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Armillaria *root rot*

A new disease of cut-flower proteas in South Africa

S. Denman[1], M.P.A. Coetzee[2], B.D. Wingfield[2], M.J. Wingfield[3] and P.W. Crous[1].

[1]Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Matieland 7602.
[2]Department of Genetics, Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002.
[3]Department of Microbiology and Plant Pathology, Tree Pathology Co-operative Programme (TPCP), Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria 0002.

Introduction

Armillaria spp. are voracious pathogens of trees and shrubs throughout the world. They survive either as pathogens, saprobes or perthobes (an organism able to utilise dead tissues in living hosts) of woody material(Gregory *et al.*,1991). As facultative necrotrophs (pathogens) these fungi kill living tissues of woody hosts and then utilise the tissues as a nutrient source (Kile *et al.*, 1991). In the saprobic mode, *Armillaria* spp. are wood decomposers aiding the recycling of nutrients in ecosystems. Pathogenic *Armillaria* spp. cause major losses to forestry (especially native forests but also to commercial plantations) world-wide, and also occur on fruit trees and ornamental plants. They have caused losses to Proteaceae in Africa (Kenya, Tanzania, South Africa), Australia, Madeira, New Zealand, United States of America (California, Hawaii) and Zimbabwe. In some of these countries where *Armillaria* root rot occurs in commercial cut-flower plantations, losses are of great economic significance to producers. This disease has never been recorded on indigenous Proteaceae in South Africa, although it was noted on Australian Proteaceae growing in this country (Doidge, 1950). Thus, in South Africa *Armillaria* has not posed a threat to either the natural flora or to commercial production of Proteaceae.

In a recent investigation into the cause of decline and death of *Protea scolymocephala* (L.) Reichard, at Kirstenbosch, we were surprised to discover typical signs of Armillaria root rot. The significance of this finding for conservation and Proteaceae farmers needs to be assessed. General information about the genus *Armillaria*, and the diseases that it causes, is provided here. Other than reporting this disease for the first time, we also discuss the significance of this record to the Protea industry in South Africa.

Taxonomy and Identification

The taxonomy of *Armillaria* has been problematic mainly because it was assumed that *Armillaria mellea* (Vahl:Fr.) Kummer *sensu lato* (in the widest sense) was a pleomorphic (multiple formed) species that occurred in Asia, Europe and North America (Singer 1956). This resulted in different species being lumped together under the name *A. mellea*. Thus *Armillaria* root- and butt-rot was frequently incorrectly attributed to *A. mellea*. Although this might seem to be a rather academic matter, it is important to know the correct species identification of the pathogen that affects specific crops. This ensures that appropriate disease control and management practices can be selected and implemented.

With the discovery of mating compatibility tests (Hintikka, 1973) and the advent of molecular techniques as an aid to species identification, the taxonomic uncertainties within *Armillaria* are being resolved. Currently, Volk and Burdshall (1995) recognise *A. mellea* as the type (or representative) of the genus and accept 38 species. According to Volk and Burdsall (2000) the genus *Armillaria*, is reserved for facultatively parasitic (non-obligate) root- and butt-rot fungi that produce rhizomorphs.

Identification

Armillaria is an Agaric (a so-called mushroom or toadstool [Kendrick 1992]) and the fruitbodies (basidiomes or, more simply, mushrooms) when produced, are one of the ways this fungus can be identified (Fig. 1a). The main component parts of mushrooms are the cap (pileus) and the stalk (stipe), and both of these may have other characteristic features. The pileus of *Armillaria* is fleshy and ranges in colour from yellow, honey-beige to tawny or cigar brown (Watling *et al.*, 1991).

Fig. 1a. Basidiomes (fruitbodies / mushrooms) of *Armillaria mellea* found on decaying oak trees in the Company Gardens in Cape Town

The stipe is centrally situated in the cap, fibrous to fleshy, often becoming hollow with maturity. An annulus (a membranous ring/veil of tissue) prevails on the stipe (Fig. 1b) and fruitbodies may arise from white fan-like mycelium (Fig. 2) or from black rhizomorphs (Fig. 3). The rhizomorphs are unusual macroscopic features that are comprised of hard, black, melanised cells which form long string-like structures under the ground, from which the common term "shoe-string" rhizomorphs, takes its name. Rhizomorphs enable the pathogen to spread from plant to plant through the soil. *Armillaria* can be cultured in a Petri dish containing culture medium where the rhizomorphs are often seen (Fig. 4).

