Husk rot disease

causal agents and disease epidemiology

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Husk rot is a fungal disease of the pericarp. Symptoms are characterised by black spots that coalesce to form soft and spongy black lesions clearly visible on green fruit (Figure 13) (Akinsanmi and Drenth, 2017). The fungal infection spreads through the husk causing nuts to drop prematurely and may cause internal discoloration. In addition, nuts affected by the disease have a shorter shelf life thus reducing the storage potential (Fitzell, 1994).

Husks are usually infected during prolonged (two to five days) wet weather conditions with air temperatures above 15°C. This suggests that high relative humidity and rain influence disease occurrence in orchards (Akinsanmi and Drenth, 2017).

Due to the irregular occurrence of husk rot, the importance of the disease is often underrated and attributed to environmental and physiological factors (Akinsanmi and Drenth, 2017). However, during the last few years, the disease has become more prevalent and has raised concerns amongst South African growers (Campbell 2015, Schoeman 2016).

Husk rot causal agents

There is uncertainty regarding the causal agents of husk rot, with multiple genera frequently isolated from diseased nuts. Studies in Australia have reported the absence of *Colletotrichum gloeosporioides sensu lato* (Anthracnose husk rot) on lesions of infected nuts and described new *Diaporthe* species *(Phomopsis)* (Phomopsis husk rot) as the causal agents of husk rot (Akinsanmi and Drenth, 2017). Reports from South Africa, however suggested the presence of *C. gloeosporioides* sensu lato (Anthracnose husk rot), *Diaporthe* (Phomopsis husk rot) as well as *Stibella* species on diseased nuts (Campbell 2015, Schoeman 2016).

The research therefore aimed to identify the causal agent and to assess pathogenicity of possible pathogens. Fully expanded macadamia nuts with husk rot symptoms were collected from different trees from three farms in Limpopo province in the 2016/2017 season. Nuts were washed with water, left to dry and then surface sterilised. Cultures obtained from isolations made on PDA plates were grouped according to morphology and identified based on the analyses of DNA sequences. From this study, *Diaporthe* spp were the most frequently isolated pathogens

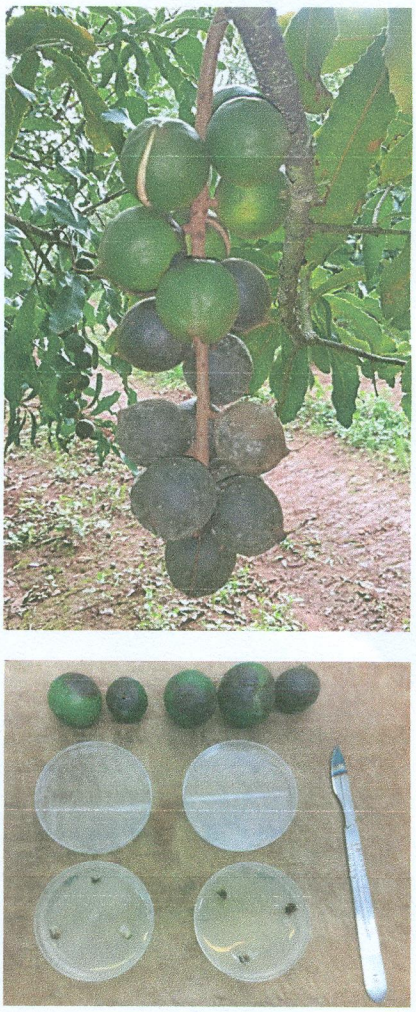


Figure 13. Husk rot disease symptoms.

(76%), followed by *Colletotrichum* (19%) with other fungi representing only 5%.

Both *Diaporthe* and *Colletortrichum* isolates were subsequently selected for pathogenicity tests. Fully expanded nuts of cultivar 816 were randomly detached from healthy looking macadamia trees and disinfected with 70% ethanol. All nuts were assessed for visible wounds or holes under a microscope. Fifteen nuts per isolate were wounded and inoculated with an agar block (5 x 5 mm) and sealed with parafilm (Figure 14). Fifteen nuts were inoculated with an agar block containing no fungus as negative control. Nuts were incubated in moist chambers at room temperature and disease incidence and severity recorded seven days after inoculation. Pathogens were re-isolated and identified by means of DNA sequencing.

