# Virulence and the genetic composition of the *Cryphonectria cubensis* population in South Africa

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## RESUME

*Cryphonectria cubensis* causes a serious canker disease of *Eucalyptus*, and particularly *E. grandis* in tropical and sub-tropical areas of the world. Where these species are grown intensively, losses can be considerable. Various strategies have thus been implemented to minimise the impact of the pathogen. The most common of these has been to select clones of *E. grandis* and particularly hybrids of this and other species such as *E. urophylla*, for deployment. Screening of these clones through inoculation is also being used in various countries, to gain an early perspective of the relative susceptibility of the planting stock. Other more rapid techniques to identify disease tolerant clones, such as the selection of genetic and molecular markers are also being developed, and these hold considerable promise for the future.

While it is critically important to plant clones tolerant to infection by *C. cubensis* in high risk areas, it is also extremely costly to select such planting stock. An important consideration in this regard relates to the likely durability of disease tolerance, and thus how long clones are likely to retain this trait. The durability of disease tolerant clones will be closely linked to the diversity of the pathogen population, and particularly, its capacity to change with time. Despite this fact, almost nothing is known regarding the genetic composition of the *C. cubensis* population in any country where the pathogen is important. In recent years, we have begun to conduct studies on populations of *C. cubensis* in South Africa, various countries of South America and in Indonesia. From these investigations, we have learned that *C. cubensis* commonly occurs in the sexual state in South America and Indonesia but that it is asexual in South Africa. Consistent with this finding, we have also found that the population of the pathogen in South America and Indonesia is highly diverse, while it is much less variable in South Africa. We thus predict that disease tolerant clones in South Africa will have a higher degree of durability than those in the former two countries, where virulent strains of the fungus are likely to adapt to disease tolerant planting stock, more rapidly.

A fascinating and potentially useful strategy to reduce the impact of *Cryphonectria* canker of *Eucalyptus* would be to implement biological control of the pathogen. This

could be achieved by introducing dsRNA - associated hypovirulence into the pathogen population. We have recently discovered dsRNA in isolates of *C. cubensis* from various parts of the world and have also shown that it can be associated with reduced virulence. DsRNA is typically spread through cytoplasmic fusion between isolates of similar genotype. Therefore, opportunities to capitalise on hypovirulence in the management of *Cryphonectria* canker will depend strongly on a thorough understanding of the diversity of the pathogen population. Thus, our findings indicating that the populations of *C. cubensis* in South America and Indonesia are genetically diverse, suggests that hypovirulence would be difficult to implement in those countries. Conversely, in countries such as South Africa where the pathogen appears to have been more recently introduced, and where it is not reproducing sexually, we believe that prospects for disease reduction through the spread of hypovirulence factors holds considerable promise.

## INTRODUCTION

*Cryphonectria* canker caused by *Cryphonectria cubensis* (Bruner) Hodges is one of the most important diseases of *Eucalyptus* in tropical and sub-tropical parts of the world (Bruner, 1916, Boerboom and Maas, 1970, Davison and Coates, 1991, Davison and Tay, 1983, Florence *et al.*, 1986, Gibson, 1981, Hodges, 1980, Hodges *et al.*, 1986, Old *et al.*, 1986, Sharma *et al.*, 1985, Wingfield *et al.*, 1989). There is a growing interest in *Eucalyptus* propagation in countries outside the native range of this tree, especially for pulp production and to provide a replacement for fibre traditionally sourced from native forests. At present it is estimated that approximately eight million hectares of *Eucalyptus* plantations have been established, chiefly in the tropics and sub-tropics and the threat of diseases to this important resource is increasingly recognised (Wingfield *et al.*, 1995 a,b).

