The dissemination of fungal pathogens on avocado trees in South Africa with reference to vector potential of insect pests

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INTRODUCTION

Fungal and plant associations have evolved over a long period, making fungi an important group of organisms of which only 10% can colonize living plants, nevertheless most plant diseases (70%) are caused by fungi. There are approximately 110000 species of fungi described to date and new species are described at a rate of 1200 species per annum (Blackwell, 2011; Kirk *et al*., 2008). Of prime importance in the spread of disease is the survival and dispersal of the reproductive propagules of pathogens. Fungi have adapted to survive by utilising several mechanisms to disperse reproductive propagules, including water, wind, soil, seeds, other vegetative plant material (i.e. fruits), their own mechanical forces, insect vectors and other organisms (Agrios, 2005). Although fungi have their own mechanical means of dispersal, they heavily rely on the abiotic and biotic factors in the environment for dissemination.

Insect vectors are one of the most important dispersal methods used by fungi. They can spread fungi as a result of chance contamination by visiting plants (pollination) or by moving around on infected and contaminated plants where sticky fungal spores stick to the abdomen or mouthparts of the insects. They transport fragments of hyphae by feeding from plant to plant and create wounds during ovipositioning and feeding where spores can be deposited (Brown & Ogle, 1997). Fungi have adapted these mechanisms to survive in diverse biotic and abiotic environmental conditions (Saaiman, 2014) and some insect-pathogen relations have coevolved into obligatory symbionts. These symbionts are nutritionally required for the survival of the insect hosts, and the survival of the pathogens depend on the insect host for dispersal (Prado & Zucchi, 2012). These pathogens and vectors can have detrimental effects on crops.

The first section of the study will focus on the insect-fungal associations and the dissemination of fungi through vectors in relation to other dispersal techniques. The second section will focus on the role of Hemiptera and Thysanopera, specifically the coconut bug, avocado bug, as well as the thrips, in damaging the avocado fruit and transmitting fungal pathogens.

Objectives

For the purposes of this study, three pests, the coconut bug, the avocado bug and the thrip complex have been identified as the most probable to influence disease dissemination or act as direct vectors. The aim of this study is to determine if any of these avocado pests indeed act as vectors for avocado disease pathogens or if they facilitate the spread or infestation of these pathogens. The study was approached in a holistic fashion to determine the biotic interactions between pathogens and insects in the orchard agro-ecosystem. As such the project was approached in several phases. The primary aim of phase 1 was to identify all relevant fungal pathogens found in the study orchards. This would act as a baseline for future comparisons and provide insight into probable vector transmitted diseases. Phase 2 focuses on investigating the vector potential of the sucking bug complex by investigating the interactions between insect pests and pathogens in the orchard environment. Phase 3 will focus on applying gathered knowledge toward refining existing management programmes with the aid of a better understanding of orchard pest/fungi dynamics.

- Survey on pathogenic fungi found in the region and identifying probable pathogens which may be disseminated via insect vectors (Phase 1)
- Investigating the role and vector potential of known sucking pests on avocados in the Nelspruit region (Phase 2)
- Investigating the bio-ecology and interaction between vectors and pathogens from a holistic point of view (Phase 2)
- Working towards a management programme for these pathogens to minimise losses (Phase 3).

MATERIALS AND METHODS

Phase 1

A survey on pathogenic fungi causing disease was investigated with primary sites located in Mpumalanga (Nelspruit), South Africa. Several sites was identified. During phase 1 of the study, samples of leaves and fruit were collected and plated out on a growth medium for fungal growth. Samples were taken throughout the year taking the entire growing season into consideration, covering from the flowering stage until the fruit are harvested. Sampling was done evenly distributed over the orchard.

- Leaf and fruit sampling: Sampling was done by randomly removing ten leaflets and ten fruits from selected trees in the orchard. Leaf samples were taken throughout the year whereas fruit sampling was done when available during the growing season. Collection of the leaflets and fruit are done actively by hand removal. Samples were placed separately in plastic bags and labelled accordingly. Samples were then transported in a cooler bag to the University of the Free State (UFS) in Bloemfontein where fungal colonies were isolated and identified.

Field trip April 2016

The first dedicated fieldwork trip was conducted during April 2016. Three sites were identified. These being at:

- The trial farm of the ARC-Nelspruit (ARC)
- An old avocado orchard belonging to Halls (Halls' farm)
- A privately owned orchard about 30 km from Nelspruit (Sakkie's farm).

The three orchards represent all different cultivars of avocado found in the region and should provide a broad overview regarding diseases and insect pests. All sampling was successfully conducted and samples isolated on agar plates to be identified.

