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THE UNIVERSITY OF ALBERTA

ARMILLARIA ROOT ROT IN ALBERTA; IDENTIFICATION, PATHOGENICITY, AND DETECTION

by

KENNETH IAN MALLETT (MSc)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

AND RESEARCH IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN FOREST PATHOLOGY

DEPARTMENTS OF FOREST SCIENCE AND PLANT SCIENCE

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Supervisors

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Date April 11, 1985

ABSTRACT

Three biological species of the North American **Armillaria mellea** complex were found in Alberta. **Biological species** I, V, and a species tentatively named the Foothills type were identified through sexual mating studies. Diploid isolates of different biological species formed black lines between their colonies when paired on malt agar medium. Isolates from the same biological species did not form a black line. This technique was used to determine species affiliation of diploid isolates collected from diseased plant material in Alberta. **Biological species** I and V and the Foothills type were found in the boreal forest. **Biological species** I and the Foothills type were the predominant species found in the subalpine forest region.

The nature of the black line was investigated by light microscopy. The line was found to be formed by melanized hyphae, from both isolates, that grew in between the pseudosclerotia of either colony.

Nine taxonomic species of the **A. mellea** complex from Europe and Australia were grown on four agar media, (potato dextrose agar, malt agar, carrot agar, and malt dextrose peptone agar). The cultures were examined for thallus growth ratio, rhizomorph branching pattern and pseudosclerotial wall type. The nine species could be divided into two groups on the basis of rhizomorph branching pattern and pseudosclerotial wall type. Using the same media as above, Alberta **biological species** V could be readily distinguished from **biological species** I and the Foothills type using rhizomorph branching pattern and pseudosclerotial type.

Two-year-old greenhouse grown seedlings of **Pinus contorta var. latifolia** Engel. were inoculated with **Armillaria mellea sensu stricto, biological species** I, V, and the Foothills type. All species were found to be pathogenic, **A. mellea sensu stricto** and **biological species** V were the most virulent. **Biological species** I and the Foothills type were similar in virulence.

A technique using trembling aspen logs was used to detect and study species of the **A. mellea** complex in the soil of a lodgepole pine stand. One hundred and twenty-one sharpened aspen logs were pounded into the ground one meter apart in a 10 x 10 m grid. Thirty-one logs showed evidence of colonization by the Foothills type of the **A. mellea** complex. A map was drawn to show the relationships of colonized logs to diseased and healthy trees as well as to stumps within the plot. Eight distinctive patches were found within the plot. This technique can be used to determine the distribution of the pathogen within the disease center and may help in epidemiological studies of Armillaria root rot.

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I. GENERAL INTRODUCTION

A. Preface

In Canada 4,364,000 km^2 or 44% of the land mass is covered by forests (Bonnor 1982). These forests support an industry that plays an integral role in the Canadian economy. In 1982, the forest industry contributed 22 billion dollars to the Canadian economy; forest products such as pulp, paper, and wood accounted for 11 billion dollars in the balance of trade (Anon. 1984). Reed (1981) called the forests Canada's most valuable natural resource.

Historically, Canadian forest products have, for the most part, been derived from virgin or natural forests. Within the forest industry and the public, there is a growing realization that the forests of Canada are being depleted faster than they are being replaced and that there may be a wood shortage in the future (Anon. 1981). Of the 2,202,050 km^2 of productive Canadian forest land, 1,964,730 km^2 are stocked with an adequate number of trees; 229,520 km^2 of potentially productive forest land are now unstocked and the productivity of 7,790 km^2 was undetermined (Bonnor 1982). In order for Canada to maintain or increase forest production, it will be necessary for forest resources to be more intensively managed. Thus, factors which cause understocking and loss, such as fire, insect, and disease damage will have to be reduced.

The Canadian annual harvest in 1982 was 276 million m^3 of wood (Anon. 1984). According to Whitney **et al.** (1983), 40.4 million m^3 of wood, (1977-1981 average), is lost annually in Canadian forests to disease. Tree disease losses are a combination of mortality, growth reduction, and wood destruction. They estimated that if losses to disease were reduced by 30%, it would allow the forest industry an additional 20 million m^3 of wood a year for future expansion, and would be worth an additional 2.9 billion dollars in forest products. In the United States, insects cause more mortality than disease, fire, weather and other causes, but diseases were responsible for the greatest growth impact (Hepting and Jamison 1958).

Tree diseases are generally grouped according to the plant organ that they affect, examples being foliage, stem and root dieases. Root diseases were reported to be responsible for 5.1 million m^3 of wood being lost in 1976 (Whitney **et al.** 1983), and are second only to stem rusts as the most important group of tree diseases in Canada (Whitney **et al.** 1982). The pathogenic fungi responsible for the major root diseases in Canada are: **Phellinus weirii** (Murr.) Gilbertson, (laminated root rot), **Armillaria mellea** (Vahl : Fr.) Kummer, (Armillaria root rot), **Heterobasidion annosum** (Fr.) Bref., (Annosus root rot), and **Inonotus tomentosus** (Fr.) Gilbertson. Armillaria root rot and **Inonotus tomentosus** root rot are found throughout Canada, laminated root rot is found only in British Columbia and Annosus root rot is found in eastern Canada and British Columbia. **P. weirii**, **H. annosum**, and **I. tomentosus** cause losses in juvenile and mature stands. **A. mellea** causes losses in newly regenerated stands as well as in juvenile and mature stands.

Armillaria root rot is one of the principal root diseases in Canada and will be the subject of study in this thesis.

B. Armillaria Root Rot

1. Incidence

Armillaria root rot has been found throughout the world on forest, orchard, and shade trees as well as on some horticultural and field crops (Raabe 1962). In Canada, losses due to Armillaria root rot have been reported for some coniferous forests. Morrison (1981) reported that mortality in British Columbia's coastal and southern interior forests was 1% and 14%, respectively. Near Robb, Alberta, the average mortality of young lodgepole pine (**Pinus contorta var. latifolia** Engelm.) in seven 0.5-acre plots was 15% (Baranyay and Stevenson 1964). In a young Douglas-fir (**Pseudotsuga menziesii** (Mirb.) Franco) stand in Waterton Lakes National park, 28% of the trees were infected and 22% were dead due to Armillaria root rot (Trip **et al** 1967). Johnstone (1981) identified this disease as the major cause of mortality in thinned seven-year-old lodgepole pine stands in west-central Alberta. Whitney (1978) found that in 76 sites in northwestern Ontario, **A. mellea** was present in 42% of the balsam fir (**Abies balsamea** (L.) Mill.), 31% of the black spruce (**Picea mariana** Mill.), and 36% of the white spruce (**Picea glauca** (Moench) Voss) examined. Some coniferous plantations in Newfoundland have had mortality as high as 32% due to this disease (Singh 1975); thus Armillaria root rot is considered to be the most important tree disease in that province. In hardwoods, Thomas **et al.** (1960) determined that **A. mellea** was one of the principal decay-causing organisms of trembling aspen (**Populus tremuloides** Michx.) and balsam poplar (**Populus balsamifera** L.) in the boreal forest of Alberta. **A. mellea** was the most frequently isolated decay fungus from butt infections of trembling aspen and the second most frequently isolated decay fungus from butt infections of balsam poplar. However, the aggregate amount of decay in both species due to this fungus was small. Approximately 50% of the mature trembling aspen in the campground at Crimson Lake Provincial Park, Alberta, were reported to have been killed or extensively decayed by **A. mellea** (Ives **et al.** 1973).

In the United States, Armillaria root rot frequently has been reported as an important disease of conifers in the western, northwestern and Great Lakes States. Zeller (1926) observed that Armillaria root rot was widespread in the Pacific Northwest, particularily west of the Cascade mountains. Childs and Zeller (1929) found Armillaria root rot to be a serious problem in orchards in the Pacific Northwest and determined that there were two physiologic strains present. The most prevalent strain was present in soils that were previously covered by Douglas-fir forests and did not seem to attack the roots of deciduous trees. The second strain, which did attack the roots of deciduous trees, was found in soils that previously had been covered with oak forests. Armillaria root rot was found by Johnson (1976) to be the most common and widely distributed root disease in forest plantations of fourteen national forests in the Pacific Northwest, west of the Cascade Mountains. Filip (1977) reported an **Armillaria** epiphytotic in a National forest in Oregon. In a 232-hectare conifer forest, composed of white fir (**Abies concolor** (Gord. & Glend.) Lindl.), shasta red fir

(**Abies magnifica** var. **shastensis** A. Murr.), incense-cedar (**Libocedrus decurrens** Torr.), western white pine (**Pinus monticola** Dougl.), lodgepole pine, and sugar pine (**Pinus lambertiana** Dougl.), Armillaria root rot was found in 7% of the trees, representing 32% of the volume on the area. Lodgepole pine was found to have the highest incidence of infection, 42% of the infected trees. In Washington state, Gregg **et al.** (1978) claimed that the St. Regis Paper Company had calculated that 6.5 million board feet of timber were lost annually on their land holdings due to Armillaria root rot. James **et al.** (1982) found that mortality centers in the northern Rocky Mountain forests of Idaho and Montana occupied 30,000 ha of productive forest. For the forests of northern Idaho and Montana, they estimated that 0.3 and 0.2 m^3/ha/yr are lost annually, amounting to 2.3 million m^3/yr or 40% of the average annual harvest. In Wisconsin, Pronos and Patton (1977) reported that Armillaria root rot had caused losses of 12, 18, and 37% in three red pine (**Pinus resinosa** Ait.) plantations.

In Kenyan pine plantations, trees grown in the montane rain forest were more frequently infected and severely affected by **Armillaria** root rot than those growing in montane conifer forests (Gibson 1960). An average of 44% of the trees in three montane rain forest plantations and 19% of trees in three coniferous montane rainforest plantations were infected.

Armillaria root rot is one of the more important root rotting diseases in Scandinavia and the USSR (Hintikka 1974, Federov and Poleschuk 1981).

Four species of **Armillaria**, closely related to **A. mellea**, have been found to cause root rot in Australian eucalypt and cool temperate rain forests (Podger **et al.** 1978). Kile (1981) recognized **Armillaria luteobulbina** Watling & Kile, as being the primary cause of decline and mortality of trees in central Victoria. Armillaria root rot was the leading source of mortality five years after planting in radiata pine (**Pinus radiata** D. Don) in New Zealand (Beveridge 1973). Shaw and Calderon ~~(1977)~~ found that 33% of the mortality in two-year-old radiata pine plantations was attributable to Armillaria root rot. Two species, **Armillaria novae-zelandiae** (Stevenson) Boeswinkel and **Armillaria limonae** (Stevenson) Boeswinkel were found to cause 16% mortality in radiata pine within 27 months of planting (MacKenzie and Shaw 1977).

2. Symptoms and Signs

Symptoms of Armillaria root rot vary with the host species, but generally include foliar discoloration, stunting of growth, resinosis or gumosis of the lower stem, and root or bole rot, (Fig. 1 and 2). The presence of a white mycelial fan beneath the bark and brown-black rhizomorphs growing on or near the root are diagnostic signs of the disease (Figs. 3 and 4). Trees may die very quickly (within several months) in the case of young seedlings, or they may die over a period of several years. Many mature trees are chronically infected with the fungus and may have only a yellow-stringy rot in the butt or roots (Fig. 5). Cause of death of trees infected by this fungus commonly has been attributed to girdling of the stem which kills the cambium and phloem tissues. Dead and dying trees are often found in patches known as disease centers (Fig. 6).

Figure 1. Foliar symptoms of Armillaria root rot on lodgepole pine.

Figure 2. Resinosis at the root collar of a lodgepole pine seedling infected by a species of the **A. mellea** complex


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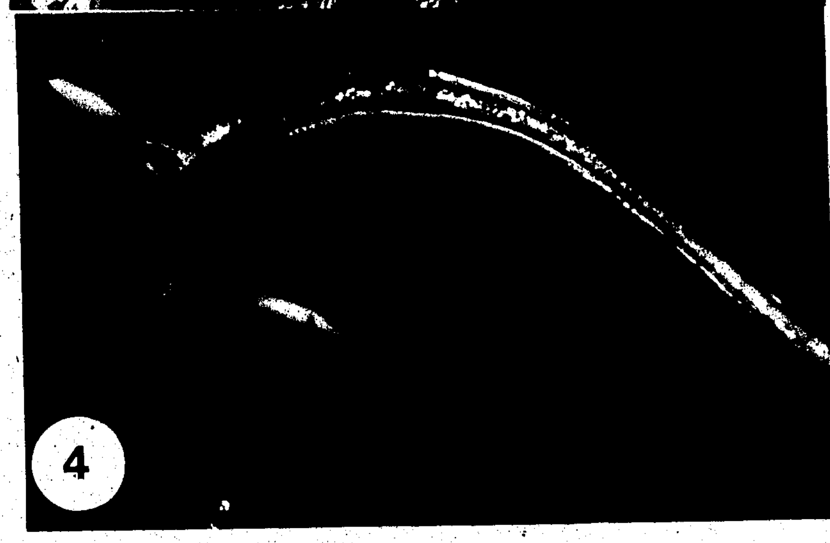
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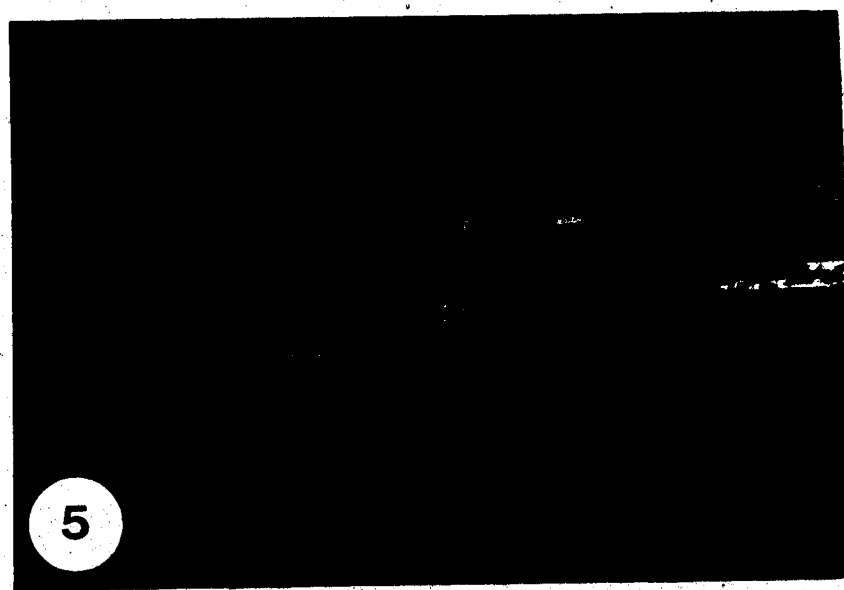
Figure 5. Yellow stringy rot, (caused by a species of the **A. mellea** complex), of trembling aspen

Figure 6. An Armillaria root rot disease center in a lodgepole pine stand.



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3. Etiology

The incitants of Armillaria root rot, **Armillaria mellea** (Vahl: Fr.) Kummer and closely related species, are basidiomycetes belonging to the order Agaricales, family Tricholomataceae. Early mycologists believed that the brown rhizomorphs growing in the soil from dead trees and the white fan of mycelium under the bark of these trees were produced by two separate fungi, **Rhizomorpha subterannea** Persoon and **Rhizomorpha subcorticalis** Persoon. Hartig (1874) established that these fungi were the vegetative structures of **Agaricus mellea** Fr. and were responsible for infection through wounds. Zeller (1926) found that infections of apple and prune roots could occur through wounds, contact between diseased and healthy roots, and at the point where lateral roots emerged from the main roots. Thomas (1934) showed that rhizomorphs could penetrate roots directly through intact periderm. He observed that penetration was by mechanical force as well as by chemical means. Once a rhizomorph had successfully penetrated the corky tissue it quickly branched into flat white rhizomorphs. These rhizomorphs grew in the periderm and cambial tissues. Thomas (1934) observed that death of cells frequently occurred well in advance of the hyphae, and thus concluded that the fungus produced enzymes and/or toxins. Successful invasion does not always lead to death of the host; the fungus often causes a butt rot with no apparent foliar symptoms in mature trees.

A. mellea can spread by rhizomorphs growing through the soil from food bases, such as diseased trees or stumps or, less frequently, by root to root contact (Redfern 1970). Basidiospores are not thought to play an important role in the spread of the fungus (Rishbeth 1970). Rate of spread of Armillaria root rot disease centers has been determined by several workers. Rishbeth (1968) calculated that the yearly rate of spread of a disease center in a Douglas-fir stand was 1.1 m. Shaw and Roth (1976) determined that the average rate of radial growth of a disease center in a ponderosa pine (**Pinus ponderosa** Dougl.) stand was one meter per year. The authors concluded that this particular clone of **A. mellea** had been growing vegetatively for at least 460 years.

The status of **Armillaria mellea** as an important pathogen has been a source of considerable controversy. Many reports have cited the fungus as being a secondary pathogen that attacks trees which have been stressed in some way (Day 1928, 1929, Cooley 1943, Peace 1962, Gremmen 1976). A variety of environmental factors which can predispose the host to infection have been suggested. Suppressed trees or those exposed to reduced light intensity have been found to be quite susceptible to infection by the fungus (Redfern 1978). Other workers have found that factors such as drought, waterlogging, insect and herbicide defoliation, frost, poor soil conditions and unfavorable sites can predispose trees to attack (Biraghi 1949, Huntly **et al.** 1961, Ono 1965, Staly 1965, Ritter and Pontor 1969, Wargo 1972). However, several studies have found the fungus to be a primary pathogen capable of killing healthy trees (Gibson 1960, Filip 1977, Shaw **et al.** 1976, Kile 1981). Shaw and Roth (1978) suggested that damage is probably determined by a combination of factors including host susceptibility, stand structure and composition, environment, fungal strain, and inoculum characteristics.

