Identification of the Armillaria root rot pathogen in Ethiopian plantations

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Summary

Armillaria root rot is a well-known disease on a wide range of plants, world-wide. In Ethiopia, the disease has previously been reported on *Pinus* spp., *Coffea arabica* and on various native hardwoods. The causal agent of the disease has been attributed to *Armillaria mellea*, a species now known to represent a complex of many different taxa. The aim of this study was to determine the extent of Armillaria root rot and the identity of the *Armillaria* sp. in Ethiopian plantations. As part of a plantation disease survey in 2000 and 2001, samples were collected in plantations at and around Munessa Shashemene, Wondo Genet, Jima, Mizan and Bedele, in south and south-western Ethiopia. Basidiocarps were collected and their morphology studied. Morphological identification was confirmed by sequencing the intergenic spacer (IGS-1) region of the ribosomal rRNA operon and comparing data with published sequences of *Armillaria* spp. *Armillaria* isolates were collected from *Acacia abyssinica*, *Pinus patula*, *Cedrela odorata* and *Cordia alliodora* trees. Sporocarps were found on stumps of native *Juniperus excelsa*. Basidiocarp morphology and sequence data suggested that the fungus in Ethiopia is similar to that causing disease of *Pinus* spp. in South Africa and previously identified as *A. fuscipes*. This identification was confirmed for all isolates, based on sequence data. *Armillaria fuscipes* is known to be common in southern Africa. Its widespread occurrence in Ethiopia suggests that it is also the major cause of Armillaria root rot in that country.

1 Introduction

Armillaria species cause root rot on a wide range of hosts, worldwide. These include many species such as *Eucalyptus*, *Pinus*, *Acacia* and *Cupressus* that are utilized in plantations (Wargo and Shaw 1985; Hood et al. 1991; Kile et al. 1991). *Armillaria* spp. have been regarded as primary pathogens, stress-induced secondary invaders and saprophytes (Wargo and Shaw 1985; Shaw and Kile 1991). Group death, wilting and yellowing of tree tops, resin exudation, as well as the occurrence of white mycelial fans under the bark of infected trees are common symptoms of *Armillaria* infections (Morrison et al. 1991). In many cases, rhizomorphs are found associated with Armillaria root rot, facilitating spread of *Armillaria* through the soil (Morrison et al. 1991). However, in Africa these structures are rare (Swift 1968) and when present, occur mainly at higher altitudes where cooler climates prevail (Mohammed and Guillaumin 1993).

The morphology of *Armillaria* spp. including the mycelium, rhizomorphs and basidiocarps has been important for species identification. Morphological characteristics of the basidiocarps have provided valuable taxonomic characters and were traditionally used for species delimitation (Shaw and Kile 1991). However, the seasonal and irregular production of these structures, coupled with their scarcity, complicate identification of *Armillaria* spp., based on morphology (Watling et al. 1982; Wargo and Shaw 1985;

Mohammed et al. 1994). Differences in vegetative mycelium and rhizomorph growth characteristics have been useful in discriminating between most *Armillaria* spp. (Morrison 1982; Rishbeth 1986; Guillaumin et al. 1989; Tsopelas 1999). However, vegetative morphology is influenced by growth medium composition and environment (Guillaumin et al. 1989) and some species exhibit limited interspecific variation, restricting the use of vegetative morphology as a primary characteristic for species identification (Watling et al. 1982; Garraway et al. 1991).

Problems associated with basidiocarp morphology have necessitated employing alternative identification techniques in *Armillaria* taxonomy. These methods include sexual compatibility studies (Korhonen 1978; Ullrich and Anderson 1978; Anderson and Ullrich 1979), biochemical comparisons (Morrison et al. 1985; Mwangi et al. 1989; Agustian et al. 1994; Mwenje and Ride 1996) and DNA-based techniques (Anderson et al. 1987; Jahnke et al. 1987; Smith and Anderson 1989; Anderson and Stasovski 1992; Harrington and Wingfield 1995). Sexual compatibility and DNA-based comparisons are now routinely used to study the biology, taxonomy and phylogeny of *Armillaria* spp.

DNA sequence and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) data have been shown to provide informative characters for discriminating between *Armillaria* spp. (Anderson and Stasovski 1992; Harrington and Wingfield 1995; Coetzee et al. 2001). These methods apply data generated from the intergenic spacer (IGS-1) and internal transcribed spacer (ITS) regions of the rDNA operon, of which the former is most commonly used. A large DNA sequence and RFLP profile database has now been compiled for many *Armillaria* spp. originating in different parts of the world. This database now provides an effective means of identifying most *Armillaria* isolates.