Field trip August 2016

A follow-up fieldwork excursion was conducted during late August 2016. The same sites were visited and collection was repeated in the same manner. As the trees were in flower, thrips were also collected at all three sites and have been plated for identification of fungal spores present. Collection was done successfully and material transported to UFS for plating and identification.

Field trip April 2017

During 2017 a third field trip was conducted at the end of April, leaf and fruit samples were plated out for fungal identification.

Phase 2

Using the data gathered from phase 1, phase 2 focusses on investigating the vector potential and interactions of known insect pests on avocado in the region. As such, this phase focusses on the collection of insect specimens to investigate the presence of any fungal reproductive structures and investigate whether insect feeding can aid in the dissemination of fungal diseases. Also, Koch's postulates will be performed on all isolated pathogenic fungi from phase 1.

- Insect specimen collection: Coconut bug collection was done by actively searching for bugs on the avocado trees and then placed in ventilated insect-proof containers. For the collection of thrips, a beating technique will be used and samples stored in a cooler box with foliage for the insects to feed on. The samples are placed in a cooler box to prevent further fungal growth on the insects and to keep the integrity of the samples intact.

Field trip November 2017

Sampling was during November 2017 in the Nelspruit region. Three farms were visited: the Agricultural Research Council (ARC), Halls and Sons and Burgers Hall. Ten trees were sampled that included avocado leaves, fruit, branches and coconut bugs. Plant material was placed in brown paper bags that allows air and absorbs all excess water. Samples were then transported to Bloemfontein for processing and identification at the University of the Free State.

Coconut bugs were sampled randomly throughout the orchard and placed in poly tops. Insects were stored in a fridge to preserve the fungi and euthanize the specimens.

RESULTS AND DISCUSSION

Phase 1

A variety of fungi have been identified thus far of which four are of pathogenic importance: *Alternaria alternata*, *Cladosporium cladosporioides, Neofusicoccum* sp. 1 and *Pestalotiopsis* sp. 1. As indicated in Figure 1 and Table 1, all four pathogenically important fungi that causes disease and crop losses were found; 0 indicating no occurrence and 5 indicating 100% occurrence.

Alternaria alternata and *Cladosporium cladosporioides* were frequently present in high numbers in almost all samples at the three farms. *Cladosporium cladosporioides* is omnipresent, and with air samples taken, 50% of the spores collected in the atmosphere were identified as *Cladosporium* sp. A wider range of fungi was identified from the ARC orchard, presumably because fungicides and other substances are not as frequently used, as is the case on commercial farms.

Other fungi occurring in high numbers such as *Fusarium* spp. and *Acremonium* sp. did not occur frequently enough in high numbers to be considered significant, or are not of pathogenic importance to plants.

Alternaria alternata is an endophyte living inside plants and emerge when the plant is weak or under stress. This fungus is associated with the dieback of tree branches and anthracnose on fruit, identified as concentric rings and sunk-in lesions. Other symptoms such as *Alternaria* leaf spot (brown spots on

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leaves) are also associated with this fungus. *Alternaria alternata* is a postharvest pathogen in avocados and have previously been isolated out of fruit and stem-end rot after harvesting.

Cladosporium cladosporioides is a fungus with a wide host range and well known throughout the world. On plants, *C. cladosporioides* causes *Cladosporium* rot, associated with "black spot" on fruits. *C. cladosporioides* was isolated out of black blotches on the fruit and leaves. However, primary cause of "black spot" are attributed to physical or chilling damage, with colonisation of *C. cladosporioides* being secondary (Fig. 2).

***Neofusicoccum* species** (previously known as *Botryosphaeria* spp.) are associated with the dieback of tree branches. *Neofusicoccum parvum* was previously found on pecan nut trees causing 100% crop loss in wounded nuts in a matter of two weeks, and 100% crop loss in non-wounded nuts in a matter of 23 days. It is suspected that this fungus is disseminated through insect vectors with piercing-sucking mouthparts.

***Pestalotiopsis* species** are ascomycete fungi and occur commonly as plant pathogens. In avocado this fungi are causing postharvest stem-end rot. It is also associated with fruit rot and brown leaf spot. Symptoms can appear as small, irregular and brown lesions on the peel at the end of the stem, typical of anthracnose. Lesions are soft and sunken in and expand rapidly, comprising the entire fruit as it ripens. Other signs and symptoms that occur are white mycelium that are often found around the stem cavity, and dark brown necrosis of the fruit pulp as the fruit matures.

This fungus also produces black spores (conidia), collecting in wet masses outside the aecervulus (fruiting structure) called sporodogia. These type of fruiting structures are specially adapted for insect dispersal. As insects crawl over it, the spore masses smear of on them and are dispersed to another plant on which the insect feed on.