Results from the pathogenicity assay confirmed that both *Diaporthe* and *Colletortrichum* spp were pathogenic to macadamia pericarp, although results indicate that different isolates

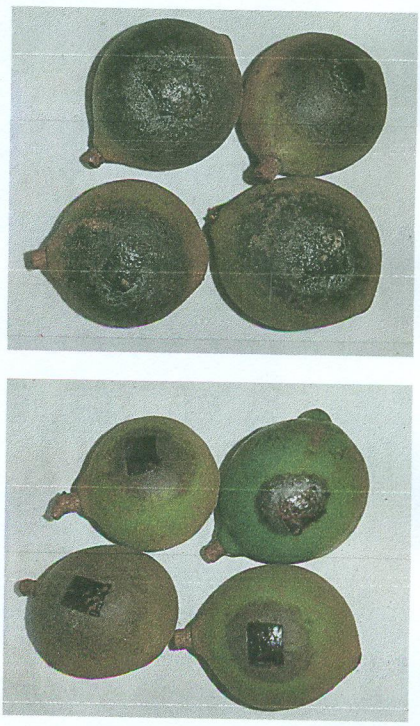


Figure 14. Pathogenicity assay on cultivar 816 nuts (detached) inoculated with Colletotrichum *and* Diaporthe *species.*

differ in their ability to cause disease, in terms of incidence and severity. Overall the results are in agreement with Wrona et al. (2020), not only in terms of a number of newly described *Diaporthe* spp causing husk rot from Australia and South Africa but also varying disease severity both within and between species. However, our study has shown that both *Diaporthe* and *Colletortrichum* species can simultaneously occur on husk tissue and this should be taken into consideration when implementing management options since different causal agents may have different inoculum sources as well as modes of disease infection and development rates.

Disease epidemiology

It is hypothesised that the most common mode of infection is through wounds caused by insect damage and/or mechanical damage such as wind rub (Akinsanmi and Drenth, 2017). Infection can occur during nut development and remain latent or dormant until disease development is triggered by stress (Fitzell, 1994; Schoeman, 2016). Disease management would therefore be improved by knowing at which nut stage macadamia is more susceptible to natural husk rot infection, guiding the timing of fungicide application (Miles et al., 2010). The abundance of old, diseased husks within the tree canopy has also been reported to influence disease incidence and severity and knowledge regarding this will greatly improve management.

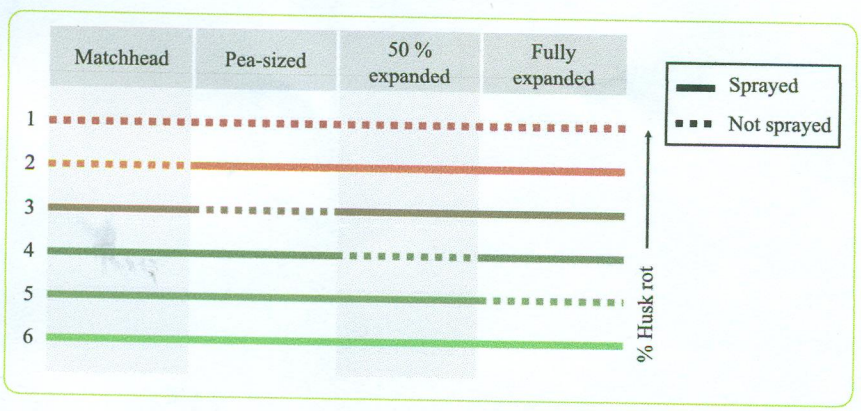
Research in the programme therefore aimed to determine:

1. the correlation between insect damage and husk rot incidence
2. the physiological stage at which nuts are most susceptible to natural husk rot infection in the field, and
3. disease incidence in relation to sticktights in macadamia orchards.