In recent years, several other species of **Armillaria**, capable of causing the disease, have been identified (Mackenzie and Shaw 1977, Podger **et al.** 1978, Korhonen 1978, Rishbeth 1982, Kile and Watling 1983). These species are seemingly closely related and are often referred to as the **Armillaria mellea** complex (Watling **et al.** 1982). In addition, biological species or intersterility groups, (no species have been morphologically identified) belonging to the the **A. mellea** complex have been found in North America (Anderson and Ullrich 1979). Different species of the **A. mellea** complex may vary in their ability to attack and kill different tree species.

C. Objectives of the Thesis

With the advent of intensively managed forest plantations in Canada, the need to control losses from damaging agents has become more important. Root diseases have been identified as a major problem affecting many Canadian forests. There can be no doubt of the significant impact that Armillaria root rot has on young conifer plantations, particularly pine, in the world's forests. In Alberta, Armillaria root rot is one of the principal diseases of lodgepole pine.

Although Armillaria root rot has been studied to some extent, knowledge concerning disease processes, host-parasite interactions, epidemiology, and control, is limited. This is due in part to the controversy concerning **Armillaria**'s ability to act as a parasite and to the difficulties associated with studying the disease. With the recent findings that there are several species of **Armillaria** capable of causing Armillaria root rot, the controversy over pathogenicity may be solved. However, these findings dictate that much of the literature concerning **A. mellea** will have to be re-evaluated, as no distinctions were made between different species or biological species.

In Alberta, little is known about the **Armillaria mellea** complex other than that it is associated with mortality and decay in lodgepole pine and Douglas-fir stands and that it causes butt rot in trembling aspen and balsam poplar. Before meaningful studies into the management and control of Armillaria root rot can be accomplished it is imperative to first know which biological species of the **A. mellea** complex are found in Alberta and where they are located. This will facilitate development of techniques allowing rapid identification of each biological species and the determination of their relative pathogenicity towards important tree species.

The objectives of this study were:

1) To identify the biological species of of the **Armillaria mellea** complex in Alberta, to determine the approximate ranges of the biological species and to develop a quick and reliable means of identifying them.

2) To determine the virulence of each biological species towards lodgepole pine.

3) To develop a method of detecting **Armillaria mellea** complex members in forest soils.

II IDENTIFICATION OF THE ALBERTA ARMILLARIA MELLEA COMPLEX

A. Introduction

The purposes of this study were to (i) identify the members of the **A. mellea** complex in Alberta, (ii) determine their approximate range, (iii) develop a quick and reliable means to identify them.

B. Literature Review

1. Taxonomy and Nomenclature

Armillaria root rot is found throughout the world and has been shown to be incited by several species of the so-called **Armillaria mellea** complex. The genus **Armillaria** and the species that cause Armillaria root rot, have been a source of taxonomic and nomenclatural instability.

According to Watling **et al.** (1982), John Ray (1704) was the first botanist to recognize and describe an **Armillaria sp.**. Micheli, in 1729, illustrated the fungus in **Novae Plantarum Generum** and both Bolton (1789, 1791) and Buillard (1780) had studied it (Watling et al. 1982). Vahl (1790), in **Flora Danica**, first described and named **Agaricus melleus**. Fries (1821) placed **A. melleus**, as well as twelve other **Agaricus** species, in the tribe **Armillaria**. Staude (1857) raised Fries's tribe **Armillaria** to generic level (Donk 1949, 1962). He did not make the combination **Armillaria mellea**; however, Kummer (1871) and Quelet (1872) did. Karsten (1881) erected the genus **Armillariella** and placed three species into it, including **Armillaria mellea**.

Two different specific epithets and three authorities have been used by myclogists and forest pathologists for this fungus; **Armillaria mellea** (Vahl : Fries) Kummer, **Armillaria mellea** (Vahl : Fries) Quelet, and **Armillariella mellea** (Vahl : Fries) Karst. Singer (1975) rejected the genus **Armillaria**, in the Friesian sense, because he considered the genus to be very artifical. In his opinion many species which did not fit into existing genera were lumped together in the genus **Armillaria**. Singer (1955 a) did not accept Staude's genus **Armillaria**, nor that Kummer (1871) had raised **Armillaria** from Fries's tribe to a genus. He instead contended that Kummer (1871) created his own genus **Armillaria** (Singer 1955 b) and that **A. melleus** was not the type species for the genus **Armillaria**. Singer (1940) separated the genus **Armillaria** into nine genera: **Armillaria sensu stricto**, **Tricholoma**, **Calocybe**, **Armillariella**, **Melanoleuca**, **Leucocortinarius**, **Pleurotus**, **Cathelasma**, and **Oudemansiella**. Only **Armillaria subcaligata** Smith & Rec. and **Armillaria luteovirens** (A. & S. : Fr.) Gillet were left in this genus. These two species are entirely unrelated to the present day concept of the **A. mellea** complex.

Watling **et al.** (1982) argued that Staude's description had all the requirements for valid publication and so cited Staude as the authority for the genus **Armillaria**. They rejected Singer's notion that Kummer (1871) set up his own genus, stating that there was valid proof that Kummer did follow Fries (1821) when he elevated **Armillaria** to a genus. Thus, they credited Kummer as the authority for **Armillaria mellea**. Watling **et al.** (1982) collected basidiocarps from the area in which Vahl (1790) originally collected the fungus he called **A. melleus**, and found that the specimens agreed with the description given by Vahl. Since no type specimen of **A. mellea** collected by Fries (1821) was in existance, and since the specimens collected by the authors were in agreement with the descriptions given by Vahl, Watling **et al.** (1982) suggested that one of these specimens could be used as a neotype for the genus. Singer's (1940) dismemberment of the genus **Armillaria** in the Friesian sense, was undoubtably necessary but because of the now established priority of **Armillaria mellea**, changes will have to be made. The species which Singer placed in the genus **Armillariella** clearly belong in the genus **Armillaria**. Therefore, the genus **Armillaria** in the Singer sense, will have to be transferred to a different genus. Table 1 lists **Armillaria** species in the Watling and Singer nomenclatorial systems.

A modern taxonomic approach to describing members of the genus **Armillaria** closely related to **A. mellea** was initiated by Singer (1956) when he described several South American species. Singer (1956) was one of the first mycologists to allude to the **Armillaria mellea** complex. Although he described **A. mellea** in Europe, Asia, and North America as being extremely polymorphic, he re-

TABLE 1. List of the A. mellea Complex Species: Comparing the Singer and Watling Nomenclature Systems

Singer (1975)	Watling (1982)
Annulate	
Armillariella mellea (Vahl:Fr.) Karst	Armillaria mellea (Vahl:Fr.) Kummer
A. bulbosa (Barla) Romag.	A. bulbosa (Barla) Kile & Watling
A. ostoyae Romag.	A. ostoyae (Romag.) Herink
A. novae-zelandiae Stevenson	A. novae-zelandiae (Steven.) Boes.
A. limonea Stevenson	A. limonea (Steven.) Boes.
A. griseomellea Sing.	A. griseomellea (Sing.) Kile & Wat.
A. montagnei Sing.	A. montagnei (Sing.) Kile & Wat.
A. sparrei Sing.	
A. melleorubens (Berk. & Curt) Sing.	
A. procera (Speg.) Sing.	
A. obscura (Scaeff.:Secr.) Romag.	
A. polymyces (Pers.:Letellier) Sing. & Clc.	
A. saviczii Sing.	
A. puigarri (Speg.) Sing.	
A. olivacacea (Rick) Sing.	

TABLE 1. cont.

A. fuscipes Petch	
A. omnituens (Berk.) Sing.	
A. yungenis Sing.	
A. elegans Heim	
A. mori (Paul:Fr.) Sing.	
	A. borealis Marx.& Kor.
	A. hinnulea Kile & Wat.
	A. luteobubalina Wat. & Kile.
	A. fellea Kile & Wat.
Exannulate	
A. tabescens (Scop: Fr.) Sing.	*A. tabescens* (Scop:Fr.) Sing.
A. ectypa (Secr.) Sing.	
A. nigropunctata (Secr.) Sing.	
A. watsonii (Murr.) Sing.	

cognized that there were several distinct species similar to **A. mellea**. He also recognized that there were two groups of species within the genus, species that were annulate and those that were exannulate, (Table 1). Subsequently, workers have found other species of **Armillaria** capable of causing Armillaria root rot. MacKenzie and Shaw (1977) found that **A. limonea** and **A. novae-zelandiae** attacked **P. radiata**. **A. luteobubalina** caused root rot in Australian **Eucalyptus regnans** F. Muell. plantations (Podger **et al.** 1978). Kile and Watling (1983) described two other species capable of causing root rot, **Armillaria hinnulea** Kile & Watling, and **Armillaria fumosa** Kile & Watling. These authors stated that the color of the pileus, stipe and lamellae as well as the annulus structure and viscidity were the only characters that were different between the Australian species. Micromorphological characters such as spore size and hymenophoral trama structure were of little use in distinguishing these species and this, according to the authors, seemed to be a similar observation for other **Armillaria** species found elsewhere. Korhonen (1978) found five species of **Armillaria** in

Finland. He considered two of the species to be predominantly saprophytic and the others to be pathogenic. In Britain, Rishbeth (1982) collected five species of **Armillaria**. **A. mellea** and **Armillaria ostoyae** (Romag.) Herink were highly virulent; **Armillaria bulbosa** (Barla) Kile & Watling caused a butt rot in stressed broad-leaved trees. **Armillaria tabescens** (Scop. ex Fr.) Emel. was not considered to be pathogenic, and the fifth species, called **Armillaria sp. B.**, was found only once.

2. Sexual Mating System

Kniep (1911) studied the mycelium of **A. mellea** and concluded that the vegetative hyphae were uninucleate. He also observed that the basidia could arise directly from the vegetative mycelium, but this observation has not since been confirmed, Hintikka (1973) observed that single spore isolates of **A. mellea**, when grown on agar media, had a fluffy aerial mycelium, whereas the colonies that resulted from tissue taken from the stipe of the basidiocarp, rhizomorphs, or infested wood, were flat and crusty. Clamp connections were not found in either type of colony. Hintikka (1973) found that hyphal tips from both types of colonies were uninucleate. This evidence, and the lack of clamp connections in the vegetative mycelium, suggested that the vegetative mycelium was diploid. Hintikka and Korhonen (1974) found a transient dikaryotic phase after two compatible single spore isolates were paired and plasmogamy had taken place. This was followed by nuclear fusion, which they interpreted as diploidization. Tommerup and Broadbent (1975) examined the cells of rhizomorphs, stipes, and caps. They found that most of the cells were uninucleate except for the cells in young gill folds of the basidiocarp primordia, which were dikaryotic and had clamp connections. The cells from which gill folds originated contained five to ten nuclei. In mature basidia, nuclei fused and underwent meiosis, with each of the daughter nuclei migrating into one of the four basidiospores. The nuclei in the basidiospores divided mitotically once more and one daughter nucleus migrated from the basidiospore back into the basidium.

Peabody **et al.** (1978), Franklin **et al.** (1983), and Peabody and Peabody (1984) confirmed, by measuring the nuclear DNA content, that the vegetative hyphae of **A. mellea** were diploid and that the hyphae orignating from basidiospores were haploid. Further evidence for the vegetative state being diploid came from pairing auxotrophic single spore isolates, which became prototrophic if compatible mating types were paired (Ullrich and Anderson 1978, Anderson and Ullrich 1979, Anderson and Ullrich 1982). Anderson (1982), using similar techniques, found that **A. tabescens** also exhibited vegetative diploidy.

From experimental evidence it would appear that members of the **A. mellea** complex have an aberrant nuclear cycle relative to those of many homobasidiomycetes. The vegetative state is diploid rather than dikaryotic. There are, however, reports that suggest that other fungi also have a diploid life cycle (Caten and Day 1977). Yeasts and the Myxomycetes are thought to have a prolonged diploid life cycle and there is increasing evidence that this is true for the Oomycetes as well (Caten and Day 1977). The **Armillaria mellea** complex seems to be anomalous in that there are two diploidizations and two dediploidizations in one life cycle. The first dediploidization occurs in the subhymenial cells, the two dikaryotic nuclei move into the basidium where they fuse again. The second dediploidization occurs in the basidium during meiosis. The reason for the first step is unknown and would seem, when compared to gametogenesis in other organisms, to be unneccessary. A further complication in the understanding of the nuclear cycle was shown by Korhonen (1980), who was able to produce basidiocarps of **A. ostoyae** in culture and compared them to those produced in nature. He found that the basidiocarps produced in the laboratory did not produce the dikaryotic clamped subhymenial cells but did undergo normal meiosis and produced viable basidiospores. However, **A. ostoyae** basidiocarps produced in nature did have dikaryotic clamped subhymenial cells.

Hintikka (1973) discovered that when single spore isolates from one **A. mellea** basidiocarp were paired, the results of certain pairings were flat crustose colonies. These colonies resembled colonies made from the stipes of basidiocarps, rhizomorphs and infested wood. By pairing the single spore isolates in every possible combination, he showed that the fungus had a tetrapolar mating system. In other homobasidiomycetes that exhibit heterothallism, compatible mating is recognized by examining the mycelia of paired cultures for clamp connections. Since **A. mellea's** vegetative mycelium does not have clamp connections, Hintikka used colony morphology, fluffy vs. crusty, as the criterion for compatibility. Ullrich and Anderson (1978) studied single spore progeny from 27 basidiocarps and confirmed that a tetrapolar mating system did exist in **A. mellea**. They also demonstrated that multiple alleles existed for both loci and when the "testers" (mating type genotypes) from each of the 27 basidiocarps were paired, six intersterile groups, which they termed biological species, were found. Concurrently, Korhonen (1978) in Finland confirmed Hintikka's (1973) findings. Korhonen used single spore isolates from approximately 430 basidiocarps, including some from two distinct species, **A. mellea sensu stricto** and **A. bulbosa**. He found that there were five intersterile biological species in his collection.

Anderson and Ullrich (1979) have subsequently found that ten biological species of the **A. mellea** complex exist in North America. These biological species had a wide geographical range and were often found in close proximity to each other. No studies into taxonomic species affiliation have yet been done. Korhonen (1978) demonstrated that species shown to be biological species could also be recognized as taxonomic species since **A. mellea sensu stricto** and **A. bulbosa** were intersterile. Guillaumin and Berthelay (1981) found that at least four of the European biological species occurred in France. They observed, however, that the taxonomic species **A. obscura**, and **A. polymyces** were interfertile with **A. ostoyae** and so, are probably variants of **A. ostoyae**. Marxmuller (1982) found the five European species in Germany and France and named Korhonen's **A. sp. A** as **Armillaria borealis** Marxmuller-Korhonen. She too recognized that **A. ostoyae** and **A. obscura** were closely related. Anderson **et al.** (1980) paired North American biological species with European species of the **A. mellea** complex. They found that certain European species were, in some cases, partially or completely interfertile with some North American biological species, (Table 2). The taxonomic relationships of the North American biological species still remains unclear because morphological studies have yet to be done. For this reason, the system which Anderson and Ullrich (1979) used to designate North American biological species shall be used to identify the Alberta isolates of the **A. mellea** complex in this thesis.

Anderson (1983) was able to induce somatic segregation in diploid mycelia of **A. mellea**, the mechanism of which is poorly understood. Somatic segregation was produced by growing prototrophic isolates of North American biological species I, heterozygous at mating type and nutritional loci, on benomyl ammended malt medium. Segregants were recognized by a fluffy colony appearance and by the auxotrophic nature of the colony when grown on media deficient in certain nutrients. When paired with a single spore isolate of appropriate mating type, colonies with crusty morphology were produced. Anderson and Yacoob (1984) subsequently has shown that somatic segregation can occur in other North American biological species as well.

TABLE 2. Interfertility Relationships Between North American and European Biological Species of the *A. mellea* Complex According to Anderson et al. (1980).

North American Species[1]	European Species[2]
I	C = *Armillaria ostoyae*
II	
III	
IV	B
V	
VI	D = *A. mellea*
VII	E = *A. bulbosa*
VIII	
IX	
X	B
	A = *A. borealis*
	A. obscura

1 Anderson and Ullrich (1979)
2 Korhonen (1978)

Efforts to study the nuclear cycle in **A. mellea** have been hampered by the inability of workers to produce basidiocarps **in vitro** on a reliable basis. However, basidiocarps have been produced in the laboratory for some species of **Armillaria**, such as **A. ostoyae** (Korhonen 1980), **A. novae-zelandiae** (Shaw **et al.** 1981) and the Australian **Armillaria** species (Kile and Watling 1983). Rykowski (1974) and Raabe (1984) have also been able to produce basidiocarps of **A. mellea** in the laboratory.

3. Intraspecific and Interspecific Incompatibility

The phenomenon of incompatibility between vegetative mycelia of different species of fungi and different strains of the same species of fungus has been observed frequently but is still poorly understood. The reaction that occurs between two opposing mycelia has been termed "barrage", "aversion phenomenon", "interaction zones", "demarcation zone", "black line", and "zone lines". Rayner and Todd (1979) reviewed this subject in relation to the population and community structure of fungi in decaying wood. It was their contention that zone lines, the narrow black lines that are found in transverse, radial, and tangential sections of decayed wood, represent the interfaces between different fungal colonies. There are two types of zone lines. The first type was described by Campbell and Munson (1936) as being the pseudosclerotial wall, a fungal tissue made up of tightly packed bladder-like cells that are melanized. The second type according to Rayner and Todd (1979) is a zone of discolored undecayed wood that lies between the confronting fungal thalli.