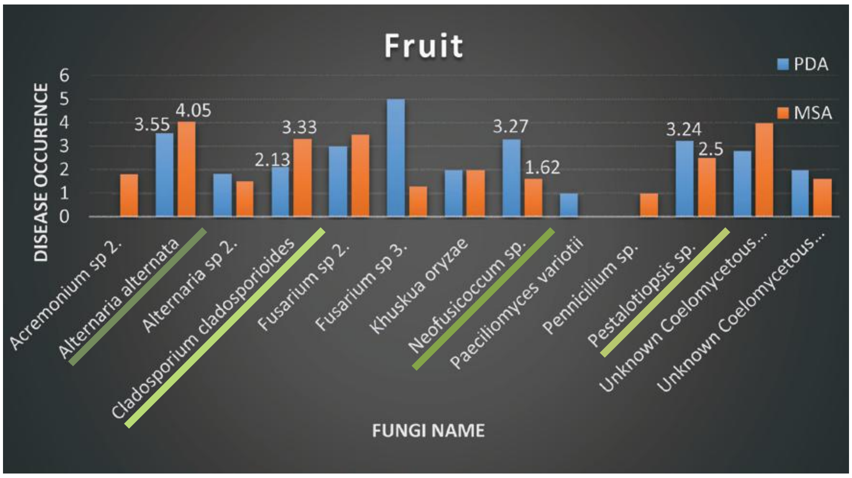

Figure 1: Four important pathogens identified from fruit collected at Sakkie's farm.

Table 1: Identified fungi indicating the four most important pathogens identified to date.

Acremonium kiliense	*Cladosporium cladosporioides*	*Pestalotiopsis* sp. 1	Unknown cream
Acremonuim sp. 1	*Epicoccum sorginum*	*Pithimyces chartarum*	Unknown white hyphomycetous fungus
Acremonuim sp. 2 (ketting spore)	*Fusarium oxysporum*	*Rhizopus oryzae*	
Altenaria alternata	*Fusarium* sp. 1	*Scytalidium* sp. 1	
Altenaria sp. 2 (donker)	*Fusarium* sp. 2	Unknown brown 1	
Aspergillus flavo furcatus	*Khuskua oryzae*	Unknown brown 2	
Aspergillus flavus	*Neofusicoccum* sp. 1	Unknown brown 3	
Aspergillus niger	*Paecilomyces variotii*	Unknown *Coelomycetous* fungus	

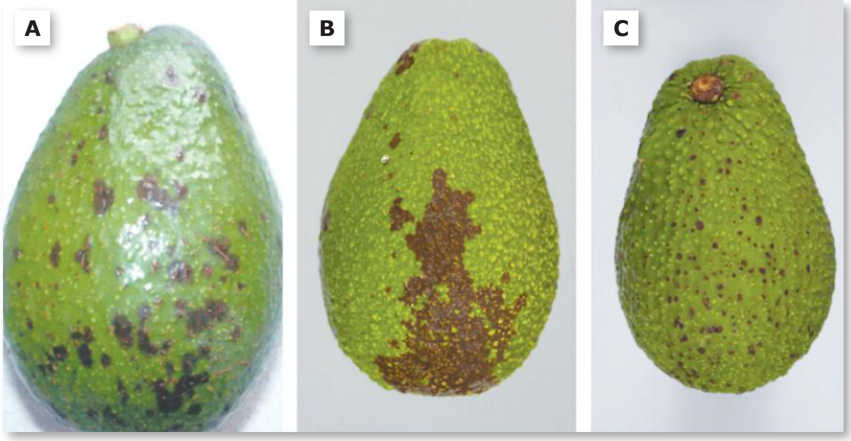

Figure 2: Symptoms of a) damaged avocado fruit nodules, b) chilling injury (blotches with sharp borders) and c) blotches with "fuzzy" borders.

Phase 2

Fungal survey

Samples were surface sterilised using 76% ethanol for 1 minute and rinsed twice with sterile water. Samples were plated out on two different growth media, Potato dextrose agar (PDA) and Malt salt agar (MSA), and incubated at 25 °C for 7-14 days for fungal growth. Fungi were then identified based on morphological structures and reproductive propagules under the light microscope.

Insect pests

Collected insect specimens were placed ventral side down on grown media to encourage fungal growth. The aim was to investigate any fungal propagules isolated from the body surface or mouthparts of the insect pests collected in the orchard.

