13

Diseases of plantation *Eucalyptus* in the Republic of Congo

J. Roux[a*], T.A. Coutinho[a], M.J. Wingfield[a] and J-P. Bouillet[b]

Plantation forestry, based on *Eucalyptus* species and clones, has been practised in the Republic of Congo since the early 1950s. A survey of plantations in the Pointe-Noire area undertaken in 1998 identified the diseases affecting plantation eucalypts. These included Cryphonectria canker, Botryosphaeria canker, Cylindrocladium leaf blight and bacterial wilt. A new and serious disease of eucalypts, caused by *Ceratocystis fimbriata*, was reported for the first time during this survey. A number of secondary, opportunistic fungi were also isolated from diseased tissue. We believe that the impact of these diseases will increase in future, as clonal forestry is practised more extensively. Disease management programmes should thus be established to reduce losses.

Background

Plantation forestry, based on *Eucalyptus* species, forms an important component of the economies in many developing countries in the tropics and subtropics. In 1991, approximately 8 million hectares of forestry areas were estimated to be planted to eucalypts.[1] Wood from these trees is used for the manufacture of paper, viscose and rayon, construction timber, as mine props and for fuel.[1–3]

The use of high-yielding *Eucalyptus* clones and clonal hybrids is a predominant trend in many countries. As a result, the occurrence of associated pests and diseases is important.[4,5] Clonal forestry in many countries has been extensively influenced by diseases. For example, in South Africa, *Cryphonectria cubensis* (Bruner) Hodges has had a serious impact on clonal forestry and has necessitated careful selection of clones for commercial planting.[6–8] Owing to the genetic uniformity characteristic of clonal forestry, there is risk in maintaining extensive plantings without careful consideration of the effect of pests and diseases. Evaluation of clones must be continuously undertaken and knowledge of the genetic base of resistance is important since pathogens rapidly adapt to new environments.[9,10] The success of clonal forestry is not based on clonal mixing alone, but is also strongly dependent on the number and proportion of resistance alleles in trees.[11]

Since *Eucalyptus* was introduced to the Republic of Congo during the 1950s, more than 70 species have been tested on the coastal plains around Pointe-Noire (4°45′S 11°54′E). Only a few pure species, such as *E. urophylla* S.T. Blake, are well adapted to the local conditions (mean annual rainfall 1200 mm) (Table 1). Natural hybrids that appeared in the 1960s, however, exhibited good growth and adaptation. As a result of much work in the early 1970s, viable methods of rooting cuttings were developed, which enabled hybrids to be reproduced extensively. The first commercial cutting nursery in the world entered production in 1978 in the Congo.[2] Its success led Unité d'Afforestation Industrielle du Congo (UAIC) to plant the first clonal plantations in the same year. These plantations included the natural hybrids *E.* PF1 (= *E. alba* × *E.* unknown) and *E.* 12ABL × *saligna* (= *E. teriticornis* Sm. × *E. grandis* Hill ex. Maid) and now cover 43 000 ha. These belong to ECO SA (Eucalyptus du Congo Société Anonymes) and currently encompass 17 000 ha of first-rotation sites, 17 000 ha of coppice, and 6000 ha of replanted sites.

A future strategy is to replace natural *Eucalyptus* hybrids with an artificial hybrid of *E. urophylla* × *E. grandis* produced in the Congo. Unité de Recherche sur la Productivité des Plantations Industrielles (UR2PI) continues development of this hybrid through a reciprocal recurrent selection scheme. The plantations are primarily designed to produce pulp wood, which is mainly exported as logs to Europe (France, Norway, Portugal) and Africa (Morocco). More than 50% of the fuel wood used in Pointe-Noire comes from the by-products of these plantations (bole tops, big branches). It also represents an efficient instrument in the integration of local populations with commercial forestry operations.

Diseases of plantation *Eucalyptus* species have been recognized by UR2PI for many years. No detailed investigation into the possible causes of these diseases has, however, previously been undertaken. We report the results of a survey of plantation *Eucalyptus* in the Pointe-Noire area to identify the predominant diseases, which provides a foundation for ensuring the continued success of plantation forestry in the region.

