Ceratocystis quercicola sp. nov. from Quercus variabilis in Korea

Sung-Eun Cho, Dong-Hyeon Lee, Michael J. Wingfield & Seonju Marincowitz

To cite this article: Sung-Eun Cho, Dong-Hyeon Lee, Michael J. Wingfield & Seonju Marincowitz (2020): Ceratocystis quercicola sp. nov. from Quercus variabilis in Korea, Mycobiology, DOI: 10.1080/12298093.2020.1766649

To link to this article: https://doi.org/10.1080/12298093.2020.1766649

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group on behalf of the Korean Society of Mycology.

Published online: 02 Jun 2020.

Submit your article to this journal

View related articles

View Crossmark data
**Ceratocystis quercicola** sp. nov. from *Quercus variabilis* in Korea

Sung-Eun Cho, Dong-Hyeon Lee, Michael J. Wingfield and Seonju Marincowitz

1. Introduction

The ascomycete genus *Ceratocystis* (Microascales, Ceratocystidaceae) represents an economically important group of fungal pathogens, occurring globally on a wide range of hosts [1–3]. The genus *Ceratocystis* was introduced to accommodate *C. fimbriata*, the causal agent of black rot on sweet potato [4]. *Ceratocystis* spp. are characterized by dark, globose ascomatal bases, elongated ascomatal necks, sticky masses of ascospores accumulating at their tips, hat-shaped ascospores, and thielaviopsis-like asexual states [1].

*Ceratocystis* includes important fungal pathogens, some of which cause serious diseases of agricultural and forestry crops worldwide [5]. Symptoms of infection by *Ceratocystis* spp. include branch and stem cankers, vascular staining, wilt, root disease, die-back, and fruit rot [1,5]. There has been a recent and unprecedented surge of novel diseases caused by *Ceratocystis* spp. in various parts of the world. These include rapid death of *Metrosideros polymorpha* in Hawai‘i caused by *C. lukuohia* and *C. huliohia* [6], canker and wilt disease of plantation-grown *Acacia* spp. in South East Asian countries caused by *C. manginecans* [7] and by *C. albifundus* in various countries of Africa [8,9].

*Ceratocystis* spp. require freshly made wounds as entry points to initiate infection [10–12]. The primary means of transmission to new tree hosts is by casual insects such as flies (Diptera) and nitidulid beetles (Coleoptera: Nitidulidae) visiting wounds [5,13–16]. These insects are attracted by the volatiles emanating from fresh tree wounds on which *Ceratocystis* spp. grow and sporulate rapidly, producing sticky spore masses that adhere to the bodies of the insects that carry them to new wounds [12,13,17,18].

A routine survey in a natural forest located in Gangneung (Gangwon province, the Republic of Korea) was conducted in 2017. This was to establish an inventory of potentially pathogenic fungi threatening the health of *Quercus* spp. One of the objectives of this study was to isolate fungi that had colonized wounded tissues resulting from severe storm damage in the summer of 2017. Among the isolated fungi was a *Ceratocystis* sp. that was consistently isolated from wounds on *Q. variabilis* (Oriental cork oak). The aim of this study was to identify this *Ceratocystis* sp. to compare it with other known *Ceratocystis* spp. and to consider its possible pathogenicity.

2. Materials and methods

2.1. Isolation

The *Ceratocystis* sp. was collected from fresh wounds on *Q. variabilis* growing in a natural forest located in Gangneung, a city in the Gangwon province of South Korea (37° 43'41.7"N 128° 47'56.1"E, 37° 43'37.4"N 128° 47'53.6"E) in July and August, 2017. Characteristic ascomata of *Ceratocystis* spp. were
recognized using a hand lens, and pieces of bark bearing ascomata were placed in individual paper bags and transported to the laboratory for further study.

Isolations were made on 2% malt extract agar (MEA; Franklin Lakes, NJ, USA) supplemented with and transported to the laboratory for further study.

In the dark for 14 days at temperatures ranging from 5 to 35°C at 5 degree intervals. The study was repeated once with five replicate plates for each isolate at each temperature. The diameters of colonies perpendicular to each other were measured after 14 days and an averages were computed.

