

The root rot fungus *Armillaria mellea* introduced into South Africa by early Dutch settlers

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Abstract

Dead and dying oak (*Quercus*) and numerous other woody ornamental trees and shrubs showing signs and symptoms of *Armillaria* root rot were identified in the Company Gardens, Cape Town, South Africa, which were established in the mid-1600s by the Dutch East Indies Trading Company. Nineteen isolates from dying trees or from mushrooms were collected and analysed to identify and characterize the *Armillaria* sp. responsible for the disease. The *AluI* digestion of the amplified product of the first intergenic spacer region (IGS-1) of the rRNA operon of 19 isolates from the Company Gardens was identical to that of some of the European isolates of *A. mellea* s. s. The IGS-1 region and the internal transcribed spacers (ITS) were sequenced for some of the Cape Town isolates. Phylogenetic analyses placed the Cape Town isolates in the European clade of *A. mellea*, which is distinct from the Asian and North American clades of this species. Identification based on sexual compatibility was conducted using *A. mellea* tester strains in diploid–haploid pairings, which showed some compatibility between the Cape Town isolates and testers from Europe. Somatic compatibility tests (diploid–diploid pairings) and DNA fingerprinting with multilocus, microsatellite probes indicated that the Cape Town isolates were genetically identical and may have resulted from vegetative (clonal) spread from a single focus in the centre of the original Company Gardens (c. 1652). The colonized area is at least 345 m in diameter. Assuming a linear spread rate underground of 0.3 m/year to 1.6 m/year, the genet (clone) was estimated to be between 108 and 575 years old. These data suggest that *A. mellea* was introduced into Cape Town from Europe, perhaps on potted plants, such as grapes or citrus, planted in the Company Gardens more than 300 years ago.

Keywords: *Armillaria*, genet, IGS-1, ITS, phylogeny, RFLPs, rRNA operon

Received 18 May 2000; revision received 2 September 2000; accepted 12 September 2000

Introduction

With the increasing globalization of the world's economies and international trade, there is mounting concern over the movement of plant pathogens and other pests (Harrington & Wingfield 1998). Phylogenetics and population genetics allow for tracking the movement of fungal populations and the identification of their putative origins. Some noteworthy examples of introductions of important

tree pathogens that have been studied with genetic tools include *Ophiostoma ulmi* and *O. novo-ulmi* (the Dutch elm disease pathogens) in North America and Europe (Brasier & Mehrotra 1995), *Cryphonectria parasitica* (the chestnut blight fungus) in North America and Europe (Milgroom *et al.* 1992), and *Cronartium ribicola* (the white blister rust fungus) in North America and Canada (Et-touil *et al.* 1999). The dispersal of the potato blight pathogen, *Phytophthora infestans*, in the 1800s and 1900s has also been studied with genetic tools (Goodwin 1997). In this study, we examined an anomalous occurrence of the root-infecting fungus *Armillaria mellea* in central Cape Town, South Africa.

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Armillaria (Fr.:Fr.) Staude (Agaricales, Basidiomycetes) is a genus of root pathogens on woody plants throughout temperate as well as most tropical regions of the world (Hood *et al.* 1991). Many of the species of *Armillaria* are serious pathogens of a wide range of native and planted conifer and hardwood trees and shrubs in forests, orchards and gardens (Raabe 1962; Hood *et al.* 1991). Many plant pathologists had attributed *Armillaria* root rot to a presumed pleiomorphic species, *A. mellea* (Vahl. Fr.) Kummer (Singer 1956), but this misconception was resolved in the late 1970s with the recognition of 'biological species' within *A. mellea* by Korhonen (1978) and Anderson & Ullrich (1979). Numerous species are now recognized (Volk & Burdsall 1995), with *A. mellea sensu stricto* perhaps the most important species on ornamental and orchard crops in the Northern Hemisphere.

Although the species of *Armillaria* in southern Africa are not well characterized, *Armillaria* root rot has been recorded on a variety of hosts in South Africa (Doidge *et al.* 1953), including *Eucalyptus* and *Pinus* spp. from the northern parts of the country (Wingfield & Knox-Davies 1980). It was also reported on *E. globulus* in the southern coastal area of South Africa, in the Eastern Cape in 1915, but these reports were questioned (Lundquist 1987). Isolates from pines and other woody hosts in the northern part of the country have been identified as *A. fuscipes* Petch (Coetzee *et al.* 2000b).

Early in 1996, *Armillaria* root rot was observed on diseased oak (*Quercus* spp.) trees in the historically important Company Gardens in Cape Town, South Africa. Oak trees were first planted there along Government Avenue in 1795, but woody plants were introduced to these gardens from Europe as early as the mid-1600s by the Dutch East Indies Trading Company. This is the only record of *Armillaria* root rot from southwestern South Africa, and because a number of fruit and ornamental tree species had been introduced to these gardens, there was a possibility that a species of *Armillaria* had been introduced in potted plants. The objective of this study was to identify the species of *Armillaria* in the Company Gardens based on morphology, molecular comparisons, and mating tests; to determine the phylogenetic relationships between the Cape Town fungus and other *Armillaria* species; and to determine the number and size of the genets of *Armillaria* using somatic incompatibility tests and DNA fingerprinting.