Materials and methods

Samples and isolation techniques

Disease symptoms from which samples were collected in 1998 were divided into four categories, namely, (1) root diseases, (2) stem cankers, (3) wilts and (4) leaf diseases. Trees of all ages were sampled and included *E. grandis, E. urophylla, E. urophylla* × *E. grandis* (UG), *E. urophylla* × *E. pellita* F. Muell. (UP) and *E. territicornis* × *E. grandis* (TG).

Representative samples were collected from all *Eucalyptus* clones showing symptoms of disease. Isolates from symptomatic tissue were cultured on different isolation media, including malt extract agar (MEA), MEA amended with 0.1% streptomycin (MEAS) and PARP (selective media for the isolation of *Pythium* and *Phytophthora* spp.).[12] Where fungal fruiting bodies were found associated with diseased tissue on the surface of the bark, isolations were made directly from these structures. Small pieces of diseased material (1–2 mm) were also transferred from symptomatic material and incubated in Petri dishes containing either 2% MEA or PARP. Small pieces (5–10 cm) of diseased material were also incubated in Petri dishes containing moistened filter paper (humidity chambers), to induce sporulation of the fungi.

Soil samples were baited for pathogenic fungi using two techniques. For the isolation of oomycetous root-rot pathogens, soil was placed in plastic containers and flooded with distilled water. Pieces of wounded citrus leaf were placed on the surface of the

Table 1. Selected forestry conditions in the Republic of Congo.

Mean annual temperature	25°C
Mean annual minimum temp.	22°C
Mean annual maximum temp.	27°C
Dry season	May–October
Soil classification	Ferralsols
Soil texture	Sand: >90%; silt: <5%
pH H_2O	4.5–5.0
Cation exchange capacity	<1 cmolc/kg soil

[a]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria, 0002 South Africa.

[b]Unité de Recherche sur la Productivité des Plantations Industrielles, Pointe-Noire, République du Congo.

*Author for correspondence. E-mail: jolanda.roux@fabi.up.ac.za

water for three days to act as a bait for the oomycetes.[13] The leaf discs were then transferred to a selective medium (PARP) and incubated to induce fungal growth. For the isolation of *Ceratocystis* spp., slices of carrot were lightly covered with soil and incubated in Petri dishes for 4–5 days. The soil was then removed and the carrots incubated for a further 5–10 days to induce sporulation.

Results

Root diseases: a root disease of one-year-old UG clones, characterized by the death of branches from the base upwards on affected trees, was found in the Livuiti plantation. The root collars and stems, for approximately 10–20 cm above soil level exuded kino and small cracks developed in depressed lesions, spreading from the roots to the root collar and rest of the stem. In most cases these cankers had completely girdled the affected trees, which explained the rapid wilt and death of the branches. Most of the main roots were diseased and rotting. All the investigated root and root collar material yielded isolates of *Lasiodiplodia theobromae* (Pat.) Griff. and Maubl. (teleomorph: *Botryosphaeria rhodina* (Cooke) von Arx.).

Stem cankers: symptoms of stem cankers included extensive cracking of the stems, accompanied by the copious exudation of kino and the formation of gum pockets in the xylem. These symptoms occurred at all sites visited during the survey. In many of these trees, the heartwood was dead and discoloured. *Lasiodiplodia theobromae* was commonly isolated from these, as well as most other diseased tissue associated with discoloured wood and basal cankers. Various other fungi were also isolated from symptomatic tissue. These included a *Cytospora* sp. and *Valsa* spp.

In the Kissoko plantation, *Cryphonectria cubensis* caused severe cracking of stems, resulting in the formation of swollen areas on the stems of *E. grandis* and *E. urophylla* trees. These symptoms were seen at only two sites and did not appear to be affecting hybrid clones.

