ERGOT OF NUT SEDGE IN SOUTH AFRICA

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DECLARATION

I, the undersigned, declare that the thesis, which I hereby submit for the degree of Doctor of Philosophy to the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Ella Johanna van der Linde

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RESUMÉ

Several cases of bovine ergotism ascribed to the intake of fodder contaminated with yellow nut sedge (*Cyperus esculentus*) ergotised by *Claviceps cyperi* have been reported since 1996 from the eastern Highveld region in South Africa. These were the first incidents of ergotism associated with a *Claviceps* species infecting a non-poaceous host. *Claviceps cyperi* was described in 1967 from herbarium specimens collected between 1940 and 1944 in and around Pretoria in the former Transvaal Province, South Africa, and has not been recorded elsewhere in the world. Besides the above taxonomic account of *C. cyperi* and its apparent noxiousness, no information is available on the fungus. This study was undertaken to elucidate, in part at least, the symptomology and epidemiology of the disease, and the pathology, toxicology and phylogenetic relationship of *C. cyperi*.

Symptoms of ergot on nut sedge, germination of sclerotia of *C. cyperi*, and the morphology of live specimens of the pathogen were described for the first time. Honeydew associated with the disease is inconspicuous and the initial symptom of infection was a black sooty layer on inflorescences of infected plants due to colonisation of the honeydew by the saprophytic fungus *Cladosporium cladosporioides*. Ergot sclerotia started to develop in March and April and could be discerned as small protuberances on inflorescences in the place of seed. Mature sclerotia were purplish-

black and required a resting period of about two months before germinating. Germination occurred without prior cold treatment, though exposure of the sclerotia to 5 °C for 21 days significantly increased the germination rate. Dimensions of sclerotia, stipes, capitula, asci and ascospores of live specimens were somewhat larger than in the original description, but the general morphology supported treatment of *C. cyperi* as a distinct species. Comparison of *C. cyperi* with 15 other *Claviceps* species available in the GenBank sequence database by means of multilocus PCR fingerprinting of genomic DNA and sequence analysis of the ITS1-5.8 rDNA-ITS2 and ß-tubulin gene intron 3 regions confirmed that it is a separate species, phylogenetically the closest related to *Claviceps zizaniae*, the ergot fungus of wild rice (*Zizania* spp.).

The sphacelial state of *C. cyperi* was isolated and grown in culture on various media at different temperatures. Optimal growth occurred at 24 °C, with no growth evident at 5 °C and 32 °C. The anamorph conformed to the description of *Sphacelia*, but an enteroblastic mode of conidiogenesis could not be confirmed and placement of the species in *Sphacelia* is therefore *nomen provisorium*.

Infection of yellow nut sedge by *C. cyperi* could not be achieved in the greenhouse. Microscopic examination of material collected in the field indicated that infection by *C. cyperi*, unlike most other ergot species, not necessarily mimics the pollination process, as infection of ovaries in some florets seemed to have already occurred when stylodia only started protruding. The dark layer of the omnipresent *C. cladosporioides* covering the honeydew appeared to cause a physical barrier preventing florets from opening, hence impeding development of sclerotia. *Fusarium heterosporum* was also often present in the honeydew but did not seem to have any effect on disease development. Large numbers of spotted maize beetle (*Astylus atromaculatus*) were commonly observed visiting nut sedge inflorescences, whereas larvae of an unidentified thrips species invaded and consumed the ovaries and anthers. These insects possibly contributed to the dissemination and/or natural control of the disease.

The main ergopeptine alkaloid in sclerotia of *C. cyperi* was identified by HPLC and tandem mass spectroscopy as α-ergocryptine, with small amounts of ergosine, ergocornine and ergocrystine also present. This alkaloid profile corresponds with the

alkaloid content of the fodder implicated in the outbreaks of bovine ergotism and is typically associated with "summer syndrome" symptoms observed in affected cattle. Although α-ergocryptine is toxic to humans and animals, its brominated derivative, 2-bromo-α-ergocryptine, is a valuable drug with various pharmaceutical applications. Unfortunately, all attempts at inducing *C. cyperi* to synthesise α-ergocryptine in culture for commercial use have failed.

LIST OF FIGURES

LIST OF TABLES

CONTENTS