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A Biosystematic Study of Three Plant Pathogenic Fungi

(TITLE)

BY

Richard James Langham

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY CHARLESTON, ILLINOIS

1994

YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING THIS PART OF THE GRADUATE DEGREE CITED ABOVE

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Abstract

Isolates of *Chalara australis*, Eucalypt *Ceratocystis*, and *Ceratocystis virescens* were grown on Potato Dextrose Agar, Malt Extract Agar, and Czapek Agar and examined as part of a biosystematic study on these fungi. Basic micromorphological and macromorphological data on the cultures were recorded and the taxa were found to be morphologically similar. Tissue isolates and single spore isolates were crossed within these taxa and an interspecific cross performed. *Chalara australis* was found to be either asexual or have only one mating type present in the cultures studied. Eucalypt *Ceratocystis* was found to have a mating system characteristic of bipolar heterothallism. *Ceratocystis virescens* was found to have no specific mating system. The interspecific cross generated data that indicates that these taxa are distinct species despite their morphological similarities.

Acknowledgements

I would like to thank Dr. Andrew Methven for his patience and guidance during this entire project. I would also like to thank him for introducing me to the world of mycology. I would like to thank Dr. Mark Boudreau for introducing me to the field of plant pathology. I would also like to thank Dr. John Speer and Dr. John Ebinger for their advice and comments on this thesis. I would like to thank my family for their encouragement and support both psychologically and financially.

I wish to thank Dr. T.C. Harrington, Department of Plant Pathology, Iowa State University who introduced me to this specific project and provided the cultures needed to pursue it. I would like to acknowledge the Eastern Illinois University Botany Department for equipment and supplies needed to carry out my project.

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I. Introduction

The ophiostomatoid fungi comprise a ubiquitous group of more than 100 species distributed in the genera *Ceratocystis* Ellis & Halstead and *Ceratocystiopsis* Upad. & Kendrick (Ophistomataceae, Microascales, Plectomycetes, Ascomycota; Upadhyay, 1993). Although the ophiostomatoid fungi were originally described by Nannfeldt (1932), the following definition includes new characteristics brought to light by subsequent workers. Ascocarps globose, subglobose or flask-shaped, dark, hyaline or lightly colored in whole or in part, leathery or ornamented, ostiolate (perithecial) or entirely closed (cleistothecial). Ostiolate ascocarps usually have long necks that may or may not be terminated with fringes of ostiolar hyphae. The asci are produced irregularly throughout the centrum, are clavate, subspherical, spherical or fusiform, deliquesce early in development, and lack a crozier. The ascospores are hyaline, usually unicellular, variable in shape, lack germ pores, are produced in a mucilaginous matrix, and exude through the neck into a mucilaginous droplet at the apex of the perithecia (Upadhyay, 1993).

Due to the complexity of taxonomic and phylogenetic relationships among the ophiostomatoid fungi there is a continuing debate regarding generic concepts within the

family Ophiostomataceae. Because a discussion of generic concept within this family is beyond the scope of this project, only the genus *Ceratocystis* will be recognized as a member of the Ophiostomataceae.

Several mycologists have studied perithecial development in *Ceratocystis* and reported unique ascus formation. Elliot (1925) noted that a stalked, one-celled "oogonium" with a trichogyne fused with an antheridium prior to nuclear fusion in *Ceratocystis fimbriata* Ellis & Halstead and that asci subsequently arose as binucleate swellings from the ascogenous hyphae. Mittmann (1932), Andrus (1936), Andrus and Harter (1933), and Gwynne-Vaughan and Broadhead (1936) reported that perithecia in *C. fimbriata* arose as a side branch of hyphae which became coiled and divided into uninucleate cells. No antheridia or definite nuclear fusion was observed although several cells of the ascogenous hyphae enlarged and became multinucleate. Lateral branches from the lower cells of the archicarp envelop the ascogenous hyphae to form the perithecial wall. The central cells of the archicarp become lobed, fragment as nuclear division proceeds, and "lie free" in a nutrient medium in the perithecial cavity. The nuclei of the central cells increase in number as the cells divide to form a number of binucleate cells which develop directly into asci while lying free in the perithecial cavity. The delicate walls of the asci break down to release the ascospores which are subsequently pushed

up and collect in a mass in the penicillate hairs at the apex of the ostiole (Wehmeyer, 1975). This sticky mass of ascospores is well-adapted for dispersal by a number of insect vectors (Harrington et. al., 1992).

In the genus Ceratocystis both homothallic and heterothallic species have been described. In heterothallic individuals the production of perithecia is dependent upon pairing of compatible strains as demonstrated in C. fagacearum (Bretz) Hunt and C. paradaxa (Dade) Moreau. Heterothallic fungi can be further subdivided into bipolar (unifactorial) or tetrapolar (bifactorial) groups. Bipolar taxa exhibit two mating types or groups of individuals that differ in their allelic composition for a compatibility factor or gene. Only thalli or hyphal systems whose nuclei carry different alleles of the same gene are compatible. Tetrapolar fungi are composed of four mating types or groups of individuals. Compatibility is governed by two pairs of genes located on different chromosomes. Only thalli or hyphal systems whose nuclei carry different alleles for both genes are compatible (Alexopoulos and Mims, 1979). In homothallic individuals like C. fimbriata, sexual reproduction takes place within a single thallus or hyphal system that is essentially self-fertile.