Wilt diseases: a first rotation coppice of a TG clone, approximately four years old, was reported to be healthy until a few months before the survey, when the trees rapidly wilted and died in the Mengo plantation. Approximately half of the trees in this stand were dead or dying at the time of sampling. Many of the affected trees exuded resin extensively from their main stems. Kino pockets were abundant in the wood. Most trees also showed streaking of the xylem. The pith of affected trees was dead and discoloured and epicormic shoots were common on many stems. Isolations from this material consistently yielded *Ceratocystis fimbriata* Ell. and Halst.

At another site (Kissoko plantation), a wilt disease of a UP hybrid was observed. Here, two-year-old trees were dead or dying in large numbers, with approximately half of the stand affected. Apart from the wilting and death of these trees, no external symptoms were apparent on the stems or branches. Extensive dark brown streaks were, however, observed in exposed xylem. These streaks were more intense and concentrated towards the base of the tree. The symptoms did not resemble any known *Eucalyptus* disease. Adjacent to this UP stand, a stand of young UP trees (less than one year in old) showed extensive stem mortality. No external symptoms were noted, but exposed xylem had distinct dark brown streaks. These symptoms were similar to those on the adjacent, two-year-old, UP trees. The cause of this wilt disease was also identified as *C. fimbriata*, which sporulated abundantly in the brown discoloured areas in the xylem.

A third wilt disease investigated affected trees less than one year old (Kissoko and Livuiti plantations) and was caused by the bacterium *Ralstonia solanacearum* (Smith) Yabiuchi *et al.* Symptoms associated with this disease included general chlorosis and wilt. The first symptoms were either the death of one or two branches, slight discolouration of the leaves on a few branches, or general discolouration and rapid wilting of trees. The roots of affected trees were rotten and wood in the region of the root collars was commonly cracked. The xylem of all affected trees showed extensive discolouration. This was more pronounced towards the base of the tree. Black 'streaks' were also evident in the discoloured xylem of many of the trees examined. A creamy to white 'bacterial ooze' appeared on the surface of cut stems. Trees affected by this disease were often found in patches within a clonal stand. The bacterium responsible was characterized as Biovar 3 using the method of Hayward.[14]

Leaf diseases: a serious leaf blight, characterized by leaves dying, initially on one or two branches, caused by *Cylindrocladium theae* (Petch) Subramanian, was observed in the Livuiti plantation. In severe cases, all the leaves on a tree died. The disease started with small spots or with lesions developing from the edges of the leaf, until the entire leaf was affected. Twig and branch cankers were also observed, leading to wilt and death of young branches. The affected trees were approximately 18 months old in this plantation, but minor occurrences of the disease were observed in other plantations on trees of different age. Other minor leaf spots and lesions were also observed in most of the plantations investigated and on a range of different clones. These diseases did not appear to cause significant damage. *Coniella fragariae* (Oud.) Sutton, was also isolated from leaf spots on many of the older leaves examined.

Discussion

At least five distinct microbial diseases of plantation *Eucalyptus* in Pointe-Noire were identified. Most of these are well-known diseases of *Eucalyptus* elsewhere in the world. Three of the diseases, Cryphonectria canker, Botryosphaeria canker and Ceratocystis wilt, are already having an impact on forestry in the Congo. Apart from the obvious and extensive mortality of trees caused by pathogens, many clones are also showing severe gummosis and cracking of the main stems, which is apparently associated with fungal infection. At least one serious leaf disease was also evident and this resulted in the defoliation of large, well-established trees.

Ralstonia solanacearum (syn.: *Pseudomonas solanacearum*) is an important pathogen of a number of plants, including *Eucalyptus* spp. It has been reported as the cause of *Eucalyptus* mortality in China, Australia, Brazil, and, in 1997, in South Africa.[15–17] Isolates found in the Congo belong to Biovar 3, which is similar to that identified in South Africa, Australia and China. Although the bacterium was found on both UG and UP clones in this study, there appeared to be differences in susceptibility of clones. There is currently no control measure for this disease and it affects a wide range of clones.[16]