*Cryphonectria cubensis* was first described in by Bruner (1916) and has thus been known for a considerable time. In contrast, recognition of the fungus as an important pathogen has emerged relatively recently (Hodges, 1980, Hodges *et al.*, 1979, Alfenas *et al.*, 1982). This change in the status of the pathogen has been coincidental with the greater intensity of *Eucalyptus* propagation, but also with the onset of vegetative propagation of *Eucalyptus* clones and hybrids. Thus, the first report of the disease was from Surinam (Boerboom and Maas, 1970) and since that time, it has been reported in reasonably rapid succession from many other countries (Hodges and Reis, 1974, Hodges *et al.*, 1979; Gibson, 1981; Davison and Tay, 1983; Florence *et al.*, 1986; Old *et al.*, 1986; Swart and Wingfield, 1991; Wingfield *et al.*, 1989).

Avoidance of *Cryphonectria* canker in highly desirable *Eucalyptus* clones and clonal hybrids is an issue of high priority, for groups growing these trees. The risks associated with the inadvertent deployment of disease susceptible clones are great, and some serious losses have already been experienced. Selection and breeding strategies generally include the need to eliminate this disease and some substantial progress has already been made in this regard.

While breeding and selection of *Cryphonectria*- tolerant planting stock is being actively pursued, relatively little attention has been given to the pathogen, and its role in

disease. Thus, questions as to the origin of the pathogen have as yet not been fully resolved. Furthermore, the related question regarding the genetic diversity of the *C. cubensis* population in areas where the associated disease is serious, has hardly been considered. Questions of this nature will be critically important in any programme to produce *Cryphonectria* tolerant planting stock, and thus deserve urgent attention. In this paper we briefly review progress in our understanding of the virulence and population diversity of *C. cubensis*.

## ORIGIN OF *C. CUBENSIS*

Based on morphological and isozyme comparisons, it has been suggested that the clove pathogen *Endothia eugeniae* is conspecific with *C. cubensis* (Alfenas *et al.*, 1984, Hodges *et al.*, 1986, Micales and Stipes, 1987). This finding has furthermore, led to the suggestion that the pathogen might have originated in Indonesia where clove is native. This might further imply that the fungus, in areas such as South and Central America, is introduced. If this were so, it could further imply that the populations of the pathogen in these countries might be of relatively limited diversity and thus subject to a limited capacity to change.

In a recent study (Myburg *et al.*, 1998) a reasonably diverse collection of isolates of *C. cubensis* were compared based on ribosomal DNA sequence data. The results of this study confirmed that *C. cubensis* and *E. eugeniae* from clove, cannot be separated based on this part of the genome, which is known to be highly variable, and taxonomically valuable at the species level (Chambers *et al.*, 1986). Some minor differences could be found between isolates of *C. cubensis* from the Western and Eastern hemispheres. Unfortunately, only a single clove isolate was available for this study and this originated from Indonesia. It will now be most interesting to include additional clove isolates from S. America, Africa and South East Asia, and to see whether these also reside in two separate clades.

Very closely related species can share similar sequence data, even in the highly variable ITS (Chambers *et al.*, 1986) region of the rDNA. An interesting example has recently been encountered in the bark beetle associated fungi, *Ceratocystis polonica* and *Ceratocystis laricicola*. These fungi are morphologically indistinguishable and share identical ITS sequence (Visser *et al.*, 1995, Witthuhn *et al.*, 1998). They, are, carried by different but very similar bark beetles that infest spruce (*Piceae* spp.) and Larch (*Larix* spp) respectively. They can also be separated from each other based on isoenzyme comparisons (Harrington *et al.*, 1996). These fungi are, therefore, believed to be distinct but very similar, and to have undergone recent speciation.

Myburg *et al.*, 1998 has recently noted the very close similarity between *C. cubensis* and the Chestnut blight pathogen *Cryphonectria parasitica*. The implication here is that the two fungi might have had a common ancestor in the relatively recent past. This hypothesis is based not only on the morphological similarity of the two fungi, but on the fact that *C. parasitica* is able to cause cankers on *Eucalyptus* (Old and Kobayashi, 1988) and likewise, *C. cubensis* will kill artificially inoculated chestnut (*Castanea dentata*) saplings (Wingfield, unpublished). Sequence data (Myburg *et al.*, 1998) comparing these

fungi has confirmed that the fungi are very closely related, although they can also be easily distinguished from each other based on analysis of rRNA sequence data.

