

Macadamia

branch dieback and decline in South Africa

ANGEL MADUKE (FORESTRY AND AGRICULTURAL BIOTECHNOLOGY INSTITUTE (FABI) AND ARC-PLANT HEALTH AND PROTECTION), PROF BERNARD SLIPPERS (FABI), DR ELNA VAN DER LINDE (ARC-PLANT HEALTH AND PROTECTION), PROF MICHAEL WINGFIELD (FABI) AND DR GERDA FOURIE (FABI)

Botryosphaeriaceae species are important latent fungal pathogens that cause diseases on many trees. There has been an increase in incidence and severity of branch dieback on macadamia trees in South Africa.

The aim of this study was to identify species of *Botryosphaeriaceae* on branches collected from both healthy and diseased trees and to consider their relative importance by conducting pathogenicity trials. Eight species of the *Botryosphaeriaceae* were identified. Macadamia seedlings inoculated with *Botryosphaeriaceae* isolates showed no dieback symptoms nine weeks post inoculation. However, disease expression of dieback symptoms became severe following water stress events for an additional three weeks. This study was the first to consider the role of different species of the *Botryosphaeriaceae* in macadamia dieback and decline in South Africa. It thus provides a foundation for knowledge regarding the role of stress on the pathogenicity and/or aggressiveness of *Botryosphaeriaceae* species.

Botryosphaeria branch dieback
The *Botryosphaeriaceae* fungal

Pathogenicity trials are used to determine a micro-organism's ability to cause disease

family has a cosmopolitan distribution and is mostly found in tropical and subtropical regions. Species in this family cause a disease known as *Botryosphaeria* branch dieback (Fig 1). Symptoms include leaves turning brown that remain attached (Fig 1A), discolouration of tissue underneath the bark (Fig 1B), while the roots remain relatively healthy. In severe cases, it can result in tree death (Fig 1C).

During our survey of both diseased and healthy macadamia branches in Limpopo, KZN and Mpumalanga, eight species of *Botryosphaeriaceae* were obtained. There was no obvious pattern of species presence per region and/or cultivar, with most species

present in all areas and isolated from all cultivars surveyed (Beaumont, Nelmak D, 814, 816, 788 and 344). *Lasiodiplodia pseudotheobromae* and *Neofusicoccum parvum* were, however, the most dominant species in all three regions. In addition, these were the only two species detected on nursery seedlings. Most of the species were isolated from healthy as well as diseased trees, which supports the theory that *Botryosphaeriaceae* as endophytes are present in woody tissue (in other words, present without causing disease).

Botryosphaeriaceae as latent pathogens

Botryosphaeriaceae species usually switch to a pathogenic lifecycle when the host plant is experiencing stress, such as drought, nutrient deficiencies, mechanical damage, damage caused by pests, or infection from other plant pathogens (Mehl et al., 2013; Slippers & Wingfield, 2007). Our pathogenicity trials on Beaumont cuttings showed that trees were able to maintain a healthy status (vigorous leaves without evidence of dieback), with only small internal necrosis (tissue dearth) for a period of nine weeks under normal watering conditions (Fig 2A). This suggests a low pathogenic effect under a non-stress scenario and is in agreement with results obtained from other *Proteaceae* trees inoculated with



Figure 1. (A) Branch dieback symptoms; (B) stem discoloration from the upper part of the stem towards the root collar of a seedling obtained via the disease clinic; (C) macadamia decline

Botryosphaeriaceae species (Swart et al., 2000).

Dieback symptoms were however visible at 12 weeks (Fig 2B) after seedlings were exposed to drought stress caused by irregular watering over three weeks. The symptoms resulting from artificial inoculations were similar to those observed in orchards (Fig 2B), while the control plants did not develop any symptoms. All species included in the pathogenicity assay were able to cause

disease, although *Neofusicoccum luteum* was the most aggressive.

Results from this study provide a foundation in terms of disease development and symptom expression of branch dieback. Our ongoing research aims to obtain more in-depth knowledge regarding the specific role of environmental stress and its impact on disease. This will be carried out in collaboration with Dr Nicky Taylor from the University of Pretoria and will include other commercially planted cultivars.

Recommendations to industry

In terms of disease control, we recommend that growers remove infected branches to the point where you can no longer see discoloration in the wood tissue. Pruned branches should be painted at the end, to prevent reinfection with *Botryosphaeriaceae* species. It is also important to ensure that pruning equipment is clean. Finally, management should focus on general orchard health, given that disease expression is associated with stress.

Acknowledgements

We would like to thank the macadamia growers that allowed us to sample on their farms. We would also like to acknowledge the University of Pretoria, the Forestry and Agricultural Biotechnology Institute (FABI), the DSI Centre of Excellence in Plant Health Biotechnology (CPHB), Macadamias South Africa NPC (SAMAC), the Agricultural Research Counsel (ARC) and NRF Thuthuka for their financial support.

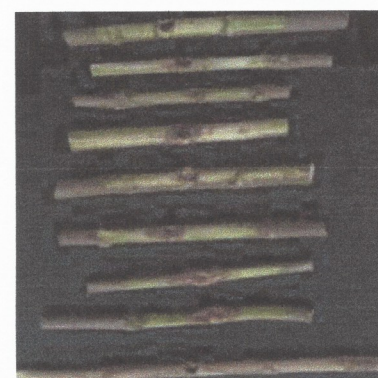


Figure 2. (A) Seedlings nine weeks post inoculation forming a callus; (B) and; (C) Seedlings twelve weeks post inoculation showing typical branch dieback symptoms.

References:

- Mehl JWM, Slippers B, Roux J, and Wingfield MJ. 2013. Cankers and Other Diseases Caused by the *Botryosphaeriaceae*. *Infectious Forest Diseases* **298**.
- Slippers B, and Wingfield MJ. 2007. *Botryosphaeriaceae* as endophytes and latent pathogens of woody plants: diversity, ecology and impact. *Fungal Biology Reviews* **21**: 90–106.
- Swart L, Crous PW, Petrini O and Taylor JE. 2000. Fungal endophytes of *Proteaceae*, with particular emphasis on *Botryosphaeria proteae*. *Mycoscience* **41**: 123–127.