Ceratocystis fimbriata is known to be the cause of *Eucalyptus* mortality only in South America and the Congo.[18] There has been only one previous report of a *Ceratocystis* species from *Eucalyptus*, namely from Australia, where it was isolated from stem wounds, in native forests.[19] A number of other *Ceratocystis* spp. are, however, known to be serious pathogens of woody hosts, and cause extensive mortality.[20] Artificial inoculation trials in the laboratory confirmed the pathogenicity of this fungus, also on South African *Eucalyptus* clones,[18] leaving no doubt as to its role in wilt disease in the Congo. The widespread occurrence of this disease in different plantations and on different clones suggests that many of the previously reported deaths of mature trees could have been caused by this pathogen. Selection for tolerance to infection by *C. fimbriata* in different clones should be afforded a high priority.

Botryosphaeria rhodina and its anamorph, *L. theobromae*, were found associated with diseased material of virtually all samples collected. This fungus is an opportunistic stress-related pathogen of woody plants in the tropics.[21–23] Although canker caused by *B. rhodina* (*L. theobromae*) is not thought to be the most severe disease of *Eucalyptus* in the Congo, *Botryosphaeria* spp. can cause serious damage to *Eucalyptus* trees.[24–27] *Botryosphaeria* spp. are cosmopolitan fungi, causing disease in many tree species, including *Eucalyptus* spp. *Botryosphaeria dothidea* (Moug.) Ces. et de Not., for example, is a well-known canker and die-back pathogen of *Eucalyptus*, leading to top-death, cracking of stems and branches, the accumulation and exudation of resin from affected trees, and in saplings, top-death or the death of entire trees.[24,26,27] It is also a latent pathogen, able to infect healthy trees.[28]

Cryphonectria cubensis is one of the best-known canker pathogens of eucalypts in the world.[29–31] This fungus is a serious pathogen in South Africa, South America and Asia where it has already caused large-scale losses and greatly influenced breeding programmes.[8,30] Its discovery in the Congo is viewed with concern and strategies to reduce its impact are required.

A number of *Cylindrocladium* spp. have been associated with leaf blight of eucalypts grown in the tropics and subtropics.[16,32–34] *Cylindrocladium* spp. can also cause shoot blight and severely damage trees. They are amongst the most serious foliage pathogens of eucalypts and can cause extensive damage to plantations.[34,35] They are also known to cause stem and root disease of plantation eucalypts.[32,34,36] Programmes to assess the susceptibility of planting stock in the Congo should be a priority.

Species of *Valsa* and *Cytospora* are believed to be mostly secondary or opportunistic pathogens closely associated with stress, such as fire damage evident on many of the sampled trees in the Tchittanga plantation.[37,38] They probably do not cause, but could contribute to, tree death. A species commonly described from eucalypts is *C. eucalypticola* van der Westhuizen, which has a teleomorph known as *V. ceratosperma* (Tode:Fr.) Maire.[38,39] Isolates of *Cytospora* and *Valsa* from Congo probably represent this fungus. Further studies are required to confirm this.

Our investigation revealed several serious diseases of *Eucalyptus* in the Congo. If left unmanaged, these diseases will increase in importance and will cause substantial economic losses. Effective measures to reduce losses due to fungal and bacterial pathogens exist and can be implemented successfully. Research and careful tree breeding are now needed to prevent the planting of disease-susceptible clones and thus avoid further economic losses due to microbial diseases.

We gratefully acknowledge the assistance of Rosalie Safou with the disease surveys. We also thank UR2PI and the Tree Pathology Cooperative Programme for funding, and Karl-Heinz Riedel for identifying the *Ralstonia* bacterium and Pedro Crous for identifying the *Cylindrocladium* sp.

Received 12 October 1999. Accepted 16 April 2000.