Armillaria root rot has been reported from several countries in South, Central, East and western Africa (Mohammed et al. 1989). In these regions, the disease is known to infect both cash crop plants such as coffee, tea, rubber and cacao as well as forest plantation species including those of *Pinus*, *Eucalyptus*, *Acacia* and *Grevillea* (Shaw and Kile 1991). The disease has generally been ascribed to *A. mellea* (Vahl. : Fr.) P. Kumm. and *A. heimii* Pegler (Ivory 1987; Mohammed et al. 1989).

The taxonomic literature on *Armillaria* from Africa includes a variety of species other than *A. heimii* and *A. mellea*. Brief accounts have been published of *A. camerunensis* (Henn.) Volk & Burdsall and *A. pelliculata* Beeli in Cameroon and the Democratic Republic of the Congo (Zaire), respectively (Hennings 1895; Beeli 1927, *loc. cit. fide* Singer 1986; Mohammed et al. 1989; Volk and Burdsall 1995). Mwenje and Ride (1996) recognized four taxonomic groups based on differences in pectic enzyme patterns; one of the groups was assigned to *A. heimii*, another to *A. mellea*, and the remaining groups that are apparently restricted to Zimbabwe were not identified. Recently, Coetzee et al. (2000) concluded that isolates from South Africa represent *A. fuscipes* Petch based on basidiocarp morphology and differences in IGS-1 sequence data between the South African isolates and isolates identified by other researchers as *A. heimii*. However, studies by various research groups suggest that *A. heimii*, *A. mellea* subspecies *africana* [= *A. mellea* subspecies *nipponica* Cha & Igarashi, (Ota et al. 2000)] and an unknown but distinct *Armillaria* sp. are the prevailing taxa in Africa (Mwangi et al. 1989; Agustian et al. 1994; Guillaumin et al. 1994; Mohammed et al. 1994; Mwangi et al. 1994; Mwenje and Ride 1996; Abomo-Ndongo and Guillaumin 1997; Otieno et al. 2003).

In Ethiopia, damage due to Armillaria root rot has been reported from *Pinus patula* Schiede & Deppe plantations at various sites (Mengistu 1992; Dagne 1998; Alemu et al. 2003). Tree death in plantations due to this disease has been estimated to be between 5 and 20% (Dagne 1998). Eshetu et al. (2000) also noted that Armillaria root rot caused minor damage in coffee (*Coffea arabica* L.) plantations. Despite this, little attention has been

given to the disease. It has generally been assumed that Armillaria root rot is caused by *A. mellea* (MENGISTU 1992; ESHETU et al. 2000) but no detailed study has been conducted to identify the *Armillaria* spp. found in Ethiopia. However, a recent study using somatic incompatibility, isozyme comparisons and random amplified polymorphic DNA (RAPD) analyses has suggested the presence of *A. mellea* subspecies *nipponica* on hard woods in the Kerita and Jima areas of Ethiopia (OTA et al. 2000).

During a survey of plantation forestry diseases in Ethiopia, conducted in 2000 and 2001, Armillaria root rot was identified as a common cause of tree mortality (ALEMU et al. 2003). However, the species identity of the causal agent was not known. The aim of this study was thus to identify the *Armillaria* isolates obtained from the surveys and to consider their phylogenetic relationships with other *Armillaria* species. To accomplish these objectives morphological characteristics of the basidiocarps and DNA-based comparisons including RFLP and DNA sequencing of the IGS-1 region of the rRNA operon, were used.

2 Materials and methods

2.1 Sample collection and isolation

Surveys were conducted in forestry plantations at Munessa Shashemene, Jima, Bedele, Aman/Mizan and Wondo Genet in western and south-western Ethiopia. Typical symptoms of Armillaria root rot were used to recognize centres of infection. Samples were collected from roots, stumps and stems of dead and dying trees. Small pieces of mycelium from the white mycelial fans between the bark and the wood were transferred to a selective medium containing benomyl, dichloran and streptomycin (HARRINGTON et al. 1992). Cultures were incubated at 25°C in the dark for 3 weeks. Pieces of mycelium from the tips of the cultures were then transferred to 2% MEA (2% Biolab malt extract, 1.5% Biolab agar; Biolab Diagnostics, Tonetti str., Midrand, South Africa) plates to multiply them for further use. Stock cultures of all the isolates used in this study are maintained on 2% MEA slants at 5°C in the culture collection (CMW) of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa (Table 1).

2.2 Basidiocarp morphology

Basidiocarps collected from stumps of felled Juniper trees were used to study their morphology. Morphological characteristics of these structures were compared with those published for other species. Characters examined included the colour of the basidiocarps and size of the pileus and stipes. RAYNER's (1970) colour chart was used to determine colours.

