firmer and darker brown rotten area. *Botryosphaeria rhodina* isolates showed very little variation in pathogenicity (Fig. 2; p115). Little variation was also seen for most *B. parva* isolates, other than for two isolates [BOT 2400, BOT 2350], which

produced significantly smaller lesions (Pr = 0.0936). One isolate of *F. indigoticum* [BOT 2315] produced similar sized lesions to isolates of *B. rhodina* and *B. parva*. The other isolate of this species [BOT 2355] produced lesions that were significantly smaller than the former isolate (Fig. 2; p115). One *F. bacilliforme* isolate produced lesions smaller than those of the other species, while the other isolate did not produce lesions. No lesions developed in any of the control inoculations (Fig. 2; p115).

Potted tree assay

All four species of *Botryosphaeria* were pathogenic and produced lesions on inoculated mango stems in both trials. In all cases, lesion lengths differed significantly from the controls (Fig. 3; p117). External symptom development was minimal with no dieback, cankering or bark cracks developing in any tree during the six week incubation period of both trials. Lateral movement of all *Botryosphaeria* isolates inoculated into mango stems was limited. All inoculated fungi could easily be re-isolated from lesions but not from control inoculations.

Pathogenicity of isolates did not vary greatly within species (Fig. 3; p117). Isolates of the *F. parvum* [BOT2413, BOT2302, BOT2353], *B. rhodina* [BOT2399, BOT2376] and *F. indigoticum* [BOT2351, BOT2355] were equally pathogenic (Fig. 3; p117). One isolate of *F. bacilliforme* [BOT2421] produced lesions of similar size to the other *Botryosphaeria* spp. inoculated (Fig. 3; p117). The second *F. bacilliforme* isolate [BOT2417] used in this study, however, produced significantly smaller lesions than all other isolates. These were not significantly larger than the lesions produced in the control inoculations (Fig. 3; p117). Control inoculations resulted in small lesions that were ascribed to a wound reaction.

Lesions on mango trees in the first trial were significantly (Pr = 0.0001) longer than those produced in the second trail for all isolates and species (Fig. 4; p119). The greatest variation in lesion size between the two trails was seen for *F. indigoticum* [BOT2355, BOT2351] and *B. rhodina* [BOT2399, BOT 2376] (Fig. 4; p119). These species produced approximately one third smaller lesion lengths in the second trial, compared to those associated with the same isolates in the first trial.

Both mango cultivars were susceptible to all *Botryosphaeria* spp. tested. Lesions were slightly larger on the Keitt compared to the Tommy Atkins cultivar, but this variation was not statistically significant (Fig. 3; p117). The relative pathogenicity of all isolates remained the same, regardless of the cultivar inoculated.

Isolates of *B. rhodina* [BOT 2399] and [BOT 2376] that were most and least pathogenic respectively in the apple inoculation trials (Fig 2; p115), were equally pathogenic during both potted tree trials (Fig. 4; p119). *Botryosphaeria parva* isolates [BOT 2302], [BOT 2353] and [BOT 2413] tree trials (Fig. 4; p119). *Botryosphaeria parva* misolates [BOT 2302], [BOT 2353] and [BOT 2413] were most, intermediately and least pathogenic respectively in the apple inoculation trials (Fig. 2; p115). BOT 2353 were, however, more pathogenic than BOT 2302 in the potted tree trials. The isolate, BOT 2413, was the least pathogenic isolate in both apple and tree trials (Fig. 4; p119). Significant differences were found in lesion sizes produced by isolates of *F. indigoticum* [BOT 2315, BOT 2355] when inoculated on apples (Fig. 2; p115), but lesion sizes between these isolates in inoculated trees did not differ significantly (Fig. 4; p119). *Fusicoccum bacilliforme* isolate BOT 2417 was significantly more pathogenic than BOT 2421 (Fig. 2; p115), but BOT 2421 seemed

significantly more pathogenic that isolate BOT 2417 during the tree inoculation trials (Fig. 4; p119).

DISCUSSION

Results of this study have shown that all four *Botryosphaeria* species recently recognised as occurring on mango in South Africa are pathogenic. These fungi were also found to be pathogenic on the two most commonly grown commercial cultivars in this country. The symptoms arising from inoculation also suggest that these species have the ability to colonise and spread rapidly within mango trees. Results are similar to those with other *Botryosphaeria* spp., which have been shown to spread through the vascular system by causing tissue discoloration and clogging vessels with tyloses and mycelium (Maas & Uecker, 1984; Ramos *et al.*, 1991).