Intraspecific incompatibility has been recognized in the Ascomycetes and some of the Basidiomycetes. Croft and Jinks (1977) found that in wild-type isolates of **Aspergillus nidulans** Eidam, there existed heterokaryon compatibility groups. Members of the same compatibility group could readily form heterokaryons with each other but not with isolates from the other groups. The compatibility groups were not found to have any particular geographic distribution and often were found in the same area. **Endothia parasitica** (Murr.) A. & A. has also been found to have compatibility groups (Anagnostakis 1977). In the

Basidiomycetes, intraspecific incompatibility has been reported for many species. Parmeter **et al.** (1969) grouped isolates of **Thanatephorous cucumeris** (Frank) Donk into anastomosis groups. Mycelia of isolates belonging to the same anastomosis group readily anastomosed, whereas no anastomosis took place between isolates of different groups. Punja and Grogan (1983) found antagonism zones formed between various isolates of **Athelia rolfsii** (Curzi) Tu & Kimbr. The authors found that they could assign isolates to 25 interaction groups. **Fomes pinicola** (Swartz) Cke., **Phellinus tremulae** (Bond.) Bond. et Boriss., **Fomes cajanderi** Karst., **Phaeolus schweinitzii** (Fr.) Pat., **Phellinus weirii**, **Coriolus versicolor** (L.: Fr.) Quel., **Bjerkandera adusta** (Fr.) Karst., **Stereum hirsutum** (Willd.: Fr.) S.F. Gray, and **Oudemansiella radicata** (Relhan : Fr.) have all been demonstrated to have intraspecific incompatibility (Mounce 1929, Verrall 1937, Childs 1963, Adams and Roth 1967, Barrett and Uscuplic 1971, Rayner and Todd 1979). Todd and Rayner (1980) regarded intraspecific incompatibility as a mechanism to maintain individualism within a natural population. The mechanism was assumed to be under heterogenic control and operated independently of the homogenic system regulating sexual compatibility. The physiological mechanisms underlying intraspecific incompatibility are not well understood. Barrett and Uscuplic (1971) and Rayner and Todd (1979) have observed that hyphae at the confronting margin often died or became distorted thus giving rise to a zone of incompatibility.

Interspecific incompatibility has also been recognized by the formation of zone lines in wood and by incompatibility zones in agar media. Hartig (1874) was the first to study the zone lines of **A. mellea**. Campbell (1933, 1934) and Campbell and Munson (1936) showed that these zone lines were part of the pseudosclerotium, a fungal tissue that envelops the wood or substrate. The formation of a pseudosclerotium, or the pseudosclerotial plate was described by Lopez-Real (1975).

Adams (1974) demonstrated, on the basis of pure culture tests, that clones of **A. mellea** could be recognized by what he thought was intraspecific incompatibility. He recognized incompatibility by the formation of a black line between two colonies paired on agar media. Clones were recognized by the colonies growing into one another and by the absence of black lines. Korhonen (1978) showed that the black line phenomenon occurred between different species of **Armillaria** rather than clones, and therefore indicates interspecific incompatibility. When different isolates of **A. sp.** A were paired, a zone of demarcation without pigment developed. If the same clone was paired the colonies grew into one another. Rishbeth (1982) reported identical results with **A. mellea**, **A. bulbosa**, **A. ostoyae**, **A. tabescens**, and **A. sp.** B. Kile (1983) also had similar results when investigating clonal distribution of **A. luteobubalina** in Australian eucalypt forests. A pigmented zone was also noted to occur between paired single spore isolates of different North American biological species of **A. mellea** (Ullrich and Anderson 1978).

Hood and Morrison (1984) could distinguish different species of the **A. mellea** complex by inoculating wood pieces. Black lines were formed in the wood pieces that were inoculated with different species.

Although the black line phenomenon has been reported by several researchers working with **Armillaria** species, the nature of the black line has never been adequately investigated.

3. Cultural Characteristics

The use of cultural characteristics in the identification and taxonomy of higher basidiomycetes is not common. Davidson **et al.** (1938) and Nobles (1948, 1964, 1971) have provided schemes for the identification of the Aphylophorales and other wood inhabiting hymenomycetes, based upon cultural characteristics. Miller (1971) has provided some characters for the identification of the Agarics when they are grown in culture.

Brefeld (1877) was one of the first researchers to grow **A. mellea** in pure culture. Since then many workers have studied the physiology of **A. mellea** grown in culture (Reitsma 1932, Hamada 1949, Benton and Ehrlich 1941, Bliss 1941, Raabe 1953, Snider 1959). Benton and Ehrlich (1941) reported on the variation in several isolates of **A. mellea** and found that they differed in appearance and rhizomorph production. Gibson (1961) studied morphological variation in 140 isolates of **A. mellea** from several continents. He found that he could group isolates according to mycelial type, rhizomorph morphology, and vigor of growth. He recognized four mycelial types and four rhizomorph types. Raabe (1967a) studied 84 isolates of **A. mellea** isolated from plants and 84 single spore isolates collected from one basidiocarp. He concluded that there was wide variation in cultural characteristics among the isolates from different plants as well as the single spore isolates. Unfortunately, at the time these studies were conducted the differences between the haploid and diploid isolates were not recognized.

Shaw et al. (1981) could readily distinguish **A. novae-zelandiae** and **A. limonea** in culture, by mycelial and rhizomorph characteristics. Kile and Watling (1983) found that isolates of **A. hinnulea** and **A. novae-zelandiae** were recognizably different from **A. fumosa** and **A. luteobubalina** on the basis of mycelial and rhizomorph morphology. Morrison (1982) grouped isolates of the **A. mellea** complex according to rhizomorph branching patterns.

Certain isolates produced rhizomorphs with a monopodial type of growth (Type I); others produced rhizomorphs with regular dichotomous branching (Types IIA and IIB). Guillaumin and Berthelay (1981) found that **A. sp. B** and **A. bulbosa** had cylindrical rhizomorphs when grown in culture, whereas, **A. mellea** and **A. ostoyae** had ribbon shaped rhizomorphs. Rishbeth (1982) found that **A. mellea, A. bulbosa, A. ostoyae,** and **A. tabescens** isolates all had significantly different growth rates when grown on 3% malt agar at 30^{o}C.

C. Materials and Methods

1. Mating System

i) Basidiocarp collection

Basidiocarps were collected in the fall of 1982, 1983, and 1984 from various locations in Alberta. The basidiocarps were dried in paper bags using a drying cabinet operated at 60°C. Specimens were retained at Edmonton, Alberta in the Northern Forest Research Centre Mycological Herbarium.

ii) Diploid isolation

Diploid isolates of the **A. mellea** complex were collected by two methods. The first was by plating tissue from the stipes of the collected basidiocarps onto acidified malt extract agar (AMA), (3% malt extract (Difco), 1.8% agar, 1 ml/l 25% lactic acid). The second method, was by isolating the fungus from diseased trees, which were identified by typical foliar symptoms and the presence of a mycelial fan underneath the bark in the root collar region. Pieces of wood and bark tissue were cut from the tree, or if the tree was small the entire plant was pulled from the ground. Specimens were wrapped in paper and brought back to the laboratory where they were kept frozen until used. Isolations were made from the infected tissue by cutting small pieces of wood (2-4 mm) that contained mycelia and placing them on AMA. Petri plates were incubated in the dark at 20^{o}C and examined every two days. Pieces of agar containing hyphae that grew from the wood were transferred to fresh 3% AMA. Isolates were identified as belonging to the **A. mellea** complex if pseudosclerotia and/or rhizomorphs typical of the **A. mellea** complex developed after several weeks of incubation. Isolates identified as belonging to the **A. mellea** complex were entered into the Northern Forest Research Centre Fungal Cuture Collection, (Appendix 1).

iii) Monospore isolation

Basidiocarps collected in the field were brought back to the laboratory where pieces of the gill tissue were removed from the pileus and suspended over 1.6% water agar in Petri plates for three hours. The bottom of the Petri plate was rotated slightly every 20 minutes. The gill tissue was then removed and the Petri plates were incubated in the dark for two to three days. The Petri plates were examined under the stereoscope for germinating basidiospores. Germ tubes and hyphae from the isolated basidiospores were cut out of the agar using the tip of a sterile hypodermic needle and transferred to 1.25% MA (1.25% malt extract (Difco), 1.5% agar) (Anderson and Ullrich 1982). Petri plates were sealed with masking tape and incubated in the dark at 20°C for seven days.

iv) Mating type identification

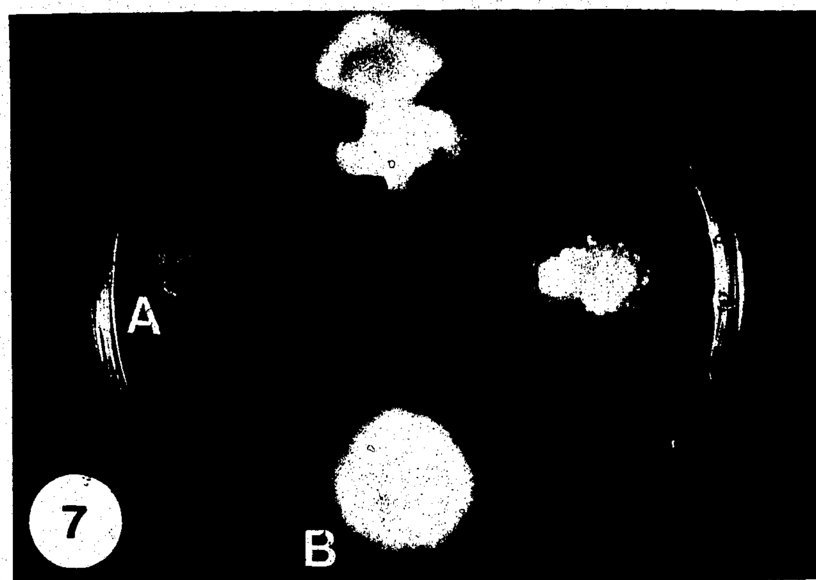
Cubes of agar (approximately 1 mm^3) were cut from the margins of actively growing cultures of single spore isolates. The cubes from different isolates were paired, 5 mm apart, on 1.25% MA. Three unique pairings were made per Petri plate. The Petri plates were sealed and incubated in the dark at 20°C for 30 days. Cultures were examined every 10 days for evidence of compatibility. Compatible pairings were recognized by a flat crustose colony, (Fig. 7). Incompatible pairings were recognized by the fluffy colonial morphology of at least one of the isolates (Fig. 7). A genotypic designation was arbitrarily assigned to each of the isolates in the four mating type groups.

v) Somatic segregation

The somatic segregation technique of Anderson (1982) was attempted on diploid isolates of C-621[1], and C-827. Isolates were grown on 1.25% MA ammended with Benzimidazole (Benomyl), 25 µg/l, for 30 days at 20°C. For each isolate, five colonies were macerated for two minutes in a Waring blendor with 800 ml of sterile distilled water. The macerate was filtered through a 10 µm nylon mesh using vacuum filtration. One ml of the filtrate was diluted 10 x with sterile distilled water and 0.1 ml was spread evenly over the surface of 1.25% MA. One hundred Petri plates were inoculated with each isolate. The Petri plates were incubated for 14 days in the dark at

[1] Northern Forest Research Fungal Culture Collection isolate number.

Figure 7. Mating interactions between testers of C-894. A compatible mating is recognized by a crusty colony morphology, A. An incompatible mating is recognized by a fluffy colony morphology, B.



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Images en couleur

20^{o}C. Colonies that had a fluffy morphology were transferred to 1.25% MA. Tester typing was then attempted for the somatic segregants in the same manner as for the single spore isolates.

vi) Biological species determination

Testers from each of the biological species and putative haploids from the somatic segregation experiment were paired with eight of the known North American biological species (provided by Dr. J. B. Anderson, Department of Botany, University of Toronto) and the biological species found in British Columbia (provided by Dr. D. J. Morrison, Pacific Forest Research Centre, Victoria, British Columbia). The isolates were also paired with single spore testers of **A. obscura**, **A. borealis**, and **A. sp. B** from Europe and Scandinavia (provided by K. Korhonen, Finnish Forest Research Institute, Helsinki, Finland), (Table 3). The isolates were examined for evidence of compatibility after 30 days of incubation.

TABLE 3. List of North American and European *A. mellea* Biological Species Haploid Tester Cultures Used in This Study.

culture collection	date	location	biological species
C-864	03/09/77	Finland	*A. borealis*
C-865	19/09/77	Finland	*A. borealis*
C-868	05/09/74	Finland	*A. sp. B*
C-869	28/08/77	W. Germany	*A. sp. B*
C-872	22/09/77	Finland	*A. obscura*
C-873	27/10/74	Finland	*A. obscura*
C-940	unknown	Vermont	I
C-941	unknown	Vermont	I
C-942	unknown	Vermont	I
C-943	unknown	unknown	II
C-944	unknown	Vermont	II
C-945	unknown	Vermont	II
C-946	unknown	Vermont	III
C-947	unknown	Vermont	III
C-948	unknown	Vermont	III
C-949	unknown	New York	V
C-950	unknown	New York	V
C-951	unknown	Massachusetts	VI
C-952	unknown	Massachusetts	VI
C-953	unknown	Massachusetts	VI
C-954	unknown	Michigan	VII
C-955	unknown	Michigan	VII
C-956	unknown	Vermont	VII
C-957	unknown	Idaho	IX
C-958	unknown	British Columbia	IX
C-959	unknown	British Columbia	IX
C-960	unknown	Idaho	X
C-961	unknown	Idaho	X
C-962	unknown	Idaho	X

isolates C-864 - C-873 obtained from K. Korhonen

isolates C-940 - C-962 obtained from J.B Anderson

2. Interspecific Incompatibility

i) Identification using interspecific incompatibility

Diploid isolates of the **A. mellea** complex were grown on 3% MA,(3% malt extract (Difco) and 1.5% agar) and malt dextrose peptone agar (MDPA), (3% malt extract (Difco), 2% dextrose, 0.5% peptone, and 1.5% agar), at 20°C for 14 days. Cubes of agar, approximately 1 mm^3 in size, were cut from the actively growing margins of colonies and paired with cubes of agar from other isolates, 5 mm apart. Three replications of each pairing were done per Petri plate on each medium. Plates were sealed with masking tape and incubated in the dark at 20°C. Observations were made every two days after inoculation for 30 days. Incompatibility was recognized by a black line that formed in the agar at the confronting margins of the two colonies. Compatible reactions were recognized by the absence of the black line at the confronting margins.

ii) Nature of the black line

The nature of the black line was investigated by carefully cutting out small pieces of agar containing the black line and mounting them in lactophenol on glass microslides. The black line was then observed under the light microscope.

3. Cultural Characteristics

Nine known species of the **A. mellea** complex were obtained, (Table 4). Isolates of these species were grown on the following four agar media:

a.) potato dextrose agar (PDA)

b.) malt agar (MA)

c.) malt dextrose peptone agar (MPDA)

d.) carrot agar (CA)

a.) PDA was made by dissolving 39 g of PDA (BBL) in one l of deionized glass distilled water and autoclaving for 20 minutes at 121°C.

b.) MA was made by dissolving 30 g of malt extract (Difco) and 17 g agar in one l of deionized glass distilled water and autoclaving for 20 minutes at 121°C.

TABLE 4. A List of A. mellea Complex Species Diploid Cultures Obtained From Other Countries.

culture collection	date	location	species
C-734	unknown	Britain	A. bulbosa
C-735	unknown	Britain	A. bulbosa
C-736	unknown	Britain	A. mellea
C-737	unknown	Britain	A. mellea
C-738	unknown	Britain	A. ostoyae
C-739	unknown	Britain	A. ostoyae
C-860	25/04/77	Australia	A. fumosa
C-861	04/77	Australia	A. luteobubalina
C-863	14/04/77	Australia	A. novae-zelandiae
C-866	05/09/79	Norway	A. borealis
C-867	27/09/80	Finland	A. borealis
C-870	29/09/79	Norway	A. sp. B
C-871	23/10/77	Finland	A. sp. B
C-874	09/74	Finland	A. obscura
C-875	unknown	Sweden	A. obscura

isolates C-734 - C-739 obtained from D.J. Morrison
isolates C-860 - C-863 obtained from G.A. Kile
isolates C-866 - C-875 obtained from K. Korhonen

c.) MDPA was made by dissolving 30 g malt extract (Difco), 20 g Dextrose, 5 g peptone, and 17 g agar in one l of deionized glass distilled water, and autoclaving for 20 minutes at 121°C.

d.) CA was made by grinding 300 g of washed carrots in 400 ml of deionized glass distilled water in a Waring blender for 45 seconds. The ground carrot slurry was autoclaved for 20 minutes at 121°C. In a second flask, 16 g agar was added to 300 ml of deionized glass distilled water; the agar and the ground carrots were autoclaved for 20 minutes at 121°C.

The four media were dispensed into plastic Petri plates (100 x 15 mm), approximately 25 ml per Petri plate.

All isolates were grown on the four types of media in the dark at 20°C for 14 days. A cube of agar 1 mm^3 in size was cut from the margin of the colony and placed at the center of a Petri plate. Inoculum used to inoculate PDA, MA, MDPA, and CA was taken from colonies grown on the corresponding media. Ten replications per isolate were made for each agar medium. All Petri plates were sealed with masking tape and incubated in the dark at 20°C for 21 days. Observations and measurements were then made on thallus growth ratio (the ratio of length of rhizomorphs produced to colony diameter), rhizomorph branching pattern, and pseudosclerotial type.

D. Results and Discussion

1. Sexual Mating System

i) Basidiocarp collection

Basidiocarps of the **A. mellea** complex were collected from nine locations in Alberta. Most of the basidiocarps were collected in the Boreal forest region (Table 5). One basidiocarp was collected in the sub-alpine forest of the Canadian Rocky Mountains. No basidiocarps were collected from the eastern slopes of the Rocky Mountains. The reason for so few basidiocarps being found is uncertain, but may relate to the environmental conditions necessary for fruiting initiation. Ginns (personal communication) has observed that basidiocarp production in eastern Canada may follow a cyclic pattern. Whether these cycles follow a climatic pattern is unknown. Mycophagists in Alberta collect basidiocarps of the **A. mellea** complex from late August to early October. They have often observed that basidiocarps are found in abundance if there has been a "wet" summer and a warm "wet" fall. There is, however no experimental evidence to support these observations.

TABLE 5. Basidiocarps of the *A. mellea* Complex Collected in Alberta.