2.2. Microscopy
To study the morphological characteristics, 3-week-old cultures maintained at optimum growth temperature were used. Fungal structures were mounted on microscope slides in water that was later replaced with 85% lactic acid for further observation. The structures were examined under Nikon microscopes (Eclipse Ni, SMZ18; Nikon, Tokyo, Japan) mounted with Nikon camera (DS-Ri2). Fifty measurements were made for taxonomically relevant structures whenever possible. Colony color (upper and reverse surfaces) was determined using the color charts of Rayner [19].

2.3. Growth in culture
Growth of the Ceratocystis sp. was conducted for two isolates serving as holotype and paratype, respectively. A 5 mm mycelial plug from a 14-day-old culture was placed at the center of 90 mm Petri dishes containing 2% MEA. These cultures were incubated in the dark for 14 days at temperatures ranging from 5 to 35°C at 5 degree intervals. The study was repeated once with five replicate plates for each isolate at each temperature. The diameters of colonies perpendicular to each other were measured after 14 days and an averages were computed.

2.4. Genomic DNA extraction, PCR amplification, and sequencing
To extract genomic DNA, cultures were incubated for two weeks to allow sufficient mycelial growth. Mycelium was scraped from the surfaces of the agar with sterilized surgical scalpel blades and transferred to 1.5 mL Eppendorf tubes. Genomic DNA was then extracted using ZR Fungal/Bacterial DNA MiniPrep kit (Zymo Research; Irvine, CA, USA) following the manufacturer’s instructions. The quantity and quality of DNA extracted was evaluated with a spectrophotometer (NanoDrop ND-1000; Thermo Fisher, Walthum, MA, USA) to calibrate the concentration and purity of DNA as PCR templates.

The PCR amplification reactions were conducted on a T-100 thermal cycler (Bio-Rad, Hercules, CA, USA). The total volume of each PCR reaction mixture was 15 μL, containing 1 μL of genomic DNA, 0.5 μL (10 pM) of each primer (forward and reverse), 0.5 μL of MyTaq PCR buffer (Bioline), and 0.5 μL of MyTaq DNA polymerase (Bioline). The PCR cycling profile consisted of an initial denaturation stage at 95°C for 5 min; 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min; and a final extension at 72°C for 7 min.

PCR amplifications were made for two gene regions, including part of the β-tubulin 1 (BT1) using primers Bt1a and Bt1b [20], and part of the translation elongation factor-1 alpha (TEF-1α) regions with primers TEF1F and TEF2R [21]. The resulting PCR products were submitted to Macrogen (Seoul, Korea) for forward and reverse sequencing reactions.

2.5. Multi-gene phylogenetic analyses
The sequences of Ceratocystis spp. closely related to the one from Q. variabilis were retrieved from GenBank. Phylogenetic trees based on a concatenated data set of the BT1 and TEF-1α gene regions were computed. Sequences for each of the two gene regions were aligned using the online interface of MAFFT v. 7 (http://mafft.cbrc.jp/alignment/server) [22], with the iterative refinement method (FFT-NS-i settings) selected. Sequence alignments were manually edited in MEGA7 [23]. Two different phylogenetic analyses were employed, including maximum parsimony (MP) analyses using MEGA7 and maximum likelihood (ML) tests using RAxML HPC BlackBox ver.8.1.11 [24,25], using the default option with the GTR substitution model implemented in the CIPRES cluster server (https://www.phylo.org/) at the San Diego Supercomputing Center. For both MP and ML analyses, Ceratocystis albifundus isolate CMW 4068 (culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa) was used as the outgroup taxon.

2.6. Pathogenicity test
Pathogenicity studies were conducted on twenty freshly excised 15-cm-long × 5-cm-diameter logs of Q. variabilis. Bark was wounded to expose the cambium using a 10 mm cork borer, and discs of agar bearing mycelium taken from the margins of actively
growing, 2-week-old cultures, (isolate CDH2017-8), were placed with the mycelium facing the cambium. Five logs were inoculated with clean agar disks to serve as controls. All inoculation points and the ends of the logs were covered with masking tape and polyethylene films, respectively, to prevent desiccation of the inoculum and cambium, and to reduce contamination. The logs were placed in a moist chamber for 8 weeks. The pathogenicity test was performed twice for statistical analysis. Analysis of variance (ANOVA) and Tukey's honestly significant difference (Tukey's HSD) test were used to determine whether there were significant differences in aggressiveness of the fungus based on a P-value computed using R v.3.4.3 (R Core Team, 2017; https://www.R-project.org/).