The taxa selected for this study are Ceratocystis virescens (Davidson) Moreau, Chalara australis Walker & Kile and an undescribed taxon referred to as Eucalypt

Ceratocystis. Ceratocystis virescens was originally described by Davidson as a fungus which causes a sapstreak disease of maple in eastern North America (Harrington et. al., 1992). Hartig (1878) demonstrated that discolorations in the wood were caused by darkly pigmented fungal hyphae. In maple, the fungus moves through the xylem and induces symptoms similar to a vascular wilt pathogen such as Ceratocystis fagacearum, the cause of oak wilt (Harrington et. al., 1992). Eucalypt Ceratocystis was discovered by G. A. Kile and appears to grow as a saprophyte on Eucalyptus L'Hérit in the states of Victoria and Tasmania in Australia (Harrington et. al., 1992).

The genus Chalara (Corda) Rabenh. was originally described from conifers in Breznia, Czechoslovakia, and found to be the anamorph of Ceratocystis. All members of Ceratocystis have Chalara anamorphs (Harrington et. al., 1992). An anamorph is the nonsexual or conidial stage of the fungal life cycle (Moore-Landecker, 1990). Chalara australis is responsible for a widespread, but locally severe, disease of Nothofagus cunninghamii (Hook.f.) Oersted in the rain forests of Tasmania, Australia. Symptoms of disease in individual trees include attack by the mountain pinhole borer Platypus subgranosus Schedl, chlorosis-necrosis, abscission of foliage, sudden wilting of shoots and foliage, discoloration of sapwood from the roots to the upper stem, and the formation of dark gray-black fungal felts on the

bark of infected stems (Kile and Walker, 1987). Lindau (1907) recognized conidial ontogeny within the conidiogenous cells as the characteristic feature of *Chalara* (Nag Rag and Kendrick, 1975). Lindau noted that "the mechanism by which the conidia are formed in the interior of the conidiogenous cells is not precisely known, but it is probably similar to endogenous spore formation in many other fungi. Probably the upper cells are hollow and the conidia are formed in the interior by ascogenous septation, emerging in the form of chains from the aperture above."

The purpose of this study was to identify asexual and sexual cultural characteristics present in *Ceratocystis virescens*, Eucalypt *Ceratocystis*, and *Chalara australis* and to elucidate their mating systems. The morphological characteristics observed will be used to identify these taxa in culture. Once the mating systems are elucidated, the life cycles of these fungi will be better understood which could lead to techniques for biological control of these plant pathogens.

II. Materials and Methods

Isolates of Eucalypt *Ceratocystis*, *Ceratocystis virescens*, and *Chalara australis* were obtained from Dr. Thomas Harrington at Iowa State University, Ames, Iowa. These cultures were transferred by car from Ames to Charleston, Illinois in sealed malt extract culture slants and stored in the dark at 8-10°C until needed.

A. Cultures utilized

Chalara australis

Australia

Tasmania: Peak Rivulet Road, Esperance Valley, on *Nothofagus cunninghamii*, March 1989, collected by Kile, isolates C448, C453, C454 (ISC); Boyd River, on *Nothofagus cunninghamii*, September 1989, collected by Kile, isolate C451, C452 (ISC); same location, unknown collection date, isolates C620, C621, C628 (ISC); Little Florentine River, on *Nothofagus cunninghamii*, May 1988, collected by Kile, isolates C455, C456 (ISC); same location, unknown collection date, isolate C619 (ISC); Arve Valley, on *Eucalyptus regnans*, March 1988, collected by Kile, from freeze dried spore suspension made 1980-1986, isolate C457 (ISC); same locality, on *Nothofagus cunninghamii*, unknown date,

collected by Kile, from freeze-dried spore suspension made 1980-1986, isolate C625 (ISC); Smithton, on Nothofagus cunninghamii, unknown date, collected by Kile, from freeze-dried spore suspension of isolate made between 1980-1986, isolate C614 (ISC); Christmas Hills Rd, on Nothofagus cunninghamii, unknown date, collected by Kile, isolate C615, (ISC); Blackwater Rd, on Nothofagus cunninghamii, unknown date, collected by Kile, isolate C616 (ISC); Mt. Arrowsmith, on Nothofagus cunninghamii, unknown date, collected by Kile, isolate C617 (ISC); Tarraleah, on Nothofagus cunninghamii, unknown date, collected by Kile, from freeze-dried spore suspension made 1980-1986, isolate C618 (ISC); Simon's Rd, on Nothofagus cunninghamii, unknown date, collected by Kile, from freeze-dried spore suspension made 1980-1986, isolate C622 (ISC); Lottah Rd, on Nothofagus cunninghamii, unknown date, collected by Kile, isolate C623 (ISC); Howard's Rd, on Nothofagus cunninghamii, unknown date, collected by Kile, from freeze-dried spore suspension made 1980-1986, isolate C624 (ISC); Esperance River, on Nothofagus cunninghamii, unknown date, collected by Kile, isolate C626 (ISC); unknown location, on Nothofagus cunninghamii, unknown date, collected by Kile, isolate C629 (ISC).

Victoria: Central Highlands, on Nothofagus cunninghamii, unknown date, collected by Kile, isolates C631, C632, C633, C634, C635, C636 (ISC); Strzelecki Range, on Nothofagus cunninghamii, collected by Kile, isolate C637

(ISC); Otway Range, on *Nothofagus cunninghamii*, collected by Kile, from freeze-dried spore suspension made 1980-1986, isolate C638 (ISC).

Eucalypt *Ceratocystis*

Australia

Victoria: unknown location, on *Eucalyptus sieberi*, unknown date, collected by Kile, isolates C639, C642, C643, C644, C645, C646 (ISC); unknown location, on *Eucalyptus globoidea*, unknown date, collected by Kile, isolates C640, C641 (ISC); unknown location, on *Eucalypt* sp., unknown date, collected by Kile, isolate C449 (ISC).

Ceratocystis virescens

Unknown location, unknown date, collected by Shigo, isolate C70 (ISC).