A fascinating report by Davison and Coates (1991) has recently suggested that *C. cubensis* occurs in Australia. What is most interesting, and perhaps enigmatic about this report is the fact that the fungus was isolated from roots of *E. marginata* in western Australia. The climate of Western Australia is wholly atypical of that usually associated with *C. cubensis* and it is also unusual for a typical stem canker pathogen to be found only on the roots of trees. Despite these ecological inconsistencies, comparison of Australian and other isolates of *C. cubensis* based on sequence data have shown that the fungi are the same (Myburg *et al.*, 1998).

## DIVERSITY OF *C. CUBENSIS* POPULATIONS

The genetic diversity of isolates of a pathogen, in a contained geographic area, should provide some clues as to its origin. Although only preliminary studies have thus far been completed, we have shown (Van Zyl *et al.*, 1998b) that a relatively large collection of isolates of *C. cubensis* from Brazil represents a population that has a high level of genetic diversity. Similar results have been obtained in preliminary studies of the diversity of *C. cubensis* isolates from Indonesia and from Venezuela (Van Heerden *et al.*, 1997). In contrast, a preliminary study has shown that isolates of *C. cubensis* in South Africa represent a much more uniform genetic base than those from other areas studied. This would imply that the pathogen has been introduced into South Africa relatively recently.

In South Africa, the sexual state (teleomorph) of *C. cubensis* is extremely rare (M.J. Wingfield, unpublished data). Where it has been seen, we suspect that it has arisen through homothalism and that it does not represent outbreeding. This is in contrast to the situation in various other parts of the world (Indonesia, Brazil, Venezuela) where the sexual state of the fungus has been found on virtually every infected tree examined (M.J. Wingfield, unpublished). In the latter countries, sexual reproduction is common and must, likewise, have led to a great diversity in the populations of the pathogen. This makes it difficult to determine, based on population structure, where the fungus might have originated. At the present time, we can only note that the fungus is well established and diverse in population structure in both South East Asia and South America. Based on population structure, we can not offer any resolution to the question of origin of the fungus.

A wide range of *C. cubensis* isolates from diverse geographic locations has recently been compared using RAPDS and rRNA sequence data. In these studies (Myburg, 1997) it has been shown that there are at least two distinct clades of the fungus. One of these includes South American and South African isolates and the other groups together isolates from S.E. Asia. The relevance of this observation is presently not clear to us, but it does suggest that *C. cubensis* in South Africa probably originated in South America, rather than in South East Asia.

## OPPORTUNITIES TO CAPITALISE ON HYPOVIRULENCE

A reduction of virulence in isolates of fungal pathogens, also known as hypovirulence, and which is conferred by cytoplasmically transmitted dsRNA hypoviruses, is a well known phenomenon (Nuss and Koltin, 1990) Indeed the bulk of research on hypovirulence has been conducted on the chestnut blight pathogen, *Cryphonectria parasitica* (Anagnostakis and Jaynes, 1973, Choi and Nuss, 1992, Smart and Fulbright, 1996) which is very similar in both biology and taxonomy to *C. cubensis*. Opportunities to capitalise on hypovirulence to reduce the impact of *Cryphonectria* canker of eucalypts appear to be increasing rapidly.