Materials and methods

Fungal isolation

Dead and dying trees were inspected for the presence of mycelial fans between the bark and wood. In addition, numerous basidiomes of *Armillaria* were found at the base of dead and dying trees throughout the garden in May

1997. Small pieces (2 mm²) of the white mycelial fans or portions of the basidiomes were surface sterilized, placed on selective benomyl-dichloran (BD) medium (Harrington *et al.* 1992) and incubated at 22 °C for 2 weeks in the dark. The tips of developing rhizomorphs in BD medium were transferred to MYEA (2% Biolab malt extract, 0.2% Biolab yeast extract and 1.5% Biolab agar) plates and incubated at 22 °C for 2 weeks in the dark. Attempts to recover single-basidiospore progeny from the basidiomes failed. Collection information for the other *Armillaria* isolates used in this study can be found in Coetzee *et al.* (2000a). A representative collection of dried basidiomes has been deposited in the National Collection of Fungi, Pretoria, South Africa (PREM 57132). The cultures used in this study are stored in the culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa and a subset with the Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands.

Diploid-haploid compatibility tests

Diploid-haploid pairings for species identification were made on 1 or 2% MYEA medium (Rizzo & Harrington 1992). Mycelial plugs of the diploid isolates of *Armillaria* from Cape Town, England (B176 and B201), North America (B927 and B253), Japan (B731) and South Korea (B608) were paired with haploid tester strains of *A. mellea* from France (B525) and North America (B497) by placing plugs from each culture 10 mm apart. The cultures were incubated at 22 °C in the dark for 6 weeks, after which three plugs of mycelium were taken from the haploid tester side of the paired cultures. One plug was taken from close to the zone of confrontation, a second from the middle of the haploid tester, and a third from the advancing margin of the tester. These subcultures were placed on 2% MYEA medium and incubated at 22 °C in the dark for 2 weeks. The mycelial morphology of the subcultures was compared with that of the haploid testers, which remained fluffy and white if they were not diploidized. Diploidization of the mycelium of the haploid testers was suggested if the subcultures were flatter or darker than the unpaired haploid tester strains.

DNA extraction for polymerase chain reaction (PCR)

Isolates were grown in liquid MYE (2% Biolab malt extract and 0.3% Biolab yeast extract) at 22 °C in the dark for 2 weeks. Mycelium was harvested by centrifugation (17 900g, 20 min) and freeze dried. DNA was extracted using a modification (Coetzee *et al.* 2000a) of the DNA extraction protocol of Raeder & Broda (1985). DNA quantification was performed by ultraviolet spectroscopy using a Beckman Du Series 7500 spectrophotometer.

Amplification of the first intergenic spacer (IGS-1) and internal transcribed spacer (ITS) regions

Extracted DNA was used as a template in PCR to amplify the IGS-1 and ITS of the rRNA operon. The IGS region was amplified using primers P-1 (5'-TTGCAGACGACT-TGAATGG-3') (Hsiao 1996) and O-1 (5'-AGTCCTATGG-CCGTGGAT-3') (Duchesne & Anderson 1990). Primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-GCTGCGTTCTTCATCGATGC-3') were used to amplify the ITS region (White *et al.* 1990). All PCR reactions were carried out on a Hybaid Omnigene Temperature Cycler. The PCR reaction mixture and reaction conditions were as described in Coetzee *et al.* (2000a).

Restriction enzyme digestion of PCR products

Amplified IGS PCR products in the PCR mixture (20 µL) were digested with the restriction endonuclease *AluI* (10 U) (Harrington & Wingfield 1995) in the presence of 1 µL (10 mg/mL) of acetylated bovine serum albumin (BSA; Promega). DNA was digested at 37 °C for 6 h. The resulting PCR–restriction fragment length polymorphism (RFLP) fragments were separated on a 3% agarose gel (Promega) stained with ethidium bromide and photographed under ultraviolet illumination.

DNA sequencing

The IGS-1 and ITS DNA sequences of two *A. mellea* isolates (CMW3975 and CMW3978) from the Company Gardens were determined using an ABI PRISM™ 377 DNA sequencer. The IGS-1 region was sequenced as described previously (Coetzee *et al.* 2000a) using primers P-1 and O-1 as well as two internal primers, MCO-2 (5'-CTTGATATC-GGCCTTATGG-3') and MCO-2R (5'-CCATAAGGCCGAT-ATCAAG-3'). The ITS region was similarly sequenced with the primers ITS1, ITS4, CS2 (5'-CAATGTGCGTTCAAA-GATTTCG-3') and CS3 (5'-CGAATCTTTGAACGCACTTG-3') (Visser *et al.* 1995).