Inside the cap of *Armillaria* fruiting bodies, the lamellae or gills that carry the spores of the fungus are ivory coloured initially but become dark with age (Watling *et al.*, 1991). The spore print (made by placing the cap of the mushroom, gills facing downwards, on a piece of coloured paper overnight) is white. The basidiomes usually arise at the base of infected trees and shrubs and occur as solitary individuals or in clusters.

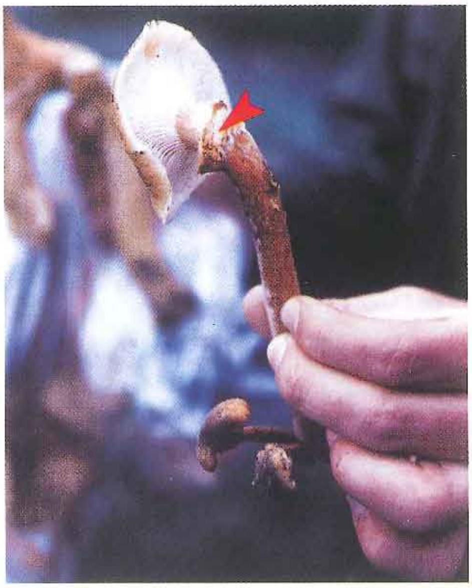

Fig. 1b. A basidiome (fruitbody / mushroom) of *Armillaria mellea* – note the annulus (or veil of tissue) on the stipe

However, basidiomes are produced seasonally, and therefore are often not present for identification purposes. The web of tough, white mycelium that is found below the bark of infected plants (Fig. 2) is characteristic of *Armillaria*.

Recognition of the taxonomic significance of mating compatibility between single spore cultures of *Armillaria*, and the development of molecular techniques have recently aided species differentiation in this genus (Harrington and Wingfield, 1995, Coetzee *et al.*, 2000a). The lack of formation of rhizomorphs and scarcity of basidiomes in African species makes identification difficult in these regions (Swift, 1968). Therefore, the morphological features that are present must be studied, and mating tests between single spore isolates and sequencing of various parts of the pathogen's genome must be carried out to reliably identify the fungus to species level. The latter of these techniques is expensive and specialised expertise is required to carry out tests.

Fig. 2. White, fan-like mycelium of *Armillaria* below the bark of an infected *Protea scolymocephala* plant found in Kirstenbosch Gardens, Cape Town ➤

Fig. 3. Black shoe-string / boot-lace rhizomorphs of *Armillaria* below the bark of an infected Spruce tree in Scotland

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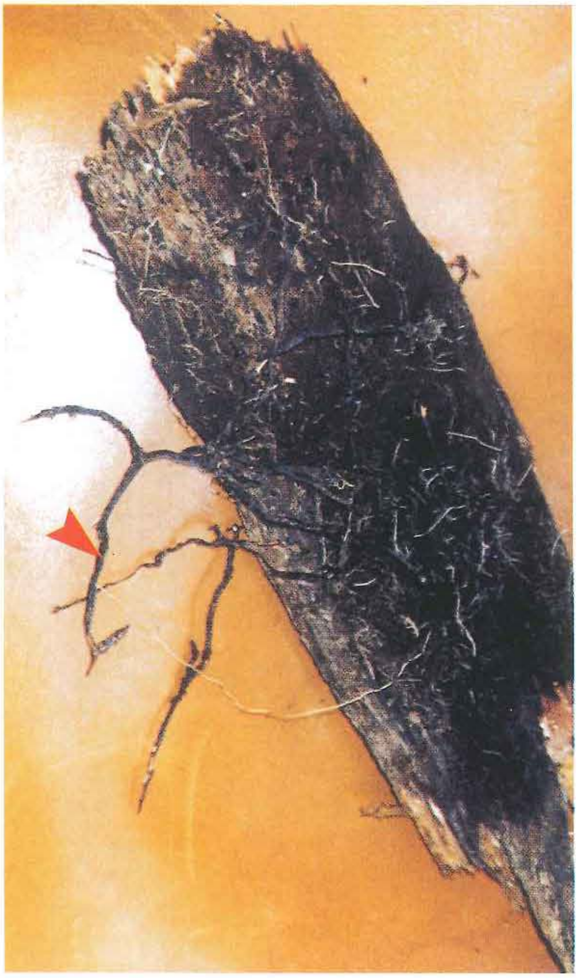