Hypovirulence has recently been reported, for the first time, to occur in isolates of *C. cubensis* from South Africa (van der Westhuizen *et al.*, 1994) as well as Brazil (van Zyl *et al.*, 1998a). Very little is known about the dsRNA associated with this hypovirulence or how it might compare with that known in *C. parasitica*. These questions are currently the basis of a concerted research effort. The most significant problem relating to hypovirulence lies in the fact that the dsRNA hypoviruses are naturally transferred only between isolates representing similar vegetative compatibility groups also known as VCG's (Anagnostakis and Kranz, 1987). These VCG's represent distinct genets in species of fungi and thus make up the genetic diversity of a fungal population. New VCG's are generated through sexual recombination and thus, in a natural ecosystem, one would expect to find a wide diversity of VCG's present. These would then also prevent the ready spread of hypoviruses within the population. Where a pathogen has been introduced into a new environment, and where it also does not undergo ready sexual recombination, it is assumed that excellent opportunities might exist to reduce to reduce its impact through hypovirulence. We feel optimistic that this scenario reflects the South African *C. cubensis* situation.

An exciting development in the very recent past has been research leading to the transfer of hypovirulence factors from one fungal species to another through transfection and transformation (Chen *et al.*, 1996). Thus the hypovirus CHV1-713 L-dsRNA from *C. parasitica* was, for example transferred to *C. cubensis*. We are currently exploring opportunities to transfer a *C. parasitica* hypovirus to dominant VCG's of *C. cubensis* in South Africa and, potentially, to use this for biological control.

## DURABILITY OF DISEASE TOLERANCE

Currently, the most effective means to reduce the impact of *Cryphonectria* canker is to select *Eucalyptus* clones that display high degrees of tolerance to the disease. This approach is being used effectively in South Africa and elsewhere in the world. It is also leading to an improved understanding of the disease tolerance trait and to breeding programmes incorporating this feature (M. and B. Wingfield, unpublished data).

The selection and testing of *Eucalyptus* clones tolerant to diseases such as *Cryphonectria* canker can be extremely time consuming. In some cases, forestry companies require up to twelve years of testing before new clones are commercially deployed. Thus, the durability of disease tolerance in these clones is a matter of crucial

concern. As has been mentioned previously in this paper, the *C. cubensis* population in South Africa appears to have a relatively limited genetic base. Although the data on which we base this conclusion are of a relatively preliminary nature, all evidence available to us suggests that *C. cubensis* in South Africa has a relatively limited capacity to change. This would then suggest that newly developed disease tolerant clones will exhibit a high degree of durability to *Cryphonectria* canker.

## CONCLUSION

*Cryphonectria* canker is one of the most important diseases of eucalypts where these trees are grown in plantations. It is likely to become more important in the future, especially with a world-wide increase in the utilisation of *Eucalyptus* for pulp production. Although it has been suggested that *C. cubensis* might have originated on clove in Indonesia, there is no firm scientific data to support this hypothesis. Populations of the fungus are diverse in both S.E. Asia and South and Central America and the pathogen has been found on native plants in both regions. Further research to resolve this question is required.

In contrast to the situation in South East Asia and South America, the genetic diversity of the *C. cubensis* population in South Africa appears to be relatively limited. In addition, sexual recombination in the fungus is common in the former areas, but virtually non existent in South Africa.

Outstanding opportunities appear to exist to utilise dsRNA-mediated hypovirulence to reduce the impact of *Cryphonectria* canker in South Africa. In South America and S.E. Asia, where the populations of *C. cubensis* are diverse, this might not be a practical option.

The durability of tolerance to *Cryphonectria* canker in *Eucalyptus* clones should be high in areas such as South Africa, where the pathogen appears to have a limited capacity to adapt. In countries where many VCG's of the pathogen exist, and where sexual outcrossing is common, a relatively rapid breakdown in disease tolerance might be expected.

## REFERENCES


ALFENAS A.C., HUBBES M., COUTO L., 1982. Effect of phenolic components from *Eucalyptus* on the mycelial growth and conidial germination of *Cryphonectria cubensis*. *Can. J. Bot.*, 60, 2535-2541.

ALFENAS A.C., HODGES C.S., JENG R., 1984. Similarities in physiological characters between *Endothia eugeniae* and *Cryphonectria cubensis*, causal agents of cankers in clove and *Eucalyptus*, respectively. *Phytopathology*, 74, 841 (Abst).