Correlation between insect damage and husk rot incidence

Nuts were collected on a weekly basis from 12 weeks post anthesis in 2016/2017and 16 weeks post anthesis during the 2017/2018 season. Monitoring continued throughout the season until harvest. Nuts were inspected for husk rot symptoms and nut borer damage. 30 trees were monitored in each season. A strong positive correlation for both seasons was found, although disease incidence varied greatly between seasons. Overall, the results suggest that damage to nuts by insects enhances the infection of nuts with husk rot pathogen(s), and that insect control in orchards is crucial for the effective management.

Figure 15. Graphical representation of the nut physiological stage and husk rot natural infection trail that where each developmental stage were exposed to natural infection and other stages prevented by means of Copper oxychloride application. Higher level of incidence were found in the earlier stages of development.



Correlation between nut physiological stage and husk rot infection

Developing nuts of cultivar 816 were exposed to natural infection at match-head size (1), pea-size (2), 50% expanded (3), fully expanded (4), fully exposed (5) and not exposed to natural infection throughout the development stages i.e. fully covered (6). The husk rot incidence was compared at each stage (Figure 15) (Miles et al. 2010).

Infection at stages was prevented by spraying Copper oxychloride (Demildex) each time the next developmental stage was reached (Miles et al., 2010). At each stage samples were collected and fungi isolated. At termination of the trial, nuts were evaluated for their husk rot incidence by harvesting all nuts and then determining the percentage of nuts infected with husk rot for each stage.

Results suggested that differences in nut sizes (maturity) resulted in differences in husk rot infection in the field. In the 2016/2017 trial, a higher level of husk rot incidence was found on nuts that received no spray "protection" (1.11%), followed by nuts that were exposed to natural infection when they were pea-sized (0.82%), 50% expanded (0.80%), then fully expanded (0.41%). Similar results were obtained during the 2017/2018 season where fully exposed nuts (i.e. no spray protection) had highest disease incidence (8.41%), followed by nuts exposed to natural infection at pea-size (4.66%), then those exposed at match-head size (3.77%), 50% expanded nut (1.78%), fully expanded nuts (1.51%) and lowest disease incidence for fully covered nuts (1.34%). We conclude that nuts in the earlier stages of development (matchhead and pea size) are more susceptible to natural infection than 50% expanded or mature nuts. Therefore, application of fungicides should target (but not be limited) to the early development stages. Both *Diaporthe* and *Colletotrichum* were also present throughout the season. However, isolates were not further investigated in this study and the occurrence of these fungi in the orchard requires further investigation.

Disease incidence in relation to sticktights

Fifty trees were rated before nuts were picked and the rating of sticktights in trees were done according to the rating scale from Miles et al. (2010) for two seasons. For assessing correlations, trees were stripped, and nuts were counted individually. The collected nuts for each tree were grouped into three categories: green nuts, sticktights and those with husk rot symptoms.

Sticktight ratings varied between location and season. Although sticktights have been associated with other nut diseases such as husk spot, caused by *Pseudocercospora macadamia*, no correlation between the percentage of husk rot and the percentage of sticktights were observed for both farms and both seasons. In this study the presence of old or desiccated nuts in a husk that remained attached to the tree after maturity did not necessarily lead to a higher incidence of husk rot during the following season.

Husk rot is becoming more important in South African macadamia orchards and could significantly reduce yield if not controlled effectively. Research has shown evidence of the presence of causal agents from different genera. It has also highlighted that nuts are more susceptible during early development, providing guidelines for fungicide treatments for effective control.

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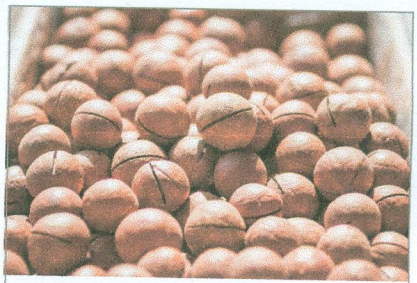
Sticktights are nuts that don't drop and remain in the canopy



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