Pathogenicity trials

Pathogenicity trials with a pathogen identified as *Neofusicoccum* sp. 1 were done on avocado fruit running for approximately 2 weeks. The fruit were surface sterilised with 76% ethanol and rinsed twice with sterile water. Ten fruit from each site was placed in a container on a grid, 5 were pricked to simulate insect piercing damage and 5 fruit were not pricked to act as control. A fungal plug was placed on each fruit and incubated at 25 °C for 12 days. The length and width of each lesion were measured with a digital calliper in 3-day intervals. The trial was terminated after 12 days and each avocado's length and width was measured to calculate the % colonisation of the fungus on the fruit. The fungus was re-isolated from the fruit and identified as *Neofusicoccum* sp. 1 (Koch's Postulates).

Fungal survey

Fungi isolated out of plant material and insects on field trip in November 2017:

In Figure 3 the pathogen *Cladosporium cladosporioides* was the most abundant in the leaf samples with a fungal occurrence of almost 3/5. This fungus is known to cause leaf spot on avocado leaves, known as scab, and does well where there is high humidity and poor air flow in orchards. This pathogen is wind dispersed.

Figure 4 shows that the only significant pathogen that poses a threat to the avocado fruit is *Cladosporium cladosporioides* that is associated with scab lesions on the fruit. This fungus occurred approximately 3/5 times in the fruit samples that were plated out.

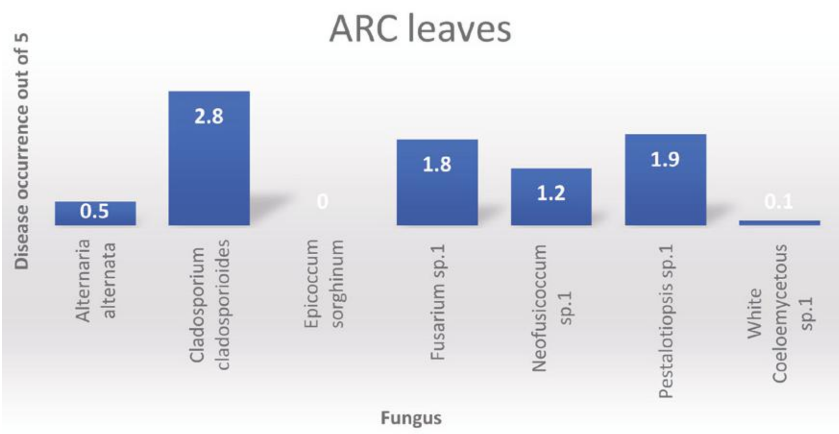

Figure 3: Fungi isolated out of leaves sampled at the ARC farm in Nelspruit, and fungal occurrence.

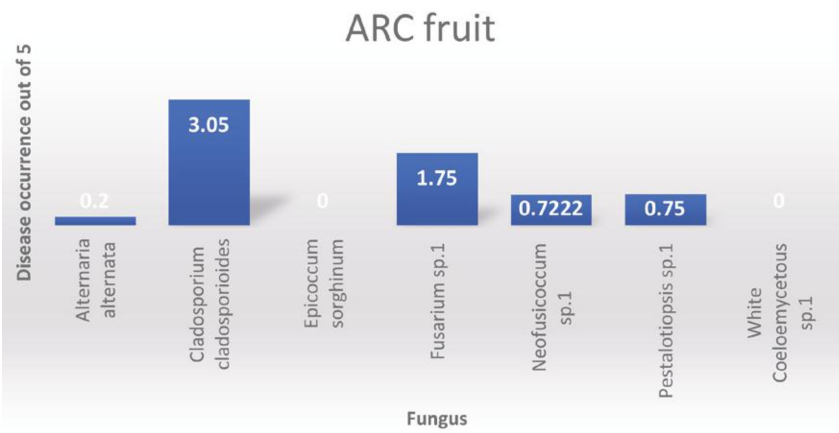

Figure 4: Fungi isolated out of fruit sampled at the ARC farm in Nelspruit, and fungal occurrence.

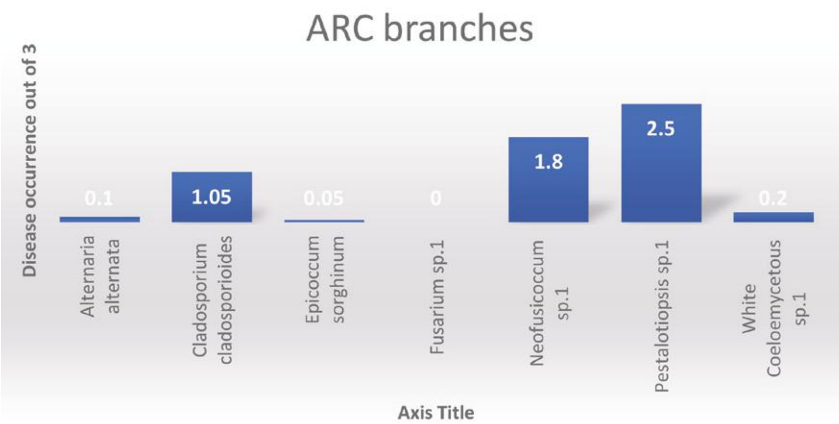

Figure 5: Fungi isolated out of branches sampled at the ARC farm in Nelspruit, and fungal occurrence.