Sequence analysis

Sequences of the Cape Town isolates were aligned with those of *A. mellea* s. s. from across the Northern Hemisphere (Coetzee *et al.* 2000a) by inserting gaps, and a phylogenetic analysis of these data was conducted using PAUP* version 4.1b (Swofford 1998). Parsimony analysis utilized heuristic searches with TBR (tree bisection reconnection) swapping and MULPAR effective. Gaps were treated as a 'fifth character' (newstate). Indels (insertions/deletions) of more than one character were treated as a single evolutionary event by binary scoring the presence (as 1) or absence (as 0) of an insertion. Ambiguously aligned regions in the

IGS-1 and ITS DNA sequences were excluded from the analysis, and changes that occurred with high frequency were down weighted by successive weighting according to the mean consistency index (CI) of each character (Coetzee *et al.* 2000a). Bootstrapping was performed to assess the confidence of the branching points. Trees generated from the analysis were rooted to *A. ostoyae* and *A. sinapina* as the outgroup taxa (Coetzee *et al.* 2000a).

DNA fingerprinting

Microsatellite fingerprinting was conducted on 13 European isolates of *A. mellea* and 16 isolates from the Company Gardens. The isolates were grown on MYEA plates overlaid with one layer of sterile Spectra/Por membrane MWCO 6–8000 (Spectrum Medical Industries) at room temperature (approximately 21 °C) until the colonies were approximately 4 cm in diameter. It was found that when grown in this manner, the mycelium and medium did not turn dark brown and fewer rhizomorphs formed than when the fungus was allowed to grow directly into MYEA. The DNA extracted from the mycelium over membranes proved to be of higher quality (less degraded) and was more suited for DNA fingerprinting. The mycelial mats were frozen with liquid nitrogen and ground with a mortar and pestle to a fine powder. Extractions were performed as described by DeScenzo & Harrington (1994), except that phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1) extractions were conducted after the first spin.

Extracted DNA (25 µg) was digested with 125 U of *PstI* in a volume of 500 µL following the manufacturer's (Promega) recommendations. The digested DNA was brought to 0.4 M NaCl and precipitated with two volumes of ethanol, washed with two volumes of 70% ethanol, dried and resuspended in water to a final concentration of 200 ng/µL before electrophoresis. Hybridization (in gel) with the labelled oligonucleotide probes (CAT)₅ and (CAC)₅, washing, and electrophoresis followed the protocols of DeScenzo & Harrington (1994). In addition to the fingerprinting probes, the gels were hybridized to a ³²P-labelled lambda marker. The gels were then exposed to a phosphor screen for 2–5 days and scanned (Storm 840 Phosphor Imager, Molecular Dynamics).

Somatic incompatibility tests

The number of genotypes (genets) among the isolates of *Armillaria* from the Company Gardens was determined by somatic incompatibility interactions. Fourteen isolates from the Company Gardens and five diploid *A. mellea* isolates from Europe (B201, B1240, B1241, B1243 and B1247) were paired in all combinations on MYEA in 90 mm diameter plastic Petri dishes. The plates were examined

after 2 months for the formation of barrage lines as seen from the underside of the plates (Harrington *et al.* 1992). These lines formed between isolates of differing genotype and were initially white, later turning brown in most cases (Shaw & Roth 1976; Korhonen 1978; Rizzo *et al.* 1998).

Results

Fungal identification

The basidiomes found in the Company Gardens in May 1997 were typical for those of *Armillaria mellea* s. s.; the caps were honey coloured, there was a prominent annulus, and the stipes tapered. Also typical of *A. mellea* s. s., numerous ornamental species were severely infected, as indicated by crown die-back and dead roots with white mycelial fans under the bark. Substantial mortality of woody species had occurred. Host genera with signs of *Armillaria* root rot included *Strelitzia*, *Quercus*, *Hydrengea*, *Morus*, *Albizia*, *Ficus*, and *Aesculus*. Mycelial fans were detected on 20 different trees. Isolates were obtained from 19 trees: one from *Aesculus* sp., two from *Hydrengea* sp., 11 from *Quercus* spp., one from a basidiome, and four isolates from unknown tree species.

The highest concentration of infected trees was observed in the vicinity of trees 4 and 5 (Fig. 1). Searches were made for diseased trees surrounding the area shown in Fig. 1, but infected trees were only observed around the Company Gardens, the garden at the House of Parliament, and along Government Avenue. The absence of *Armillaria* beyond these garden areas may be attributed to the presence of surrounding streets and buildings that prevented the underground spread of the fungus through root contacts and rhizomorphs.

Diploid-haploid compatibility tests

No compatible interaction (no diploidization of haploid testers) was observed between the diploid isolates from Cape Town and the haploid tester strains of *A. mellea* s. s. from eastern (B497) or western North America (B931). The original culture morphology of the North American tester strains was white with fluffy aerial mycelium, and this morphology was retained in the subcultures taken after pairing with the diploid Cape Town isolates.