3. Results

3.1. Multi-gene phylogenetic analyses and sequence comparisons

Two gene regions, BT1 and TEF-1α, were successfully sequenced, and these were deposited in GenBank with accession nos. MT121108–9 for BT1 and MT124072–3 for TEF-1α. The sequences obtained from BT1 and TEF-1α gene regions were aligned with closely related *Ceratocystis* spp., based on the BLAST search results from the NCBI nucleotide database BT1 (13 taxa, 545 characters), TEF-1α (13 taxa, 755 characters). Phylogenetic analyses using the concatenated BT1 and TEF-1α gene sequences resulted in a tree (Figure 1). Although the overall topologies generated from both ML and MP analyses were slightly different from each other, they consistently showed that the isolates from *Q. variabilis* represent a previously undescribed species. This species was most closely related to *C. ficicola* [26], but it was distinct from that species and all previously described *Ceratocystis* spp.

Sequence comparisons revealed that the *Ceratocystis* sp. differed from *C. ficicola* at 17 of 581 characters (about 29.3%) in the BT1 (Accession no. KY685078) and 29 of 780 characters (about 3.7%) in the TEF-1α sequences (Accession no. KY685079).

### Figure 1.
Phylogenetic trees based on maximum likelihood (ML) analysis of datasets of a combined dataset of BT1 and TEF-1α gene sequences for *Ceratocystis* species in the Asian-Australian clade of *Ceratocystis*. Isolates in bold and highlighted are the new species of *C. quercicola* described in this study. Bootstrap values >50% for MP and maximum likelihood (ML) are presented above branches as ML/MP, bootstrap values absent are not shown. Scale bar indicates 0.01 changes.
characterized by dark, globose ascomatal bases with long necks and hat-shaped ascospores. Cylindrical, hyaline conidia produced in flask-shaped conidiophores, and dark aleuriospores were also common in culture (Figure 2).

3.3. Taxonomy

Morphological comparisons and phylogenetic inference based on two gene regions provided conclusively sufficient evidence to show that the Ceratocystis sp. isolated from Q. variabilis represents an undescribed species residing in the Asian-Australian clade of Ceratocystis [27]. This species is described as follows:

*Ceratocystis quercicola* D. Hyeon Lee, S.E. Cho, Marinc, M.J. Wingf., sp. nov. (Figure 2) MycoBank No. MB834642

**Etymology:** The epithet refers to the host genus *Quercus*, from which the fungus was collected.

**Typus:** Gangneung, Korea 37° 43’41.7”N 128° 47’56.1”E, isolated from freshly made wounds (less than one month) on *Quercus variabilis*. The holotype, KA20-0012, dried culture of CDH2017-8 was deposited in the herbarium collection (KH) of Korea National Arboretum. The ex-holotype culture (CDH2017-8 = KACC 48669) was deposited in the culture collection of the National Institute of Forest Science (CDH) and Korean Agricultural Culture Collection for Type Cultures (KACC).

**Additional specimen examined:** Mt. 293-1, Eoheul-ri, Seongsan-myeon, Gangneung-si, province. Isolated from fresh wounds on *Q. variabilis*, August 2017, D.H. Lee, culture CDH2017-7.

**Habitat:** Freshly wounded tissues (less than one month) of *Quercus variabilis* trees.

**Known distribution:** The Republic of Korea (Gangwon province)

On 2% MEA, sexual and asexual states present. Sexual state. *Ascomatal base* subglobose to obpyriform, densely covered with dark hyphae, 178–269 × 141–251 μm; *ascomatal neck* straight, brown, becoming paler toward apex, 283–427 μm long, 27–41 μm wide near base, 20–29 μm wide near apex, base of neck occasionally thickened; *ostiolar hyphae* divergent, base sub-hyaline becoming hyaline toward tip, 24–58 μm long, 1–3 μm wide near base, 1–2 μm wide near apex; *ascomatal hyphae* brown, fertile or vegetative, producing 3 shapes of conidia when fertile. *Asci* evanescent. *Auscopores* hyaline, ellipsoidal to oblong with parallel sides, covered with a sheath which gives in side view a shape of hat, 5–6 × 3.5–4.5 μm (avg. 5.7 × 4.1 μm) without sheath.