United States

New York: Mantle, on *Acer saccharinum*, unknown date, collected by Houston, isolate C68 (ISC); same location, on *Acer saccharum*, unknown date, isolated from sapstreak by Houston, isolates C252, C253 (ISC); Forward Truck Trail, on *Acer saccharum*, unknown date, isolated from sapstreak by Houston, isolate C259 (ISC).

North Carolina: on *Liriodendron tulipifera*, unknown date, collected by Hepting, isolate C203 (ISC).

Wisconsin: on Acer saccharum, unknown date, isolated from sapstreak by Houston, isolate C256 (ISC).

B. Analysis of cultural characteristics

Isolates of each of the fungi used in this study were grown on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), and Czapek's Agar (CZ) in order to observe individual morphological characteristics (Appendix A). Plates were inoculated by placing @ 0.5 cm x 1 cm blocks of hyphae from an actively growing culture in the center of 90 mm Pyrex Petri Plates using a sterile probe. Five replicates from two isolates of each species were inoculated on each of the three types of media. Cultures were sealed with parafilm and incubated in the dark at 20° C. Each of the cultures were observed after 3, 9, 14, and 21 days and the following characteristics recorded: Growth rate (colony diameter), macroscopic appearance (color, texture, etc.), and microscopic morphology (presence or absence of conidia, length and diameter of conidia, presence or absence of conidiophores and conidiogenous cells, morphology of conidiophores and conidiogenous cells, and the formation of perithecial initials). Perithicial initials are referred to as protoperithecia in this study.

C. Crossing procedure

Prior to the initiation of controlled crosses, 10-12 stock isolates of each species to be crossed were grown on 90 mm Pyrex Petri Plates containing MEA in the dark at 21°C for 7-10 days or until the surface of the plate was covered. Eight of the isolates were subsequently selected for crossing based on cultural morphology and geographical distribution of the isolates. Nine recipient cultures and nine donor cultures were prepared for each of the eight isolates. Donor cultures were grown on MEA while recipient cultures were grown on malt extract twig agar (META). META was prepared by cutting the twigs of various oak species into 2 cm long and 1 cm diameter segments with pruning shears. Each segment was subsequently cut in half longitudinally and the segments sterilized in an autoclave for 1 hour. One autoclaved twig segment was placed in each 90 mm Pyrex Petri Plate as MEA was poured into the plates.

Donor cultures were allowed to grow until the mycelium colonized the entire surface of the substrate. A conidial and mycelial or "donor" suspension was prepared by flooding the surface of the donor plate with 5.0 ml of sterile distilled water and lightly scraping the surface of the agar and the mycelium with a sterile spatula. Two to three drops of the "donor" suspension was placed on the surface of the recipient culture using a sterile Pasteur Pipette and a small squeeze bulb. The recipient plates were gently

agitated to spread the "donor" suspension over the surface of the mycelium, sealed with parafilm, and placed in an incubator at 21°C in the dark. Each of the eight isolates were crossed in every possible combination so that each of the eight recipient cultures were crossed with each of the eight donor cultures. After 5-7 days the cultures were removed from the incubator and checked for the formation of protoperithecia and perithecia using a dissecting microscope in a laminar flow hood.

If perithecia were observed, the presence or absence of a droplet exuding from the ostiole of the perithecia was assessed by examining the cultures with a dissecting microscope. If a droplet was observed the droplet was allowed to turn milky-white before it was checked for the presence or absence of ascospores. A wet mount of a milky-white droplet was prepared by using a dissecting needle to suspend the droplet. in a drop of light oil (isopar M) on a slide and covering the suspension with a coverslip. The slide was examined using a compound microscope at 430x for the presence of ascospores. If ascospores were observed, single spore isolates were prepared as follows.

D. Single Spore Isolates

To obtain single spore isolates, 30 ul of isopar M oil was placed into a well of a double depression slide using a Pipetman micropipetter. Using a sterile dissecting needle a

milky-white droplet was removed from the apex of a perithecium, placed in the same well, and mixed with the oil. Using a sterile inoculating loop, one loopful of the spore suspension was streaked over the surface of a plate of MEA. 15 ul of isopar M oil was then placed in the other well of the double depression slide and 15 ul of the suspension in the first well was added to it using a micropipetter. This suspension was streaked on a plate of MEA in the same manner. Both plates were sealed with parafilm and stored in the dark at 21° C. Streaked plates were checked after 18-24 hours for the presence of germlings (i.e., germinating ascospores) using a compound microscope at 100x. Germlings or monokaryons were removed from streaked plates using a sterile probe, transferred to META plates, and allowed to grow for 10-14 days in the dark at 21°C.

Single spore isolates were mated amongst each other using the crossing technique described above. Matings of the F_1 progeny were then scored for successful (compatible) or unsuccessful (incompatible) matings. This procedure was performed within all three of the species under study (intraspecific crosses), between all three species (interspecific crosses), and the F_1 single spore isolates of each species (intraspecific crosses).

III. Results and Discussion

A. Analysis of cultural characteristics

Chalara australis isolate C624

Macromorphology: PDA: Radius day 3, 2-5 mm, day 9, growth covering entire plate. Hyphae dark gray, submerged, tufts of dark gray aerial mycelium forming with area around inoculum becoming fluffy. Few protoperithecia produced between day 14 and 21. **MEA:** Radius day 3, 3-6 mm, day 9, growth covering entire plate. Hyphae gray, submerged with little aerial mycelium forming. No protoperithecia produced. **Cz:** Radius day 3, 1-6 mm, day 9, growth covering entire plate. Hyphae light gray, submerged, growth sparse, little aerial mycelium produced. Few protoperithecia produced between day 14 and 21.