A positive reaction (diploidization) was found between diploid isolates from Cape Town and the *A. mellea* s. s. haploid tester strain from France (B525). The tester B525 was white and fluffy, with abundant aerial mycelium, but subcultures of isolate B525 had depressed crusts and pigmented mycelium, indicating successful diploidization, after being paired with each of 10 isolates from Cape Town.

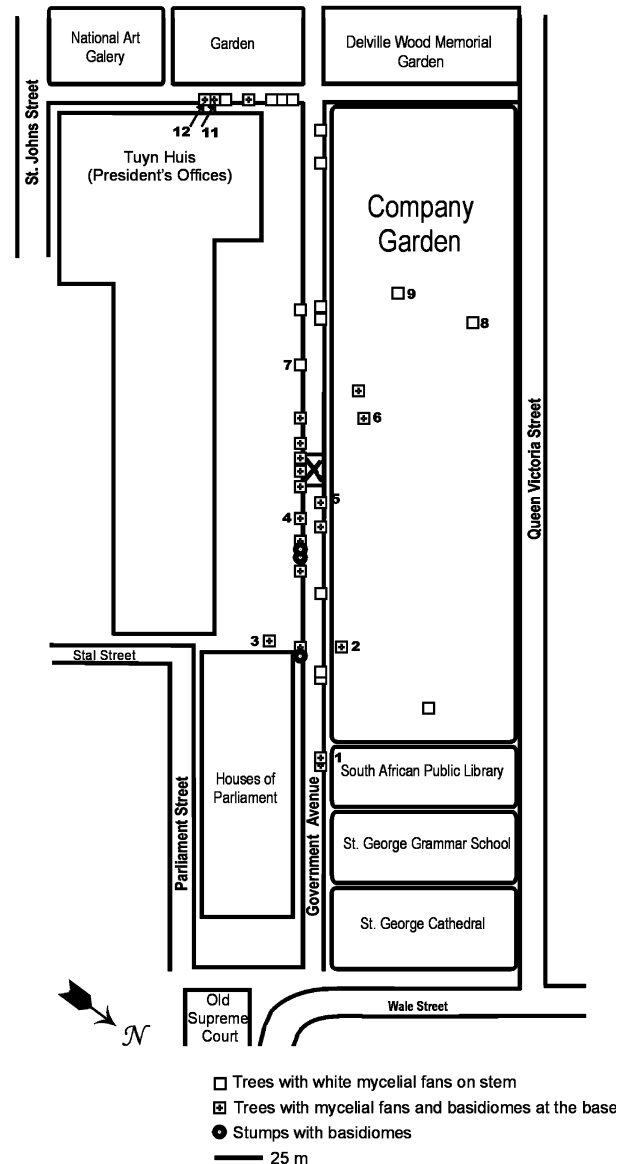


Fig. 1 Map of the Company Gardens area showing the relative position of trees with signs of *Armillaria*. The numbers given next to tree locations are where isolates of *Armillaria* were collected: (1) CMW3341, (2) CMW4303, (3) CMW3340, (4) CMW3788, (5) CMW3787, (6) CMW4304, (7) CMW4307, (8) CMW3328, (9) CMW4302, (10) CMW4306 and (11) CMW4305.

PCR/RFLP analysis

The 19 isolates from the Company Gardens had identical IGS-1/RFLP profiles. Fragment sizes of 215, 175 and 150 bp in length were observed after digestion with *AluI*. This RFLP profile was identical to that observed for some European isolates of *A. mellea* s. s. (Harrington & Wingfield 1995; Coetzee 1997).

