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Fungal Diseases Occurring on Trees of Namibia

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Abstract

During the past few years, disease symptoms in many *Acacia* trees in the Windhoek Municipality area and the rest of Namibia have been observed. This observation triggered the investigation of phytopathological aspects that are reported in this chapter. The importance of indigenous trees of the Namibia flora is apparent considering that Namibia has two old deserts within its borders: the Namib Desert and the Kalahari Desert. Namibia's tourism and meat industry are dependent on the indigenous trees of Namibia flora. The trees are the primary source of vegetation land cover (attracting tourists), and they provide browsing food matter to domestic livestock and wild animals (sources of meat). Hence, it is important to ensure that a healthy vegetation is maintained in this area.. This survey is the first dedicated step to find ways of protecting them from disease-causing agents. The aim of this survey is to investigate the possible causes of disease symptoms in trees. It is important to understand the biology of the pathogenic agents to propose a possible method to control the diseases. The survey involved sampling leaves, stems and roots from dying trees that show symptoms such as branch girdling, gum oozing and defoliation, suspicious general twig wilting and die-back. The survey was carried out in places where symptoms were observed. The tree surveys were done on *Aloe zebrina*, *Tylosema esculentum*, *Syzygium* and *Acacia* species. Primary isolations from plant material and then single-spore pure cultures were made for identification. In this chapter, we report isolation and identification of *Microsphearopsis* sp., *Dreschelra* sp., *Botryosphaeria* spp., *Acremonium* spp., *Coniothyrium* sp., *Phellinus* spp., *Cytospora* sp., *Fusarium* sp., *Scytalidium* sp., *Phoma* spp., *Gliomastix* sp., *Trichoderma koningii*, *Peecilomyces variotii*, *Alternaria citri* and *Curvularia palescens* from the diseased trees. This work is still ongoing. This study paves way for proper designing of control methods to protect crops, trees and their biodiversity. The protection of plant biodiversity ensures better reaping of food products and other ecosystem services and products. Without knowledge of the identity of these disease-causing agents, it is not possible to accurately identify and manage threats to food production and threats to the native botanical biodiversity of Namibia.

Keywords: *Acacia mellifera*, *Acacia karroo*, *Syzygium*, fungal pathogens, Namibia tree diseases

1. Introduction

Fungal plant pathogens, if not controlled one way or the other, can have devastating effects on biodiversity, forest structure and dynamics, commercial plantations, agro forestry and urban environments. This is especially the case with introduced (exotic) pathogens. A well-known example is that of *Cryphonectria parasitica*, a fungus native to Asia, which was introduced into the United States in the early 1900s. This fungus, a mild pathogen in its areas of origin, after introducing it to the United States caused the near extinction of North American chestnut trees (*Castanea dentata*). Today, a once-dominant canopy tree in the Eastern United States has been reduced to a low-growing shrub and the entire ecology of the forests has been changed, impacting on animals, other trees and humans. Another example is that of sooty baobab disease, which has killed a lot of baobabs in Southern Africa [1-4]. The impact on plantation forestry species and agricultural crops can be equally severe and with the increased movement of humans and plant products around the world, more and more pests and diseases are being moved to areas where they previously did not occur.

Die-back on *Acacia* trees in areas in and around the city of Windhoek in Namibia, especially in the Dorado Park area, was observed and since then, disease symptoms and tree deaths have been increasing in Namibia forest lands [3]. Life-threatening basal cankers on *Syzygium guineense* trees have been observed along the Zambezi River in the Katima Mulilo area of Namibia. *Acacia* species are particularly important for their use as fodder and fuel wood (see Figure 1) for enhancing soil fertility through biological nitrogen fixation and gum production, whereas *Syzygium guineense* is important source of edible fruits and fuel wood. In this study, there are two main objectives: firstly, to investigate the cause of decline and death of *Acacia* species trees around Windhoek, to develop management strategies to reduce the impact of the disease and secondly, to survey fungal pathogens occurring in selected habitats of *Syzygium* and *Acacia* species in Namibia. These surveys will serve as the groundwork for future investigations on tree health in Namibia, that is, in expanding our knowledge pertaining to the nationwide distribution, impact and origin of these pathogens. This knowledge is also essential to understand the functional dynamics of these native ecosystems at a basic level, which is potentially important for the conservation of these ecosystems.