1. Turnbull J.W. (1991). Future use of *Eucalyptus*: opportunities and problems. In *Intensive Forestry: The Role of Eucalyptus. Proceedings of the IUFRO Symposium, Durban, South Africa, September 1991.*
2. Leakey R.R.B. (1987). Clonal forestry in the tropics — a review of developments, strategies and opportunities. *Commonw. For. Rev.* **66**, 61–75.
3. Brooker I. and Kleinig D. (1996). *Eucalyptus. An Illustrated Guide to Identification.* Reed Books, Port Melbourne, Australia.
4. Elstand N.C. and Elam D.R. (1993). Population genetic consequences of small population size: implications for plant conservation. *Ann. Rev. Ecol. Syst.* **24**, 217–242.
5. Wingfield M.J., Swart W.J. and Kemp G.H.J. (1991). Pathology considerations in clonal propagation of *Eucalyptus*with special reference to the South African situation. In *Proceedings of the IUFRO International Symposium on Intensive Forestry: the role of Eucalypts. Durban, September 1991*, pp. 811–820.
6. Denison N.P. and Kietzka J.E. (1993). The use and importance of hybrid intensive forestry in South Africa. *S. Afr. For. J.* **165**, 55–60.
7. Wingfield M.J., Swart W.J. and Abear B.J. (1989). First record of *Cryphonectria* canker of *Eucalyptus* in South Africa. *Phytophylactica* **21**, 311–313.
8. Wingfield M.J., Van Zyl L.M., Van Heerden S., Myburg H. and Wingfield B.D. (1999). Virulence and the genetic composition of the *Cryphonectria cubensis* population in South Africa. In *Proceedings of the IUFRO Symposium on Physiology and Genetics of Tree Phytophage Interactions, Arcachon, France, August 31–September 5, 1997.* INRA, Paris.
9. Mcdonald B.A. (1997). The population genetics of fungi: tools and techniques. *Phytopathology* **87**, 448–453.
10. Milgroom M.G. and Fry W.E. (1997). Contributions of population genetics to plant disease epidemiology and management. *Adv. Bot. Res.* **24**, 1–30.
11. Roberds J.H., Namkoong G. and Skrøppa T. (1990). Genetic analysis of risk in clonal populations of forest trees. *Theor. Appl. Gen.* **79**, 841–848.
12. Ribeiro O.K. (1978). *A Source Book of the Genus* Phytophthora. Strauss and Cramer, Lehr, Germany.
13. Grimm G. and Alexander A. (1973). Citrus leaf pieces as traps for *Phytophthora parasitica* from soil slurries. *Phytopathology* **63**, 540–541.
14. Hayward A.C. (1964). Characteristics of *Pseudomonas solanacearum*. *J. Appl. Bact.* **27**, 265–277.
15. Wang W.Y. (1992). Survey of *Eucalyptus* diseases in Taiwan. *Bull. Taiwan For. Res. Inst.* **7**, 179–194.
16. Ciesla W.M., Diekmann M. and Putter C.A.J. (1996). *FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm. No. 17. Eucalyptus spp.* FAO, Rome/International Plant Genetic Resources Institute, Rome.
17. Coutinho T.A., Roux J., Riedel K-H., Tereblanche J. and Wingfield M.J. (2000). First report of bacterial wilt caused by *Ralstonia solanacearum* on eucalypts in South Africa. *Eur. J. for. Path.* **30**, 1–8.
18. Roux J., Wingfield M.J., Bouillet J-P., Wingfield B.D. and Alferas A.C. (2000). A serious new wilt disease of *Eucalyptus* caused by *Caratocystis fimbriata* in Central Africa. *For. Path.* **30**, 175–184.
19. Kile G.A. (1993). Plant diseases caused by species of *Ceratocystis sensu stricto* and *chalara*. In Ceratocystis *and* Ophiostoma: *Taxonomy, Ecology and Pathogenicity*, eds M.J. Wingfield, K.A. Seifert and J.A. Webber, chap. 19, pp. 173–183. APS Press, St Paul, Minnesota.
