GENERAL DISCUSSION

In this study, it is clearly shown that four *Botryosphaeria* spp. occur on mango in South Africa. This is the first time that the taxonomy of these fungi on mango has been studied in South Africa. Results for this study will facilitate further research and more effective management of *Botryosphaeria* diseases associated with mango. The four species can be identified relatively easy based on morphological characteristics combined with ITS and ß-tubulin gene sequences. The development of the PCR-RFLP identification system will also facilitate future identification of these *Botryosphaeria* spp. from mango. These species represent *F. parvum*, *Lasiodiplodia theobromae* (*B. rhodina*) and two undescribed *Fusicoccum* spp., for which names are provided here, namely *F. indigoticum* and *F. bacilliforme*.

The majority of isolates collected from symptomatic and asymptomatic tissues in this study reside as *B. parva*. *Botryosphaeria parva (*previously known as *B. ribis)* is a well-known pathogen of many woody plants world-wide (Von Arx, 1987; Punithalingham, 1980). On mango, this fungus has commonly been described as *Dothiorella dominicana*, but has now been correctly identified as *F. parvum* in South Africa, which is most frequently encountered in nature (Johnson, 1992; Slippers *et al.*, 2001). *Fusicoccum parvum* has regularly been isolated from mango in various countries and is considered the primary causal agent of pre- and postharvest disease (Darvas, 1991; Ramos *et al.*, 1991; Johnson, 1992). When evaluating the dominance and pathogenicity of this species on mango, it became evident that this is the most important causal agent of mango diseases in any area and on any cultivar in South Africa. *Botryosphaeria parva* was more frequently isolated than any other *Botryosphaeria* spp. from all plant parts, but mostly from symptomatic fruit

and branches. We therefore, consider *B. parva* to be the main cause of *Botryosphaeria* diseases on mango trees in orchards and on fruit in South Africa. When developing control strategies and other management practices, the presence of this species should, therefore, be closely considered.

Botryosphaeria rhodina was the second most dominant species isolated from mango in South Africa. This species was mostly obtained from fruit rots and asymptomatic plant tissue. The fact that this species was infrequent or absent from any symptomatic plant parts other than the fruit, suggests that it is probably insignificant in causing tree diseases. These findings are similar to those from previously published literature, where *B. rhodina* is well-documented as an endophyte and is described as the most common fruit rot pathogen of many fruit crops, including mango (Punitalingham, 1980; Sanchote, 1991; Johnson, 1992). The *B. rhodina* isolates were easily identified based on morphological characters and identifications were easily confirmed using DNA sequence data and PCR-RFLP. Although this species is commonly isolated together with *Botryosphaeria* spp. having hyaline conidia, it tends to dominate only in warmer, tropical regions (Brown & Britton, 1986; Johnson, 1992). This suggests that different *Botryosphaeria* spp. may respond to the environment and host differently. Pathogen reaction to seasonal variation should thus be considered before final conclusions are made regarding the role of this and other *Botryosphaeria* spp. in disease (Britton & Hendrix, 1986; Brown-Rytlewski & McManus, 2000). The dominant occurrence, together with the pathogenicity of *B. rhodina,* especially on fruit is thus important to consider when developing disease control strategies.

Both molecular and morphological data confirmed that a unique *Botryosphaeria* spp. was isolated in this study, which represent a previously undescribed taxon, which was assigned

the name *F. indigoticum*. The conidial morphology of this new species resembles that of *B. parva* to some degree, but it remains distinct in cultural and conidial morphology. Using morphology on its own to distinguish *Botryosphaeria* spp. may be confusing and it is recommended that the PCR-RFLP system additionally be used for reliable identification. *Fusicoccum indigoticum, B. parva* and *B. rhodina* were also found to be equally pathogenic to mango trees in this study. Variation in virulence of this species between the two pathogenicity trials can be attributed to the fact that symptom expression can be influenced by environmental conditions, as is the case with *B. rhodina* (Johnson, 1992). *Fusicoccum indigoticum* is, however, rarely found in mango orchards or on fruit and is therefor not considered an important *Botryosphaeria* spp. affecting mango in South Africa.