The most and least pathogenic isolates for each of the four *Botryosphaeria* spp., identified using the apple assay, did not produce significantly different lesion sizes on inoculated mango stems. Our results are similar to those of Brown-Rytlewski & McManus (2000) who reported a lack of correlation between the pathogenicity of *Botryosphaeria* isolates inoculated on fruit and stems. This lack of correlation between apple and potted-tree assays was suggested to be attributable to variation in incubation temperature, fruit ripeness, fruit size and tree vigour (Sutton, 1983; Brown-Rytlewski & McManus, 2000). Clearly, lesions on apple fruit do not reflect relative pathogenicity on mango plants. Mango fruit have been used in pathogenicity and cultivar resistance trials (Johnson,1992; Sanchote, 1991), however, fruit are not readily available and fruit ripeness levels at inoculation and endophytic colonisation can confuse results. However, we chose not to use mango fruit in

this study because *Botryosphaeria* spp. are commonly isolated from mango fruit and these may have influenced our results.

Botryosphaeria parva, B. rhodina and *F. indigoticum* were equally pathogenic to mango trees in this study. *Botryosphaeria parva* is the most frequently encountered fungus in mango orchards and appears to be the most important *Botryosphaeria* spp. causing mango decline in South Africa. Both *B. parva* and *B. rhodina* are also common on mango fruit (Darvas, 1991; Chapter 2). *Fusicoccum indigoticum* is, however, very rarely found in mango orchards or on fruit (Chapter 2). The dominant occurrence, together with the pathogenicity of *B. parva* and *B. rhodina* in orchards and on fruit is thus important to consider when developing disease control strategies. The smaller lesions, lower virulence and low isolation frequency (Chapter 2) of *F. bacilliforme*, as well as the variation in pathogenicity of isolates, suggests that this species does not contribute significantly to mango diseases in South Africa under these conditions.

Botryosphaeria rhodina and *F. indigoticum* gave rise to significantly smaller lesions in the second inoculation trial on mango stems. It is possible that these differences were due to physiological changes in the mango trees (Britton & Hendrix, 1986; Johnson, 1992). The fact that *B. parva* and *F. bacilliforme* produced lesions of similar length in both trials, suggests that different *Botryosphaeria* spp. may respond differently to the environment and host. Pathogen reaction to seasonal variation should thus be considered before final conclusions are made regarding the role of different *Botryosphaeria* spp. in disease (Britton & Hendrix, 1986; Brown-Rytlewski & McManus, 2000).

There were no obvious differences in the extent of vascular discoloration resulting from the four *Botryosphaeria* spp. on the two mango cultivars used in this study. Keitt has, however, previously been noted as more tolerant to infection by *Botryosphaeria* spp. under field conditions (Lonsdale, personal communication). Our results may suggest that, in the absence of environmental stress, the cultivars express the same level of susceptibility to infection by *Botryosphaeria* spp. It is, however, possible that under field conditions, cultivar Keitt may tolerate environmental stress more effectively, and this may give rise to an impression of disease resistance. Variation in tolerance to *Botryosphaeria* infection of different cultivars under field conditions has been reported with other woody hosts (English & De Vay, 1975; Sutton, 1983).

The pathogenic ability of *Botryosphaeria* spp. on mango in South Africa suggests that most of these species have the ability to cause diseases. *Botryosphaeria parva* and *B. rhodina* are, however, the most important to consider when management strategies are implemented. Resistance of cultivars to these pathogens should be tested under field conditions, as greenhouse trials do not accurately reflect this in a cultivar. Currently, the most effective means of control of *Botryosphaeria* diseases can be achieved through increasing plant vigour by reducing stress. This can minimise disease incidence due to *Botryosphaeria* spp., which will possibly impact on mango quality and production both pre- and postharvest.