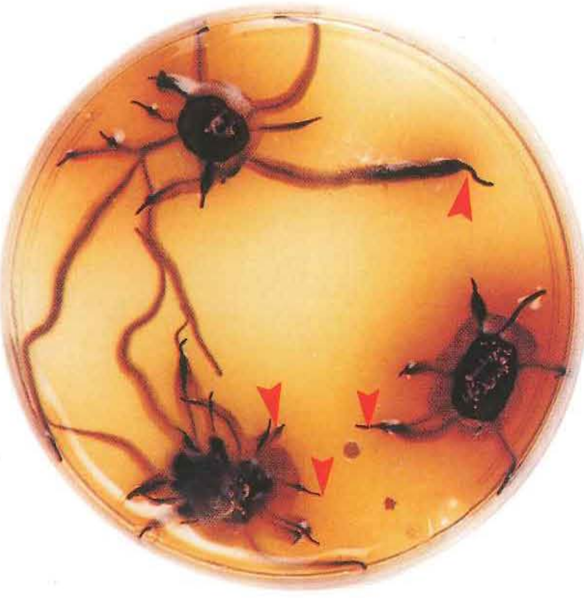

Fig. 4. *Armillaria* from infected *Protea scolymocephala*, cultured in a Petri dish. The long spidery outgrowths are the rhizomorphs

Epidemiology

Armillaria thrives in moist conditions (Porter *et al.*, 1996) but is restricted by excessively wet, waterlogged, cold, or dry conditions (Kile *et al.*, 1991). Infection can take place through the soil by rhizomorph contact with roots, or by mycelium from infected roots colonising healthy roots that are in close proximity to the infected ones (via root to root contact) (Garraway *et al.*, 1991; Morrison *et al.*, 1991). This fact holds serious implications for farmers planting young plants in old stands where plants have died as a result of infection by this pathogen, and the infected roots remain in the soil. Although rhizomorphs do not require wounds or weakened points in root tissues to initiate infection, roots with these afflictions can all serve as infection courts (Morrison *et al.*, 1991). Stumps of felled trees can be infected by basidiospores (Rishbeth, 1970) but this is considered a fairly uncommon event and there is no evidence to support the idea that basidiospores infect roots directly (Redfern and Filip, 1991).

Armillaria on Proteaceae throughout the world

There are not many records of *Armillaria* spp. on Proteaceae throughout the world. However, this pathogen has been recorded from a number of countries. These include Australia (von Broembsen, 1989; Forsberg, 1993; Porter *et al.*, 1996), California (Farr *et al.*, 1989), Hawaii (Laemmlen and Bega 1974), Kenya (Anonymous, 1960) Madeira (Rodriguez, 2000), New Zealand (Pennycook, 1989), South Africa (Doidge, 1950), Tanzania (Anonomous, 1960) and Zimbabwe (Musuka *et al.*, 1998).

In Australia *Armillaria luteobubalina* Watling & Kile is the main species that causes root rot of Proteaceae (Kile *et al.*, 1983, Pearce *et al.*, 1986, Shivas, 1989, von Broembsen *et al.*, 1989). The distribution of this disease on Proteaceae in Australia is very wide, ranging from Dwellingup, Manjimup, Pemberton and Perth in the west, to the south and east regions. In Sydney, South African Proteaceae are especially susceptible to *A. luteobubalina* (P.W. Crous pers. obs.). The first above ground symptoms often shown by infected plants are undersized leaves that drop prematurely (Porter *et al.*, 1996). Signs of the pathogen include the cream-coloured web of mycelium below the bark, black "shoe-string" rhizomorphs that also form below the bark and run along the roots and in the soil. Honey coloured mushrooms may occur at the base of infected plants (Porter *et al.*, 1996). Proteaceous hosts known to be infected with *A. luteobubalina* in Australia include *Banksia grandis* Willd and *B. seminuda* (A.S. George) Rye, (Pearce *et al.*, 1986, Shivas, 1989), *Grevillea robusta* R. Br., (Kile *et al.*, 1983, Shivas, 1989) and *Hakea prostrata* R. Br. (Kile *et al.*, 1983, Shivas, 1989), and several members of the genus *Protea* (P.W. Crous pers. obs.). An unidentified *Armillaria* sp. has also been recorded on *Dryandra polycephala* Benth (Shivas, 1989).