Micromorphology: PDA: Hyphae branched, septate, hyaline, thin-walled, 2.5-8.8 um diameter. Conidia produced between day 9 and 14, phialospores, differentiated inside a phialide or conidiogenous cell, produced in long, fragile chains. Conidia (n=50) 4-11 x 2-4 um (L_m= 7.7 um; W_m= 3.1 um), non-septate, barrel-shaped, swollen on occasion, hyaline, thin-walled. **MEA:** Hyphae branched, septate, hyaline, thin-walled, 2.5-10 um diameter. Conidia produced between day 9 and 14, formed as on PDA. Conidia (n=50) 4-8 x 2.5-13.5 um (L_m= 6.5 um; W_m= 2.7 um),

morphology as on PDA. **Cz:** Hyphae branched, septate, hyaline, thin-walled, 2.5-7.5 um diameter. Conidia produced between inoculation and day 3, formed as on PDA. Conidia (n=135) 4-11 x 2-3.5 um (L_m= 6.5 um; W_m= 2.6 um) morphology as on PDA (Table I).

Chalara australis isolate C635

Macromorphology: PDA: Radius day 3, 5-12 mm, day 9, growth covering entire plate. Hyphae dark gray to black with areas of fluffy white aerial mycelium. No protoperithecia produced in 21 days. **MEA:** Radius day 3, 4-10 mm, day 9, growth covering entire plate. Hyphae hyaline, becoming dark gray with age, little aerial mycelium formed. No protoperithecia produced in 21 days. **Cz:** Radius day 3, 4-11 mm, day 9, growth covering entire plate. Hyphae white to light gray, growth sparse, irregular, little aerial mycelium. No protoperithecia produced in 21 days.

Micromorphology: PDA: Hyphae branched, septate, hyaline, thin-walled, 2.5-10 um diameter. No conidia produced in 21 days. **MEA:** Hyphae as on PDA. Conidia as on PDA. **Cz:** Hyphae branched, septate, hyaline thin-walled, 2.5-7.5 um diameter. Conidia as on PDA (Table I).

Eucalypt Ceratocystis isolate C639

Macromorphology: PDA: Radius day 3, 6-13 mm, day 9, growth covering entire plate. Hyphae light gray, turning dark gray with age, light gray aerial mycelium present over

Table I. Summary of conidia measurements of *Chalara australis* isolates. (n) = number of conidia measured; (L_m) = mean length; (W_m) = mean width; (---) = no conidia observed.

Isolates	Media	n	Range(um)	L_m(um)	W_m(um)
C624	PDA	50	4-11 x 2-4	7.7	3.1
	MEA	50	4-8 x 2.5-13.5	6.5	2.7
	Cz	135	4-11 x 2-3.5	6.5	2.6
C635	PDA	---	---	---	---
	MEA	---	---	---	---
	Cz	---	---	---	---

the entire plate. Protoperithecia produced between day 3 and 9. **MEA:** Radius day 3, 6-10 mm, day 9, growth covering entire plate. Hyphae light gray, turning dark gray with age, thin aerial mycelium covering entire plate. Protoperithecia produced between day 3 and 9. **Cz:** Radius day 3, < 1 mm, day 9, growth covering entire plate. Hyphae white, becoming light gray, little aerial mycelium covering entire plate. Protoperithecia produced between day 14 and 21.

Micromorphology: PDA: Hyphae branched, septate, hyaline, thin-walled, 2.5-12 um diameter. Conidia produced between day 1 and 3, phialospores, differentiated inside a phialide or a conidiogenous cell, produced in long, fragile chains. Conidia (n= 150) 3-26 x 2.5-5 um (L_m= 10.2 um; W_m= 3.2 um) non-septate, barrel-shaped, hyaline, thin-walled. **MEA:** Hyphae as on PDA. Conidia produced between day 1 and 3, formed as on PDA. Conidia (n=127) 4-17 x 2-4 um (L_m= 8.8 um; W_m= 2.9 um), morphology as on PDA. **Cz:** Hyphae as on PDA. Conidia produced between day 1 and 3, produced as on PDA. Conidia (n= 125) 3-15 x 2-5 um (L_m= 10.0; W_m= 3.2 um), morphology as on PDA (Table II).

Eucalypt Ceratocystis isolate C644

Macromorphology: PDA: Radius day 3, 6-11 mm, radius day 9, 30-40 mm, day 14, growth covering entire plate. Hyphae light gray, turning dark gray with age. Tufts of white aerial mycelium turning light gray over the entire plate.

Protoperithecia produced between day 3 and 9. **MEA:** Radius day 3, 6-15 mm, day 9, 31-41 mm, day 14, growth covering entire plate. Hyphae white, becoming light gray with age, thin, light gray aerial mycelium covering entire plate. Protoperithecia produced between day 3 and 9. **Cz:** Radius day 3, 2-7 mm, day 9, growth covering entire plate. Hyphae gray over the entire plate, thin white aerial mycelium growing irregularly over entire plate. Protoperithecia produced between day 9 and 14.

Micromorphology: PDA: Hyphae branched, septate, hyaline, thin-walled, 2.5-10 um diameter. Conidia produced between day 1 and 3, phialospores differentiated inside a phialide or conidiogenous cell, produced in long, fragile chains. Conidia (n=100) 2.5-26 x 2-4.5 um (L_m= 12.8; W_m= 3.1 um), non-septate, barrel-shaped, hyaline, thin-walled. **MEA:** Hyphae as on PDA. Conidia produced between day 3 and 9, formed as on PDA. Conidia (n=100) 6-19 x 2-4 um (L_m= 10.6 um; W_m= 2.9 um), morphology as on PDA. **Cz:** Hyphae as on PDA. Conidia produced between day 3 and 9, formed as on PDA. Conidia (n= 100) 4-25 x 2-4 um (L_m= 12.7; W_m= 2.9), morphology as on PDA (Table II).