Asexual state. Three different shapes of spores produced, cylindrical, barrel-shaped conidia, and aleuriospores. (1) Cylindrical; *conidiophores* macro-nematous, seldom branched, straight or flexuous, septate, subhyaline to hyaline, 65–195 μm long, 3–5 μm wide near base; *conidiogenous cells* endoblastic, integrated, cylindrical or tubular shape, often gradually tapering toward apex, 33–65 μm long, 3–5 μm wide near base; *conidia* abundant, hyaline, cylindrical, when produced in ascomatal hyphae, often with ends slightly bulged like a dumbbell, in chain, 8–18 × 3–4.5 μm (avg. 13 × 3.7 μm). (2) Barrel-shaped; scarce, observed only from fertile ascomatal hyphae, *conidiophores* macronematous, pigmented, straight, septate, sub-hyaline to pale brown; *conidiogenous cells* enteroblastic, integrated, cylindrical or tubular shape, becoming wider toward apex; *conidia* hyaline, in chain, barrel-shaped, 8–22 × 6–8 μm (avg. 10.3 × 7.1 μm). (3) Aleuriospores; *conidiophores* macronematous, straight or flexuous, occasionally branched, septate, 23–123 μm long, 3–4 μm wide near base; *conidiogenous cells* holoblastic, integrated, hyaline or subhyaline, cylindrical or tubular, 11–23 μm long, 3.5–4 μm wide; *conidia* abundant, terminal, in chain, basauxic, ellipsoidal to sub-globose, occasionally with base elongated, hyaline when young, becoming pigmented with age, 11–16 × 7.5–12 μm (avg. 13.5 × 9 μm).

**Culture characteristics:** Colonies on MEA greenish olivaceous, reverse greenish olivaceous. Mycelium immersed and superficial. Hyphae smooth, septate, without constriction at septa. Colony surfaces scattered with black ascomata. Optimal temperature for growth 25° C reaching 75 mm in 14 days, followed by 30° C (53 mm) and 20° C (51 mm). No growth at 5 or 35° C.

**Notes:** *Ceratocystis quercicola* was phylogenetically closely related to *C. ficicola* which first reported in Japan causing canker on *Ficus carica* (fig) [26]. However, *C. ficicola* had much larger structures than *C. quercicola*; larger ascomata (*C. ficicola*: 280–640 μm wide, *C. quercicola*: 141–251 μm wide), longer ascomatal neck (*C. ficicola*: 890–2,460 μm long, *C. quercicola*: 283–427 μm long), longer ostiolar hyphae (*C. ficicola*: 140–300 μm, *C. quercicola*: 24–58 μm long), and larger ascospores (*C. ficicola*: 6.5–8 × 4–5.5 μm, *C. quercicola*: 5–6 × 3.5–4.5 μm).

3.4. Pathogenicity test

The isolate used in the pathogenicity study produced distinct lesions on the cambium of the *Q. variabilis* logs 8 weeks after the inoculation (Figure 3). This is in contrast to the control inoculations where there were no signs of infection (p value < 0.01, at the 95% confidence level). Re-isolations from the lesions resulted in *C. quercicola* isolates morphologically indistinguishable from those inoculated onto the logs.
Figure 2. Field and microscopic features of *Ceratocystis quercicola* sp. nov. (ex-holotype: KACC 48699). (A) The wounded oak tree, *Quercus variabilis* from which the fungus was isolated. (B) Close-up of wound showing dark stain and fungal growth. (C) Fruiting structures on 2% MEA showing asexual states (white masses and thread-like) and a cluster of ascomata characterized by masses of dark hyphae (arrow). (D) Ascoma with a neck with thickened base. (E) Ascoma densely covered with dark fertile hyphae. (F,G) Divergent Ostiolar hyphae. (H,J) Ascospores with evident of gelatinous sheath (arrow). (I,K) Conidiogenous cell producing barrel-shaped conidia. (L) Chain of barrel-shaped conidia. (M) Aleuriospores showing basauxic generation. (N) Some aleurioconidia on 2% MEA with elongated base. (O) Chain of Aleuriospores. (P) Conidiophores producing cylindrical shape conidia. (Q,R) Conidiogenous cell producing cylindrical shape conidium. (S,T) Cylindrical shape conidia. Scale bars: C = 500 μm; D, E = 100 μm; F, G, L–P, S, T = 10 μm; H–J, Q, R = 5 μm.
4. Discussion