ANAGNOSTAKIS S.L., JAYNES R.A., 1973. Chestnut blight control: Use of hypovirulent cultures. *Plant Dis. Rep.*, 27, 225-226.

ANAGNOSTAKIS S.L., KRANZ J., 1987. Population dynamics of *Cryphonectria parasitica* in a mixed hardwood forest in Connecticut. *Phytopathology*, 77, 751-754.

BOERBOOM J.H.A., MAAS P.W.T., 1970. Canker of *Eucalyptus grandis* and *E. saligna* in Surinam caused by *Endothia havanensis*. *Turrialba*, 20, 94-99.

BRUNER S.E., 1916. A new species of *Endothia*. *Mycologia*, 8, 239-242.

CHAMBERS C., DUTTA S.K., CROUCH R.J., 1986. *Neurospora crassa* ribosomal DNA: sequence comparison of internal transcribed spacer and comparison with *N. intermedia* and *N. sitophila*. *Gene*, 44, 159-164.

CHEN B., CHEN C.H., BOWMAN B.H., NUSS D.L., 1996. Phenotypic changes associated with wild-type and mutant hypovirus RNA transfection of plant pathogenic fungi phylogenetically related to *Cryphonectria parasitica*. *Phytopathology*, 86, 301-310.

CHOI E.H., NUSS D.L., 1992. A viral gene confers hypovirulence-associated traits to the chestnut blight fungus. *EMBO*, 11, 473-478.

DAVIDSON E.M., COATES D.J., 1991. Identification of *Cryphonectria cubensis* and *Endothia gyrosa* from Eucalypts in Western Australia using isozyme analysis. *Austral. Plant Path.*, 20, 157-160.

DAVISON E.M., TAY F.C., 1983. Twig, branch and upper trunk canker of *Eucalyptus marginata*. *Plant Dis.*, 67, 1285-1287.

FLORENCE E.J.M., J. K. SHARMA C. MOHANAN., 1986. A stem canker disease of *Eucalyptus* caused by *Cryphonectria cubensis* in Kerala. *Kerala Forest Research Institute Scientific Paper*, 66, 384-387.

GIBSON I. A.S., 1981. A canker disease of *Eucalyptus* new to Africa. FAO, *Forest Genetics Resources Information*, 10, 23-24.

HARRINGTON T.C., STEIMEL J.P., WINGFIELD M.J., KILE G., 1996. Isozyme variation in the *Ceratocystis coerulescens* complex. *Mycologia*, 88, 104-113.

HODGES C.S., REIS M.S., 1974. Identificacao do fungo causador de cancro de *Eucalyptus* spp. no Brasil. *Brasil Florestal*, 5, 19.

HODGES C.S, GEARY T.F., CORDELL C.E., 1979. The occurrence of *Diaporthe cubensis* on *Eucalyptus* in Florida, Hawaii and Puerto Rico. *Plant Dis. Rep.*, 63, 216-220.

HODGES C.S., 1980. The taxonomy *of Diaporthe cubensis*. *Mycologia*, 72, 542-548.

HODGES C.S., ALFENAS A.C., FERREIRA F.A., 1986. The conspecificity of *Cryphonectria cubensis* and *Endothia eugeniae*. *Mycologia*, 78, 343-350.

MICALES J.A., STIPES R.J., 1987. A re-examination of the fungal genera *Cryphonectria* and *Endothia*. *Phytopathology*, 77, 650-654.

MYBURG H., WINGFIELD B.D., WINGFIELD M.J., 1998. Phylogeny using DNA sequence of geographically diverse isolates of *Cryphonectria cubensis* and allied species. *Mycologia*, (submitted).

MYBURG H., *1997 Cryphonectria cubensis, a molecular taxonomic and population study.* MSc. Thesis. Univ. of the Orange Free State, Bloemfontein, South Africa. 109p.