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Table 1 Isolates used for potted-tree inoculations in this study

Culture nr.[1]	Identity[2]	Isolated from	Mango cultivar	Isolation area	Location	Isolator
BOT 2413	*Fusicoccum parvum*	Branch	Tommy Atkins	Hoedspruit	Mpumalanga, SA	R. Jacobs
BOT 2302	*F. parvum*	Side branch	Tommy Atkins	Hoedspruit	Mpumalanga, SA	R. Jacobs
BOT 2353	*F. parvum*	Fruit	Sensation	Letsetele Valley	Mpumalanga, SA	R. Jacobs
BOT 2351	*F. indigoticum*	Leaf stem	Tommy Atkins	Hoedspruit	Mpumalanga, SA	R. Jacobs
BOT 2355	*F. indigoticum*	Fruit	Sensation	Letsetele Valley	Mpumalanga, SA	R. Jacobs
BOT 2417	*F. bacilliforme*	Main stem	Heidi	Malelaan	Mpumalanga, SA	R. Jacobs
BOT 2421	*F. bacilliforme*	Side branch	Heidi	Malelaan	Mpumalanga, SA	R. Jacobs
BOT 2376	*Botryosphaeria rhodina*	Fruit	Sensation	Hoedspruit	Mpumalanga, SA	R. Jacobs
BOT 2399	*B. rhodina*	Side branch	Sensation	Mariepskop	Mpumalanga, SA	R. Jacobs

[1]Culture collections where isolates are kept: BOT = Forestry and Agricultiral Biotechnology Institute (FABI), University of Pretoria.

[2]Identities as determined in this study

Figure 1. Symptom development after apple fruit were inoculated with *Botryosphaeria parva* [BOT 2302]. (A) represents the inoculation wound with symptom development (B) after two days, (C) four days, (D) six days and (E) seven days incubation at 25°C.

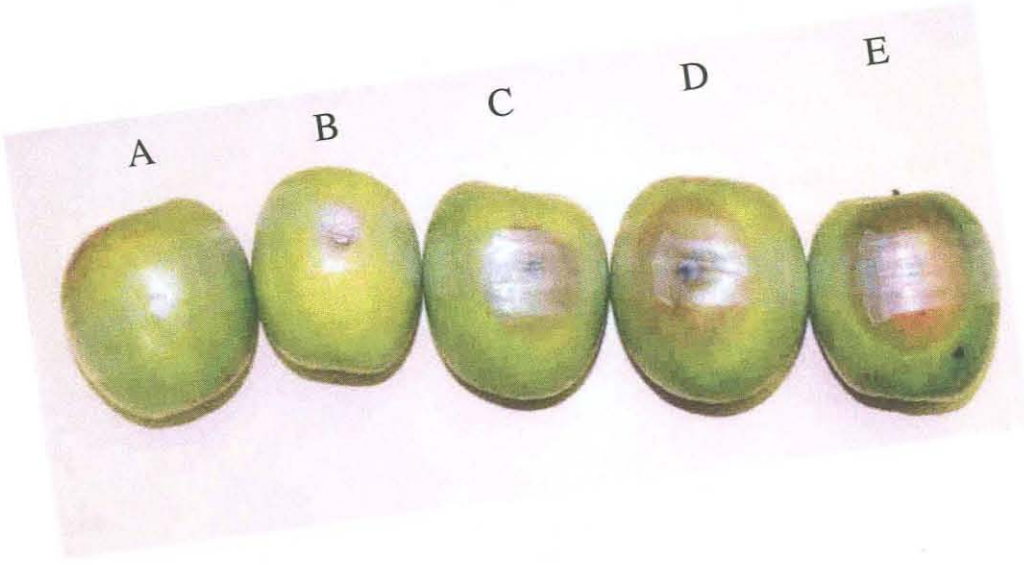


Figure 2. Graph illustrating variation in pathogenicity (y-axis) of *Botryosphaeria* isolates (x-axis) screened for pathogenicity on apple fruit (Pr = 0.0936). (A) represents isolates of *Botryosphaeria rhodina*, (B) represents *F. parvum*, (C) represent *F. indigoticum* and (D) represent isolates of *F. bacilliforme*.