2.3 DNA extraction

Representative isolates (CMW5838, CMW5846, CMW5844, CMW 8971, CMW8969, CMW8967) from different sites and hosts were grown in liquid MY medium (2% Biolab malt extract; 0.3% Biolab yeast extract agar) in the dark at 25°C, for approximately 3 weeks. Mycelium was harvested from cultures by centrifugation (8000 g, 30 min) and freeze dried. The dried mycelial samples were ground to a fine powder in liquid nitrogen using a mortar and pestle. DNA was extracted using a modified version of the DNA extraction method of RAEDER and BRODA (1985). Extraction buffer (200 mM Tris–HCl pH 8; 25 mM EDTA; 250 mM NaCl) (1000 μl) was added to approximately 0.5 g of powdered mycelium and incubated at 60°C for 30 min. This was followed by a phenol-chloroform extraction step. Cell debris was removed by centrifugation at 13 000 g for 1 h. Further phenol-chloroform extractions were performed on the aqueous phase until a clean

Table 1. Armillaria isolates used in this study

Identity	Culture number[1]	Alternative number	Host	Origin	Collector	GenBank
A. fuscipes[2]	CMW5838		Pinus patula	Wondo Genet, Ethiopia	Alemu Gezahgne & Roux, J.	AY172029
A. fuscipes[2]	CMW5844		P. patula	Wondo Genet, Ethiopia	Alemu Gezahgne & Roux, J.	AY172032
A. fuscipes[2]	CMW5846		P. patula	Wondo Genet, Ethiopia	Alemu Gezahgne & Roux, J.	AY172030
A. fuscipes[2]	CMW8967		Cordia alliodora	Aman/Mizan, Ethiopia	Alemu Gezahgne & Roux, J.	AY172031
A. fuscipes[2]	CMW8969		Acacia abyssinica	Bedele, Ethiopia	Alemu Gezahgne & Roux, J.	AY172034
A. fuscipes[2]	CMW8971		P. patula	Belete/Jima, Ethiopia	Alemu Gezahgne & Roux, J.	AY172033
A. fuscipes[3]	CMW2717	A01-SA	P. elliottii	Sabie, South Africa	Wingfield, M.J.	AF204821
A. fuscipes[3]	CMW2740	B07-SA	P. patula	Entabeni, South Africa	Wingfield, M.J.	AF204822
A. fuscipes[3]	CMW3167	B934, S1	P. elliottii	Sabie, South Africa	Ivory, M.	AF204823
A. heimii[3]	CMW3152	B935, CA1	Unknown	Western Province, Cameroon	Watling, R.	AF204826
A. heimii[3]	CMW3164	B933, LR3	Pelargonium asperum	Saint-Denis, La Reunion	Fabrègue, C.	AF204824
A. heimii[3]	CMW3173	B932, ZM1	Tectona grandis	Dola Hill, Zambia	Ivory, M.	AF204825
A. heimii[3]	CMW3955	F01-Zim	Acacia xantbophloea	Harare, Zimbabwe	Wingfield, M.J. & Coetzee, M.P.A.	AF204827

[1]CMW numbers refer to the culture collection numbers of the Tree Protection Co-operative Programme (TPCP), FABI, UP, Pretoria.
[2]Isolates sequenced in this study.
[3]Sequence of Armillaria, in FABI database, identical to those submitted to GenBank (COETZEE et al. 2000).

interphase was obtained. Chloroform extractions were carried out to remove the traces of phenol. Sodium acetate (3 M NaAc, pH 5) and absolute ethanol were added to precipitate the nucleic acids and they were collected by centrifugation at 13 000 g. The nucleic acid pellet was washed with 70% ethanol, vacuum dried and dissolved in 50 μl sterile water. RNase A (0.01 mg/ml) (Roche, Penzberg, Mannheim, Germany) was added to the DNA and water suspensions to remove RNA and incubated overnight at 37°C in a water bath. The resulting DNA was visualized under UV illumination after electrophoresis on a 1% agarose gel (Promega, Madison, WI, USA) stained with ethidium bromide.

2.4   DNA amplification

The IGS-1 region of the ribosomal RNA (rRNA) operon was amplified using the PCR. This region was amplified with Primers P-1 (5′-TTG CAG ACG ACT TGA ATG G-3′) (HSIAU 1996) and 5S-2B (5′-CAC CGC ATC CCG TCT GAT CTG CG-3′) (COETZEE et al. 2000). The PCR mixtures used included dNTPs (200 μM of each), MgCl$_2$ (2.66 mM), 10X buffer containing MgCl$_2$ (supplied with enzyme), 0.375 μM of each primer, *Taq* polymerase (2.6 U) and approximately 80 ng template DNA. The final reaction volume was adjusted to 50 μl with H$_2$O. The PCR programme consisted of an initial denaturation step at 96°C for 2 min. This was followed by 35 cycles of annealing at 58°C for 30 s, elongation at 72°C for 2 min, a ramp time of 1.5 s and another denaturation at 94°C for 30 s. A final elongation step was allowed for 7 min at 72°C. Prior to sequencing, the PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany). The fragment sizes of the PCR products were determined after electrophoresis on a 1% agarose gel stained with ethidium bromide and visualized under UV illumination.