NUSS D.L., KOLTIN Y., 1990. Significance of dsRNA genetic elements in plant pathogenic fungi. *Ann. Rev. Phytopathology*, 28, 37-58.

OLD K.M., KOBAYASHI T., 1988. Eucalypts are susceptible to the chestnut blight fungus *Cryphonectria parasitica*. *Austral. J. of Bot.* 36, 599-603.

OLD K. M., MURRAY D.I.L., KILE G.A., SIMPSON J., MALAFANT K., 1986. The pathology of fungi isolated from eucalypt cankers in South eastern Australia. *A. For. Res.* 16, 21-36.

SHARMA J.K., MOHANAN L., FLORENCE E.J.M., 1985. *Disease survey in nurseries and plantations of forest tree species grown in Kerala.* Research report 36 Kerala Forest Research Institute, India.

SMART C.D., FULBRIGHT D.W., 1996. Molecular Biology of fungal diseases. *In*: M. Gunasekaran M. and Weber D.J. (Eds.) *Molecular Biology of the Biological Control of Pests and Diseases of Plants.* CRC Press, Inc., Boca Raton, Florida.

SWART W.J., WINGFIELD M.J., 1991. Cryphonectria canker of *Eucalyptus* spp. in South Africa. Proceedings of the IUFRO International Symposium. Intensive Forestry: The Role of Eucalypts. pp. 806.

VAN DER WESTHUIZEN I.P., SMIT W.A., WINGFIELD M.J., KEMP G.H.J., 1994. First report of hypovirulence associated with dsRNA in *Cryphonectria cubensis*. *S.A. J. Sci.* 91, 7 (Abst).

VAN ZYL L.M., WINGFIELD M.J., ALFENAS A.C., CROUS P.W., 1998a. First report of hypovirulence in Brazilian isolates of *Cryphonectria cubensis*. *Pl. Pathology* (in press).

VAN ZYL L.M., WINGFIELD M.J., KEMP G.H.J., CROUS P.W., 1998b. Population diversity among isolates of *Cryphonectria cubensis*. *Forest Ecology and Management*. (submitted).

VAN HEERDEN S.W., WINGFIELD M.J., COUTHINO T., VAN ZYL L.M., WRIGHT J.A., 1997. Diversity of *Cryphonectria cubensis* isolates in Venezuela and Indonesia. Proceedings of IUFRO Conference on Silviculture and Improvement of Eucalypts. Salvador, Bahia, Brazil. b,142-146.

VISSER C., WINGFIELD M.J., WINGFIELD BD., YAMOAKA Y., 1995. Generic placement of *Ophiostoma polonicum* amongst the ophiostomatoid fungi. *Syst. & Appl. Micro.* 18, 403-409.

WINGFIELD M. J., SWART W. J., ABEAR B., 1989. First record of *Cryphonectria* canker of *Eucalyptus* in South Africa. *Phytophylactica* 21, 311-313.

WINGFIELD M.J., SWART W.J., KEMP G.H.J., 1991. *Pathology considerations in clonal propagation of Eucalyptus with special reference to the South African situation.* Proceedings of the IUFRO International Symposium. Intensive Forestry: The Role of Eucalypts. pp. 811

WINGFIELD M.J., HARRINGTON T.C., SOLHEIM H. 1995. *Do conifer bark beetles require fungi to kill trees?* Proceedings of the IUFRO International Symposium on Bark Beetle, Fungus, Tree Interactions. As, Norway.

WINGFIELD M.J., WINGFIELD B.D., COUTINHO T.A., 1995. Management of Eucalyptus diseases in subtropical areas of South Africa. Proceedings of IUFRO Conference on Silviculture and Improvement of Eucalypts. Salvador, Bahia, Brazil pp171-172.

WITTHUHN R.C., WINGFIELD B.D., WINGFIELD M.J., T.C. HARRINGTON T.C., 1998. Monophyly of the conifer species in the *Ceratocystis coerulescens* complex based on DNA sequence data. *Mycologia*, (in press).