The results in Figure 5 indicates that *Pestalotiopsis* sp. 1 and *Neofusicoccum* sp. 1 were the most abundant in the branches with 2.5/5 and 1.8/5 fungal occurrence respectively. Both these pathogens are associated with twigs and branches. *Pestalotiopsis* is associated with stem-end rot in avocado and causes the fruit to start rotting at the end where the fruit and stem comes together. *Neofusicoccum* sp. 1 is associated with dieback of branches and the black discolouration of the pith tissue. This block the xylem and phloem vessels and obstruct the flow of water and nutrients in the plant. Trees start to die back from the top branches downwards.

In Figure 6 the results show that the pathogen *Neofusicoccum* sp. 1 were mostly isolated out of the leaf samples of the Halls farm. This fungus was isolated 3.35/5 times out of the leaves. *Neofusicoccum* spp. is known to be endophytes that are always present in the plant material and attack the plant when the plant is weakened by either insect damage, nutrition deficiencies, environmental conditions or a combination of these factors. This pathogen causes the disease known as dieback, as previously mentioned.

As in the leaves, the results of the fruit show the same tendency with the pathogen *Neofusicoccum* sp. 1 being the most abundant in the Halls samples (Fig. 7). Compared to the leaves, the fungal occurrence declined in the fruit. This may be because the leaves are exposed to the surrounding environment for longer periods than the fruit, which are seasonal. Another factor that plays a role is the health of the plant. Most of the sugars and nutrients are translocated from the leaves to the fruit during fruit development. *Neofusicoccum* was isolated 2/5 times out of the fruit samples at the Halls farm.

When the leaves, fruit and branches of the Halls farm are compared to each other, it is clear that *Neofusicoccum* sp. 1 is the most abundant throughout the samples (Fig. 6, 7 & 8). As previously mentioned, this pathogen causes dieback of the branches.

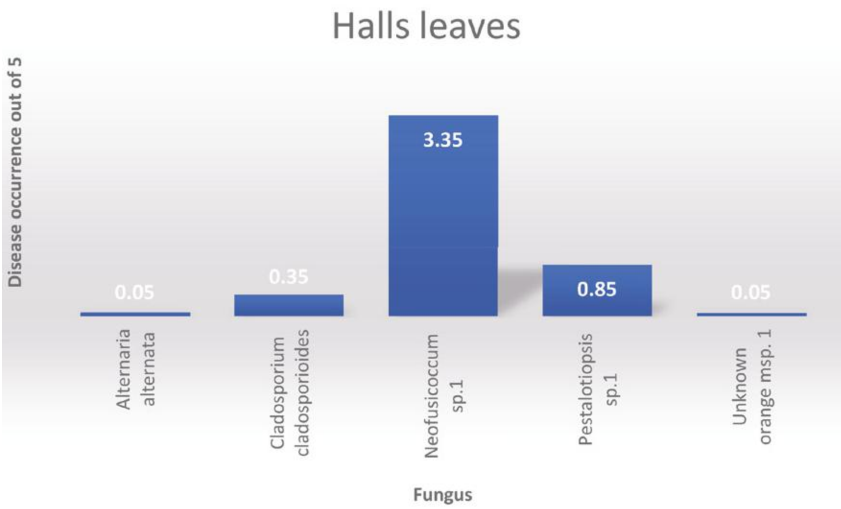

Figure 6: Fungi isolated out of leaves sampled at the Halls farm in Nelspruit, and fungal occurrence.

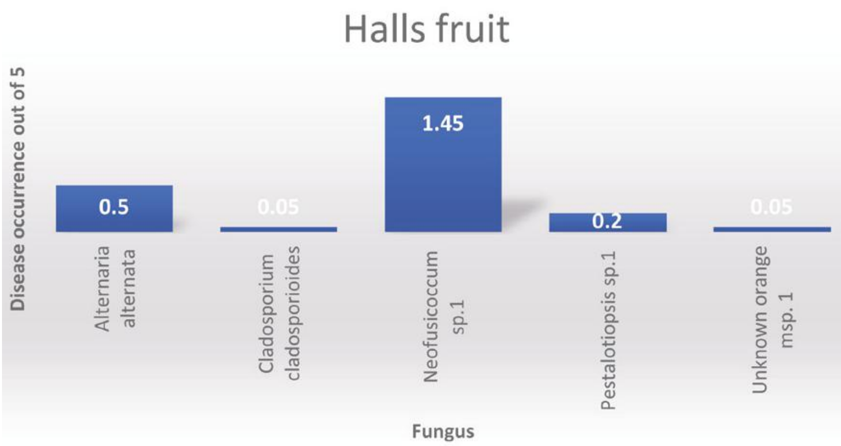

Figure 7: Fungi isolated out of fruit sampled at the Halls farm in Nelspruit, and fungal occurrence.