Yet another close example is that of the baobab disease threat. Baobab trees are very resilient and can survive an extraordinary amount of abuse by humans, animals and natural conditions. Very little, however, is known about the diseases that affect these trees. In recent years there have been numerous reports on baobab tree mortality. In most cases the causes of these deaths are not known. The first reports of diseased baobabs are from 1944, when it was reported that baobabs were dying subsequent to being infected with “a brown black smut” after prolonged drought in Zimbabwe [5]. Numerous isolations were attempted from diseased trees but no pathogens were obtained, and it was concluded that the symptoms were a result of environmental stresses that made trees susceptible to colonization by sooty mould fungi (Hopkins 1950 as cited by [5]). The same symptoms were noted in Zimbabwe in the 1960s and again in the 1980s [5], as well as in South Africa in the early 1990s [2]. In 2002, a pilot study was conducted to determine the cause of death of baobab trees in the Musina nature reserve [6]. Die-back of branches and exudation of large amounts of sap were noted, but no sooty mould

was evident. Internal discoloration of diseased branches was also observed and diseased trees died within a few months.

The death of several large baobabs [5] in the Nyae Nyae conservancy in Namibia: Both sooty mould and the exudation symptoms were observed on the trees. It, therefore, appears that the cause of death may be a combination of diseases or an unidentified pathogen. *Lasiodiplodia theobromae* was isolated from these trees. This fungus belongs to the Botryosphaeriaceae family, in which many of the species included are plant pathogens [7]; however some of the species in this family are endophytes that live in plants and only start to cause disease when the right conditions occur [8].

The increasing number of reports of diseased and dying trees in Namibia [9-13] emphasizes the need for urgent investigation of the causes of these deaths. It is necessary to determine whether these reports are isolated incidences or if there is an epidemic underway that can threaten the survival of indigenous trees. Furthermore, it is important to understand the fungal assemblages of these trees to understand possible future disease outbreaks. One of the greatest challenges to tree health management is the fact that so little is known regarding the fungal biodiversity on trees and the role these fungi play in tree health. In this chapter, the symptoms observed on dying trees and potential causal agents of death are reported. In plant health biology and forestry conservation, the first dedicated step is to determine causes of plant diseases, which is a primary objective of this investigation. Yearly, calls have been received from farmers about plants dying or manifesting life-threatening symptoms [9-14] (see Figure 1). Some of these reports have been partially investigated and yet others remain to be investigated.



Figure 1. Ecosystem services from indigenous plants. In panel A: edible fruits of the monkey oranges on the road side in Kavango region of Namibia, panel B: fire wood harvested from local forests in Kavango region being sold on the road side, panel C: bird nests habitat on *Acacia karroo* trees, panel D: pods of *Acacia erioloba* packed in bags for sell on the roadside for feeding livestock, panel E: twig die-back symptoms manifesting on *Acacia karroo*, panel F: *Acacia karroo* tree totally collapsing after fungal infection.

2. Materials and methods

2.1. Description of study site and population

The study was carried out in Oshikoto and Otjozondjupa regions of Namibia, where the disease reports on trees have been received from. In addition, the survey was carried out on forest stands where some of the species occur in Erongo, East and West Kavango and Zambezi regions.



Figure 2. Symptoms observed on selected indigenous trees of Namibia. On panel A: discoloration of the vascular tissue due to fungal infections; panel B: severe malformations of pods on *Acacia karroo*; panel C: malformation of flower inflorescences of *Aloe zebrina*; panel D: *Acacia erioloba* infected with a bracket fungus; panel E: *Welwitschia mirabilis* infected with fungi; panel F: basal canker on *Syzygium guineense* showing sporulation of fungi on the stems along the banks of the Zambezi river in Zambezi region of Namibia.

The study was exploratory in nature and used standard mycological methods to investigate the identity of fungi causing diseases on plant species based on previous report calls received and reports existing in the media. In cases where no reports have been made, field surveys were carried out to observe symptoms in native forest stands.