2.5   Restriction enzyme digestion

The RFLP profiles of isolates included in this study were obtained by digesting the IGS-1 amplicons with the restriction endonuclease *Alu*I (HARRINGTON and WINGFIELD 1995). IGS-1 amplicons were digested by adding 3 U of *Alu*I restriction endonuclease to 18 μl of unpurified PCR product. Digestion was allowed to occur overnight at 37°C. DNA fragments were separated on a 3% (w/v) agarose gel (Promega) stained with ethidium bromide and profiles were visualized under UV illumination. A 100 base molecular weight marker was used to determine the fragment sizes. The absolute fragment sizes were determined using the programme Gelreader 20.5 (National Center, Supercomputing Applications, University of Illinois at Urbana-Champaign, 1991). RFLP patterns and sizes of IGS-1 amplicons for the Ethiopian *Armillaria* isolates were compared with those of *Armillaria* spp. published by COETZEE et al. (2000).

2.6   Cloning

It was not possible to sequence the IGS-1 amplicons directly and they were subsequently cloned to resolve this problem. Ligation of the PCR products was conducted using the pGEM® T Easy Vector System (Promega), 2X Rapid Ligation Buffer, T4 DNA Ligase, PCR products and deionized water according to the protocols outlined by the manufacturer. This reaction was incubated for 1 h at room temperature. For transformation, JM109 High Efficiency Competent cells provided with the pGEM® T EASY Vector System II were used. Two microlitres of the ligation reactions were transferred to 1.5-ml Eppendorf tubes and 25 μl competent cell solution added to each tube. Isolation of recombinant plasmid DNA was accomplished using a standard plasmid miniprep procedure, using the instructions provided by the company.

2.7   DNA sequencing

Plasmid DNA was used as template to sequence the inserted IGS-1 region of the *Armillaria* samples. DNA sequences were determined using an automated (ABI PRISM™ 3100; Applied Biosystems/HITACHI, Foster City, California, USA) DNA sequencer. The inserted region was sequenced in both directions using primers T7 (5′-ATT ATG CTG AGT GAT ATC CC-3′) and SP6 (5′-ATT TAG GTG ACA CTA TAG AA-3′) (Promega 1999). The sequencing reactions were prepared using the Big Dye sequence system (ABI Advanced Biotechnology Institute, Perkin Elmer) as recommended in the manufacturer's protocols.

2.8   Analysis of DNA sequence data

Sequence Navigator version 1.01 (ABI PRISM™) was used to manually align the sequence data by inserting gaps. Analysis of the sequence data was performed using PAUP* version 4.0b2 (Swofford 1998). In the sequence data analysis, indels of more than 1 base were excluded and substituted with multi-state characters and gaps treated as a fifth character. IGS-1 DNA sequences obtained in this study were aligned against the data matrix published by Coetzee et al. (2000) (Table 1). Phylogenetic trees were rooted to *A. heimii* as the monophyletic sister group to the taxa. Analyses were carried out using Neighbour-joining distance analysis and the total character difference was used to generate the tree. The confidence levels of the branching points were determined by 1000 bootstrap replicates.

3   Results

3.1   Sample collection and isolation

Symptoms of Armillaria root rot were found in plantations at Wondo Genet, Munesa Shashemene, Belete/Jima, Bedele and Aman/Mizan. These localities vary in altitude, mean annual temperature and rainfall (Table 2). The characteristic symptoms of infection included groups of dead trees, wilting and chlorosis, as well as the occurrence of white mycelial fans under the bark of diseased trees. At Wondo Genet, mycelial mats were found on 10–13-year-old *P. patula* and *Acacia abyssinica* Hochest and fruiting bodies were obtained from native *Juniperus excelsa* Hochest. Ex. Endl. stumps. At the time of sample collection, the incidence of Armillaria root rot damage was most pronounced on *P. patula* at this location. At Aman, white mycelial growth was found on *Cordia alliodora* (Ruiz & Pav) Oken and *Cedrela odorata* L. trees. Masses of light brown rhizomorphs were found only on diseased *C. alliodora* trees in a research plot at Aman. At Belete forest, *Armillaria* isolates were obtained from *P. patula*, while in the Bedele area *Armillaria* isolates were obtained from *P. patula* and *A. abyssinica*.