A routine tree health survey in a natural forest of Korea resulted in the discovery of a new Ceratocystis sp., commonly occurring on fresh wounds of Q. variabilis. Phylogenetic inference based on sequence data for parts of the BT1 and TEF-1α gene regions showed that this species resides in the Asian-Australian Clade of Ceratocystis [27]. The new species was provided with the name Ceratocystis quercicola, which was shown to be most closely related to C. ficicola. Other than being distinct from C. ficicola based on DNA sequences, C. quercicola can easily be distinguished from other Ceratocystis spp. based on its morphological characteristics.

The presence of C. quercicola on freshly induced wounds on trees is typical for Ceratocystis spp. as well as various other genera in the Ceratocystidaceae as defined by de Beer et al. [1]. We assume that, as with other Ceratocystis spp. and their relatives [5,13–16], C. quercicola was transferred to the wounds on Q. variabilis by insects such as flies and nitidulid beetles. This hypothesis needs to be tested.

The relatedness of C. quercicola and C. ficicola was not surprising given the fact that both fungi occur on trees in southeast Asia. However, C. ficicola is an aggressive pathogen of Ficus carica in Japan [26] and the disease that it causes in that country suggests that it could be an introduced pathogen. The pathogenicity test conducted in this study showed evidence that C. quercicola might be a pathogen, given the fact that lesions on inoculated trees of Q. variabilis are developed by infection of the fungus with very low level of aggressiveness. However, the preliminary pathogenicity test was conducted on freshly excised Q. variabilis bolts and the results should be interpreted with caution. Ideally, living trees would be inoculated but these could not be sourced and a proxy for pathogenicity [28].

Very little is known regarding species of Ceratocystidaceae or other insect related fungi such as the Ophiostomatales in Korea. The results of this study illustrate the fact that there is a significant reason to establish a baseline of understanding regarding these fungi in the country. Further surveys and research is planned to achieve this goal in the future.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by a project, the development of the effective control for oak wilt diseases and the research on applications of the damaged trees (Project No. FE0700-2017-2017), from the National Institute of Forest Science, Republic of Korea.

ORCID

Dong-Hyeon Lee http://orcid.org/0000-0002-6400-6132
Michael J. Wingfield http://orcid.org/0000-0001-9346-2009
Seonju Marincowitz http://orcid.org/0000-0002-4726-1211

References

[1] de Beer ZW, Duong TA, Barnes I, et al. Redefining Ceratocystis and allied genera. Stud Mycol. 2014;79:187–219.
[2] Seifert KA, De Beer ZW, Wingfield MJ. *Ceratocystis* and *Ophiostoma*: international spread, new associations and plant health. In Wingfield MJ, Roux J, Wingfield BD, Slippers B, editors. Ophiostomatoïd fungi: expanding Frontiers. Utrecht, The Netherlands: CBS-KNAW Biodiversity Centre, CBS Biodiversity Series, vol. 12; 2013. p. 191–200.

[3] Marin-Felix Y, Groenewald JZ, Cai L, et al. Genera of phytopathogenic fungi: GOPHY 1. Stud Mycol. 2017;86:99–216.

[4] Halsted BD. Some fungous diseases of the sweet potato. The black rot. New Jers Aes Bull. 1890;76:1–32.

[5] Roux J, Wingfield MJ. *Ceratocystis* species: emerging pathogens of non-native plantation *Eucalyptus* and *Acacia* species. South Forests. 2009;71(2):115–120.