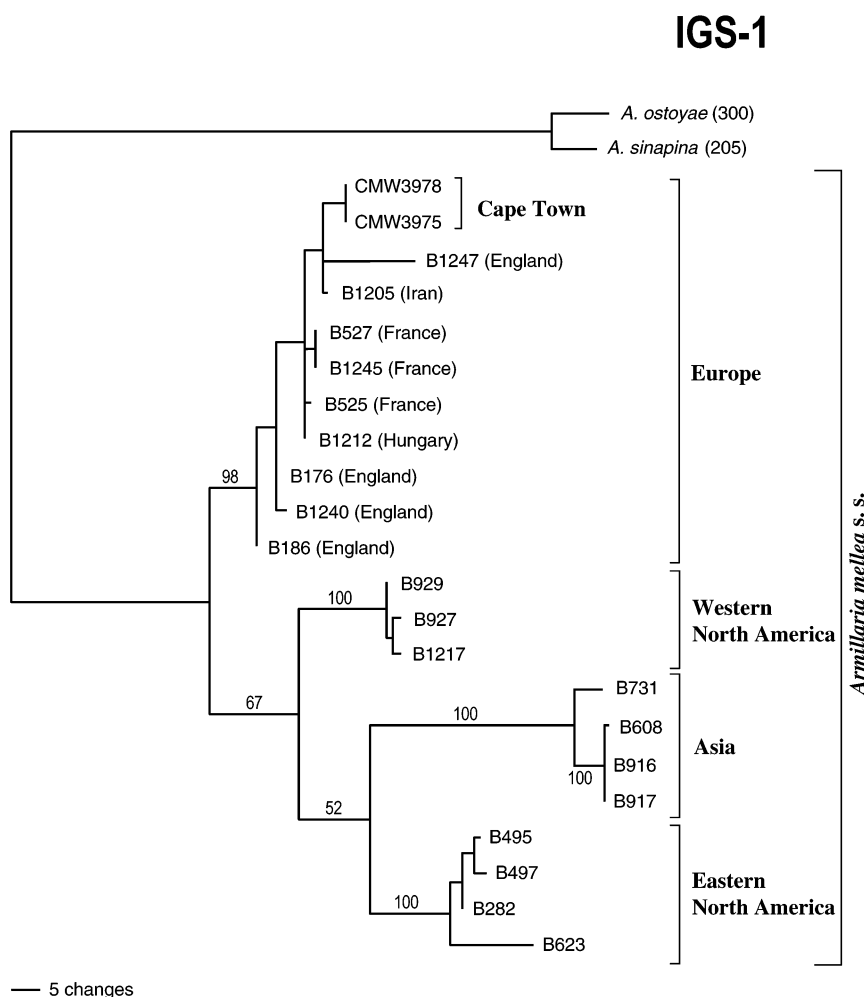


Fig. 2 One of the five most-parsimonious trees generated with a heuristic search of first intergenic spacer region (IGS-1) DNA sequence data of isolates of *Armillaria mellea*. Confidence levels generated using bootstrap analysis (1000 replicates) are indicated above the branches of the tree. (GenBank numbers: CMW3975 = AF310327; CMW3978 = AF310326.)

Sequence data and analysis

The sequences of the IGS-1 region for the two Cape Town isolates (CMW3975 and CMW3978) were identical. These sequences and those of *A. mellea* s. s. isolates from Asia, Europe, eastern North America, and western North America (Coetzee *et al.* 2000a) were aligned by inserting gaps. A total alignment of 781 characters was obtained, but 27 indels of more than one base were excluded, which reduced the nucleotide data set to 614 characters. A total of 641 characters (27 binary-coded indels included) was used in parsimony analysis. Of these, 375 characters were constant and 79 characters were parsimony uninformative.

Analysis of the IGS-1 DNA sequence data through heuristic searches yielded five most-parsimonious trees with a length of 317 steps (Fig. 2). The topologies of the trees were similar with regard to the position of the inferred clades relative to one another. The CI and retention index (RI) for the trees were 0.855 and 0.926, respectively. As found previously (Coetzee *et al.* 2000a), parsimony analysis of the IGS-1 region grouped *A. mellea* s. s. into four lineages:

European, Asian, western North American, and eastern North American. The Cape Town isolates grouped within the European lineage.

The ITS sequences of isolates CMW3975 and CMW3978 from Cape Town were aligned with those published by Coetzee *et al.* (2000a) for *A. mellea*. A total alignment of 938 nucleotide characters was obtained. Replacement of 29 indels with binary characters reduced the data set by 161 characters. Ambiguously aligned data were excluded from the data set, leaving 716 characters (including binary-coded indels) for parsimony analysis. Of the 716 characters, 548 characters were constant, and 125 characters were parsimony uninformative.

Eighteen most-parsimonious trees of 254 steps were generated from the ITS data set (Fig. 3). In all phylogenetic trees, the Cape Town isolates grouped among the European isolates of *A. mellea* s. s. The ITS trees had similar topologies, but, like the IGS-1 trees, differed in the position of the Cape Town isolates relative to other isolates in the European clade and in their branch lengths. The CI and RI of the ITS trees were 0.87 and 0.929, respectively.