Ceratocystis virescens isolate C68

Macromorphology: PDA: Radius day 3, 13-19 um, day 9, growth covering entire plate. Hyphae light to dark gray, aerial mycelium white turning gray at the edges of the

Table II. Summary of conidia measurements of Eucalypt *Ceratocystis* isolates. (n) = number of conidia measured; (L_m) = mean length; (W_m) = mean width.

Isolates	media	n	Range(um)	L_m(um)	W_m(um)
C639	PDA	150	3-26 x 2.5-13.5	10.2	3.2
	MEA	127	4-17 x 2-4	8.8	2.9
	Cz	125	3-15 x 2-5	10.0	3.2
C644	PDA	125	6-26 x 2-4.5	12.8	3.1
	MEA	100	6-19 x 2-4	10.6	2.9
	Cz	100	4-25 x 2-4	12.7	2.9

culture. Protoperithecia produced between day 3 and 9. **MEA:** Radius day 3, 10-18 mm, day 9, growth covering entire plate. Hyphae white, turning brown with age, forming a small area of light gray aerial mycelium around the edges of the culture. Protoperithecia produced between day 14 and 21. **Cz:** Radius day 3, 10-20 mm, day 9, growth covering entire plate. Hyphae white, turning light gray with age, thin white aerial mycelium covering entire plate. No protoperithecia produced.

Micromorphology: PDA: Hyphae, branched, septate, strangulated, hyaline, thin-walled, 2.5-7.5 um in diameter. Conidia produced between day 9 and 14, phialospores, differentiated inside a phialide or conidiogenesis cell, produced in long, fragile chains. Conidia (n=75) 5-17 x 2-3.5 um (L_m= 9.6 um; W_m= 2.7 um), non-septate, barrel-shaped hyaline, thin-walled. **MEA:** Hyphae as on PDA. Conidia produced between day 14 and 21, formed as on PDA. Conidia (n=50) 4-19 x 2-4 um (L_m= 8.5 um; W_m= 2.8 um), morphology as on PDA. **Cz:** Hyphae as on PDA. Conidia produced between day 14 and 21, formed as on PDA. Conidia (n=100) 5-22 x 2-3.5 um (L_m= 9.3 um; W_m= 3.1 um), morphology as on PDA (Table III).

<u>Ceratocystis</u> <u>virescens</u> isolate C261

Macromorphology: Radius day 3, 23-34 mm, day 9, growth covering entire plate. Hyphae white, turning dark gray with age, very thick gray aerial mycelium produced. Protoperithecia produced between day 9 and 14. **MEA:** Radius

day 3, 9-23 mm, day 9, growth covering entire plate. Hyphae dark gray, turning light gray at edges of the culture, light gray aerial mycelium covering entire plate. Protoperithecia produced between day 9 and 14. **Cz:** Radius day 3, 7-13 um, day 9, growth covering entire plate. Hyphae light gray over the entire culture, thin aerial mycelium over entire plate. Protoperithecia produced after day 9.

Micromorphology: PDA: Hyphae branched, septate, hyaline, thin-walled, 2.5-10 um diameter. Conidia produced between day 1 and 3, phialospores, differentiated inside a phialide or conidiogenous cell, produced in long, fragile chains. Conidia (n=150) 4-16 x 2-4 um (L_m= 9.0 um; W_m= 2.7 um), non-septate, barrel-shaped, hyaline, thin-walled. **MEA:** Hyphae as on PDA. Conidia produced between day 3 and 9, formed as on PDA. Conidia (n=75) 4-16 x 2-3 um (L_m= 9.0 um; W_m= 2.6 um), morphology as on PDA. **Cz:** Hyphae as on PDA. Conidia produced between day 1 and 3, formed as on PDA. Conidia (n=75) 4-16 x 2-3.5 um (L_m= 8.5 um; W_m= 2.5 um), morphology as on PDA.

The taxa characterized in this study are indistinguishable using solely macromorphology and micromorphology with as much difference between various isolates of the same species as between the species themselves. The fungi did develop noticeably different on the three media types used. Growth was faster and more

Table III. Summary of conidia measurements of *Ceratocystis virescens* isolates. (n)= number of conidia measured; (L_m)= mean length; (W_m)= mean width.

Isolates	media	n	Range(um)	L_m(um)	W_m(um)
C68	PDA	75	5-17 x 2-3.5	9.6	2.7
	MEA	50	4-19 x 2-4	8.5	2.8
	Cz	75	5-22 x 2-3.5	9.3	3.1
C261	PDA	150	4-16 x 2-4	9.0	2.7
	MEA	75	4-16 x 2-3	9.0	2.6
	Cz	75	4-16 x 2-3.5	8.5	2.5

vigorous on PDA than MEA or Cz, with more aerial mycelium forming on the PDA as well.

B. Mating Analyses

Mating systems have become an important tool for determining species concepts in fungal systematics. Boidin (1986) summarized the uses of intercompatibility testing as follows: 1) to determine if morphologically similar taxa are reproductively isolated; 2) to determine the range of variability within a single biological species; 3) to demonstrate genetic isolation of closely related species; 4) to detect reproductive isolation of a single morphospecies and to demonstrate gradual speciation; and, 5) to determine the geographical range of a species.

In this project the mating systems of Chalara australis, Ceratocystis virescens, and Eucalypt Ceratocystis were determined by crossing tissue isolates of each species as well as single ascospore isolates obtained from the tissue crosses. In each of the crosses performed, a successful mating was indicated by the formation of fertile perithecia which produce ascospores. Sterile perithecia, which were perithecia in which ascospores did not develop, indicate that a successful mating did not occur. Based on these data it was possible to determine if the taxa were asexual, homothallic, bipolar heterothallic or tetrapolar heterothallic.