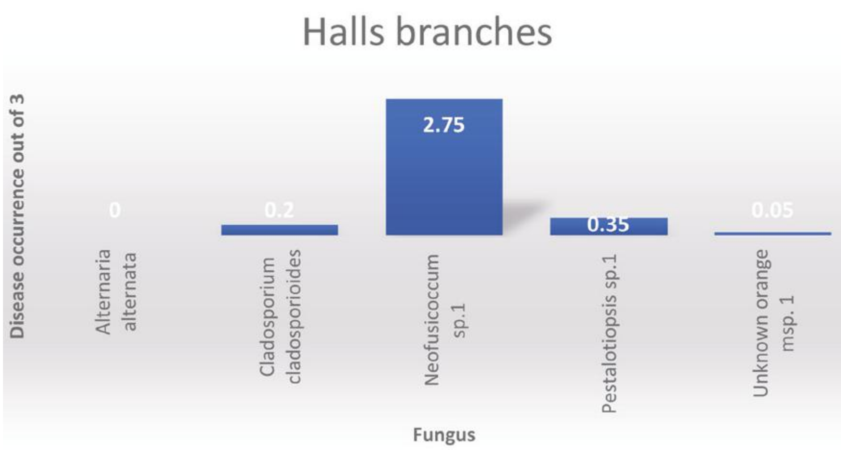

Figure 8: Fungi isolated out of branches sampled at the Halls farm in Nelspruit, and fungal occurrence.

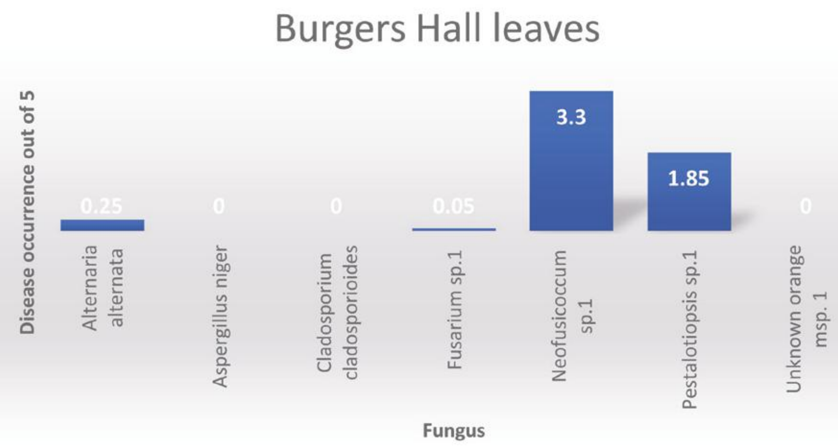

Figure 9: Fungi isolated out of leaves sampled at the Burgers Hall farm in Nelspruit, and fungal occurrence.

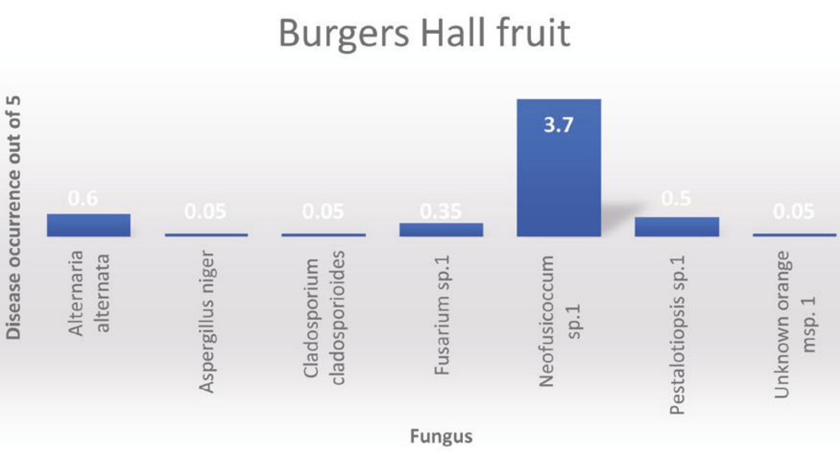

Figure 10: Fungi isolated out of fruit sampled at the Burgers Hall farm in Nelspruit, and fungal occurrence.