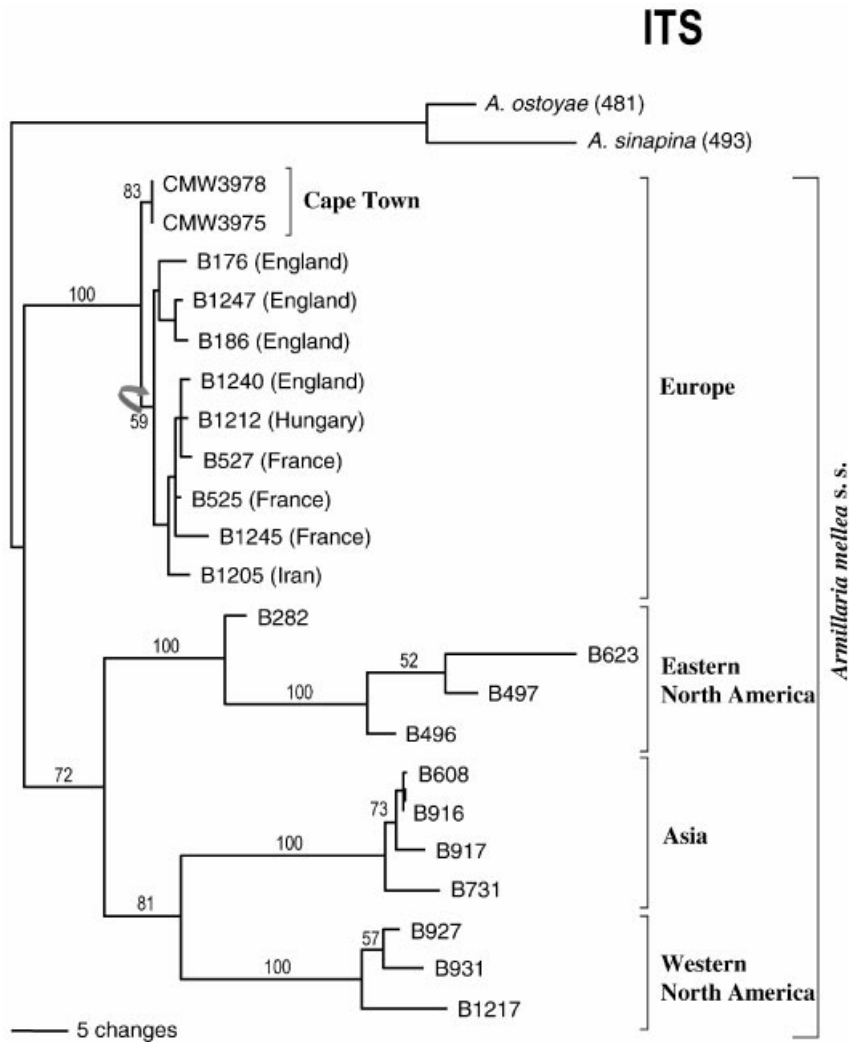


Fig. 3 Phylogenetic tree (one of the 18 most-parsimonious trees) generated after the reweighting of characters and a heuristic search of internal transcribed spacer (ITS) DNA sequence data of isolates of *Armillaria mellea*. Confidence levels generated using bootstrap analysis (1000 replicates) are indicated above the branches of the tree. (GenBank numbers: CMW3975 = AF310329; CMW3978 = AF310328.)

DNA fingerprinting

Each of the 13 European isolates that were tested had unique DNA fingerprints with both the (CAT)₅ and (CAC)₅ probes (Fig. 4), thus showing that the markers were highly polymorphic and sufficient to differentiate individual genotypes of *A. mellea*. The 16 tested isolates from the Company Gardens in Cape Town had identical fingerprints with both probes (Fig. 4), strongly suggesting that the Cape Town isolates represent a single genotype or genet.

Somatic incompatibility tests

When 14 Cape Town diploid isolates were paired in all combinations, they generally fused with no sign of barrage formation. However, faint barrage lines were sometimes observed when isolates of similar or identical genotypes

were paired. For instance, isolate CMW3328 formed a barrage line when paired with itself. In 91 pairings between Cape Town isolates, 81 showed no barrage line, indicating somatic compatibility. Ten pairings showed faint lines or strong barrage reactions suggestive of somatic incompatibility. In pairings between European isolates, no barrage lines formed when isolates B1240, B1241, B1243, and B1247 were paired with themselves, but a barrage line was seen when B201 was paired with itself, and faint barrage lines were seen in 10 of 10 pairings between different European isolates. In the 70 pairings between European and Cape Town isolates, 65 showed barrage lines, and five pairings showed no reaction or only faint lines.

Genet size and age

Genet size was determined by measuring the distance between the most northern and southern as well as the