According to Laemmlen and Bega (1974), Bega (1962) made the first report of *A. mellea* in Hawaii, when he found this pathogen on *G. robusta*. However, no basidiomes (mushrooms) of *Armillaria* have ever been found on the island (Laemmlen and Bega, 1974). In view of the recent advances in the taxonomy and species identification of this genus, the identification of this pathogen on Proteaceae in Hawaii, probably needs to be reassessed. On another island where Proteaceae are cultivated, namely Madeira, *Armillaria* has been isolated from Proteaceous hosts, but the pathogen has not yet been identified to species level (Moura and Rodrigues, 2000).

Interestingly, a number of different species of *Armillaria* have been reported on Proteaceae from New Zealand. Both *Armillaria limonea* (Stevenson) Boesewinkel and *Armillaria novae-zelandiae* (Stevenson) Herink. were found on *Knightia excelsa* R.Br. and the latter was also reported on *G. robusta* (Pennycook, 1989).

African records of *Armillaria* on Proteaceae are very scant but the fungus has been recorded on *G. robusta* in Kenya and Tanzania (Anonomyous, 1960) as well as in South Africa (Doidge, 1950). In Zimbabwe an unidentified *Armillaria* sp. has been reported from a *Protea* sp (Musuka *et al.*, 1998).

Armillaria in South Africa

Until now the only record of *Armillaria* on Proteaceae in South Africa, was on *G. robusta* (Doidge, 1950,). According to Coetzee *et al.*, (Coetzee *et al.*, 2000a) only two species of *Armillaria* are thus far known occur in South Africa, *Armillaria fuscipes* Petch and *A. mellea*. In view of the recent advances in the taxonomy of *Armillaria*, previous records (pre-1996) of this fungus should be referred to merely as *Armillaria* sp. because the species identity is no longer certain. *Armillaria fuscipes* is an important pathogen of pines (Coetzee *et al.*, 2000a), especially in the northern parts of the country. The only reliable record of *A. mellea* in South Africa is on oak trees in the Company Gardens in the centre of Cape Town where it was apparently introduced by early settlers (Coetzee *et al.*, 2000b). *Armillaria* sp. (originally as *Armillaria mellea*) has been recorded as a pathogen on *Araucaria braziliana* A. Rich. (monkey puzzle trees) (Doidge 1950), on *Cedrela odorata* Linn. (West Indian cedars) (Doidge 1950), *Cupressus lusitanica* (cypress) (Doidge 1950), *Delonix regia* Raf. (flamboyant trees) (Doidge 1950), *Eriobotrya japonica* (Thunb.) Lindl. (loquats)) (Doidge 1950), *Eucalyptus paniculata* Sm. (Doidge 1950), *Grevillea robusta* (Doidge 1950), *Parinari curatellifolia* (Mobola plum) (Doidge 1950), *Musa cavendishii* Lamb. (bananas) (Brodrick, 1971), *Pinus* spp. (Doidge 1950), Quercus spp. (oaks) (Doidge 1950), *Syzygium gerrardii* (Harv. Ex Hook. f.) Burtt. Davy (forest waterwood) (Doidge 1950), and *Toona ciliata* M.J. Roem. (toon trees) (Doidge 1950). *Armillaria* sp. (originally as *A. mellea*) was also reported by Doidge (1950) on several other hosts, but as these reports were unconfirmed they were not listed by Doidge *et al.*, 1953, Gorter, 1977 or Crous *et al.*, 2000

In 1996 *A. mellea*, a devastating pathogen of many woody species world-wide was identified in the "Company Gardens" in the centre of Cape Town on oak trees (Coetzee *et al.*, 2000b). An intriguing story of how this fungus must have been introduced into Cape Town during the early Cape Dutch settlement (1652) on one of the potted plants (such as citrus or grapevine) is presented by Coetzee *et al.* (2000b). If the hypothesis proposed by Coetzee *et al.* (2000b) is correct, and this fungus has not been reported else where in the Western Cape until now, the spread of this pathogen has obviously been contained up to the present.