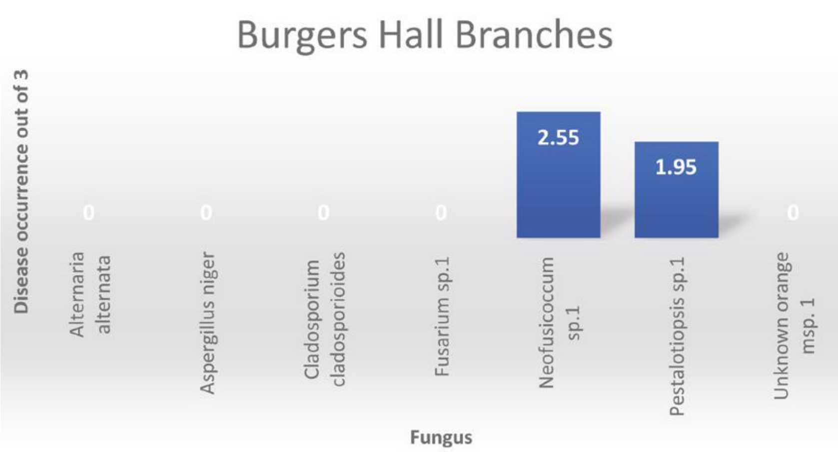

Figure 11: Fungi isolated out of branches sampled at the Burgers Hall farm in Nelspruit, and fungal occurrence.

Comparing Figures 9 & 10, a high occurrence of *Neofusicoccum* sp. 1 is seen in both the leaf and fruit samples at 3.3 and 3.7, respectively. This fungus is a very fast-growing organism and seems to compete out the other fungi. The fungus is an endophyte and is usually not a problem in orchards, until the trees are under environmental, nutritional or insect pressure.

In Figure 11 it shows that the branches were dominated by two pathogens. Both these pathogens, *Neofusicoccum* and *Pestalotiopsis*, are associated with dieback of branches and stem-end rot respectively. *Pestalotiopisis* sp. causes stem-end rot in avocado fruits at the place where the fruit and twig comes together, then moves through to the rest of the fruit.

Insect pests

The results in Figure 12 shows that four of the coconut bug samples that were plated out were 100% colonised by *Neofusicoccum* sp. The other samples could not be used, since they were contaminated with bacteria. This fungus produces reproductive propagules called pycnidia that are adapted for insect dispersal.

Pathogenicity trials

When comparing Figures 13 & 14 it seems that the pathogen colonised the fruit at a faster rate on the pricked fruit than on the un-pricked fruit. After 6 days of inoculation, the un-pricked fruit still showed almost no fungal colonisation whereas the pricked fruit already showed good fungal colonisation (Fig. 15). Both pricked and un-pricked fruit reached 100% fungal colonisation after 12 days before the trial was terminated. The pricked fruit is to simulate insect damage, since this pathogen is adapted for insect dispersal.

This results clearly indicate that wounding from insect feeding will facilitate fungal infection in damaged fruit. The wound acts as an easy access point for fungal reproductive propagules to enter and infect the fruit. This can have severe effects, including post-harvest.

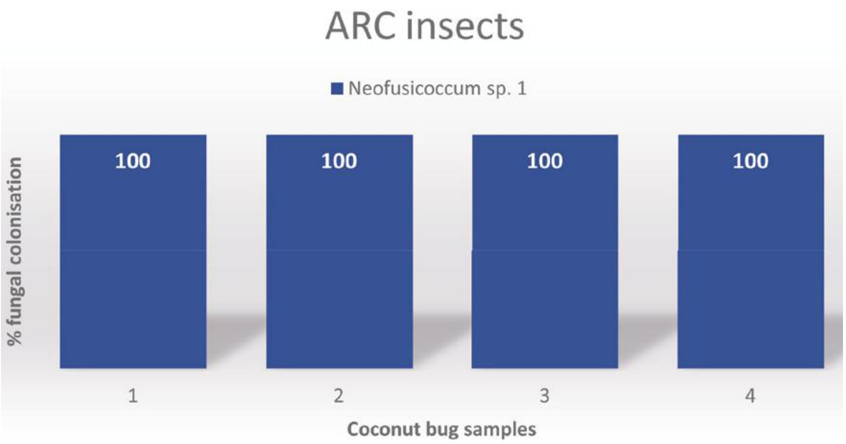

Figure 12: Coconut bugs collected at the ARC farm in Nelspruit.