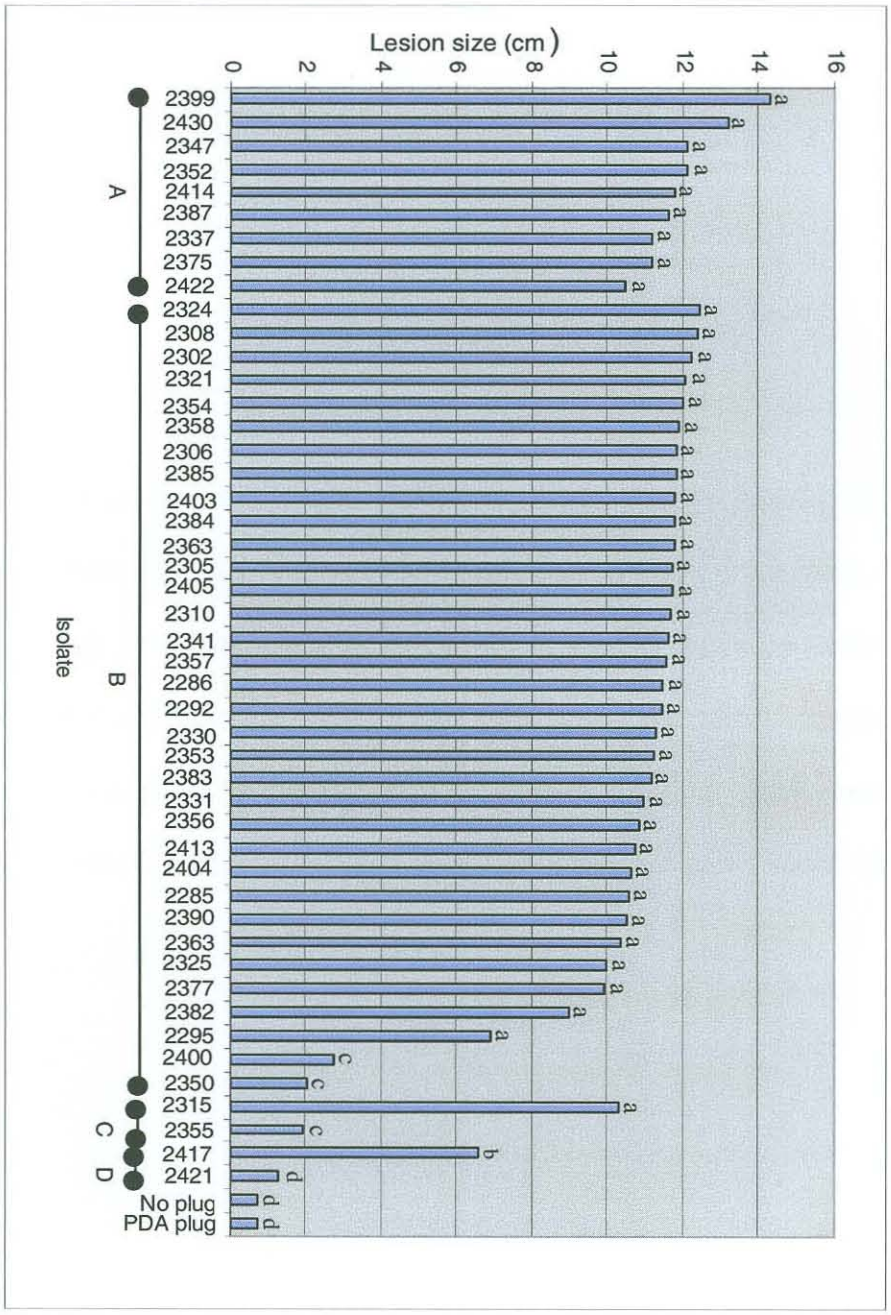

Lesion size (cm)
0
2
4
6
8
10
12
14
16
Isolate
A
B
C
D
2399
2430
2347
2352
2414
2387
2337
2375
2422
2324
2308
2302
2321
2354
2358
2306
2385
2403
2384
2363
2305
2405
2310
2341
2357
2286
2292
2330
2353
2383
2331
2356
2413
2404
2285
2390
2363
2325
2377
2382
2295
2400
2350
2315
2355
2417
2421
No plug
PDA plug

Figure 3. Lesions lengths (y-axis) associated with *Botryosphaeria* isolates inoculated on two mango cultivars Keitt and Tommy Atkins. Bars with the same letter do not differ significantly for each other. Bars bearing different letters differ significantly according to Duncan's multiple range test (Pr = 0.0001). (A) represents isolates of *Botryosphaeria rhodina*, (B) represents *F. parvum*, (C) represent *F. indigoticum* and (D) represent isolates of *F. bacilliforme*.