20. Kile G.A. (1993). In Ceratocystis *and* Ophiostoma: *Taxonomy, Ecology and Pathogenicity*, eds M.J. Wingfield, K.A. Seifert and J.A. Webber, chap. 19, pp. 173–183. APS Press, St Paul, Minnesota.
21. Punithalingam E. (1976). *Botryodiplodia theobromae*. CMI Descriptions of Pathogenic Fungi and Bacteria. No. 519. Commonwealth Mycological Institute, Kew, England.
22. Punithalingam E. (1979). *Plant Diseases Attributed to Botryodiplodia theobromae* Pat. Strauss and Cramer, Lehr, Germany.
23. Cilliers A.J., Swart W.J. and Wingfield M.J. (1993). A review of *Lasiodiplodia theobromae* with particular reference to its occurrence on coniferous seeds. *S. Afr. For. J.* **166**, 47–52.
24. Davison E.M. and Tay C.S. (1983). Twig, branch and upper trunk cankers of *Eucalyptus marginata*. *Plant Dis.* **67**, 1285–1287.
25. Sharma J.K., Mohanan C. and Maria Florence E.J. (1984). A new stem canker disease of *Eucalyptus* caused by *Botryodiplodia theobromae* in India. *Trans. Br. Mycol. Soc.* **83**, 162–163.
26. Wingfield M.J. and Kemp G.H.J. (1993). Diseases of pines, eucalypts and wattles. In *Forestry Handbook*, ed. H.A. van der Sijde, pp. 231–266. South African Institute of Forestry, Pretoria.
27. Smith H., Kemp G.H.J. and Wingfield M.J. (1994). Canker and die-back of *Eucalyptus* in South Africa caused by *Botryosphaeria dothidea*. *Plant Path.* **43**, 1031–1034.
28. Smith H., Wingfield M.J. and Petrini O. (1996). *Botryosphaeria dothidea* endophytic in *Eucalyptus grandis* and *Eucalyptus nitens* in South Africa. *For. Ecol. Mngmt* **89**, 189–195.
29. Florence E.J., Sharma J.K. and Mohanan C. (1986). A stem canker disease of *Eucalyptus* caused by *Cryphonectria cubensis* in Kerala. In *Eucalyptus* in India: Past, Present and Future, 1986. *Kerala Forest Research Institute Scientific Paper* No. 66: 384–387.
30. Hodges C.S., Alfenas A.C. and Ferreira F.A. (1986). The conspecificity of *Cryphonectria cubensis* and *Endothia eugeniae*. *Mycologia* **78**, 343–350.
31. Conradie E., Swart W.J. and Wingfield M.J. (1990). Cryphonectria canker of *Eucalyptus*, an important disease in plantation forestry in South Africa. *S. Afr. For. J.* **152**, 43–49.
32. Sharma J.K. and Mohanan C. (1982). *Cylindrocladium* spp. associated with various diseases of *Eucalyptus* in Kerala. *Eur. J. For. Path.* **12**, 129–136.
33. Duarte V. (1985). Severe outbreak of leaf spot (*Cylindrocladium* sp.) in *Eucalyptus saligna* Smith in Viamao Rs. *Fitopatologia Brasil* **10**, 251.
34. Crous P.W., Phillips A.J.L. and Wingfield M.J. (1991). The genera *Cylindrocladium* and *Cylindrocladiella* in South Africa, with special reference to forest nurseries. *S. Afr. For. J.* **157**, 69–85.
35. Sharma J.K. and Mohanan C. (1991). Pathogenic variation in *Cylindrocladium quinqueseptatum* causing leaf blight in *Eucalyptus*. *Eur. J. For. Path.* **21**, 210–217.
36. Peerally A. (1974). *Cylindrocladium braziliensis. CMI Descriptions for Pathogenic Fungi and Bacteria No. 427.* Commonwealth Mycological Institute, Kew, England.
37. Shearer B.L., Tippet J.T. and Bartle J.R. (1987). *Botryosphaeria ribis* infection associated with death of *Eucalyptus radiata* in species trials. *Plant Dis.* **71**, 140–145.
38. Old K.M., Yuan Z.Q. and Kobayashi T. (1991). A *Valsa* teleomorph for *Cytospora eucalypticola*. *Mycol. Res.* **95**, 1253–1256.
39. Van Der Westhuizen G.C.A. (1965). *Cytospora eucalypticola* sp. nov. on *Eucalyptus saligna* from Northern Transvaal. *S. Afr. For. J.* **54**, 8–11.