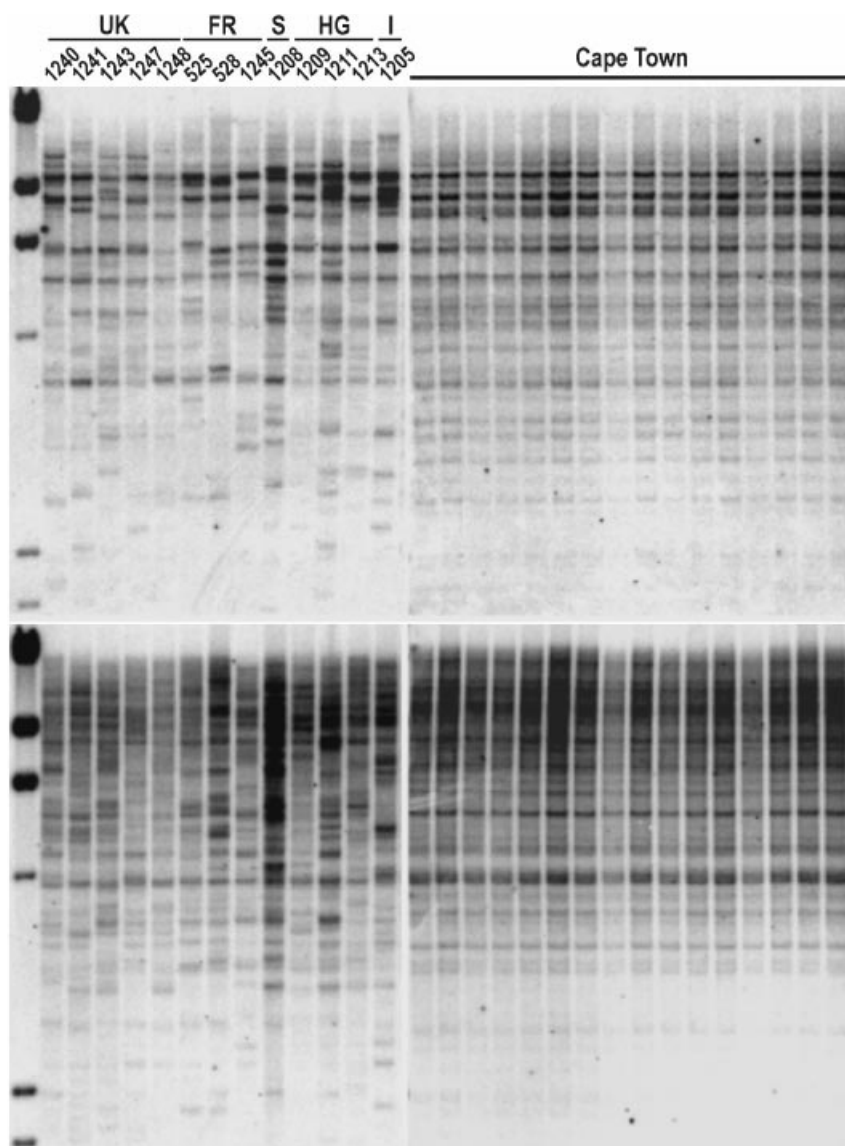


Fig. 4 DNA fingerprints of isolates of *Armillaria mellea* from Europe (UK, France, Slovakia, Hungary and Iran) and Cape Town. The top gels were probed with $(CAT)_5$ and the bottom gels with $(CAC)_5$. The first lanes are DNA markers (23.1, 9.4, 6.6, 2.3 and 2.0 Kb).

most western and eastern points at which mycelial fans or basidiomes were observed. The size of the genet was conservatively estimated at 345 m from the most southern to the most northern point and 120 m from the most eastern to the most western point (Fig. 1).

The age of the genet was calculated by using empirical growth rates of 0.3 or 1.6 m/year estimated for underground spread rates of *A. mellea* in earlier studies (Kable 1974; Rizzo *et al.* 1998). The original point of introduction is unknown; therefore, the age of the genet was estimated assuming a radial growth of 173 m from the putative centre of the present genet to the periphery. The minimum age of the genet was thus calculated to be 108 years and the maximum age of the genet to be 575 years. Assuming a radial spread rate of 0.5 m/year, the genet would be an estimated 345 years old in 1997.

Discussion

The disease syndrome found at the Company Gardens was typical for *Armillaria* root rot as caused by *Armillaria mellea* s. s. From the numerous stumps, it was evident that oaks and other trees had been dying for many decades. There were numerous oaks and other ornamental trees and shrubs showing typical symptoms of *Armillaria* root rot, such as crown die-back and discoloration of the foliage (Morrison 1981; Williams *et al.* 1986). Most trees with crown die-back had white mycelial fans characteristic of *Armillaria* under the bark (Morrison 1981; Williams *et al.* 1986). Rhizomorphs, although produced in culture after isolation, were not found in the soil or associated with the diseased trees. The absence of rhizomorphs in the field is common for *Armillaria* root rot caused by *A. mellea* s. s.

Identical IGS-1/RFLP profiles amongst the *Armillaria* isolates from the Company Gardens and those of *A. mellea* isolates from Europe (Harrington & Wingfield 1995; Coetzee 1997) suggested that the isolates were related to European strains of *A. mellea*. Analysis of the DNA sequence data for the IGS-1 and ITS regions grouped the Cape Town isolates with *A. mellea* from Europe, clearly showing it to be of the European geographical lineage (Coetzee *et al.* 2000a).

Basidiomes were present in abundance in May 1997. The morphology of the basidiomes was typical of *A. mellea* s. s. and consistent with that of the neotype from Copenhagen, Denmark (Watling *et al.* 1982). Some minor differences were found in the size of the caps, stipes and the basidiospores. Similar differences were also noted between the neotype and basidiomes of *A. mellea* from eastern North America (Motta & Korhonen 1986).

A wide range of interactions was observed between diploid isolates of *Armillaria* from Cape Town and haploid isolates of *A. mellea* from France and North America. The transition in culture morphology of the haploid testers from white/fluffy to more appressed, crustose mycelium was observed in the haploid tester isolate of *A. mellea* from France after pairing with each of the Cape Town isolates, suggesting diploidization. Diploidization was not evident when haploid testers from North America were used, perhaps because the Cape Town isolates are European, and the North American isolates represent a newly diverging species of *A. mellea* (Coetzee *et al.* 2000a).

