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 colonise 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 of the insects. They transport fragments of hyphae by feeding from plant to plant and create wounds during ovipositioning 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, as well as the thrips, in damaging the avocado fruit and transmitting fungal pathogens such as *Pseudocercospora* sp. and *Colletotrichum* sp.

OBJECTIVES

For the purposes of this study, two pests, the coconut 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 the coconut bug (*P. wayi*) and the thrip complex indeed act as vectors of the fungal pathogens *Colletotrichum* sp. and *Pseudocercospora purpurea*, both serious avocado diseases in South Africa, causing significant financial loss. The study is approached in a holistic fashion to determine the biotic interactions between pathogens and insects in the orchard agro-ecosystem. After identifying the pathogen and vector, a successful control programme must be applied from an integrated pest management view.

1. Investigating the role and vector potential of known sucking pests on avocados in the Nelspruit region.
2. Investigating the bio-ecology and interaction between vectors and pathogens from a holistic point of view.
3. Working towards a management programme for these pathogens to minimise losses.

METHODS

The association of fungi with the coconut bug and thrips on avocado is being investigated with primary sites located in Mpumalanga (Nelspruit), South Africa. Three sites have been identified. Samples of insects, soil, air, leaf and fruit were collected and plated out on a growth medium for fungal growth. Samples were taken throughout the year taking the whole growing season in consideration, covering from the flowering stage until the fruit are harvested.

Sampling was done evenly distributed over the orchard.

1. Insect specimen collection: Coconut bug collection will be done by actively searching for bugs on the avocado trees and 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.
2. 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 back to the University of the Free State (UFS) where fungal colonies were isolated and identified.

PROGRESS

Field trip April 2016:

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

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

These 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 back 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.

RESULTS

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 on 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.

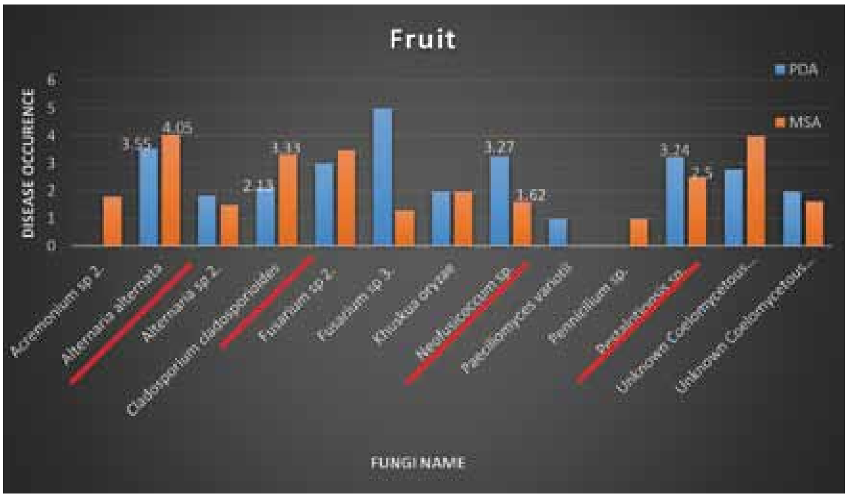

Figure 1. Four important pathogens identified from fruit collected at Sakkie's farm.

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	

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 too be considered significant, or are not of pathogenic importance to plants.

DISCUSSION

Alternaria alternata is an endophyte living inside plants and emerge when the plant is weak or under stress. This fungus is associated with the die-back 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 leaves) are also associated with this fungus. *Alternaria alternata* is a post-harvest 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 of chilling damage, with colonisation of *C. cladosporioides* being secondary (Fig. 4).

***Neofusicoccum* species** (previously known as *Botryosphaeria* spp.) are associated with the die-back 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 avocados 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.

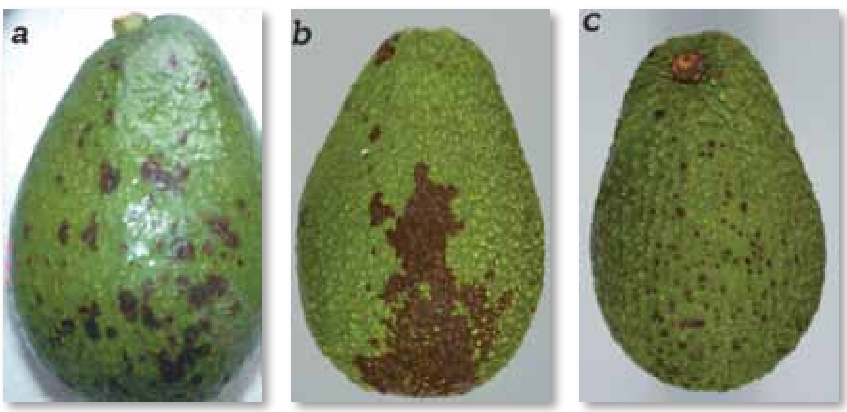

Figure 4. Symptoms of a) damaged avocado fruit nodules, b) chilling injury (blotches with sharp borders and c) blotches with "fuzzy" borders.

FUTURE WORK

This study is on going and one fieldwork trip still needs to be completed to cover the collection of insects from the sites. The fieldwork is planned for the growing season of 2017 and will target periods when insect populations (especially coconut bug) numbers are high.

Koch's postulates will be performed to see whether *Alternaria alternata*, *Cladosporium cladosporioides, Pestalotiopsis* sp.1 and *Neofusicoccum* sp. 1, can cause disease with or without the presence of a "needle like" wound, similar to the wounds caused by piercing sucking mouthparts of insects.

The identification of fungi from collected samples will continue throughout 2017, with the goal set to finish the first sampling data at the end of the year. If any correlation is found between the fungi on the insects and fruits, the coconut bug and thrips will be considered as vectors.

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