Table 2. **Climatic descriptions and altitudinal range of the collection sites in Ethiopia**

Site	Host	Mean annual temperature (°C)	Mean annual rainfall (mm)	Altitude (m)	Remarks
Wondo Genet	*P. patula*, *A. abyssinica*, *J. excelsa*	19	1200	1890	Mycelium, basidiocarps
Jima/Belete	*P. patula*	20	1500	1750	Mycelium
Aman/Mizan	*C. alliodora*	24	2200	1350	Mycelium, rhizomorphs
Bedele	*P. patula*, *A. abysinica*	19	1800	2100	Mycelium

The causal fungus was successfully isolated from symptomatic trees and grown on the selective medium. A total of 32 isolates were collected from the different hosts. Of these 25 isolates were obtained from Wondo Genet, four from Bedele, one from Belete and two from Aman. In culture, the *Armillaria* isolates produced a sparse flat whitish mycelium with brown, cylindrical rhizomorphs produced in abundance.

3.2   Basidiocarp morphology

Ten basidiocarps were collected from the stumps of *J. excelsa* trees, in a plantation at Wondo Genet. These basidiocarps were used to tentatively identify the *Armillaria* sp. in this study. When the colour and size of the stipe and the pileus of the basidiocarps were considered, they were most similar to the basidiocarps found in South African pine plantations and considered to be *A. fuscipes* (COETZEE et al. 2000). The pileus of the fungus had an average diameter of 45 mm and the length of the stipes varied between 60 and 87 mm.

3.3   DNA amplification

The IGS regions of all *Armillaria* isolates from Ethiopia were successfully amplified with primers P-1 and 5S-2B. The PCR products of all *Armillaria* isolates used in this study yielded fragments of approximately 1200 bp. This PCR fragment size is similar to that published for *A. fuscipes* (COETZEE et al. 2000).

3.4   Restriction enzyme digestion

*Alu*I restriction digestion of PCR amplicons generated identical fragment patterns for all isolates. Three distinct bands with sizes of approximately 370, 249 and 94 bp were obtained. Comparison of RFLP profiles of the Ethiopian *Armillaria* isolates with previously published profiles for African *Armillaria* did not exactly match that of any other *Armillaria* spp. However, the RFLP patterns of *Armillaria* isolates from Ethiopia were most similar to *A. fuscipes* (COETZEE et al. 2000) and group I isolates, considered to represent *A. heimii* by OTIENO et al. (2003) (Table 3).

3.5   DNA sequencing

Before alignment, the IGS sequence of the *Armillaria* isolates from Ethiopia varied between 1056 and 1100 bp. A Blast search using the IGS-1 and 5S gene sequences for these isolates against sequences in GenBank [National Centre for Biotechnology information (NCBI), US National Institute of Health Bethesda, (http://www.ncbi.nlm.Nih.gov/ BLAST)], showed that the DNA sequences of *Armillaria* isolates from Ethiopia were most similar to those identified as *A. fuscipes* and *A. heimii*. Therefore, the DNA sequences

Table 3. **Comparison of RFLP sizes of *Armillaria* isolates**

Ethiopia	*A. fuscipes*[1]	*A. heimii*[1,2]	*A. heimii* (group I)[2]
370	365	530	380
249	245	220	245
94	135	175	135

[1]Data obtained from COETZEE et al. (2000).
[2]Data obtained from OTIENO et al. (2003).

of the Ethiopian *Armillaria* isolates were aligned with these two species (COETZEE et al. 2000). A total of 1247 characters were obtained for analysis after manual alignment.