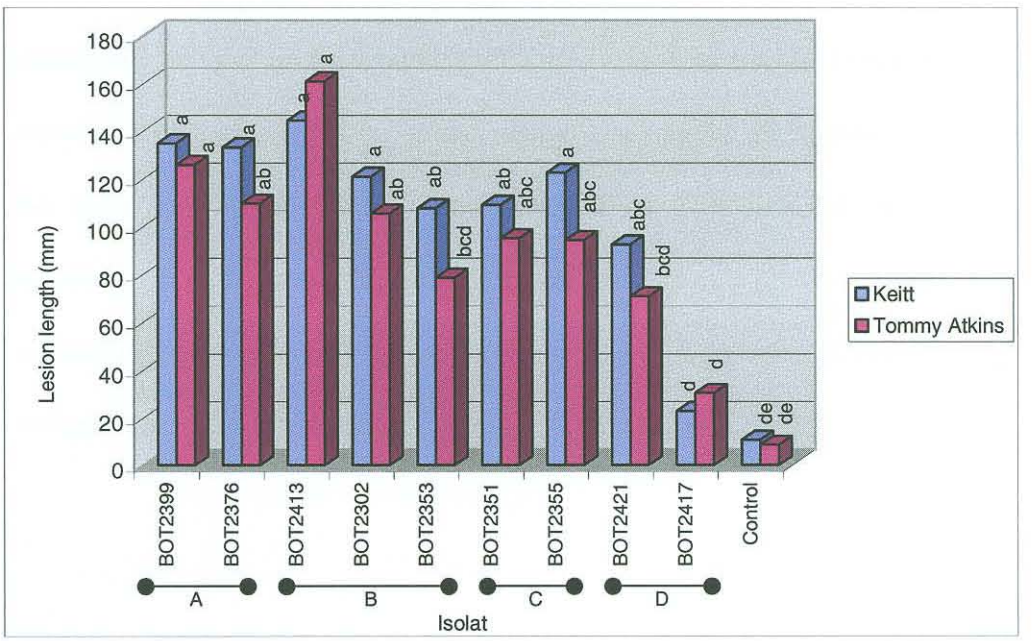
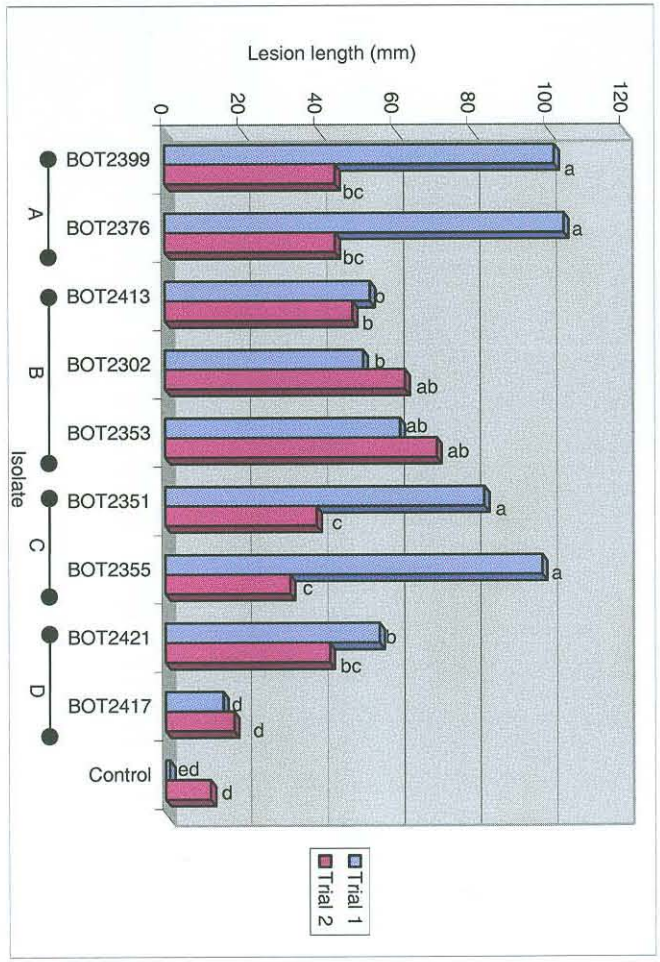


Figure 4. Lesion lengths (y-axis) associated with *Botryosphaeria* isolates (x-axis) in two separate inoculation trials on potted mango trees in the glasshouse. Bars with the same letter do not differ significantly. Bars with different letters differ significantly with Duncan's multiple range test (Pr = 0.0001). (A) represents isolates of *Botryosphaeria rhodina*, (B) represents *F. parvum*, (C) represent *F. indigoticum* and (D) represent isolates of *F. bacilliforme*.


Lesion length (mm)
0
20
40
60
80
100
120
BOT2399
BOT2376
BOT2413
BOT2302
BOT2353
BOT2351
BOT2355
BOT2421
BOT2417
Control
A
B
C
D
Isolate
a
bc
a
bc
b
b
b
ab
ab
ab
a
c
a
c
b
bc
d
d
ed
d
Trial 1
Trial 2