Two South African isolates obtained in this study closely resembled two isolates that were described by Johnson *et al.* (1991) as unknown species from a mango stem end rot pathogen survey in Australia. The fungus was not formally described, but was referred to as *Dothiorella* 'long' (Johnson *et al.*, 1991; Johnson, 1992). *Dothiorella* 'long' has, however, been shown to belong to the genus *Fusicoccum* (Slippers *et al.*, 2001). Sequence data, however, confirmed that the isolates from Australia and South Africa were not identical and could reside as different species, if more isolates are obtained. In this study, however, isolates in this group has cylindrical to bacilliform conidia and produce a yellow pigment in the growth medium and is, therefore, considered as the same species. Mycelial clumps are also produced in concentric rings, which is very different to any of the other *Botryosphaeria* sp. We have, therefore, provided the name *Fusicoccum bacilliforme* for all isolates falling within this group. This species was, however, isolated only from diseased mango branches. The smaller lesions, lower virulence and low isolation frequency of *F. bacilliforme*, as well as the variation in pathogenicity of isolates, suggests that this species

is a weak pathogen that does not contribute significantly to mango diseases in South Africa.

Botryosphaeria spp. were isolated in varying frequencies from different commercial mango cultivars cultivated in South Africa and sampled during this study. The cultivars Sensation and Tommy Atkins yielded the highest frequency of *Botryoshaeria* spp. The cultivars Keitt and Kent indicated a very low isolation frequency in this study, which correlates well with previous findings that these species are more disease tolerant in the orchards. With the inoculation trials, Tommy Atkins and Keitt were respectively chosen for their disease susceptibility and tolerance ability. Under controlled glasshouse conditions, however, these cultivars showed no difference in their susceptibility to *Botryosphaeria* diseases. The resistance of cultivars should, therefore, be tested under normal environmental conditions for a true reflection of disease resistance to various pathogens.

Botryosphaeria spp. were isolated from all mango producing regions of South Africa surveyed in this study. The highest incidence of *Botryosphaeria* spp. were found in the Letsetele Valley, Hoedspruit and Mariepskop areas. The use of weather data can attribute to the estimation of environmental conditions, which may influence the incidence of disease such as higher rainfall in the Letsetele Valley region (South African Weather Buro). Close correlation of production with environmental conditions in mango production regions may give a broader view of the optimal environmental conditions which may favour *Botryosphaeria* disease development in orchards.

A small number of asymptomatic isolations yielded the species *B. parva* and *B. rhodina*, which are known endophytes of mango and other woody hosts world-wide. The endophytic status of these fungi have been investigated previously (McPartland & Schoeneweiss, 1986; Johnson et al., 1991), where it was shown that the endophytic phase can be found in all mango plant parts. In this study, we have, however, confirmed that these fungi can also be pathogenic on all plant parts screened in this survey. Control of this fungi can, therefore, not be restricted to a specific area on the tree, due to the movement thereof, and systemic control should be the focus point.

In this study, we conclude that four *Botryosphaeria* spp. occur on mango in South Africa, of which two species are new to science. *Botryosphaeria parva*, *B. rhodina*, *F. indigoticum* and *F. bacilliforme* is easily identified with the use of a PCR-RFLP identification system developed in this study. The pathogenic ability of the *Botryosphaeria* spp. on mango in South Africa suggests that most of these species has the ability to cause diseases and should be consider when management strategies are implemented in the mango industry. Other *Botryosphaeria* spp. have, however, also been implicated as causal agents of diseases on mango in other countries, such as *F. mangiferum* (known as *D. mangiferae*) and *F. aesculi* (known as *D. aromatica*) (Johnson, 1992; Slippers *et al.*, 2001), but these species are easily distinguished from the four South African species with the PCR-RFLP identification system. The PCR-RFLP technique overcomes the difficulties experienced using morphological characteristics to identify *Botryosphaeria* spp. from mango. It is simple and rapid and negates problems experienced when needing to sequence DNA from large numbers of isolates.

No true disease tolerant cultivars could be identified in this study, but further field trials are needed to evaluate resistance under normal field conditions. Currently, however, the most effective means of control of *Botryosphaeria* diseases can be achieved through increasing plant vigour by reducing stress. This is expected to minimise disease incidence due to *Botryosphaeria* spp. and impact on mango quality and production. Care should, however, be taken to prevent the introduction of foreign species such as *F. aesculi* and *F. mangiferum* that currently do not occur in South Africa. The implementation of effective quarantine strategies and the screening of foreign material with the PCR-RFLP system may provide a useful method implemented in sanitation and management practices for these species world-wide.

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