3.6   Analysis of DNA sequence data

The *Armillaria* isolates used in this study formed two main groups in a Neighbour-joining tree (Fig. 1). Sequences of *Armillaria* isolates from South Africa and La Reunion, which were previously identified as *A. fuscipes* (COETZEE et al. 2000) grouped together with a bootstrap support of 90%. The *Armillaria* isolates from Ethiopia resided in a separate cluster with 72% bootstrap support. The Ethiopian *Armillaria* isolates grouped separately from those representing *A. heimii*, and although there were some differences, they were most closely related to *A. fuscipes*. The Ethiopian isolates differed from *A. fuscipes* in having several indels. Isolate CMW8971 differed from *A. fuscipes* with only 11 bp indels, of
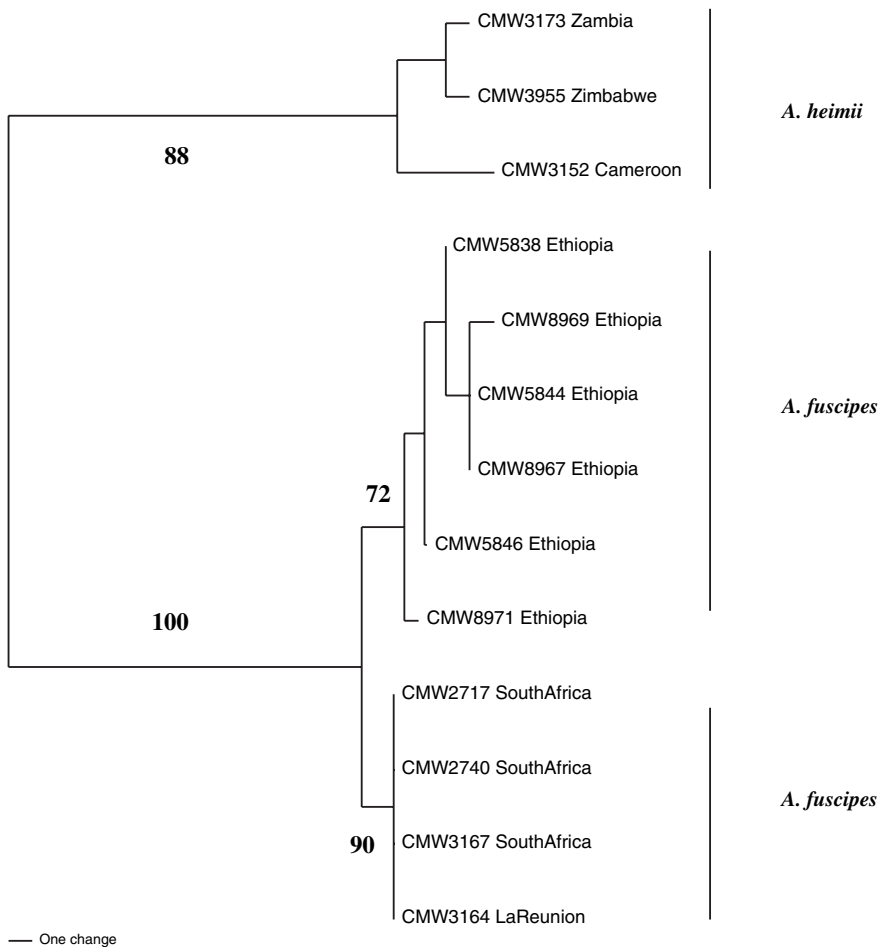

Fig. 1. Phylogram generated from Neighbour-joining analysis of the IGS-1 sequence data used in this study. Bootstrap values are shown on the branch

which 7 bp are deletions, while other Ethiopian isolates showed more variation. The most notable of these were isolates CMW5838 and CMW5846, which have 16 bp deletions, whereas isolates CMW5844, CMW8967 and CMW8969 have 33 bp deletions and contain one restriction site at position nine. Despite these differences, the Ethiopian isolates grouped in the *A. fuscipes* clade with a bootstrap of 100% and separately from *A. heimii*.

4 Discussion

Recently, the importance of plantation forestry diseases in Ethiopia has been afforded considerable attention. This study forms part of the first comprehensive plantation disease survey conducted in the country (ALEMU et al. 2003), and represents the first extensive survey of Armillaria root rot in Ethiopian forest plantations. Our data clearly show that the dominant *Armillaria* sp. causing root rot and death in plantations is very similar to the species found in South African pine plantations, considered to be *A. fuscipes* (COETZEE et al. 2000). This is the first report of *A. fuscipes* from Ethiopia and also greatly extends its known host range.

The taxonomy of *A. fuscipes* poses a problem to mycologists and plant pathologists. PETCH (1909) described this species in the early 1900s from tea plantations in Ceylon (Sri Lanka). Later, HEIM (1963, 1967) described *Clitocybe (Armillariella) elegans* R.Heim from Cameroon, Ivory Coast, Madagascar and New Guinea. PEGLER (1977) found *C. elegans* in Tanzania and Uganda, transferred it to the genus *Armillaria* and changed the epithet to *heimii*. CHANDRA and WATLING (1981), after analysing microscopic characteristics of the type specimen of *A. fuscipes*, suggested that *A. heimii* and *A. fuscipes* are conspecific, but deferred from reducing the two taxa to synonymy. PEGLER (1986) later reduced *A. heimii* to synonymy with *A. fuscipes*. He retained the older name *A. fuscipes*, and proposed that the fungus was introduced from Africa into Sri Lanka on tea. KILE and WATLING (1988), examined the microscopic morphology of the type specimen of *A. heimii* and came to the same conclusion as PEGLER (1986) that *A. heimii* and *A. fuscipes* represent the same species. Despite these results, the name *A. heimii* has continued to be used in some studies of *Armillaria* spp. from Africa. Cultural and interfertility tests have not been carried out for isolates identified as *A. heimii* and *A. fuscipes*, but from a nomenclatural perspective, there is no reason why the name *A. heimii* should be used in preference to the older *A. fuscipes*.