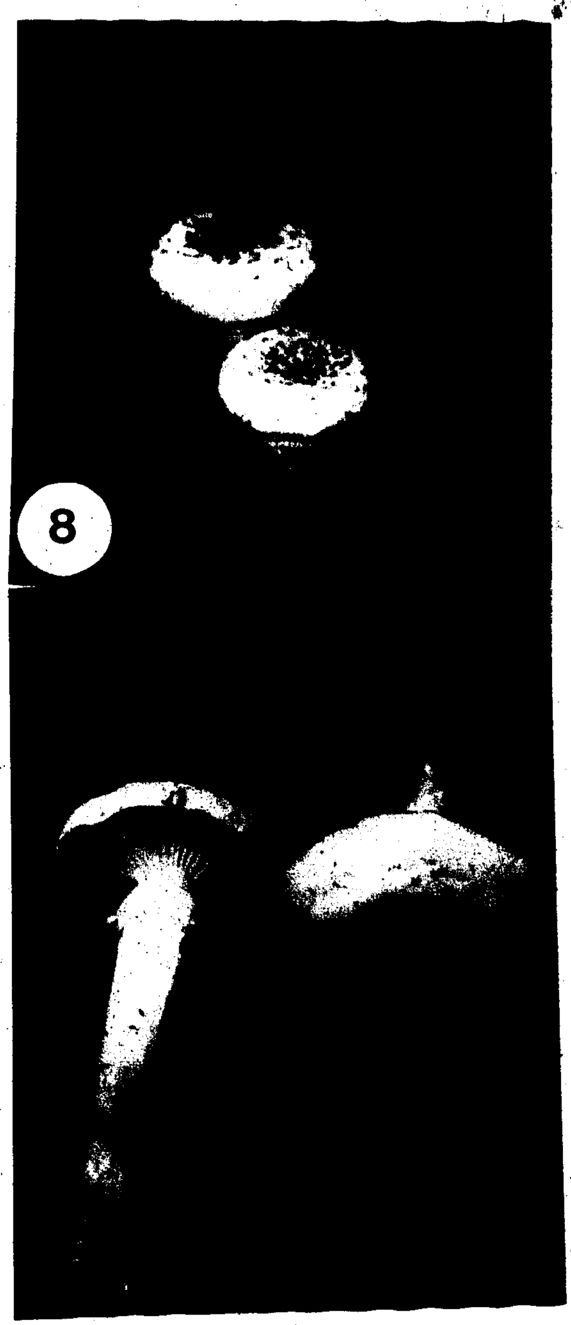
culture[1] collection	CFB #[2]	date	location	host
C-808	21325	09/82	Hasse Lake	*Populus tremuloides*
———	21333	09/82	Devon	*P. tremuloides*
———	21337	09/82	Devon	Conifer stump
———	21363	09/82	Windfall	*Pinus contorta*
C-758	21378	08/82	Maligne lake	*Salix* sp.
———	21379	09/82	Blue Ridge	Ground
C-878	21415	09/83	Devon	*Betula papyrifera*
C-892	21416	09/83	Devon	*B. papyrifera*
———	21417	09/83	Devon	*B. papyrifera*
C-889	21418	09/83	Fox Creek	*B. papyrifera*
C-890	21419	09/83	Fox Creek	*B. papyrifera*
C-830	21422	09/84	Edmonton	*P. tremuloides*
C-859	21423	09/84	Edmonton	*P. tremuloides*
C-927	21424	09/84	Saint Albert	ground
C-891	———	09/83	Fox Creek	*B. papyrifera*
C-893	———	09/83	Hasse Lake	*Populus balsamifera*
C-894	———	09/83	Hasse Lake	*P. balsamifera*
C-895	———	09/83	Edmonton	ground
C-896	———	09/83	Edmonton	*P. tremuloides*
C-897	———	09/83	Edmonton	*B. papyrifera*
C-898	———	09/83	Windfall	*P. contorta*
C-833	———	09/84	Blue Ridge	*P. tremuloides*

1. Northern Forest Research Centre Fungal Culture Collection
2. Northern Forest Research Centre Mycological Herbarium

Sixteen of the basidiocarps collected were found on stumps or trees of, trembling aspen, balsam poplar, and paper birch; two were found on the ground in trembling aspen forests. Three of the basidiocarps were found associated with conifers, two with lodgepole pine and the third on the stump of an unknown conifer. All of the basidiocarps found associated with hardwoods were on mesic to moist sites and those associated with the conifers were found on well-drained or dry sites.

Isolates C-830 and C-859 produced basidiocarps in the greenhouse on lodgepole pine seedlings inoculated with fungus-infested trembling aspen branches, (Fig. 8). The basidiocarps were produced September 14 to October 1, 1984, five months after the branch segments had been infested. Basidiocarps of C-830 were found in seven of fifteen pots. Basidiocarps of C-859 were found in four of fifteen pots. None of the other five isolates used in the pathogenicity experiment produced basidiocarps in the greenhouse.

Figure 8. Basidiocarps of C-830 produced in the greenhouse.



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The stimulus for triggering fruiting in C-830 and C-859 is unknown, but must be different than the stimuli necessary for triggering the other isolates used in the experiment. Shaw **et al.** (1981) were able to produce basidiocarps of **A. novae-zelandiae** but not **A. limonea**, by growing the fungus in flasks on agar media ammended with sodium pentachloraphenol and incubating in full light at 20-23^{o}C. Kile and Watling (1983) produced basidiocarps of **A. novae-zelandiae**, **A. hinnulea**, and **A. luteobubalina** in culture but could not produce basidiocarps of **A. fumosa** by their method. Raabe (1984) studied the day-time and night-time temperatures that occurred two weeks prior to the fruiting of an unidentified species of the **A. mellea** complex. He grew cultures of this species at the same day-time and night-time temperatures and found that basidiocarps were produced.

ii) Mating type identification

Single spore isolates of C-894 were paired in every possible combination to identify mating types, (Table 6). The tetrapolar mating system was confirmed for this isolate. Compatible matings

TABLE 6. Mating Interactions Between Eleven Single Spore Isolates From the Basidiocarp of C-894.

Isolate/	05	15	14	03	08	12	10	09	07	02	01
05	-	-	-	+	+	+	-	-	-	-	-
15	-	-	-	+	+	+	-	-	-	-	-
14	-	-	-	+	+	+	-	-	-	-	-
03	+	+	+	-	-	-	-	-	-	+	-
08	+	+	+	-	-	-	-	-	-	-	-
12	+	+	+	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	+	+	+	+
09	-	-	-	-	-	-	+	-	-	-	-
07	-	-	-	-	-	-	+	-	-	-	-
02	-	-	-	-	-	-	+	-	-	-	-
01	-	-	-	-	-	-	+	-	-	-	-

A plus (+) represents a compatible mating, recognized by the formation of a flat crustose type colony on agar medium. A minus (-) represents an incompatible mating, recognized by a fluffy type of colony on agar medium.

could be recognized after 20 days of incubation. However, 30 of days incubation was necessary so that the compatible reaction was not confused with a hemizygous pairing, (Fig. 9). Compatible matings resulted in a flat crusty type of colony that was melanized. Incompatible matings resulted in either both colonies being fluffy or one fluffy colony and the other being crusty.

The single spore isolates of C-894 were assigned mating type genotypes according to their mating reaction. One isolate from each genotype was selected as a "tester". These tester isolates were paired with one single spore isolate from the other basidiocarps collected. This experiment indicated which basidiocarps were of the same biological species as C-894. The results are shown in Table 7. Five of the single spore isolates tested were the same biological species, as C-894. Four of the isolates, C-891, C-892, C-896, and C-897 produced crusty colonies in all of the matings, which suggests that there are multiple alleles for the mating type loci in this species. Isolate C-893 produced only one compatible colony and so must have the same mating type alleles for both loci as C-894. This is not surprising since the

Figure 9. Mating interactions of single spore testers of C-894. Compatible mating A, Incompatible reaction B, Hemizygous pairing C.

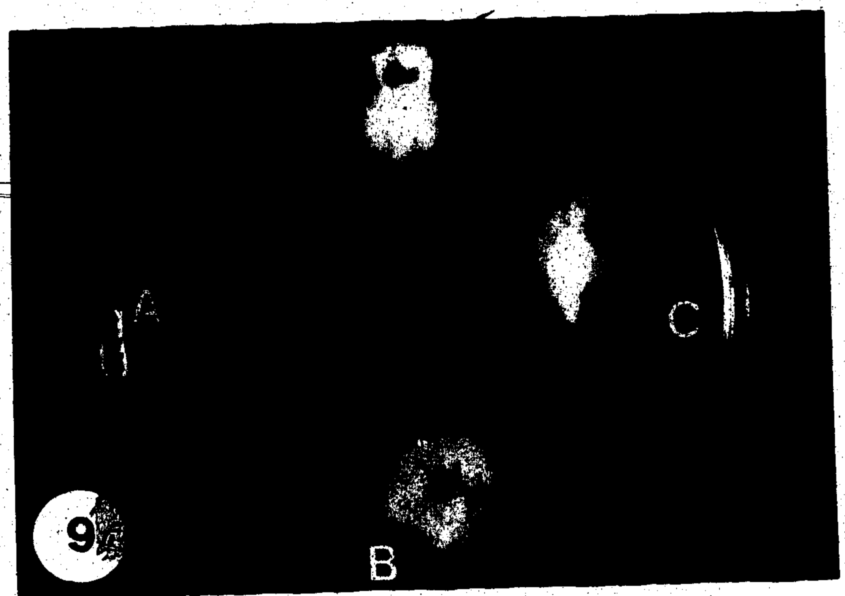

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TABLE 7. Mating Interactions Between the Four Mating Type Testers of C-894 and Single Spore Isolates From Other Alberta Basidiocarps.

Isolate/	894-09	894-10	894-12	894-14
830-32	-	-	-	-
896-02	+	+	+	+
897-05	+	+	+	+
892-06	+	+	+	+
891-04	+	+	+	+
893-13	-	-	+	-
895-11	-	-	-	-
898-03	-	-	-	-

A plus (+) represents a compatible mating, recognized by the formation of a flat crustose colony on agar medium. A minus (-) represents an incompatible mating, recognized by a fluffy colony on agar medium.

basidiocarps of both C-894 and C-893 were found within a few feet of each other. Isolates C-830, C-859, C-895, and C-898 were incompatible in all of the matings, indicating that they were different biological species than C-894.

Single spore isolates from each of C-830, C-895, and C-898 were paired with single spore isolates from their own basidiocarps in every possible combination, to identify mating types. The results are shown in Tables 8, 9, and 10. Only three mating types could be identified for C-830 although [illegible] single spore isolations were made. Four mating types were found for both C-895 and C-898. Three testers were selected from C-830 and four testers from both C-895 and C-898.

The testers from C-830, C-894, C-895, and C-898 were paired in every possible combination to confirm that C-894 was a different biological species than the others, and to determine if C-830, C-895, and C-898 were the same or different biological species. The results are shown in Table 11. C-894 was confirmed to be a different biological species than C-830, C-895, and C-898, and these three basidiocarps were found to be the same biological species.

TABLE 8. Mating Interactions Between Eight Single Spore Isolates From Basidiocarp C-895.

Isolate/	02	07	06	11	03	05	09	10
02	-	-	-	-	+	-	-	-
07	-	-	-	-	+	-	-	-
06	-	-	-	-	+	-	-	-
11	-	-	-	-	+	-	-	-
03	+	+	+	+	-	-	-	-
05	-	-	-	-	-	-	+	+
09	-	-	-	-	-	+	-	-
10	-	-	-	-	-	+	-	-

A plus (+) represents a compatible mating, recognized by the formation of the of a flat crustose colony on agar medium. A minus (-) represents an incompatible mating, recognized by a fluffy colony on agar medium.

TABLE 9. Mating Interactions Between Eight Single Spore Isolates From Basidiocarp C-898.

Isolates/	01	09	10	06	02	04	08	03
01	-	+	+	+	-	-	-	-
09	+	-	-	-	-	-	-	-
10	+	-	-	-	-	-	-	-
06	+	-	-	-	-	-	-	-
02	-	-	-	-	-	-	+	+
04	-	-	-	-	-	-	+	+
08	-	-	-	-	+	+	-	-
03	-	-	-	-	+	+	-	-

A plus (+) represents a compatible mating, recognized by the formation of a flat crustose colony on agar medium. A minus (-) represents an incompatible mating, recognized by a fluffy colony on agar medium.

TABLE 10. Mating Interactions Between Ten Single Spore Isolates From Basidiocarp C-830.

Isolate/	11	29	31	32	33	34	24	28	16	20
11	-	-	-	-	-	-	-	-	+	-
29	-	-	-	-	-	-	-	-	+	-
31	-	-	-	-	-	-	-	-	+	-
32	-	-	-	-	-	-	-	-	+	-
34	-	-	-	-	-	-	-	-	+	-
24	-	-	-	-	-	-	-	-	+	-
28	-	-	-	-	-	-	-	-	+	-
16	+	+	+	+	+	+	+	+	-	-
20	-	-	-	-	-	-	-	-	-	-

A plus (+) represents a compatible mating, recognized by the formation of a flat crustose of colony on agar medium. A minus (-) represents an incompatible mating, recognized by a fluffy colony morphology.

TABLE 11. Mating Interactions Between Alberta Biological Species Mating Type Testers.

	C-894				C-895				C-898				C-830		
	09	10	12	14	02	03	05	10	01	02	08	09	11	16	20
C-894															
09	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
10	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
14	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
C-895															
02	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+
03	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+
05	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
10	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+
C-898															
01	-	-	-	-	+	+	+	+	-	-	-	+	+	+	+
02	-	-	-	-	+	+	+	+	-	-	+	-	+	+	+
08	-	-	-	-	+	+	+	+	-	+	-	-	+	+	+
10	-	-	-	-	+	+	+	+	+	-	-	-	+	+	+
C-830															
11	-	-	-	-	+	+	+	+	+	+	+	+	-	+	-
16	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-
20	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-

[illegible] = compatible reaction. (-) = incompatible reaction

iii) Somatic segregation

No basidiocarps were collected from the foothills or eastern slopes. It was therefore impossible to determine biological species on the basis of mating single spore isolates. Anderson's (1983) technique of somatic segregation was attempted so that somatically segregated haploid isolates could be made from diploid isolates and used to determine biological species affiliation. Four of fifty C-621 somatic segregant colonies initially selected, maintained a fluffy colony morphology over repeated subculturing. These four colonies were paired in every possible combination, (Table 12). Four somatic segregant colonies of C-827 were found to maintain the fluffy colony morphology and these were paired in every possible combination, (Table 13). When isolates from both C-621 and C-827 were paired with North American biological species testers, no compatable matings occurred.

TABLE 12. Mating Interactions Between Somatic Segregants of C-621.

Isolate/	19	17	11	08
19	-	+	-	-
17	+	-	-	-
11	-	-	-	-
08	-	-	-	-

A plus (+) represents a compatible mating. A minus (-) represents an incompatible mating.

TABLE 13. Mating Interactions Between Somatic Segregants of C-827.

Isolate/	01	05	06	10
01	-	-	-	-
05	-	-	-	-
06	-	-	-	-
10	-	-	-	-

A plus represents a compatible mating. A minus (-) represents an incompatible mating.

iv) Identification of biological species in Alberta

Representative biological species testers from Alberta were paired with the testers of eight North American biological species, **A. tabescens**, **A. borealis**, **A. sp. B** and **A. obscura**. The results are shown in Table 14. The testers from C-894 formed diploid colonies with North American **biological species V**. The testers from C-830, C-895, and C-898 formed diploid colonies with North American **biological species I**. When paired with North American testers from British Columbia, C-894 formed diploids with **biological species V**, and the other Alberta isolates formed diploids with **biological species I**.

Diploid isolates C-109 and C-620, when grown on autoclaved branch stems produced a fluffy type of colony similar to the colonies of single spore isolates. When grown on agar a fluffy colony was formed. The mechanism for the origin of these type of colonies is unknown. These two isolates were paired with the North American and European species, (Table 15). No compatible matings occurred. Somatic segregant colonies C-621-17 and

TABLE 14. Mating Interactions Between Haploid Testers From Alberta and Known Species <u>Armillaria</u> <u>mellea</u> Complex.

	C-894				C-895				C-898				C-830		
	09	10	12	14	02	[illegible]	05	10	01	02	08	09	11	16	20
I															
A	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
B	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
C	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
II															
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III															
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V															
A	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
B	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-
VI															
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VII															
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IX															
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
X															
A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<u>A. tabescens</u>															
817	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
818	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<u>A. borealis</u>															
864	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
865	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<u>A. sp. B</u>															
868	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
869	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<u>A. obscura</u>															
873	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
874	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

(+) = a compatible mating. (+) = an incompatible mating.

TABLE 15. Mating Interactions Between Isolates of C-109, C-620, C-621-17 and C-827-1 and Haploid Testers of Known A. mellea Complex Species.

	C-109	C-620	C-621-17	C-827-1
I				
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
II				
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
III				
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
V				
A	-	-	-	-
B	-	-	-	-
VI				
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
VII				
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
IX				
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
X				
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
A. tabescens				
C-817	-	-	-	-
C-818	-	-	-	-
A. borealis				
C-864	-	-	-	-
C-865	-	-	-	-
A. sp. B				
C-868	-	-	-	-
C-869	-	-	-	-
A. obscura				
C-873	-	-	-	-
C-874	-	-	-	-

(+) = compatible mating. (-) = incompatible mating.

C-827-1 and the fluffy isolates of C-109 and C-620 were paired with the North American species and the European species. No compatible pairings occurred, (Table 15).

From these mating studies it can be concluded that two biological species definitely exist in Alberta, North American **biological species** I and V. A third biological species may exist but this has not been conclusively proven by sexual mating studies.

Anderson and Ullrich (1979) found **biological species** I in British Columbia, Ontario, Washington, New York, and Vermont. **Biological species** V was found only in New York, but has subsequently also been found in British Columbia (Morrison personal communication). Anderson **et al.** (1980) observed that North American **biological species** I was partially interfertile with European biological species C, which has now been identified as **A. ostoyae**. Several other North American biological species were also found at this time to be partially interfertile with European biological species, (VI = **A. mellea**, VII = **A. bulbosa**, VIII and X = **A. sp.** B).