Table IV. Chalara australis tissue isolate crosses. Donor cultures across the top, recipient cultures down left the column. (SP) = sterile perithecia; (PP) = protoperithecia; (---) = no protoperithecia or perithecia.

	C448	C449	C615	C620	C626	C631	C635	C637
C448	PP	PP	PP	PP	PP	PP	PP	PP
C449	PP	PP	SP	PP	SP	SP	PP	PP
C615	PP	PP	PP	---	---	PP	---	---
C620	---	PP	---	---	---	---	---	---
C626	PP	PP	PP	PP	---	PP	PP	---
C631	---	PP	PP	PP	PP	PP	PP	---
C635	PP	PP	PP	PP	PP	PP	---	PP
C637	PP	PP	PP	PP	---	PP	PP	---

Results from _Chalara australis_ crosses are summarized in Table IV. In all of the crosses performed no fertile perithecia were observed. Isolate C635 was then crossed both as a donor and recipient to isolates C450, C451, C452, C453, C454, C455, C456, C457, C614, C616, C617, C618, C619, C621, C622, C623, C624, C625, C628, C629, C632, C633, C634 and C638 but no positive crosses were produced. These data indicate that either this species is asexual or that only isolates of one mating type are present among the cultures studied.

Results from the crosses of Eucalypt _Ceratocystis_ tissue isolates and single spore isolates, summarized in Tables V and VI, respectively, are characteristic of bipolar heterothallism. When single spore isolates were mated in all possible combinations (Table VI) the two mating types segregated out into "recipient" cultures (mat-1) and "donor" cultures (mat-2). Single spore isolates A, E, F, and H are "recipient" or mat-1 cultures that are characterized by the formation of protoperithecia or fertile perithecia. Single spore isolates B, C, D, and G "donor" or mat-2 cultures. In these isolates, B spermatizes A and F, D spermatizes A, and G spermatizes F and H. These data indicate a mating system in which a single multiple allelic gene controls compatibility.

Results from crosses of _Ceratocystis virescens_ tissue isolates and single spore isolates are summarized in Tables

Table V. Cross of **Eucalypt** Ceratocystis tissue isolates. Donor cultures across the top, recipient cultures down the left column. (FP) = fertile perithecia; (SP) = sterile perithecia; (PP) = protoperithecia; (---) = no perithecia or protoperithecia.

	C639	C640	C641	C642	C644	C645	C646
C639	PP	FP	FP	FP	FP	PP	PP
C640	PP	---	PP	PP	---	---	---
C641	PP	---	---	PP	---	---	---
C642	FP	SP	PP	PP	PP	FP	---
C644	---	---	---	PP	---	---	PP
C645	PP	---	---	PP	---	---	PP
C646	PP	PP	PP	PP	PP	PP	PP

Table VI. Eucalypt *Ceratocystis* single spore isolate crosses. Donor cultures listed across the top, recipient cultures listed down the left column. (FP) = fertile perithecia; (PP) = protoperithecia; (---) = no perithecia or protoperithecia.

	A	B	C	D	E	F	G	H
A	PP	FP	PP	FP	PP	PP	PP	PP
B	---	---	---	---	---	---	---	---
C	---	---	---	---	---	---	---	---
D	---	---	---	---	---	---	---	---
E	PP	PP	PP	PP	PP	PP	PP	PP
F	PP	FP	PP	PP	PP	PP	FP	PP
G	---	---	---	---	---	---	---	---
H	PP	PP	PP	PP	PP	PP	FP	PP

VII and VIII, respectively. Data generated from crosses of these isolates indicated no specific mating system and the isolates were found to act both as "donor" and "recipient" cultures. For example, isolate C252 acts as a recipient culture when crossed with isolate C70 but as a "donor" culture when crossed with isolate C256 (Table VII). The single spore isolates behave in much the same manner, with isolates D, F, and H being both "donors" and "recipients" (Table VIII). This data neither confirms nor disproves the current thinking that C. virescens is homothallic (Harrington et. al., 1992).

Results from the interspecific cross are summarized in Table IX. Isolates of C. virescens that were not self-fertile were paired with each other, with an isolate of Chalara australis, and with both mating types of the Eucalypt Ceratocystis. The Chalara australis isolate (C448) was not self-fertile nor did it mate successfully with any of the other isolates. The Eucalypt Ceratocystis produced fertile perithecia when paired with other isolates of the same taxon and with a single isolate of C. virescens but did not pair with the Chalara australis isolate. Within the C. virescens isolates only C253 produced fertile perithecia when selfed and when paired with Eucalypt Ceratocystis isolate C639. The formation of fertile perithecia was likely due to a gene induction and is actually a self-cross. These data suggests that Ceratocystis virescens, Chalara

Table VII. *Ceratocystis virescens* tissue isolate crosses. Donor culture across the top, recipient cultures down the left column. (FP) = fertile perithecia; (SP) = sterile perithecia; (PP) = protoperithecia; (---) = no perithecia or protoperithecia.

	C68	C70	C203	C252	C253	C256	C259
C68	---	---	---	---	---	---	---
C70	FP	SP	FP	FP	FP	FP	FP
C203	---	---	---	---	---	---	---
C252	PP	FP	PP	PP	PP	PP	PP
C253	FP	---	FP	PP	---	PP	FP
C256	PP	PP	---	FP	---	PP	FP
C259	---	---	---	---	---	---	---

Table VIII. Ceratocystis virescens single spore isolate cross. Donor cultures across the top, recipient cultures down the left column. (FP) = fertile perithecia; (SP) = sterile perithecia; (PP) = protoperithecia; (---) = no perithecia or protoperithecia.

