Pathogenicity of *Ophiostoma piliferum* (Cartapip 97®) compared with that of other South African sap-staining fungi

C. Dunn[a], F. Wolfaardt[a,b*] and M.J. Wingfield[c]

Cartapip 97® is a commercial product consisting of inoculum from a melanin-deficient strain of *Ophiostoma piliferum*, that does not cause staining of wood. Cartapip 97® is used for the prevention of sap-stain on wood as well as in biopulping, where *O. piliferum* reduces the pitch content in wood chips before pulping. The biological removal of pitch results in stronger paper with better optical properties. Cartapip 97® used not to be imported into South Africa because of quarantine restrictions. It was therefore necessary to demonstrate that this fungus does not pose a threat to South African forestry. In this study, the pathogenicity of *O. piliferum* was compared with *Ophiostoma ips* and *Sphaeropsis sapinea*, which are common causes of sap-stain on *Pinus* species in South Africa. Different pine species, including *Pinus elliottii*, *P. patula*, *P. greggii* and *P. radiata*, were inoculated during autumn and spring at three different locations. In most cases, *O. piliferum* resulted in smaller lesions than *S. sapinea* and *O. ips* and the length of lesions caused by *O. piliferum* differed from controls in only a few instances. The development of lesions indicated that different pine species react differently to infection by the test fungi. Pathogenicity was also strongly associated with the location of the trials and season of inoculation. We conclude that *O. piliferum* should not be regarded as a virulent pathogen and that it is safe to use Cartapip 97® in South Africa.

Introduction

Cartapip 97® is produced from a strain of *Ophiostoma piliferum* (Fries) H. and P. Sydow. The inoculum is produced from a melanin-deficient strain of *O. piliferum* and cannot, therefore, stain wood.[1] *Ophiostoma piliferum* occurs commonly on softwoods throughout the United States and the taxonomy, biology and economic importance of the fungus have been well documented.[2,3] *Ophiostoma piliferum* is generally viewed by the Environmental Protection Agency (EPA) and the U.S. Department of Agriculture (USDA) as neither pathogenic nor toxic to plants and animals.[4] It has, furthermore, never been associated with bark beetle vectors and is considered to be exclusively saprobic.[5,6] The lack of sexual reproduction in the melanin-free Cartapip 97® strain and the absence of an associated insect vector reduces the potential for spread of the fungus.[1,6] In the absence of sexual reproduction, the potential for genetic recombination is also limited. Cartapip 97® has been tested and approved for release in countries with stringent quarantine requirements such as Australia, Brazil and New Zealand.[7] Since the release of Cartapip 97® in these countries, there have been no reports of any negative consequences.[8]

Ophiostoma piliferum is a primary colonist of wood[9] and can therefore play a role in the prevention of sap-stain by competing with fungi that cause sap-stain.[6] The fungus can also be used in biopulping processes. When *O. piliferum* was applied to wood chips before pulping, it was shown to utilize pitch during the colonization process.[6] The reduction in pitch resulted in stronger paper with improved optical properties.[6] Cartapip® has also been applied in biokraft pulping of wood, resulting in an increase of pulp yield and viscosity and also a reduction in consumption of chemicals.[4]

In recent years, South African forestry companies have wished to test Cartapip 97® in various industrial processes. The product could not be imported into the country, however, until it had been certified by the Department of Agriculture as safe for general release. At that time, *O. piliferum* was not known to occur in South Africa and no information was available on the effect of Cartapip 97® on *Pinus* species commonly grown here. One of the preconditions for certification of the product was that it had to be shown as non-pathogenic under South African conditions. The purpose of this study, consequently, was to consider the potential of *O. piliferum* to cause disease on *Pinus* species grown in South Africa and to compare its pathogenicity with the most common local agents of sap-stain.

Materials and methods

Three major pine-growing regions of South Africa (Table 1) were selected for the pathogenicity tests with Cartapip 97® (AgraSol Inc., Raleigh, NC). Trials were conducted during the autumn and spring of 1997 on *Pinus elliottii* Engelm. var. *elliotii*, *P. patula* Schl. & Cham., *P. greggii* Engelm. and *P. radiata* D. Don. Softwood species were targeted, because the strain of *O. piliferum* used for Cartapip 97® was isolated from *Pinus taeda*[11] and applications in South Africa would also be on softwoods. The numbers of trees of each species inoculated differed from site to site depending on availability. At Longmore, *P. radiata* (13 trees) and *P. elliottii* (20 trees) were inoculated. At Ugie, *P. patula* (15 trees) and *P. greggii* (14 trees) were used, and at Jessievale *P. patula* (16 trees) and *P. elliottii* (12 trees) were available (Table 1). In fulfilment of quarantine requirements, the trial sites were isolated from human activity and surrounded by a 4-m-wide perimeter that was free of vegetation.

Inoculum of *O. piliferum* was produced by cultivating Cartapip 97® on potato dextrose agar (PDA) (Biolab, Merck) plates. For comparative purposes, trees were also inoculated with strains of *Ophiostoma ips* (Rumb.) Nannf. (CMW 0386) and *Sphaeropsis sapinea* (Fr.:Fr.) Dyko & Sutton in Sutton (CMW 1184). *Ophiostoma ips* was chosen because it is a sap-stain fungus with a similar biology to *O. piliferum*,[2] which occurs commonly in South Africa.[11] *Sphaeropsis sapinea* is one of the most important pathogens of *Pinus* species in South Africa.[12,16] Strains of this fungus had been selected for pathogenicity testing in previous trials (unpubl. results) and all isolates used in this study are maintained in the culture collection of the Forestry and Agricultural Biotechnology Institute at the University of Pretoria. Inoculum of each strain was grown on PDA at 24°C for eight days. The control treatment consisted of sterile PDA disks.

Table 1. Location and description of different trial sites used for pathogenicity trials.

Location	Latitude	Longitude	Altitude (m)	Mean annual rainfall (mm/yr)	Tree species
Longmore (Southern Cape)	33°49′S	25°08′E	530	702	*P. radiata* *P. elliottii*
Ugie (NE Cape)	31°06′S	28°14′E	1320	875	*P. patula* *P. greggii*
Jessievale (Mpumalanga)	26°24′S	30°11′E	1700	878	*P. patula* *P. elliottii*

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[a] Department of Microbiology and Biochemistry, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa.

[b] Sappi Forest Products, Technology Centre, P.O. Box 3252, Springs 1560, South Africa.

[c] Forestry and Agricultural Biotechnology Institute, University of Pretoria, Pretoria 0002, South Africa.

[*] Author for correspondence. E-mail: wolfaarf@sci.uovs.ac.za