The behavior of the fluffy isolates of C-109, C-620, somatic segregants C-621-17 and C-827-1 suggest that two other biological species may exist in Alberta. C-109 and C-620 are likely the same biological species as segregants from C-621 and C-827. Diploid incompatibility tests indicated that all these isolates were the same. All of these isolates come from the same general location near Hinton, Alberta. This may account for the infertility between all four of the haploid isolates, since they all may share common mating type alleles. Another explanation may be that the somatic segregants are mutants and are unable to form dipoid colonies. Whether this group is truly distinct from the other biological species will remain unknown until the basidiocarps are found. This group has tentatively been named the Foothills type.

2. Diploid Interspecific Incompatibility

i) Confirmation of the black line phenomenon

To confirm that the black line phenomenon could be used to distinguish different biological species of the **A. mellea** complex, diploid isolates of the known Alberta biological species were paired. Isolates of **biological species V** when paired with isolates of **biological species I** always produced a black line in the agar between the two colonies, (Table 16). Isolates of the same species, when paired, never produced a black line. Diploid isolates of the other North American biological species were created by pairing compatible single spore isolates. These diploid colonies were paired in every possible combination. Black lines developed in the agar between different biological species.

These results are similar to those obtained by Korhonen (1978) and Rishbeth (1982) for known species of the **A. mellea** complex.

TABLE 16. Interactions Between Diploid Isolates of Biological Species I and V When Grown on Malt Agar.

	Isolate/	Biological species V C-878	C-891	C-894	Biological species I C-830	C-895	C-898
	C-878	-	-	-	+	+	+
V	C-891	-	-	-	+	+	+
	C-894	-	-	-	+	+	+
	C-830	+	+	+	-	-	-
I	C-895	+	+	+	-	-	-
	C-898	+	+	+	-	-	-

A plus sign (+) indicates a black line formed between the two colonies and represents an incompatible reaction. A minus sign (-) indicates a compatible reaction, no black line formed between the colonies.

ii) Reliability of the black line phenomenon

Reliability of the black line phenomenon was tested by pairing **biological species** V (C-878) and **biological species** I (C-859) thirty times. The black line was found in 93% of the pairings, and occurred in at least one of the three pairings per Petri plate.

iii) Diploid isolate collection

Sixty-eight diploid isolates were collected from 36 locations in Alberta, (Fig.10). Isolates, their hosts, locations and dates of collection are given in Appendix 1.

iv) Identification of Alberta diploid isolates

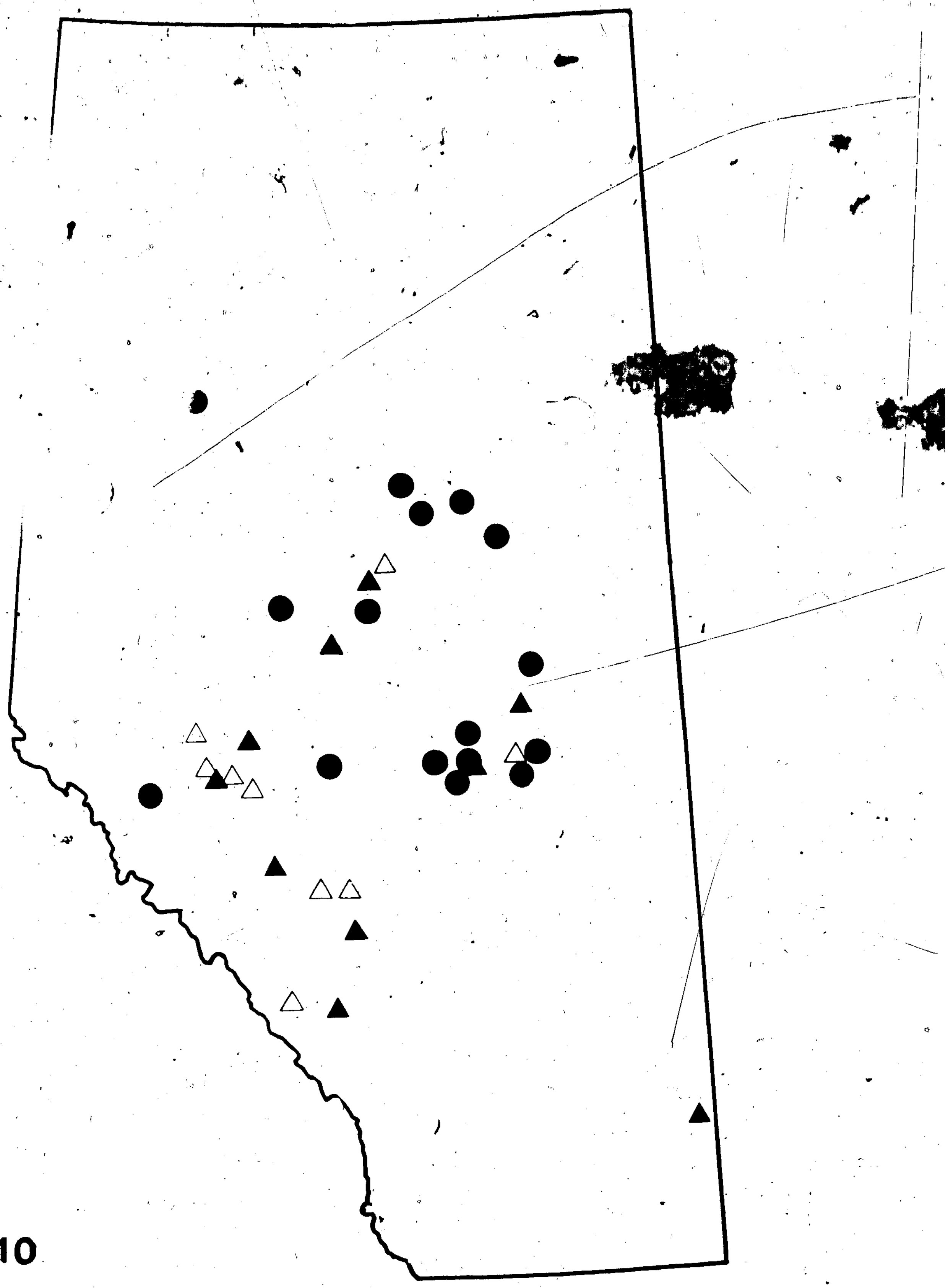
From sexual mating studies two Alberta biological species were known to exist. Diploids of these two biological species could be distinguished by the black line phenomenon, and so representative isolates (C-621, C-859, C-878, C-898,) of these biological species were paired with other Alberta diploid isolates to determine

Figure 10. Locations where biological species of the **A. mellea** complex were collected in Alberta.

▲ = **biological species I**

△ = Foothills type

● = **biological species V**



10

species affiliation. All Alberta diploid isolates were tested in this manner and assigned to either **biological species** I, V or to the Foothills type.

Of the 68 specimens isolated, 17 were found to belong to **biological species** I, 26 to **biological species** V, and 24 to the Foothills type (one isolate was indeterminable because of contamination). A summary of host species and the number of different biological species isolated from each host species is given in Table 17. The Foothills type of isolate always formed a black-line with isolates of **biological species** V, but gave an ambiguous result when paired with **biological species** I. Often pigment could be seen in the agar where the two colonies met. There were, however, no melanized cells in this zone and the hyphae appeared normal. The Foothills type rarely forms a pseudosclerotium when paired with **biological species** I or V on malt agar, but will produce pseudosclerotia when paired with other isolates of the Foothills type. The pseudosclerotium formed more often if the pairings were done on MDPA, but a thick aerial mycelium was formed by both **biological species** I and the Foothills type making it difficult to interpret the outcome of the pairing.

TABLE 17. Host Tree Species of the Three Biological Species of the *A. mellea* Complex Found in Alberta.

Tree Species	Total number of samples collected	Number of samples collected from each biological species		
		I	V	Ft
Pinus contorta	31	12	2	17
Pinus banksiana	2	1	0	1
Abies balsamea	6	1	5	0
Abies lasiocarpa	2	0	0	2
Picea glauca	2	0	1	1
Picea mariana	1	0	0	1
Pseudotsuga menziesii	1	0	0	1
Populus tremuloides	9	1	7	1
Populus balsamifera	4*	0	3	0
Betula papyrifera	7	0	7	0
Salix sp.	1	0	1	0
ground (basidiocarps)	2	1	1	0

* isolate indeterminate due to contamination

These results suggest that the Foothills type is biological species I and so contradict the results from the mating study which indicate the two are unrelated.

Diploid isolates were grouped according to the geographic areas from which they originated (Table 18).

In the Hinton area the most common group isolated was the Foothills type. Biological species I was isolated twice. A single isolate of biological species V was found in the Hinton area at Maligne lake. The Foothills type was found on lodgepole pine and subalpine fir. Biological species I was isolated from lodgepole pine. The isolate of biological species V was found on a willow.

In the Rocky Mountain House, Banff, and Cypress Hills areas biological species I was found most often. The Foothills type was found in the Banff and Rocky Mountain House areas. All of the isolates were found on lodgepole pine, except two of the Foothills type, which were found on trembling aspen and Douglas-fir. No biological species V isolates were found in any of these areas.

TABLE 18. Geographic Areas in Alberta Where Biological Species of the A. mellea Complex Were Collected.

Hinton Area

isolate	location	host	species
C-109	Robb	Pinus contorta	Ft
C-613	Trunk Road	P. contorta	Ft
C-615	Trunk Road	P. contorta	Ft
C-620	Trunk Road	P. contorta	Ft
C-621	Trunk Road	P. contorta	Ft
C-824	Robb Road	P. contorta	Ft
C-827	Robb Road	P. contorta	Ft
C-826	Robb Road	P. contorta	Ft
C-926	Gregg River	P. contorta	I
C-748	McLeod River	P. contorta	I
C-746	Luscar Road	P. contorta	Ft
C-757	Luscar Road	P. contorta	Ft
C-756	Robb Road	P. contorta	Ft
C-825	Robb Road	P. contorta	Ft
C-934	Robb Road	P. contorta	Ft
C-921	Gregg River	P. contorta	Ft
C-900	Obed	P. contorta	I
C-922	Robb Road	Abies lasiocarpa	Ft
C-929	Hinton	A. lasiocarpa	Ft
C-758	Maligne lake	Salix sp.	V

TABLE 18. cont.

Rocky Mountain House Area

isolate	location	host	species
C-828	Cow lake	*P. contorta*	I
C-829	Cow lake	*P. contorta*	I
C-830	Cow lake	*P. contorta*	I
C-859	Ram Falls	*P. contorta*	I
C-813	Rocky Mountain House	*P. contorta*	I
C-812	Ferrier	*Populus tremuloides*	Pt

Banff Area

C-876	Banff	*Pseudotsuga menziesii*	Pt
C-811	Seebe	*P. contorta*	I

Cypress Hills Area

C-908	Cypress	*P. contorta*	I
C-909	Cypress	*P. contorta*	I

TABLE 18. cont.

Edmonton Area

isolate	location	host	species
C-808	Hasse Lake	*P. tremuloides*	V
C-896	Edmonton	*P. tremuloides*	V
C-905	Warspite	*P. tremuloides*	V
C-905	Victoria	*P. tremuloides*	I
C-893	Hasse Lake	*Populus balsamifera*	V
C-894	Hasse Lake	*P. balsamifera*	V
C-925	Elk Island	*P. balsamifera*	V
C-878	Devon	*Betula papyrifera*	V
C-892	Devon	*B. papyrifera*	V
C-897	Edmonton	*B. papyrifera*	V
C-924	Elk Island	*B. papyrifera*	V
C-904	Victoria	*Pinus banksiana*	I
C-923	Elk Island	*P. banksiana*	I
C-906	Cynthia	*P. contorta*	V
C-895	Edmonton	ground	I
C-927	St. Albert	ground	V
C-931	Elk Island	*Picea glauca*	Ft

TABLE 18. cont.

Slave Lake Area

isolate	location	host	species
C-902	Calling Lake	Abies balsamea	V
C-903	Calling Lake	A. balsamea	V
C-915	Slave Lake	A. balsamea	V
C-910	Calling Lake	A. balsamea	I
C-901	Calling Lake	P. balsamifera	V
C-916	Fawcett Lake	B. papyrifera	I

Whitecourt Area

isolate	location	host	species
C-889	Fox creek	B. papyrifera	V
C-890	Fox creek	B. papyrifera	V
C-891	Fox creek	B. papyrifera	V
C-898	Windfall	P. contorta	I
C-920	Blue Ridge	P. contorta	I
C-932	Blue Ridge	P. contorta	V
C-919	Blue Ridge	P. contorta	Ft
C-918	Blue Ridge	P. tremuloides	V
C-930	Blue Ridge	P. tremuloides	V
C-933	Blue Ridge	P. tremuloides	V

Peace River Area

isolate	location	host	species
C-928	McLennon	P. glauca	V
C-937	White Mud	P. balsamifera	V
C-938	Twin Lakes	A. balsamea	V

In the Edmonton area biological species V was found at seven locations. Host species included trembling aspen, balsam poplar, paper birch, and lodgepole pine. Biological species I was found at three locations. It was isolated from jack pine, white spruce and trembling aspen. The Foothills type was found once on jack pine and once on white spruce.

In the Slave Lake area biological species V was isolated from balsam fir and paper birch, biological species I was isolated from Balsam fir.

In the Whitecourt area, **biological species** I, V and the Foothills type were found. **Biological species** I was isolated from lodgepole pine and **biological species** V was isolated from lodgepole pine, trembling aspen, balsam poplar, and paper birch. The Foothills type was found once on lodgepole pine.

In the Peace River area **biological species** V was found in three locations on white spruce, trembling aspen and balsam fir.

v) Nature of the black line phenomenon

The nature of the black line phenomenon was investigated by microscopically examining the black line that formed between **biological species** I (C-859) and a diploid of North American **biological species** II. Both colonies developed separate pseudosclerotia which extended down into the agar. The black line developed between the two pseudosclerotial walls, (Fig. 11), and was readily distinguishable microscopically, (Fig. 12). Pseudosclerotial tissue was made up of bladder-like cells, (Fig. 13), that were melanized in the case of **biological species** I or not


11

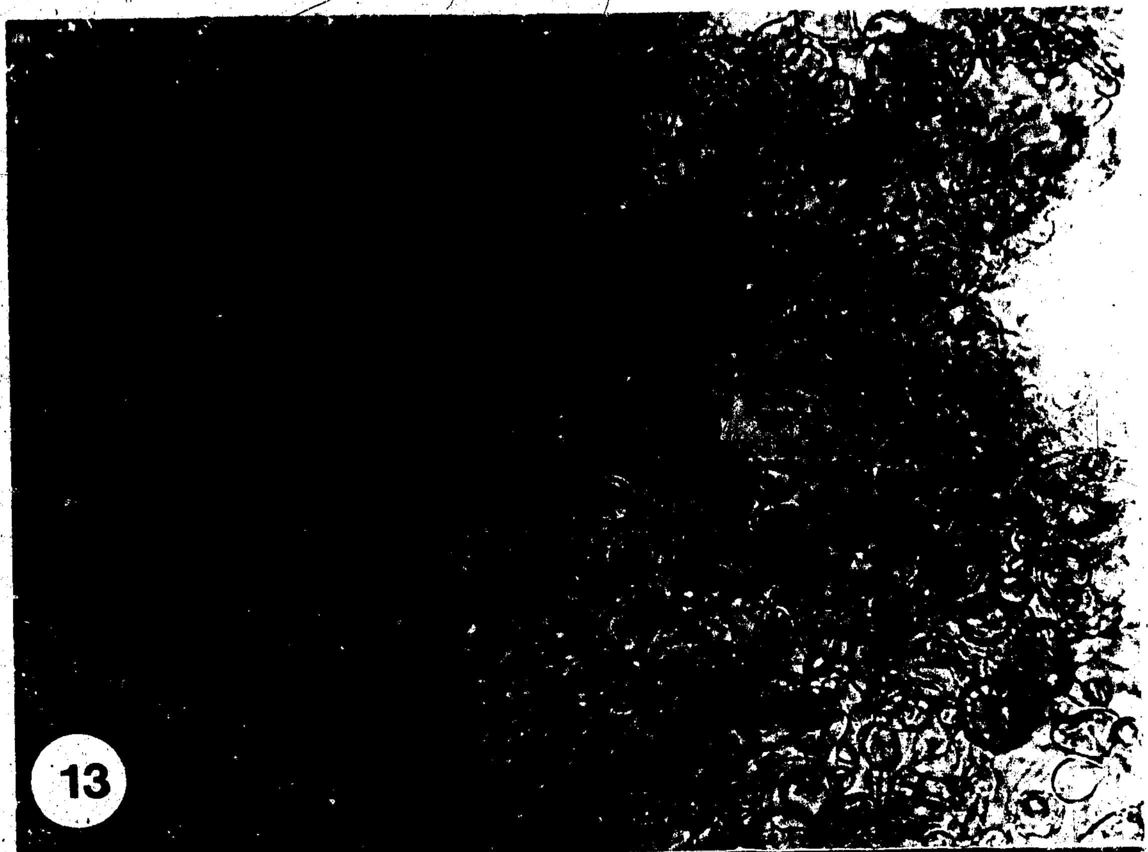

12

Figure 11. Interspecific incompatibility between biological species of the **A. mellea** complex as indicated by the black line (arrow) which forms in the agar between the two colonies. (Mag. 4.6x)

Figure 12. Photomicrograph of the black line between two different biological species of the **A. mellea** complex. (Mag. 21x)

Figure 13. Bladder-like cells of the pseudosclerotial of **A. mellea** complex species. (Mag. 83x)

Figure 14. Melanized hyphae of the black line found between two biological species of the **A. mellea** complex. (Mag. 83x)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so melanized as in the case of **biological species II.** The black line area was composed of melanized hyphae, (Fig. 14). Zones of unmelanized hyphae were found between the black line and the pseudosclerotial wall. Similar observations were made for black lines that occurred between other biological species.