During the survey, diseased samples from leaves and small branches and twigs were collected (see Figure2). These were then brought to the laboratory at the University of Namibia for isolation of fungal organisms. The pure cultures of these microbial organisms were then stored in a type culture collection facility and used for further classical microbiological analyses and genetic analyses to determine the identity of the organisms. Fungi were identified based on morphology and ITS DNA sequencing data. Further, the pure cultures were used to test Koch's postulates by inoculating the possible pathogens into healthy seedlings.

For the *Welwitschia* and *Syzygium* work, non-destructive sampling was used. Small pieces of infected/cankered tissue were removed for isolation of the fungi. The collection of these pieces of material did not result in the death or deformation of the plants. In the case of the *Welwitschia*, small pieces, less than 5 cm in length and 1 cm wide, were removed for study. The *Acacia* work was somewhat more destructive because entire sections of dying tree parts were needed

to be collected to determine the cause of death and, in the long-term, to prevent other trees from dying. This involved chopping of stems and branches and possibly digging up the roots of affected plants if required.

Survey trips were conducted in various sites in Namibia, including Windhoek, Dordabis, Grootfontein, Katima Mulilo, Omaruru, Swakopmund, Rundu, Popa Falls and Rehoboth between 2003 and 2013 to collect leaves, stems and roots of *Acacia* and *Syzygium* plants showing disease symptoms. At least 10 trees were sampled from each site. Collected samples were examined for insect damage and used for fungal isolations. Primary isolations and pure cultures of fungi were made using 2% MEA media. All isolates were identified in the laboratory using morphology and ribosomal DNA sequencing of the internal transcribed spacer (ITS) regions. The ZR Fungal/Bacterial DNA extraction kit was used to obtain DNA from the crushed powder-form mycelium of the fungi isolated from these trees. ITS 1 and ITS 4 primers were used to obtain amplicons of the extracted DNA.

3. Results and discussion

The main objective is to investigate the decline and death of forest tree species in Namibia with a hope to develop management strategies to reduce the impact of the disease and help ensure the continued survival of an important component of the Namibian plant ecosystem for continued supply of ecosystem services. For this survey, for a number of specific fungal pathogens in key Namibian ecosystems. For this, the surveys conducted served as the groundwork for future research field of microbiology, plant pathology and plant protection. The results of this research helped to answer some key questions regarding fungal plant pathogens that occur in Namibia. Results (see Table 1) will expand our knowledge pertaining to the distribution, impact and origin of these pathogens. This knowledge is also essential to understand the working of these native ecosystems at a basic level, which is potentially important for the conservation of these ecosystems.

It is anticipated that the findings reported here will articulate into the Namibia's National Forest Programme aimed at promoting the sustainable use of the country's forests. In this national aim it is important to ensure forests that are healthy. If forest tree species are declining because of diseases, then sustainability becomes impossible. These data reported here assist in creating linkages with various sectors, such as the National Forestry Research Division in Okahandja in Namibia to promote forestry research, protect and conserve forest areas against destructive diseases and pests.

Most of the fungal species recorded in Table 1 have also been recorded elsewhere in the literature [9-15] as fungal pathogens affecting similar plant species that we investigated in this study or affecting other plant or crop species, which points to the fact that it is important to keep surveillance of plant disease as these fungal pathogens can easily be transported worldwide and be able to cause diseases or death in similar or other plant species.

Area sampled	Tree species	Fungi isolated
Windhoek	A. karroo	<i>Acremonium</i> sp.
		<i>Alternariacitri</i>
		<i>Botryospheararia</i> sp.
		<i>Curvulariapalescens</i>
		<i>Cytospora</i> sp.
		<i>Dreschlera</i> sp.
		<i>Fusarium</i> spp.
		<i>Microsphearopsis</i> sp.
		<i>Paecilomyceslilacinus</i>
		<i>Phoma</i> spp.
		<i>Trichodermakonigingii</i>
	A. hebeclada	<i>A. citri</i>
		<i>Acremonium</i> sp.
		<i>Botryospheararia</i> sp.
		<i>Dreschlera</i> sp.
		<i>Fusarium</i> spp.
		<i>Microsphearopsis</i> sp.
	A. mellifera	<i>Phoma</i> spp.
		<i>Fusarium</i> spp.
		<i>Botryospheararia</i> sp.
		<i>Gliomastix</i> sp.
		<i>Paecilomycesvariotii</i>
Dordabis	Acacia reficiens	<i>Phomaglomerata</i> .
		<i>Gliocladium cibotii</i> .
	Aloe zebrina	<i>Alternaria tenuissima</i> ,
		<i>Ampelomyces</i> spp
	A. mellifera	<i>Botryosphaeria</i> sp.
		<i>Coniothyrium</i> sp.
		<i>Cytospora</i> sp
		<i>Fusarium</i> sp.
		<i>Paecilomycesvariotii</i>
		<i>Phellinus</i> sp.
		<i>Phoma</i> sp.
Grootfontein	A. erioloba	<i>Phellinus</i> sp.
	A. mellifera	<i>Botryosphaeria</i> sp.
		<i>Fusarium</i> sp.