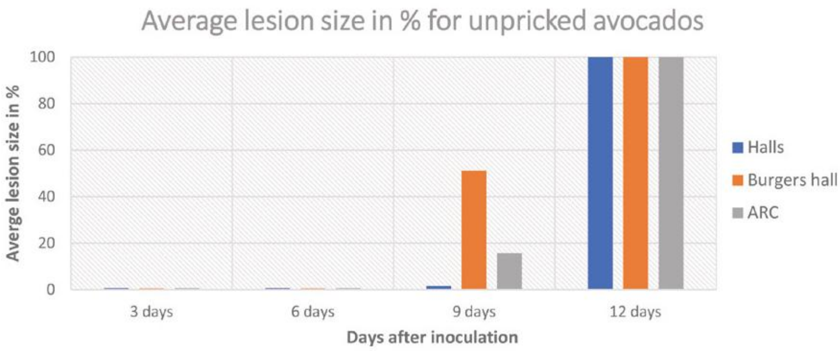

Figure 13: Average lesion size after 12 days of inoculation on un-pricked avocado fruit for all three localities.

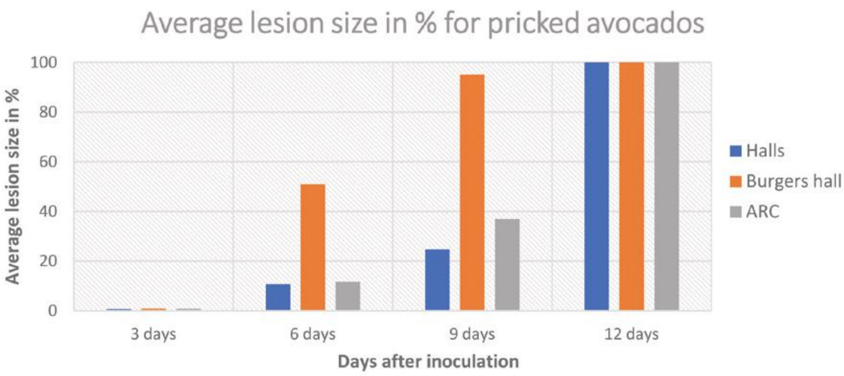

Figure 14: Average lesion size after 12 days of inoculation on pricked avocado fruits for all three localities.

Figure 15: Prick test performed using *Neofusicoccum* sp. 1 on avocados from Burgers Hall.

The collection of insect pests will continue throughout 2018, with the goal set to finish the first sampling data at the end of the growing season. If any correlation is found between the fungi on the insects and fruits, the coconut bug and thrips will be considered as vectors.

Future work

This study is ongoing and fieldwork still needs to be completed to cover the collection of insects from the sites. Fieldwork is planned for the growing season of 2018 and will target periods when insect populations (especially coconut bug) numbers are high.

Koch's postulates still needs to be performed on *Alternaria alternata*, *Cladosporium cladosporioides* and *Pestalotiopsis* sp. 1. The same approach will be followed by investigating the disease with or without the presence of a "needle like" wound, similar to the wounds caused by piercing sucking mouthparts of insects.

CONCLUSION

Initial fungal surveys indicated several important pathogenic fungi present all year round in the orchards of the Nelspruit region. Understanding how these fungi disseminate and interact with insect pests are of cardinal importance. From the fungi isolated, *Neofusicoccum* sp. 1 was very prevalent in numbers and distribution. It is also suspected that this fungi makes use of insect vectors to aid in dissemination. This fungus was also isolated from collected coconut bugs, indicating the presence of fungal propagules on the bodies of insects in the orchards. Furthermore, prick tests and Koch's postulates were performed on gathered fruit. Results clearly indicate that wounding, as would be the case with insect piercing feeding damage, greatly aids the fungi in colonizing the fruit. All the above seems to indicate a level of interaction between insect pests and fungal diseases previously underestimated in the orchard environment. Several other important fungi have also been identified and the same tests will be done with them.

REFERENCES

AGRIOS, G.N. 2005. *Plant Pathology. 5th ed*. Elsevier Academic Press, London. 922 pp.

BLACKWELL, M. 2011. The Fungi: 1, 2, 3 … 5.1 million species? *American Journal of Botany* 98: 426-438.

BROWN, J.F. & OGLE, H.J. 1997. *Plant pathogens and plant disease*. 1st Edition. Rockvale Publications, Australia. 571 pp.

KIRK, P.M., CANNON, P.F., MINTER, D.W. & STALPERS J.A. 2008. *Dictionary of the Fungi*. 10th Edition. CAB International, Wallingford, UK. 402 pp.

PRADO, S.S. & ZUCCHI, T.D. 2012. Host-symbiont interactions for potentially managing heteropteran pests. *Psyche* 2012: 1-9.

SAAIMAN, J. 2014. *Insect-fungal interactions in tree nut crop orchards in South Africa, with specific reference to pecans*. M.Sc. thesis. Department of Zoology & Entomology, Faculty of Natural and Agricultural Sciences, University of the Free State, Bloemfontein, South Africa.