Sexual and intraspecific somatic incompatibility tests between isolates thought to represent *A. heimii* from various countries in Africa have indicated that they are conspecific (ABOMO-NDONGO and GUILLAUMIN 1997; ABOMO-NDONGO et al. 1997). However, these isolates are highly variable in terms of their sexual systems, reactions to temperature, culture morphology and other criteria. Consequently *A. heimii* has been viewed as a complex species (MOHAMMED et al. 1989). Recently, COETZEE et al. (2000) used IGS-1 DNA sequence data to study *Armillaria* isolates from Southern Africa and separated those identified as *A. heimii* into two monophyletic groups. The first group was designated as *A. fuscipes* based on the similarity between basidiocarps found in South Africa and the morphological descriptions of this species (PETCH 1909; CHANDRA and WATLING 1981; PEGLER 1986). The second monophyletic group included an isolate from Cameroon and this was considered to represent *A. heimii*, mainly based on the fact that HEIM (1963) described this species from that country. This differentiation of the two species is thus in conflict with the sexual and somatic compatibility studies between isolates (ABOMO-NDONGO and GUILLAUMIN 1997; ABOMO-NDONGO et al. 1997). Although the appropriate names for the species might be debated, we consider the phylogenetic classification of COETZEE et al. (2000) as an important basis to distinguish between the fungi residing in the two monophyletic groups representing what has traditionally been considered as *A. heimii sensu lato*. Our identification of *A. fuscipes* from Ethiopia in this study is also based on this view. However, a comparison with isolates of *A. fuscipes* from Sri Lanka would be necessary to definitely establish the identity of this taxon.

Seasonal availability of the basidiocarps has limited the use of basidiocarp morphology for species identification in this study. Very few fruiting structures were obtained and these were only from Wondo Genet. The morphology of these basidiocarps was very similar to those described for *A. fuscipes* from South Africa (PETCH 1909; CHANDRA and WATLING 1981; PEGLER 1986; COETZEE et al. 2000). However, when looking at published descriptions for *A. fuscipes* and *A. heimii* it is clear that there is considerable overlap in the size of sporocarps of these fungi (PETCH 1909; HEIM 1963, 1967; PEGLER 1977, 1986), making it impossible to identify Ethiopian isolates based only on morphology. It was not possible to make a culture linked to the basidiocarps in question, but the proximity of the dying trees to others from which cultures and DNA sequences were obtained provides strong circumstantial evidence that they represent the same fungus.

COETZEE et al. (2000) showed that the 5S ribosomal rRNA gene of African *A. fuscipes* and *A. heimii* isolates are in opposite orientation in comparison with other *Armillaria* spp. Because of this, primers used to amplify the IGS-1 region of non-African isolates, failed to amplify the IGS-1 region of African *Armillaria* isolates (COETZEE et al. 2000). Therefore, primer 5S-2B was used to amplify the IGS-1 region of African *Armillaria* spp. The IGS-1 region of the *Armillaria* isolates from Ethiopia was successfully amplified with primers P-1 and 5S-2B, indicating that the 5S gene of Ethiopian *Armillaria* isolates has the same orientation as that of other African isolates.

The RFLP patterns of all Ethiopian *Armillaria* isolates differed from those of *A. fuscipes* and all other *Armillaria* spp. This difference in RFLP pattern was supported by DNA sequence data, which showed the deletion of indels within one of the restriction sites. Although the Ethiopian isolates grouped closely to *A. fuscipes*, they formed a separate sub-clade. This suggests that the *Armillaria* samples from Ethiopia could represent a distinct species, closely related to *A. fuscipes*. Macro- and micro-morphological comparison of the basidiocarps will be essential to understand the significance of this variation.

A recent population biology study on *Armillaria* spp. in Ethiopia reported that *A. mellea* subspecies *nipponica* is responsible for root rot on hard-wood trees in the Jima and Kerita areas (OTA et al. 2000). In our study, an isolate from symptomatic *P. patula* trees near Jima produced the same RFLP profile as those of other *Armillaria* isolates that we identified as *A. fuscipes*. This suggests that the causative agent of Armillaria root rot on *P. patula* around Jima is identical to other isolates included in our study and that it also represents *A. fuscipes*. Hence, the combined results of OTA et al. (2000) and this study suggest that more than one *Armillaria* spp. might be involved in causing Armillaria root rot in Ethiopia.