	A	B	C	D	E	F	G	H
A	---	---	---	---	---	---	---	---
B	---	---	---	---	---	---	---	---
C	---	SP	---	---	---	SP	---	---
D	---	FP	---	SP	---	---	---	---
E	---	FP	FP	---	PP	---	---	FP
F	FP	FP	PP	FP	PP	PP	PP	---
G	FP	PP	FP	PP	PP	PP	PP	PP
H	FP	PP	PP	PP	PP	FP	---	---

Table IX. Interspecific crosses. Donor cultures across the top, recipient cultures down the left column. (FP) = fertile perithecia; (SP) = sterile perithecia; (PP) = protoperithecia; (---) = no perithecia or protoperithecia. C448 = Chalara australis; C639, C642, C640, C654 = Eucalypt Ceratocystis; C70, C253, C203, C259 = Ceratocystis virescens.

	C448	C639	C642	C640	C645	C70	C253	C203	C259
C448	PP	PP	PP	PP	PP	PP	PP	PP	PP
C639	SP	PP	FP	FP	PP	PP	SP	PP	SP
C642	PP	PP	PP	PP	FP	PP	PP	PP	PP
C640	---	---	---	---	---	---	---	---	---
C645	---	---	---	---	---	---	---	---	---
C70	SP	SP	PP	SP	SP	SP	---	SP	SP
C253	---	FP	---	---	---	---	FP	PP	---
C203	---	---	---	---	---	---	---	---	---
C259	---	---	---	---	---	---	---	---	---

australis, and Eucalypt Ceratocystis are distinct species despite their morphological similarities.

V. Conclusions

In this study Chalara australis, Eucalypt Ceratocystis, and Ceratocystis virescens were found to be macroscopically and microscopically similar morphologically in culture. All three taxa grew most rapidly and produced the most aerial mycelium on PDA. Mating analyses performed on these taxa suggests that Chalara australis is either asexual or that only one mating type was present among the cultures studied, Eucalypt Ceratocystis displays bipolar heterothallism. The mating system present in Ceratocystis virescens is not clear at this point, additional cultures need to be assessed in the future. These data suggest that it is not possible to identify these cultures using exclusivly morphological data. The addition of these data has helped to make the mating system of these fungi better understood and has brought us closer to a method of biocontrol for these plant pathogens. The interspecific cross generated data that suggests these fungi are distinct species despite their morphological similarities.

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Appendix A: Culture media

<u>Malt Extract Agar</u>

15 grams Difco Malt Extract

20 grams Bacto Agar

1000 ml deionized, distilled water

<u>Malt Extract-Twig Agar</u>

15 grams Difco Malt Extract

20 grams Bacto Agar

1 sterile oak twig (@ 1 cm in diameter x 2 cm long)/plate

1000 ml deionized, distilled water

<u>Potato Dextrose Agar</u>

39 grams Difco Potato Dextrose Agar

1000 ml deionized, distilled water

Czapek Solution Agar

49 grams Difco Czapek Solution Agar

1000 ml deionized, distilled water

Culture Slants

20 grams Difco Malt Extract

15 grams Bacto Agar

1000 ml deionized distilled water

Decant 6 ml of agar into 14 ml tubes

Appendix B: Figures

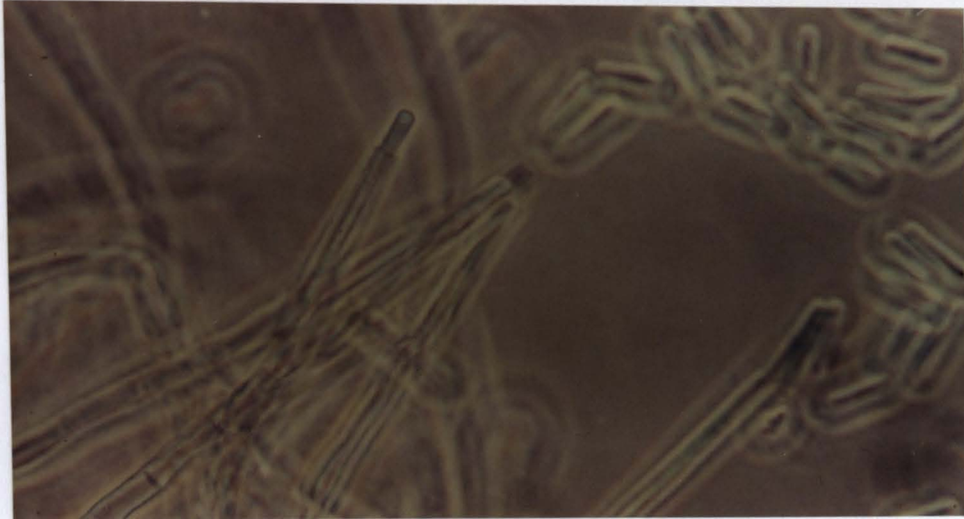

Figure I. Eucalypt Ceratocystis conidiophore with conidia. (400X)