This experiment clearly shows that the black line found between two confronting **A. mellea** complex species is not pigmented agar but a cellular response. The mechanism which causes hyphae to become melanized is unknown. Hyphal disruption or destruction of cell walls was not observed.

There must be two types of recognition systems that operate in the **A. mellea** complex species. The first system must distinguish between similar and dissimilar species. This system would allow the intermingling of the undifferentiated cells of the colony margins, possibly facilitating the exchange of nuclear material in same species pairings. If different species are paired the recognition system may prevent exchange of nuclear material.

The other system that seems to exist is one that recognizes self only. When members of the same species are paired the two colonies retain their separate identities unless they are the same clone. If two colonies belong to the same clone the pseudosclerotia will join. If the two colonies are the same species, but different clones, the pseudosclerotium from either colony grows down into the agar, separating one from the other, This also occurs when two different species are paired. This type of incompatibility has been recognized to occur with several wood rotting hymenomycetes (Rayner and Todd 1978). Hood and Morrison (1984) found that different species of the **A. mellea** complex, when grown in wood, formed zone lines where the two colonies confronted. If isolates, identified as being **A. ostoyae**, taken from disease centers within a small forested area were paired, certain pairings produced zone lines while others did not. This may be evidence that the two clones of **A. ostoyae** existing in the area were in fact different species. The same clones would not form zone lines between each other, as the clone recognized itself and grew into one large colony. A detailed examination of the zone lines that form between the two so-called clones should be done to determine if these zone lines differ from those produced between two different species. In other hymenomycetes this system can discriminate between sibling colonies of the same parent (Adams and Roth 1967, Rayner and Todd 1978). Whether this is true for **A. mellea** complex species is unknown.

3. Cultural Characteristics

i) Thallus growth ratio

The thallus of **A. mellea** complex species is defined as the mycelia formed in a colony on and in the agar, including the pseudosclerotia, and the rhizomorphs, if produced. Gibson (1961) rated isolates of **A. mellea** according to vigour of rhizomorph growth. He classed rhizomorph vigor into three categories. Vigour class 1 was defined as lacking rhizomorphs or if present were produced to a very limited extent and never exceeded the margin of the colony. Vigour class 2 rhizomorphs were those which had a radial growth that was never twice the diameter of the colony and vigour class 3 rhizomorphs had a radial diameter that exceeded twice the diameter of the colony. Since vigour is an imprecise word, the term vigour class has been replaced in this study with thallus growth ratio. The categories and their definitions used by Gibson (1961) remain the same.

The thallus growth ratio was determined for nine taxonomic species of the **A. mellea** complex, (Table 19). Thallus growth ratio was found to vary for some species depending upon the media on which the fungus was grown.

ii) Rhizomorph branching patterns

Rhizomorph branching pattern types for rhizomorphs grown in agar media were similar to those described by Morrison (1982) for rhizomorphs grown in soil. Two types of branching patterns were recognized in agar. Type I rhizomorphs had monopodial growth with occasional dichotomous branches and occasional small lateral branches (Fig. 15). Type II rhizomorphs branched frequently and most often dichotomously (Fig. 16). Rhizomorph branching patterns for the nine taxonomic species of the **A. mellea** complex which were tested are shown in Table 19.

TABLE 19. Summary of Cultural Characteristics of Nine *A. mellea* Complex Species Grown on Four Agar Media.

	Media			
	PDA	MA	CA	MDPA
A. mellea				
C-736	B II 3	B II 3	B II 3	B II 3
C-737	B II 3	B II 3	B II 3	B II 3
A. bulbosa				
C-734	- - 1	- I 2	A I 3	A I 3
C-735	A - 1	A I 2	A I 3	A I 3
A. ostoyae				
C-738	- - 1	B - 1	B II 3	B - 1
C-739	- - 1	B - 1	B II 1	B - 1
A. fumosa				
C-860	A I 3	A I 3	A I 3	A I 3
A. luteobubalina				
C-862	A I 3	A I 3	A I 3	A I 3
A. novae-zelandiae				
C-863	B II 1	B - 1	B II 3	B II 3
A. borealis				
C-866	B - 1	B II 1	A II 3	B II 1
C-867	B - 1	B II 1	A II 3	B II 3
A. sp. B				
C-870	A I 2	A I 2	A I 2	A I 2
C-871	A I 2	A I 3	A I 3	A I 3
A. obscura				
C-875	B - 1	B - 1	B II 3	B - 1

A = Crusty type of pseudosclerotial wall

B = Aerial hyphae type of pseudosclerotial wall

I = Rhizomorph branch pattern Type I

II = Rhizomorph branch pattern Type II

1 = Thallus growth ratio 1

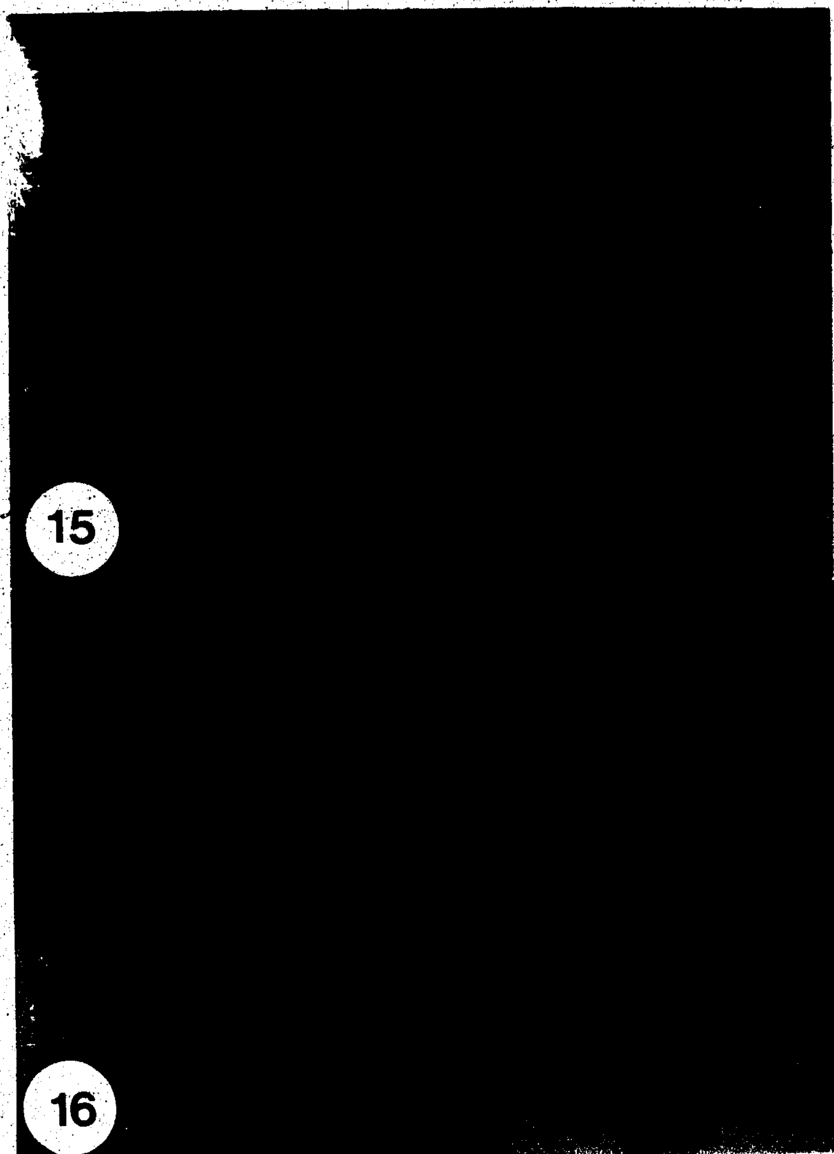
2 = Thallus growth ratio 2

3 = Thallus growth ratio 3

\- = Character not observed

Figure 15. Rhizomorphs exhibiting Type I rhizomorph branching pattern.

Figure 16. Rhizomorphs exhibiting Type II rhizomorph branching pattern.



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iii) Pseudosclerotial type

The pseudosclerotia of the nine **A. mellea** complex species, when grown on the four media, were examined and classified into three general types. Type A had a thin crusty appearance with very little aerial hyphae, (Fig. 17). The Type B pseudosclerotium was thick with an abundance of aerial hyphae, (Fig. 18). Certain species, when grown on some media, failed to produce pseudosclerotia. Instead, thin hyaline mycelium developed very close to the agar surface, (Fig. 19). The pseudosclerotial types of the nine **A. mellea** complex species are shown in Table 19.

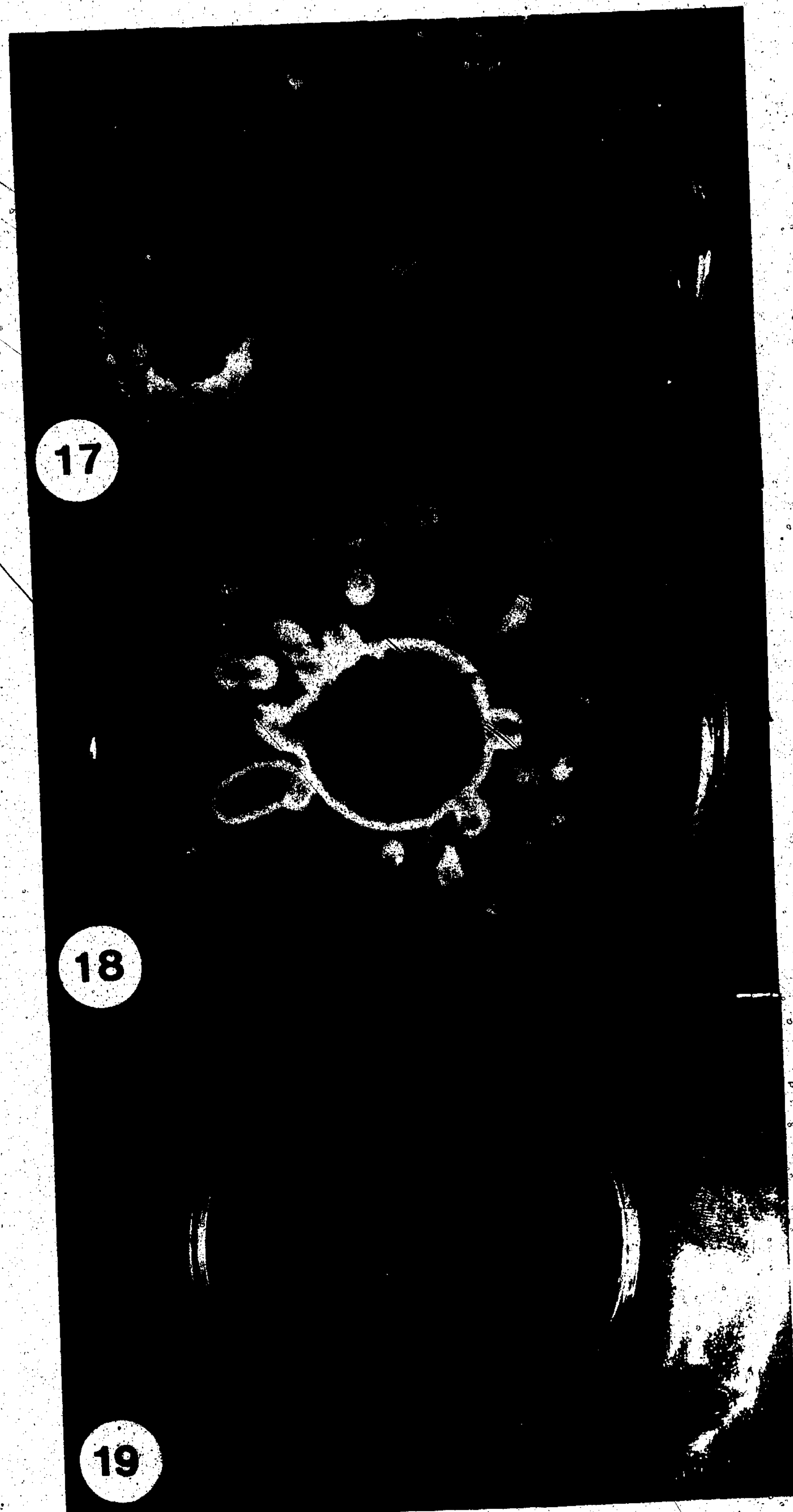
iv) Identification based upon cultural characteristics

The nine species of the **A. mellea** complex could be identified and separated into two distinct groups. Group 1 could be recognized by the production of the Type A pseudosclerotium and the Type I rhizomorph branching pattern. Group 2 was recognized by the Type B pseudosclerotium and the Type II rhizomorph branch pattern. Thallus growth

Figure 17. **A. mellea** complex species exhibiting the Type A pseudosclerotium.

Figure 18. **A. mellea** complex species exhibiting the Type B pseudosclerotium.

Figure 19. **A. mellea** complex species lacking a pseudosclerotium.



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ratio in most species of Group 1 seems to be constant, thus making it difficult to separate **A. fumosa**, **A. luteobubalina**, and **A. sp. B** from one another. The thallus growth ratio of Group 2 species is not constant between species and often is different for a particular species on different media. The use of thallus growth ratio to separate Group 2 species will depend upon further testing with more isolates of each species.

The results of the rhizomorph branching pattern for **A. mellea**, **A. bulbosa**, and **A. ostoyae** are consistant with Morrison's (1982) findings for these isolates. The pseudosclerotial types of **A. fumosa**, **A. luteobubalina**, and **A. novae-zelandiae** are in agreement with Kile and Watling (1981). Shaw **et al** (1981) recognized **A. novae-zelandiae** and **A. limonea** by pseudosclerotial wall coloration. There were differences within Group 1 and 2 species with respect to coloration and amount of aerial hyphae above the pseudosclerotium. However, because of the ambiguity of color shades, and because so few isolates of each species being tested, this was not used as a defining characteristic.

Isolates of Alberta biological species I and V, as well as the Foothills type, were grown on the different agar media and examined for thallus growth rate, rhizomorph branch pattern, and pseudosclerotial type, (Table 20). The two biological species can be readily separated and biological species V be distinguished from the Foothills type. Biological species V clearly belongs to the Group 1 species of the A. mellea complex, biological species I and the Foothills type belong to Group 2. Isolates of biological species I and the Foothills type have similar cultural characteristics when grown on the four media. The only difference that has been observed was in coloring of the pseudosclerotial wall. Even within biological species I however, C-859 and C-830 have similar coloring of the pseudosclerotial wall but are different than C-895 and C-898. Therefore coloring may be a variable genetic characteristic of biological species I. Isolates from the Hinton area that were identified as being biological species I had a different pseudosclerotial wall coloring than the Foothills type from the same area.

TABLE 20. Summary of Cultural Characteristics of the Alberta A. mellea Complex Species Grown on Four Agar Media.

	Media			
	PDA	MA	CA	MDPA
Bioloical Species I				
C-830	B - 1	B II 3	B II 3	B II 3
C-859	B - 1	B II 3	B II 3	B II 3
C-895	- - 1	B - 1	B II 1	B II 1
C-898	B - 1	B - 1	B - 1	B II 1
C-900	B - 1	B - 1	B II 3	B II 2
C-904	B - 1	B - 1	B II 3	B II 1
C-905	B - 1	B - 1	B II 3	B II 3
C-910	B - 1	B - 1	B II 3	B II 3
C-920	B - 1	B - 1	B II 3	B II 3
Biological Species V				
C-808	- I 2	A I 3	A I 3	A I 3
C-878	- I 3	A I 3	A I 3	A I 3
C-891	- I 3	A I 3	A I 3	A I 3
C-892	- I 3	A I 3	A I 3	A I 3
C-894	- I 3	A I 3	A I 3	A I 3
C-902	- I 3	A I 3	A I 3	A I 3
C-906	- I 3	A I 3	A I 3	A I 3
C-906	- I 3	A I 3	A I 3	A I 3
C-915	- I 3	A I 3	A I 3	A I 3
C-916	- I 3	A I 3	A I 3	A I 3
Foothills type				
C-621	- - 1	- - 1	B II 3	B II 3
C-876	- - 1	B II 1	B II 3	B II 1

A = Crusty type of pseudosclerotial wall
B = Aerial hyphae type of pseudosclerotial wall
I = Rhizomorph branch pattern Type I
II = Rhizomorph branch pattern Type II
1 = Thallus growth ratio 1
2 = Thallus growth ratio 2
3 = Thallus growth ratio 3
- = Character not observed

E. Conclusion

It is evident from this study that there are at least two different biological species of the **A. mellea** complex in Alberta. Both species have been found on the major conifer and hardwood tree species in the province and both seem to have a broad geographic range. A third biological species may exist. This species also has a wide geographic range and has only been found on conifers. Morrison (personal communication) has found that **biological species V** is the predominant form of the **A. mellea** complex in northern British Columbia. This may also be true for Alberta, since in the more northern regions of this survey only **biological species V** was found. The survey was by no means extensive or exhaustive and so no firm conclusions can yet be drawn as to exact ranges.

Biological species of the **A. mellea** complex can be distinguished using the black line incompatibility test with diploid isolates. The black line which forms between two different biological species has been examined and determined to be melanized hyphae, different from the bladder-like cells of the pseudosclerotial wall.

The two biological species of the **A. mellea** complex in Alberta can be readily identified when grown on agar media and certain morphologic characters such as rhizomorph branching pattern and pseudosclerotial wall type can be used to separate nine known **A. mellea** complex species into two groups.

III PATHOGENICITY OF THE ARMILLARIA MELLEA COMPLEX OF ALBERTA

A. Introduction

The objective of this study was to determine the relative pathogenicity of **A. mellea sensu stricto**, biological species I, V, and the Foothills type to lodgepole pine.

B. Literature Review

Hartig (1874) was the first to recognize that **Armillaria mellea** was a pathogen of trees. Workers have since been divided as to whether **A. mellea** is a primary pathogen, capable of attacking healthy trees (Gibson 1960, Filip 1977, Shaw **et al.** 1976, Kile 1981), or a secondary pathogen, which only attacks trees predisposed to disease by some stress factor (Day 1928, 1929, Cooley 1943, Peace 1962, Gremmen 1976).