A. mellea s. s. is generally considered to be a Northern Hemisphere species, and this is the only confirmed report of *A. mellea* s. s. from Sub-Saharan Africa (Kile *et al.* 1993; Volk & Burdsall 1995). In South Africa, *A. fuscipes* Petch has been found in the eastern and northern regions (Coetzee *et al.* 2000b), but the Company Gardens is the only location at which an *Armillaria* species has been found in the southwestern region of South Africa. We examined ornamental trees along streets in Cape Town near the Company Gardens and found no evidence of *Armillaria* root rot, and it appeared that the disease in Cape Town was restricted to this historical site.

We believe that this fungus was introduced into the Company Gardens by early European settlers. The Company Gardens are surrounded by many historical monuments, including the residence of the State President, which was erected in 1650 for the storage of tools and supplies for the fruit and vegetable garden. Produce from this site was used to supply ships travelling via Cape Town to and from the Far East and Europe. Phylogenetic analyses and the sexual compatibility of European tester strains by the Company Gardens isolates strongly support the hypothesis that *A. mellea* was introduced into the gardens from Europe, most likely as diploid mycelium on potted fruit trees or grape vines.

Somatic compatibility tests using isolates from the Company Gardens suggested that they represent a single genet of the fungus, and DNA fingerprinting confirmed that the isolates were of a single genotype. Numerous basidiomes of *A. mellea* were found in the Company Gardens in 1997, and it is possible that the fungus is reproducing by basidiospores. All of the isolates from Cape Town that were studied were, however, genetically identical, most likely the result of mycelial (asexual) spread. Once a diploid is established by basidiospore infection, *Armillaria* species are known to spread vegetatively, underground, by growth of rhizomorphs and mycelia in root wood, and such mycelia show remarkable genetic stability over time (Smith *et al.* 1990, 1992; Rizzo & Harrington 1993; Rizzo *et al.* 1995; Shulze *et al.* 1997; Shulze & Bahnweg 1998).

The genet is now at least 345 m in diameter. We do not know the precise point of introduction, but we can conservatively estimate that the introduction was at the centre of the delimited genet. Based on a range of putative growth rates through the soil and root systems, we estimated the Cape Town genet to be between 108 and 575 years old. The gardens were established in 1652, well within the range of estimated initiation dates. If *A. mellea* became established in 1652, then an average radial growth rate of 0.5 m/year would place the fungus at the edges of the infection centre as presently recognized.

In this study, we have shown that *A. mellea* s. s. is present in the Company Gardens of Cape Town. We have based our identification on basidiome morphology, IGS-1/RFLP data and sexual compatibility tests. Sequence analysis has further shown that the Cape Town fungus is most closely related to isolates of *A. mellea* s. s. from Europe. The isolates collected at the site were genetically identical based on somatic incompatibility tests and DNA fingerprinting, and they appear to represent a single genet. We believe that early Dutch settlers introduced this fungus into Cape Town in the mid to late 1600s on potted plants, rather than on seeds. This would be consistent with historical records showing that potted citrus trees were initially planted on the same site where oak trees and other woody hosts are currently infected with *A. mellea*. The fact that the fungus and the disease with which it is associated have gone undetected for so many years is intriguing. Perhaps the heavy irrigation to these trees has recently exacerbated the damage caused by *A. mellea* (Whiting & Rizzo 1999).

In this study, we have used a number of contemporary techniques to show that *A. mellea* was introduced into Cape Town by early settlers. As far as we are aware, this is one of the earliest records, verified genetically, of a fungal pathogen having been introduced into a new region by humans. Such a study would not have been possible prior to the advent of DNA sequencing and other molecular tools to analyse population structure. We believe these

tools will become increasingly important in addressing issues pertaining to biosecurity and the movement of pathogens around the world.

Acknowledgements

We thank the members of the Tree Pathology Co-operative Programme (TPCP) and the National Research Foundation (NRF), South Africa, for their financial support. The financial support of the Ernest Oppenheimer Trust in the form of the W.D. Wilson Visiting Fellowship to the third author is acknowledged. We also thank Mr Chris Buys from the Parks and Forest Gardens Board for his help and information.

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The work described in this paper formed part of the MSc study of Martin Coetzee, under the supervision of Professor Brenda Wingfield, Dr Teresa Coutinho and Professor Mike Wingfield. Martin Coetzee is currently completing a PhD in Genetics with the Forestry and Agricultural Biotechnology Institute (FABI) at the University of Pretoria, on various aspects of the taxonomy and ecology of *Armillaria* spp. Thomas Harrington, Professor of Plant Pathology, participated in this study as part of his sabbatical visit to South Africa in 1997, and Joe Steimel currently assists him at Iowa State University.