Armillaria on Proteaceae in South Africa – A new record

Declining *P. scolymocephala* plants were encountered in the planted beds at Kirstenbosch National Botanical Gardens, Kirstenbosch, Cape Town in May 2000. The leaves of affected plants were chlorotic and the plants did not look healthy (Figs. 5 and 6). When the infected plants were removed from the soil the roots were blackened (not unlike typical *Phytophthora* root rot symptoms) and there was a lack of feeder roots. Upon cutting into the crown tissue, white mycelium typical of the *Armillaria* mycelial fans was present in the cambial region (Fig. 7). Isolations were made and the pathogen was obtained in culture (Fig. 4). Cultures are maintained in the Department of Plant Pathology culture collection, University of Stellenbosch Private Bag X1, Matieland 7600 - Culture numbers STE-U 3773 and STE-U 3774. No fruiting structures (basidiomes) were present nor were rhizomorphs observed, thus identification to species level will have to be derived from molecular studies. This work is underway and the results promise to be most intriguing. One possibility is that the exotic *A. mellea* has escaped from the Company Gardens and entered Kirstenbosch. Alternatively, this could be another species of *Armillaria* and possibly even something new for South Africa.

Fig. 5. A *Protea scolymocephala* plant infected with *Armillaria* in Kirstenbosch Gardens. Note the chlorotic lower leaves and dead branches

Fig. 6. A *Protea scolymocephala* plant infected with *Armillaria* in Kirstenbosch Gardens. Note the chlorotic lower leaves, dead branches and unhealthy appearance of the plant. The white mycelial fans can be seen below the bark on the main root of the plant

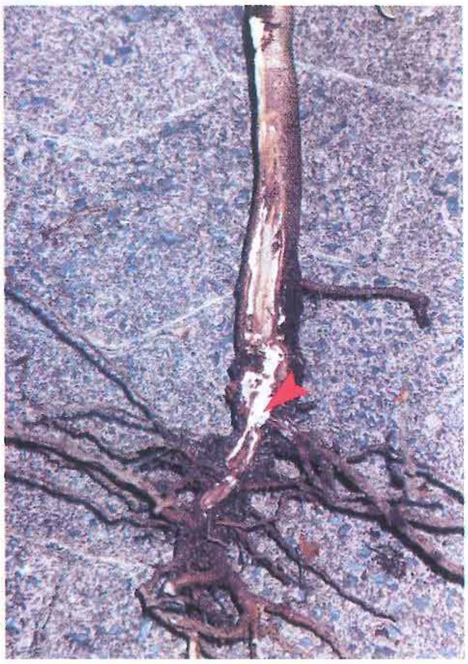

Fig. 7. White mycelial fans typical of *Armillaria* present below the bark in the crown region of *P. scolymocephala* plants infected with *Armillaria* in Kirstenbosch Gardens

Discussion

Armillaria is a serious root rot pathogen of many woody hosts and in South Africa, the most serious root pathogen on Proteaceae that we have had to contend with thus far is *Phytophthora cinnamomi* Rands, although in the past two years *Fusarium* wilt caused by *Fusarium oxysporum* Schlecht. has become a problem. Does this new record mean that we now have a third serious root pathogen that we need to keep in check? In order to answer this question a few facts need to be established. Firstly the species identification of the *Armillaria* able to infect *P. scolymocephalla* must be resolved. The host range of this pathogen must be established and then the big challenge will be to answer the question of how this pathogen appeared in Kirstenbosch Gardens. Once these facts are established it will be easier to estimate the potential impact of this pathogen to the South African Proteaceae industry. However, if the fungus is *Armillaria mellea*, there may be serious implications for indigenous vegetation on Table Mountain and it will be essential to eradicate this pathogen before it becomes a threat to both natural and cultivated Proteaceae in the Western Cape.

The flowerbeds at Kirstenbosch are covered with bark chips that help retain soil moisture and when they decompose they return nutrients to the soil, they also help prevent soil erosion and moderate the temperature of the soil. *Armillaria* spp. are common wood rotting fungi thus it is quite possible that this pathogen was introduced into Kirstenbosch Gardens on the bark chips that have been strewn over the flower beds. Growers need to be warned of the potential risk involved with the practice of covering beds with bark chips especially if they were derived from diseased, rotted or dead trees or shrubs.

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