[6] Barnes I, Fourie A, Wingfield MJ, et al. New *Ceratocystis* species associated with rapid death of *Metrosideros polymorpha* in Hawai‘i. Persoonia Mol Phylogenet Evol. 2018;40:154–181.

[7] Fourie A, Wingfield MJ, Wingfield BD, et al. Molecular markers delimit cryptic species in *Ceratocystis sensu stricto*. Mycol Prog. 2015;14:1–18.

[8] Morris MJ, Wingfield MJ, Beer C. Gummosis and wilt of *Acacia mearnsii* in South Africa caused by *Ceratocystis fimbriata*. Plant Pathol. 1993;42(5):814–817.

[9] Lee D-H, Roux J, Wingfield BD, et al. The genetic landscape of *Ceratocystis albifundus* populations in South Africa reveals a recent fungal introduction event. Fungal Biol. 2016;120(5):690–700.

[10] Walter JM. Canker stain of plane trees. Washington, DC: United States Department of Agriculture. (Circular No. 742.); 1946.

[11] DeVay JE, Lukezic FL, English H, et al. Ceratocystis canker of deciduous fruit trees. Phytopathology. 1968;58:949–954.

[12] Teviotdale BL, Harper DH. Infection of pruning and small bark wounds in almond by *Ceratocystis fimbriata*. Plant Dis. 1991;75(10):1026–1030.

[13] Møller WJ, DeVay JE. Carrot as a species-selective isolation medium for *Ceratocystis fimbriata*. Phytopathology. 1968;58:123–124.

[14] Cease KR, Juzwik J. Predominant nitidulid species (Coleoptera: Nitidulidae) associated with spring oak wilt mats in Minnesota. Can J For Res. 2001;31(4):635–643.

[15] Heath RN, Wingfield MJ, van Wyk M, et al. Insect associates of *Ceratocystis albifundus* and patterns of association in a native savanna ecosystem in South Africa. Environ Entomol. 2009;38(2):356–364.

[16] Mbenoun M, Garnas JR, Wingfield MJ, et al. Metacomunity analyses of *Ceratocystidaceae* fungi across heterogeneous African savanna landscapes. Fungal Ecol. 2017;28:76–85.

[17] Juzwik J, Skalbeck TC, Neuman MF. Sap beetle species (Coleoptera: Nitidulidae) visiting fresh wounds on healthy oaks during spring in Minnesota. For Sci. 2004;50:757–764.

[18] Mbenoun M, Wingfield MJ, Letsoalo T, et al. Independent origins and incipient speciation among host-associated populations of *Thielaviopsis ethacetica* in Cameroon. Fungal Biol. 2015;119(11):957–972.

[19] Rayner RW. 1970. A mycological colour chart. Kew, UK: Commonwealth Mycological Institute and British Mycological Society.

[20] Glass NL, Donaldson GC. Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Appl Environ Microbiol. 1995;61(4):1323–1330.

[21] Jacobs K, Bergdahl DR, Wingfield MJ, et al. *Leptographium wingfieldii* introduced into North America and found associated with exotic *Tomicus piniperda* and native bark beetles. Mycol Res. 2004;108(Pt 4):411–418.

[22] Katoh K, Misawa K, Kuma K, et al. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002;30(14):3059–3066.

[23] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol. 2016;33(7):1870–1874.

[24] Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics. 2006;22(21):2688–2690.

[25] Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web servers. Syst Biol. 2008;57(5):758–771.

[26] Kajitani Y, Masuya H. *Ceratocystis ficicola* sp. nov., a causal fungus of fig canker in Japan. Mycoscience. 2011;52(5):349–353.

[27] Li Q, Harrington TC, McNew D, et al. *Ceratocystis uchidae* sp. nov., a new species on Araceae in Hawaii and Fiji. Mycoscience. 2017;58(6):398–412.

[28] Van Wyk M, Heath RN, Tarigan M, et al. Comparison of procedures to evaluate the pathogenicity of *Ceratocystis fimbriata sensu lato* isolates from *Eucalyptus* in South Africa. South For. 2010;72(2):57–62.