The results of this study show that *Armillaria* root rot not only affects *P. patula*, but that it also kills *Cordia alliodora* and *C. odorata* trees planted in research plots at Aman, near Mizan. The fungus was also found on *A. abyssinica* and *J. excelsa*, species native to Ethiopia and growing in the *Pinus* plantations at Bedele and Wondo Genet. Most plantations in Ethiopia consist of exotic species planted on sites previously occupied by indigenous hardwoods. This suggests that stumps of the native hardwoods could be sources of the initial inoculum needed to infect exotic species. Planting of *Pinus* spp. in these areas should be avoided. The occurrence of the same *Armillaria* sp. on these different tree species implies that this pathogen could be damaging to a wider range of trees in the country. In order to better understand the distribution, diversity and host range of *Armillaria* spp., as well as to investigate its importance in other plantation areas, this study should be extended to other parts of Ethiopia.

Résumé

Identification spécifique de l'Armillaire agent de pourridié dans les plantations d'Ethiopie

Le pourridié à Armillaire est une maladie bien connue affectant une large gamme de plantes dans le monde entier. En Ethiopie, la maladie a été mentionnée sur *Pinus* spp., *Coffea arabica* et un certain nombre d'espèces indigènes feuillues. L'agent causal de la maladie a été identifié comme *Armillaria mellea*, une espèce maintenant décrite comme un complexe de nombreux différents taxons. Le but de cette étude était de déterminer l'importance du pourridié à Armillaire et l'identité des espèces dans les plantations d'Ethiopie. Dans le cadre d'une campagne de suivi de l'état sanitaire des plantations en 2000 et 2001, des isolats ont été récoltés en plantations à et autour de Munessa Shashemene, Wondo Genet, Jima, Mizan et Bedele, dans le sud et le sud-ouest de l'Ethiopie. Des carpophores ont été récoltés et leur morphologie étudiée. L'identification morphologique a été confirmée par séquençage de la région IGS1 de l'opéron ribosomique rRNA et comparaison des données avec les séquences publiées d'*Armillaria* spp. Les isolats d'*Armillaria* provenaient d'*Acacia abyssinica*, *Pinus patula*, *Cedrela odorata* et *Cordia alliodora*. Des carpophores ont également été trouvés sur des souches de l'espèce indigène *Juniperus excelsa*. La morphologie des carpophores et les données de séquence suggèrent que le champignon présent en Ethiopie est le même que l'agent causal de la maladie des pins en Afrique du Sud et précédemment identifié comme *A. fuscipes*. Cette identification a été confirmée pour tous les isolats, sur la base des données de séquence. *A. fuscipes* est connu pour être commun en Afrique du Sud. Sa large présence en Ethiopie suggère qu'il serait également la cause majeure de pourridié à Armillaire dans ce pays.

Zusammenfassung

Identifizierung des Erregers der Armillaria-Wurzelfäule aus Plantagen in Äthiopien

Die Armillaria-Wurzelfäule ist eine weltweit bedeutende Pflanzenerkrankung. In Äthiopien wurde sie an *Pinus* spp., *Coffea arabica* und verschiedenen einheimischen Laubgehölzen nachgewiesen. Als Ursache wurde *Armillaria mellea* benannt, eine Art, die heute als ein Komplex aus vielen verschiedenen Taxa betrachtet wird. Das Ziel der Untersuchung war es, den Befall mit *Armillaria* in Plantagen in Äthiopien zu erfassen und die Identität der beteiligten *Armillaria*-Taxa abzuklären. Während einer Inventur von Pflanzenkrankheiten in den Jahren 2000 und 2001 wurden in Plantagen bei Munessa Shashemene, Wondo Genet, Jima, Mizan und Bedele in Süd- und Südwest-Äthiopien Proben gesammelt. Die Fruchtkörper wurden morphologisch untersucht. Die morphologische Bestimmung wurde durch die Sequenzierung der IGS-1-Region des ribosomalen rRNA-Operons und durch Vergleich der Daten mit publizierten Sequenzen von *Armillaria* spp. überprüft. Die *Armillaria*-Isolate wurden von folgenden Bäumen gewonnen: *Acacia abyssinica*, *Pinus patula*, *Cedrela odorata* und *Cordia alliodora*. Die Fruchtkörper kamen auf Stümpfen des einheimischen *Juniperus excelsa* vor. Die Fruchtkörper-Morphologie und die Sequenzdaten zeigen, dass der Pilz in Äthiopien einer Art sehr nahe steht, die Schäden an *Pinus* spp. in Südafrika verursacht, und die als *A. fuscipes* identifiziert worden war. Dieser Befund wurde auf der Basis der Sequenzdaten für alle Isolate bestätigt. *Armillaria fuscipes* kommt in Südafrika häufig vor. Die weite Verbreitung dieser Art in Äthiopien lässt darauf schliessen, dass sie hier wesentlich zur *Armillaria*-Wurzelfäule beiträgt.

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