Historically, attempts at artificial inoculations with **A. mellea** date back to Brefeld (1877). Thomas (1934) and Bliss (1941) were successful in inoculating fruit and walnut tree seedlings, as well as various herbaceous species, with isolates of the fungus grown on autoclaved branch segments which they placed in the soil near the seedlings. Other techniques, such as mixing fungus-infested agar or bran with the potting mixture in which test plants were grown, failed (Bliss 1941). Since then, most inoculations of trees with the **A. mellea** complex have been done by placing pieces of infested wood, in the soil near the test plants (Patton and Riker 1959, Raabe 1967b, Wilbur **et al.** 1972, Shaw 1977, Redfern 1978, Podger **et al.** 1978, Singh 1980, Shaw **et al.** 1981, Kile 1981, Morrison 1982, Rishbeth 1982). Another technique that has seen limited use is the axenic culture technique of Riffle (1973). He axenically grew ponderosa pine seedlings on an agar medium and inoculated them with **A. mellea**.

Until recently, it has been relatively difficult to interpret the results of inoculation studies. Many workers used only a single isolate to inoculate one or several species of trees (Thomas 1934; Patton and Riker 1959; Riffle 1973; Singh 1980), while others have used several isolates that were assumed to be **A. mellea** (Raabe 1967a, 1967b, Wilbur **et al.** 1972, Shaw 1977, Guillaumin and Pierson 1978). Raabe (1967b) and Wilbur **et al.** (1972) found that isolates taken from different host plants and from geographically different locations varied in their virulence to different host species. The virulence of **A. novae-zelandiae** and **A. limonea** to **P. radiata** seedlings was compared by Shaw **et al.** (1981). Morrison (1982) recognized that different isolates of the **A. mellea** complex could be grouped according to rhizomorph branching pattern and that these groups exhibited differences in virulence towards coniferous tree seedlings. Rishbeth (1982) showed that there was a great deal of difference in virulence of **A. mellea, A. ostoyae, A. bulbosa,** and **A. tabescens** to inoculated **Pinus sylvestris** L. seedlings.

C. Materials and Methods

1. Inoculum Preparation

Branch segments of trembling aspen (10 x 2 cm) were autoclaved with 50 ml distilled water in 250 ml Erlenmyer flasks covered with inverted beakers, 5 segments per flask, at 121°C and 15 psi for 60 minutes. Fifty ml of malt dextrose peptone broth, (3% malt extract (Difco), 2% dextrose, 0.5% peptone), was added to each flask before autoclaving for an additional 20 minutes. Trembling aspen was used because it was readily available. Redfern (1970) has found that tree species used for the inoculum has no appreciable affect on infection.

Isolates of **A. mellea sensu stricto** (C-736), **biological species** I (C-830, C-859), **biological species** V (C-891, C-894, C-897), and the Foothills type (C-621), were grown on 3% MA in the dark at 20°C for two weeks. The agar containing the mycelium was cut into small pieces (approximately 1 cm^2) and placed onto the branch segments inside the flasks. Fifteen branch segments were inoculated with each isolate. The flasks were incubated in the dark at room temperature for three months.

2. Seedling Growth Condtions

Two-year-old, field-grown lodgepole pine seedlings were obtained from the Alberta Forest Service, Pine Ridge Forest Tree Nursery. The seedlings were planted in 2 litre plastic pots with limed peat moss, one tree per pot. An inverted 2 X 25 cm test tube was placed parallel next to the taproot of each seedling at the time of planting. The end of the test tube was left sticking out of the peat.

The potted seedlings were grown in a greenhouse compartment with supplemented light provided by high pressure sodium vapor lamps (400 watts), with an intensity of 363 µmol $m^{-2}s^{-1}$. The photoperiod was 18 hours. Daytime temperature was 25°C and nighttime temperature was 20°C. Seedlings were watered every second day and were fertilized biweekly for three months before inoculation.

3. Inoculation

The test tubes beside the seedlings were removed from the pots and replaced with infested branch segments which were prepared as above.

Fifteen seedlings per isolate were inoculated. Control seedlings had autoclaved branch segments placed beside them. Inoculated and control seedlings were watered every second day and fertilized biweekly.

Seedlings were observed for symptoms of Armillaria root rot weekly after inoculation. Roots of dead trees were carefully washed in running water and dissected. If a white fan of mycelia was observed under the bark, the tree was considered to have been infected with the pathogen. Isolations were made from the infected roots, onto AMA, and the resultant colonies were grown in the dark at 20°C for biological species identification. The experiment was terminated after six months. All seedlings were unpotted and the root systems were washed carefully and examined.

D. Results

Foliar symptoms allowed diseased seedlings to be identified before they died (Fig. 20). Foliage of these seedlings was at first a dull green with yellowing of some of the needles, usually in the top portion of the plant. Within 10 to 14 days the needles had become red brown and drooped. When the entire foliage of the seedling was brown the tree was considered dead.

The results of the pathogenicity experiment are shown in Table 21. All of the dead seedlings had a white fan of mycelium under the bark of the root and root collar. Isolations made from the infected seedlings yielded the same biological species as had been used in the inoculation. The attacked seedlings were categorized as seedlings that had lesions on the roots but no foliar symptoms. Healthy seedlings were normal in appearance.

Seedlings showed symptoms of Armillaria root rot approximately one month after inoculation. **A. mellea sensu stricto**, (C-736) caused the greatest mortality; 86.7% of the seedlings inoculated were killed. Eleven of the seedlings inoculated with

Figure 20. Lodgepole pine seedlings inoculated with **A. mellea** complex species.

A = control

B = dying

C = dead

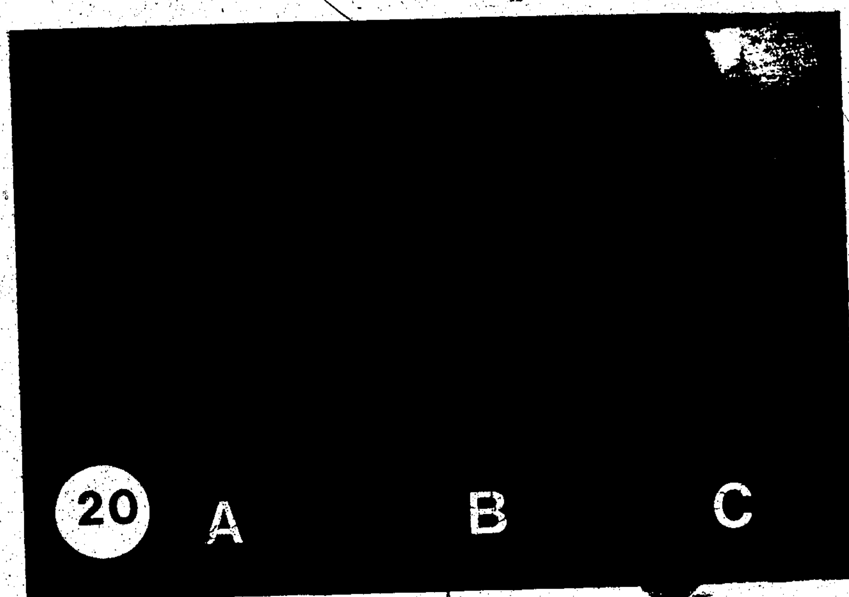

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TABLE 21. Lodgepole Pine Seedlings Inoculated With *Armillaria mellea* sensu stricto, Alberta Biological Species I, V, and the Foothills Type.

Isolate	lodgepole pine seedlings		
	% Dead	% Attacked	% Healthy
A. mellea			
C-736	86.7	13.3	0.0
Biological Species V			
C-891	60.0	33.3	6.7
C-894	26.7	73.3	0.0
C-897	13.3	66.7	20.0
Biological Species I			
C-830	13.3	13.3	73.4
C-859	6.7	33.3	60.0
Foothills Type			
C-621	6.7	26.7	66.3
Control			
	0.0	0.0	100.0

C-736 died within three months of inoculation, two other trees died later. Most of these seedlings died quickly, within 25 days. At the end of the experiment the two remaining trees were found to have lesions with resinosis and rot on the taproots. No trees were found to be healthy.

The three isolates of **biological species V** (C-891, C-894, C-897) caused less mortality than **A. mellea sensu stricto**, but produced significant amounts of mortality.

Isolate C-891 killed 60% of the seedlings within three months of inoculation. Seedling death also ocurred in a short time span, one month. All of the attacked trees had lesions at the root collar. Mycelium and resin were found underneath the bark. One tree was found to be healthy at the end of the experiment.

Isolate C-894 killed 26.7% of the seedlings inoculated with it. Mortality was first recorded two months after inoculation. All of the seedlings remaining at the end of the experiment were found to have been attacked. Lesions were found on the taproot of all the seedlings except one that had lesions on the lateral roots. Four of the trees were found to have decay.

Isolate C-897 killed 13.3 % of the seedlings inoculated with it. Mortality started almost five months after inoculation. Lesions were found on 66.7% of the seedlings. Four seedlings were lesioned and girdled at the root collar and two had lesions on the lateral roots.

Biological species I (C-830, and C-859) and the Foothills type (C-621), both caused relatively little mortality. Most of the seedlings inoculated with these isolates were healthy at the end of the experiment. C-830 and C-859 caused lesions on the lateral roots and the taproots, whereas C-621 caused lesions mostly on the lateral roots.

Infested branch segments were examined for rhizomorphs and mycelia when the seedlings were unpotted. All infested branch segments, except for one infested with C-859, were found to have either mycelium or white rhizomorphs inside the bark, and yellow stringy rot. Rhizomorphs were found growing from the segments infested with **A. mellea sensu stricto,** and **biological species V** isolates. Rhizomorphs of these isolates were also found attached to attacked seedlings roots. No rhizomorphs were found on branch segments infested with **biological species I** or the Foothills type. A rhizomorph was found attached to the root of a seedling killed by the Foothills type, but no rhizomorphs were found on seedlings inoculated with biological species I.

E. Discussion

Pathogenicity is the ability of an organism to incite disease in another organism, therefore, all isolates of the **A. mellea** complex tested in this experiment were pathogenic. Virulence, the relative measure of pathogenicity, can be determined by comparing the total infections (% dead + % attacked), (Table 21). Four conclusions can be drawn from the data. First, **biological species** V is more virulent than both **biological species** I and the foothills type under these experimental conditions. **Biological species V** is just as virulent as isolate C-736, **Armillaria mellea sensu stricto**. However, **A. mellea sensu stricto** caused the most seedling mortality, 86.7%, followed **biological species** V, 33.3% (average of the three isolates). Comparatively, **biological species** I and the Foothills type did not cause much mortality, 10% and 6.7% respectively.

Morrison (1982) and Rishbeth (1982) have shown that there are differences in pathogenicity and virulence amongst the different species of the **A. mellea** complex. Morrison (1982) inoculated three-year-old seedlings of **P. menziesii, P. sylvestris, Picea sitchensis** (Bong.) Carr., **Larix eurolepis** Henry, and **Tsuga heterophylla** (Raf.) Sarg. with isolates that had different rhizomorph branching patterns. He found that isolates with different rhizomorph branching patterns differed in the number of seedlings they killed. One isolate that Morrison (1982) used, C-736, was the same isolate of **A. mellea sensu sricto** used in this study. This isolate killed 45% of the seedlings inoculated with it. Other isolates of **A. mellea** gave similar results. **A. bulbosa**, however, did not kill any seedlings, and **A. ostoyae** isolates were variable in the number of seedling that they killed. Rishbeth (1982) found that 44% of the two-year-old **P. sylvestris** seedlings inoculated with **A. mellea** died, but only 12% of the seedlings inoculated with **A. ostoyae** were killed. Shaw (1977) inoculated **P. ponderosa** and **P. radiata** with isolates taken from hardwood species which were growing in a pine forest, and with isolates taken from diseased pine. The hardwood isolates were not pathogenic to the seedlings, but the pine isolates were. In contrast, Shaw **et al.** (1981) found virtually no difference in mortality or infection between P. radiata seedlings inoculated with A. novae-zelandiae and A. limonea.

The high level of infection caused by A. mellea sensu stricto (C-736) and biological species V (C-891, C-894, and C-897) was similar to to that found by Raabe (1967b). He inoculated P. radiata., Dahlia pinnata Cav., and Prunus persica Batsch with ten Californian isolates of A. mellea. The average amount of infection by the ten isolates on the three species ranged from 53% to 100%. Differences in results between studies are probably due to inoculation techniques, growth conditions, host susceptibility and pathogen species.The virulence differences exhibited by biological species I, the Foothills type, and biological species V may be related to the ability to produce rhizomorphs. Lack of rhizomorphs might be caused by edaphic factors, experimental conditions or the isolates being slow in forming rhizomorphs.

F. Conclusion

All three biological species were pathogenic to lodgepole pine. **Biological species V** was the most virulent. **Biological species I** and the Foothills type, although pathogenic, caused relatively low mortality.

IV. DETECTION OF THE ARMILLARIA MELLEA COMPLEX IN FOREST SOILS

A. Introduction

The purpose of this study was to develop a technique that would enable the detection of **A. mellea** complex members in forest soils.

B. Literature Review

Detection of Armillaria root rot has been primarily based upon foliar symptoms, presence of a white mycelial fan beneath the bark of the root or root collar and/or production of basidiocarps (mushrooms). Trees that have died or are dying from the disease often occur in patches known as disease centers. The movement of the fungus within disease centers and through the forest soil is not well understood.

The primary means of spread of the fungus is considered to be by rhizomorphs growing through the soil, (Redfern 1978) although root to root contact has also been found to be a means of spread in certain situations (Kile 1981). Distribution patterns of the rhizomorphs within the soil have been reported (Ono 1970, Morrison 1976, Singh 1981). The distribution of the fungus within a disease center has not been well studied. Kable (1974) examined disease patterns within an Australian peach orchard over a period of several years and deduced the rate and direction of spread of the disease by tree mortality. Mackenzie and Shaw (1977) found that the greatest number of dead and dying radiata pine seedlings occurred around stumps.

There is [illegible] well established technique to detect **A. mellea** [illegible] soils. However, Aoshima and Hyashi (1981) were able to detect **A. mellea** in an orchard soil by using wooden stakes.

C. Materials and Methods

The study area was located in the foothills of the Canadian Rocky Mountains near Hinton, Alberta. The three plots that were chosen for study were occupied by six-year-old lodgepole pine regeneration after logging. Plot one was chosen because of the presence of Armillaria root rot disease centers. A 10 x 10 m plot that included healthy and diseased trees was established at the site. Trembling aspen logs, approximately 100 cm long and 10 cm in diameter were cut, sharpened at one end, pounded approximately 30 cm into the ground, and spaced one meter apart forming a grid within the plot, (Fig. 21). The logs were labeled and the locations of seedlings, and stumps were mapped. Notes were kept concerning the health of the seedlings. The plot was established in May 1982 and the logs were carefully dug up in June 1983, at which time the logs were individually wrapped and brought back to the laboratory for examination.

In September 1983, two other plots were established in lodgepole pine regeneration that displayed no visual evidence of Armillaria root

Figure 21. Trap logs in lodgepole pine regeneration


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rot. At both of these plots, 48 trembling aspen stakes, similar to those used previously, were driven into the ground one meter apart in a 6 x 8 m grid. The logs were labeled and maps were drawn of the locations of seedlings within the sites. Notes were again kept on the health of the seedlings. The logs were removed in September 1984 and brought back to the laboratory for examination.

The logs were examined for the presence of rhizomorphs and/or a white mycelial fan underneath the bark. Confirmation of **A. mellea** was made by aseptically isolating pieces of the mycelial fan on AMA.

The diploid incompatibility method was used to determine if the isolates from the logs and the diseased seedlings in the plot were the same biological species. Three replications of the pairings were done per Petri plate and every isolate was paired with every other isolate. Petri plates were sealed with masking tape and incubated in the dark at 20^{o} C and observed every second day for three weeks.

D. Results

A. mellea was found in 31 of 121 logs in the first plot. Mycelial fans were found under the bark of the buried portion, on 27 of the logs (Fig. 22). Rhizomorphs were found attached to the bark of the other four logs but no white mycelial fans were found beneath the bark. These logs were found to have thick bark with furrows, while those with mycelial fans which did not have rhizomorphs attached, may have been colonized by contact with an infected root. Most points of entry originated in the bark just above where the logs were sharpened.

The cultural characteristics of the isolates from the logs were similar to those of isolates made from infected lodgepole pine seedlings found in the same plot. The diploid incompatibility test indicated that log isolates and diseased tree isolates were all the same biological species and probably the same clone. The clone was identified as belonging to the Foothills type.