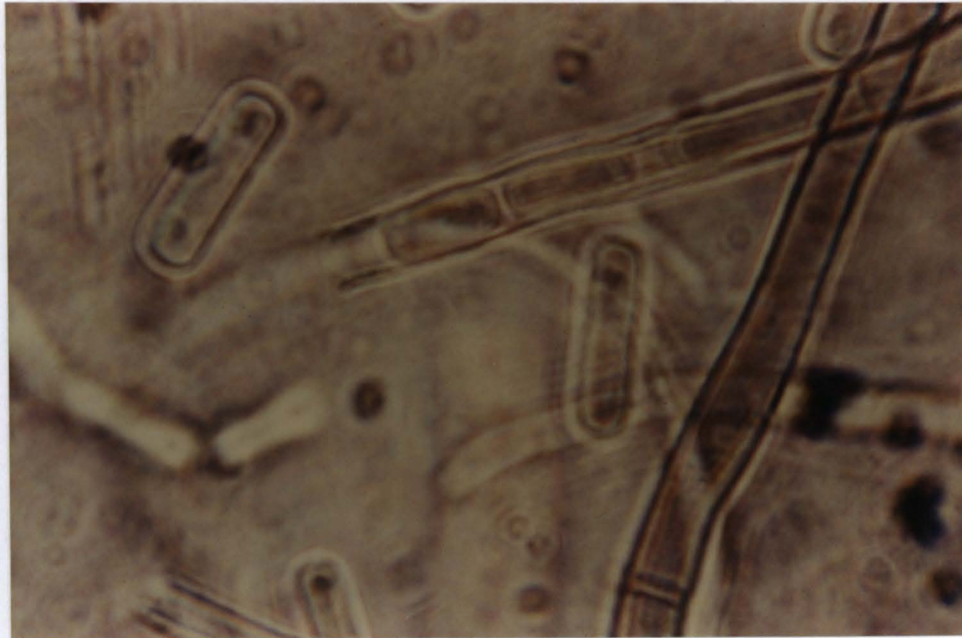

Figure II. Eucalypt Ceratocystis conidiophore with conidia. (1000X)

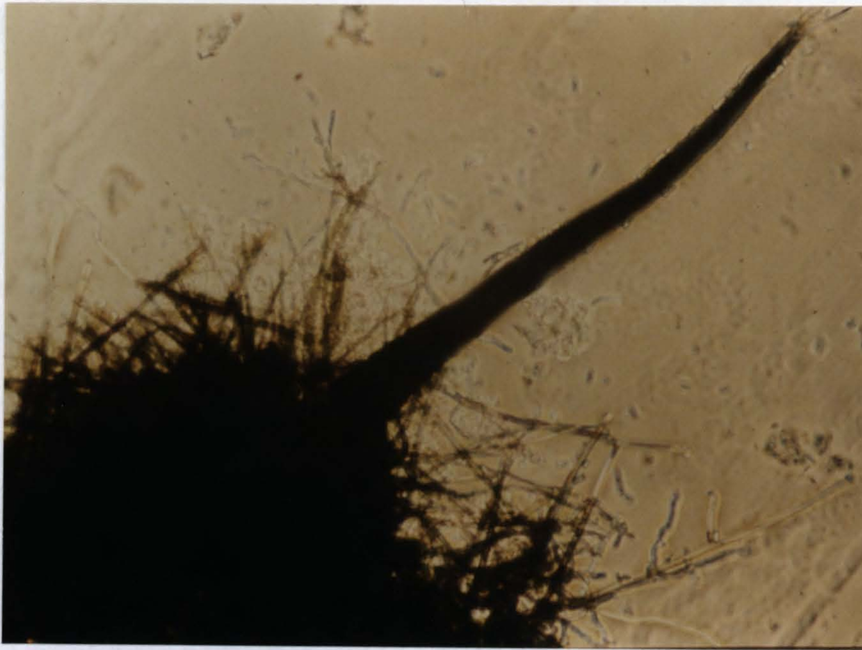

Figure III. Eucalypt _Ceratocystis_ perithecia. (200X)

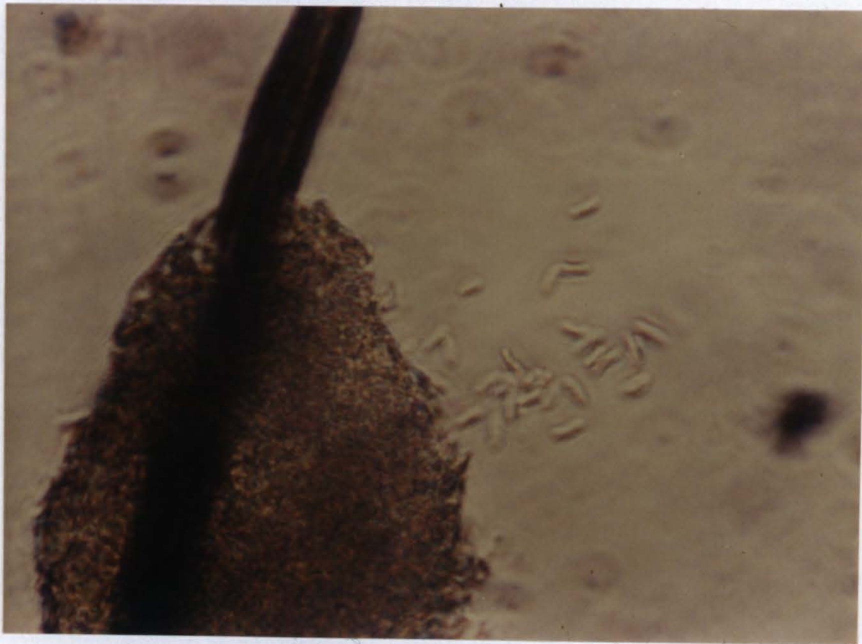

Figure IV. _Ceratocystis_ _virescens_ tip of perithecia with ascospores. (400X)

VI. Vita

Richard James Langham was born on December 20, 1969, in Rock Island, Illinois. He was raised in Peru, a city on the Illinois River, in the north central portion of the state. He received his high school education at LaSalle-Peru Township High School. He received varsity letters in tennis and wrestling and was the captain of the wrestling team. He graduated in 1988.

In August, 1988, Mr Langham enrolled at Southern Illinois University and began study in the Biological Sciences. In 1992, he graduated with a B.A. in Biological Sciences and a minor in Chemistry. He continued his education at Eastern Illinois University under Dr. Andrew Methven in the Department of Botany. He received his Master of Science degree in Botany in August, 1994.