Area sampled	Tree species	Fungi isolated
		<i>Phellinus</i> sp.
		<i>Phoma</i> spp.
		<i>Trichoderma</i> sp.
Rundu	<i>A. erioloba</i>	<i>Phellinus</i> sp.
	<i>A. mellifera</i>	<i>Phellinus</i> sp.
Popa Falls	<i>A. mellifera</i>	<i>Phellinus</i> sp.
	<i>S. guineense</i>	<i>Chrysosporthe</i> sp.
Katima Mulilo	<i>A. erioloba</i>	<i>Phellinus</i> sp.
	<i>S. guineense</i>	<i>Chrysosporthe</i> sp.
Erongo	<i>W. mirabilis</i>	<i>Botryosphaeria</i> spp
		<i>Phoma sorghina</i>
Otjiwarongo	<i>Tylosema esculentum</i>	<i>Alternaria tenuissima</i>
		<i>Alternaria alternata</i>

Table 1. Fungi isolated from diseased indigenous plants in Namibia.

Several fungal species have been isolated and some of them for the first time in diseased *Acacia* species and *Syzygium guineense* in Namibia. Visual inspections of disease symptoms on *A. karroo* trees in Windhoek showed that these trees had all been infested by a wood boring insect. This resulted in girdling of branches and subsequent wilt and death of these branches. Numerous fungal species were isolated from lesions of insect-infested trees (see Table 1). A few *A. hebeclada* trees were surveyed in Windhoek. Symptoms on these trees included stem cankers and branch die-back. No primary pathogens were, however, isolated from these trees. Those fungi isolated are opportunistic stress-associated pathogens capable of causing die-back and death of trees. Symptoms of foliage discoloration and apparent death of trees observed on *A. hebeclada* in the Rehoboth area were as the result of insect infestation. Caterpillars of an unidentified insect species, possibly of the Lepidoptera group were found feeding on the leaves and seedpods of these trees. Three sites with *A. mellifera* die-back were investigated. In Windhoek, tip defoliation and death of branch tips on *A. mellifera* trees caused by insects were observed. Extensive mortality of trees was observed on the farm near Dordabis, where numerous trees were sampled and isolated. On a farm in Grootfontein, branch die-back associated with a *Phellinus* sp. was the most common symptom observed, although many other fungi were also isolated (Table 1). *Chrysosporthe* sp. (previously *Cryphonectria*) was found on *S. guineense* trees around Popa Falls and Katima Mulilo. This fungus was found sporulating abundantly on stem and root cankers of trees.

4. Conclusions and recommendations

Several fungal species associated with diseases in *Acacia* and *Syzygium* trees in Namibia have been purified, stored and identified. There are ongoing surveys and confirmation studies of

disease causation on other parts of the country. Population studies of the isolated fungi are underway. Possible pathogens and other fungi of interest are currently being identified to species level using morphology and DNA sequence data.

The results reported in this chapter can be used to develop a catalogue of fungi associated with crop and tree diseases in Namibia forest and possible control methods. In addition, the results can be used to devise long-term strategy for breeding for disease-tolerant forest species, especially those that have immediate commercial value, e.g., *Tylosema esculentum* and marula fruit plant. Brochures for disease alerts and awareness campaign on various trees diseases of Namibia can also be easily developed from these results. Copies of the brochures would then be made available for further dissemination to communities. The results here will help to ensure that the crops and native tree species are kept healthy. When the plants are healthy, food protection by crops and all the other ecosystem services and products are readily available to communities. This reduces the national burden on import of food and other plant-based services and products. The scientific data that have been generated will be used in plant health policy development.

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