Within the plot there were a total of 93 trees, 17 of which were dead or dying at the beginning of the study. One year later 23 trees

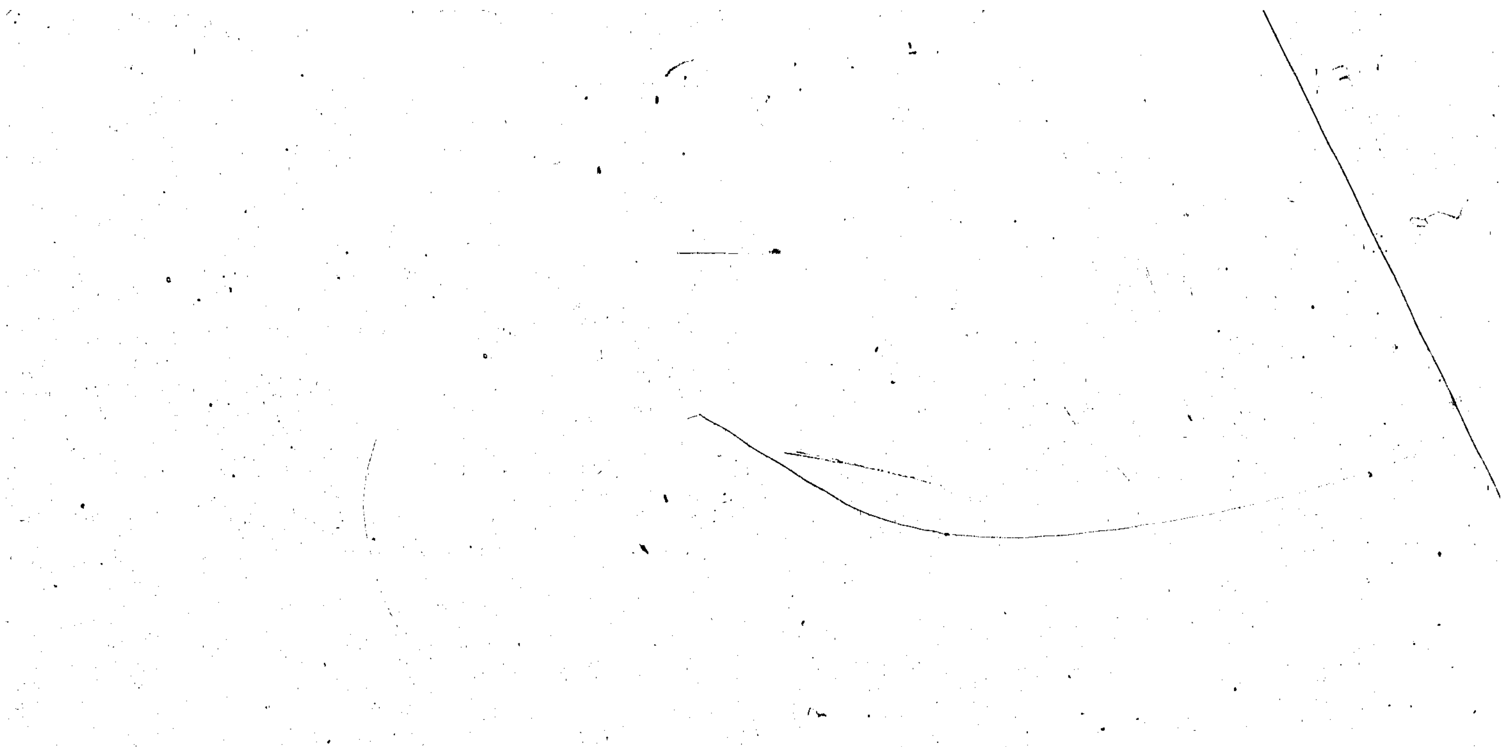

Figure 22. White mycelial fan on a trap log.

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were dead or dying. Eight areas within the plot were found to have the fungus within them, (Fig. 23). The borders of these areas were somewhat arbitrarily assigned.

No trees in plots two and three were found to have Armillaria root rot, nor were any trap logs from these two plots colonized by a species of the **A. mellea** complex.

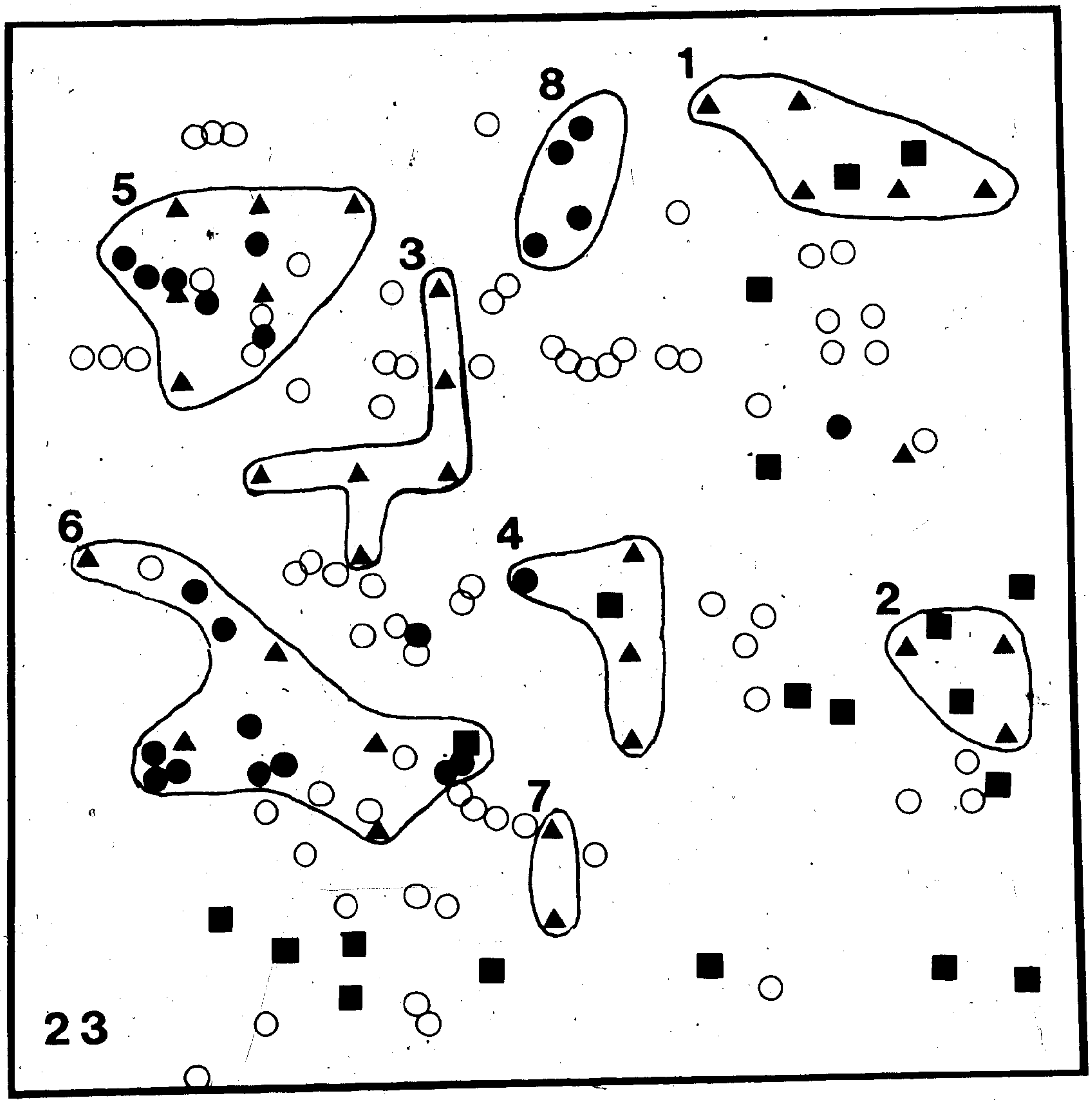
Figure 23. Schematic diagram of trap log plot 1.

■ = stumps

○ = healthy trees

● = diseased trees

▲ = colonized trap logs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23

E. Discussion

It is significant that 30 of the 31 positive logs occurred next to other infested logs, suggesting that the fungus has certain territorial patterns, (Fig. 23, areas 1-8). The fact that no trees exist in areas 1 and 2 with positive logs indicates that there may have been prior killing in these areas. Areas 5 and 6 seem to be active disease centers as they contain both healthy and diseased trees. The other five areas do not contain any healthy trees. One notable anomaly is that no positive logs were found close to four dead trees in area 8.

Stumps have been considered to be important in the initiation of infection centers Mackenzie and Shaw 1977, Roth et al. 1979, and Van der Pas 1981). There are positive logs associated with stumps, (Fig. 23, areas 1, 2, 4 and 7), however, areas with diseased trees do not seem to be closely associated with stumps, (Fig. 23, areas 5, and 8). The exception is area 6, where two diseased trees are found next to a stump. The shapes of some of the areas which include positive logs and diseased trees suggest that they may be associated with buried roots of the cutover trees and that these roots may be serving as the source of inoculum, (Fig. 23, areas 3, 6, 7, and 8). An alternative explanation, is that these patterns developed because of the pattern of rhizomorph growth through the soil. Aoshima and Hyashi (1981) found **A. mellea** in the soil surrounding diseased trees but not in the soil near healthy trees.

Mackenzie and Shaw (1977) have suggested that disease centers are initiated from stumps and that the disease center expands radially with time. The work of Pielou and Foster (1962) and Pielou (1965) indicated that patches of diseased trees coalesced through time to forn larger patches. Van der Pas (1981) studied mc :ality in radiata pine plantations in New Zealanc He found that the pattern of mortality increased ecause of a consolidation of existing patches and the formation of new disease centers. Studies in the Pacific Northwest showed that disease centers increase radially with time (Shaw 1980). It therefore seems likely that two types of disease center development can take place, the radially expanding disease center, and the coalescent disease center. It is also possible that coalescent disease centers develop initially and then once established, expand radially. Hinton plot 1 may exhibit the coalescent type of disease center, since the centers do not originate from a single stump, but give the appearance of being several centers, some of which may have originated from the same infected tree, whether it be stump or infested root.

The trap log technique may be a good method to study the movement of the fungus through the soil and for establishing boundaries of disease centers. In this study the fungus was trapped in logs that were not associated with dying trees and so therefore must have come in contact with the fungus in the soil or in buried roots. If the plot had been assessed purely on foliar symptoms, the presence of the fungus in areas 1, 2, 3, 4, and 5 would have gone undetected. This technique might be used in mature forests, where trees do not exhibit Armillaria root rot symptoms, to determine if the fungus is present in forest soils. It might also be useful in the study of clones or territories of biological species in a forest stand.

F. Conclusion

The trap log method has been successfully used to detect the presence of an *A. mellea* complex species in a forest soil. It can be used to determine the distribution of the pathogen within a disease center and may help in epidemiological studies of Armillaria root rot.

V. GENERAL DISCUSSION

A. Species Concept in the *Armillaria mellea* Complex.

Taxonomy of the higher Basidiomycetes has been based mainly upon morphological features of the basidiocarp. In North America, no serious attempts have yet been made to deliniate taxonomic species of the **A. mellea** complex, although biological species, based mainly upon sexual mating experiments, have been recognized. The **A. mellea** complex exemplifies the difficulty of the species concept in the hymenomycetes. At the 1976, Herbette symposium on species concept in the hymenomycetes, the common species concept that emerged was:

> "Populations belong to the same species when they are able to interbreed and to produce viable offspring, provided that an absence of fertility is caused only by those genetic parameters operating in the entire sexual cycle. For taxa, for which the information necessary, or the application of the criteria

mentioned above is missing, the following practical definition is to be applied: A species is a population which posses constant reproducible characters (morphological etc.) and for which a hiatus exists between this and other populations."

Biological species of the North American **A. mellea** complex do not satisfy this defintion as they have not been shown to produce viable offspring. This may only be due to the difficulty in fruiting some **A. mellea** complex members. In Europe, Korhonen (1978) and Guillaumin and Berthelay (1981) have shown that the biological species concept is compatible with the taxonomic species concept. This study has shown that there are morphological differences , such as hizomorph branching pattern and pseudosclerotial wall type, between some of the North American biological species. Therefore, it is likely that the so-called North American biological species will prove to be distinct taxonomic species.

The partial fertility between some European species and some North American species poses some interesting questions. Are they the same species that have been geographically isolated and are now showing evidence of further speciation, or are they different species whose incompatability simply breaks down in certain matings?

The evolutionary history of the **A. mellea** group seems now to be quite complex, with as many as ten biological species in North America and five taxonomic species in Europe as well as five in Australia. The ranges of the biological species in North America overlap and often different biological species are found in close proximity to each other (Ullrich and Anderson 1978). From this study, it appears that biological and taxonomic species of the **A. mellea** complex could be assigned to two major groups based upon cultural characteristics. Whether these two groups reflect evolutionary patterns is unknown, but it is interesting to note that that the two groups found on three continents suggesting that they have been in existence for a long period of time.

B. Armillaria Root Rot in Alberta

Two known biological species of the **A. mellea** complex have been identified in Alberta, North American **biological species I** and V. A third species, the Foothills type, may exist, but evidence from diploid incompatibility, cultural characteristics and pathogenicity testing suggest that it may be **biological species I.** Somatic segregants and putative haploid isolates of this group are intersterile with all North American biological species including **biological species I.** To resolve the identity of this group careful examination of the basidiocarps of each group is clearly needed.

It has been shown that the two known biological species can be readily distinguihed and identified when grown on potato dextrose agar, malt extract agar, carrot agar, or malt dextrose peptone agar. This should allow for quick identification of isolates taken from diseased trees in the field. Malt agar and carrot agar alone could be used for identification purposes.

Survey sampling suggests that both biological species are distributed throughout the forested areas of the province and are often found in the same area. There is limited evidence that **biological species** V may predominate in the northern regions and the boreal forest of the province, and that **biological species** I and the Foothills type predominate in the subalpine forest. The reasons for this are uncertain but may be the result of several factors. **Biological species** V was most often isolated from trees growing in a mesic to wet environment. **Biological species** I and the Foothills type were most often isolated from trees growing in well-drained to dry sites. It may be that **biological species** V is adapted to moist soil conditions and that **biological species** I is adapted to dry sites. This may explain why **biological species** V was seldom isolated from lodgepole pine since most lodgepole pine in the subalpine forest grow on well-drained or dry sites. **Biological species** V is pathogenic to lodgepole pine and highly virulent. **Biological species** I although pathogenic is much less virulent than **biological species** V under the experimental conditions tested. This may be due to the inherently slow growth of this species as shown in the thallus growth ratio measurements, or that it may need drier soil conditions to grow and attack.

These findings may have important implications for forest management in Alberta. With conversion of trembling aspen forest to pine or spruce forest there may be unexpected mortality of young pine seedlings due to Armillaria root rot caused by biological species V. More study is needed into the pathogenicity and ecology of biological species V to determine if this will be an important factor in aspen conversion projects. As well, pathogenicity studies involving white spruce and aspen seedlings should be conducted to determine the possible effect that biological species I and V could have in intensive forest management of these tree species.

In the past Armillaria root rot has been detected after it has caused damage. With intensive forest management, foresters will have to determine where disease centers exist before a stand is cut in order to apply control measures in regeneration. If no visible symptoms are present in the old stand a detection method such as the trap log method may be used. The trap log method would not only determine the presence of the fungus in the soil, it could also help to determine the extent of spread.

C. Suggestions for Future Studies

Future studies into Armillaria root rot must include a careful study of the morphological charactersistics of the basidiocarps of the different North American biological species. This will be neccessary to determine whether they are taxonomic species and will clarify their relationship to known taxonomic species of the **Armillaria mellea** complex. In areas where production of basidiocarps occurs infrequently, it will be neccessary to try to produce basidiocarps in culture.

Ecological studies of each of the major **A. mellea** complex biological species should be done to determine if different species occupy different niches and to understand their relationships in the forest ecosystem. Complimentary studies in the physiology of the different species may also give greater understanding of the **A. mellea** complex.

In Alberta more intensive surveys in lodgepole pine stands should be done to determine the exact distribution of the biological species and how much loss each causes. Pathogenicity studies using white spruce and trembling aspen are needed to determine the possible effects of the different biological species on these species.

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Appendix 1. The Northern Forest Research Centre
Armillaria mellea
Complex Culture Collection.

ISOLATE NUMBER	HOST	DATE COLLECTED	LOCATION
C-49	Populus tremuloides	unknown	Ontario
C-109	Pinus contorta	2/10/53	Alberta
C-612	Pinus monticola	unknown	Idaho
C-613	Pinus contorta	/68	Alberta
C-615	Pinus contorta	/68	Alberta
C-616	Tsuga heterophylla	unknown	B.C.
C-617	Pseudotsuga menziesii	unknown	B.C.
C-618	Pinus radiata	unknown	B.C.
C-619	Chamaecyparis nootkatensis	unknown	B.C.
C-620	Pinus contorta	/68	Alberta
C-621	Pinus contorta	/68	Alberta
C-622	Pinus contorta	/68	Alberta
C-623	Larix occidentalis	unknown	B.C.
C-624	Pinus ponderosa	unknown	B.C.
C-625	Pinus muricata	unknown	B.C.
C-626	Pinus monticola	unknown	B.C.
C-627	Pinus sylvestris	unknown	B.C.
C-628	Pinus resinosa	unknown	B.C.
C-630	Populus tremuloides	unknown	B.C.
C-631	Picea glauca	unknown	B.C.
C-632	Abies amabilis	unknown	B.C.
C-633	Abies lasiocarpa	unknown	B.C.
C-734	Pseudotsuga menziesii	unknown	Britain
C-735	Fagus sylvatica	unknown	Britain
C-736	Fraxinus excelsior	unknown	Britain

Appendix 1. cont.

C-737	Viburnum sp.	unknown	Britain
C-738	Pinus sylvestris	unknown	Britain
C-739	Pinus sylvestris	unknown	Britain
C-746	Pinus contorta	05/82	Alberta
C-748	Pinus contorta	29/06/82	Alberta
C-749	Quercus rubra	unknown	Ontario
C-750	Pinus banksiana	23/05/69	Ontario
C-751	Betula alleghaniensis	unknown	Ontario
C-752	Pinus resinosa	06/68	Ontario
C-753	Abies balsamea	unknown	Ontario
C-754	Pinus banksiana	06/67	Ontario
C-755	Abies balsamea	11/76	Ontario
C-756	Pinus contorta	06/82	Alberta
C-757	Pinus contorta	05/82	Alberta
C-758	Salix sp.	08/82	Alberta
C-759	Pinus resinosa	10/08/82	Manitoba
C-760	Abies balsamea	25/09/77	Newfoundland
C-761	Picea sitchensis	17/10/69	Newfoundland
C-762	Picea glauca	11/08/69	Newfoundland
C-763	Picea glauca	11/08/69	Newfoundland
C-764	Picea glauca	11/08/69	Newfoundland
C-765	Picea sitchensis	01/10/68	Newfoundland
C-766	Picea sitchensis	24/10/68	Newfoundland
C-767	Picea sitchensis	24/10/68	Newfoundland
C-768	Picea abies	25/09/68	Newfoundland
C-769	Picea rubra	25/09/69	Newfoundland
C-770	Populus tremuloides	28/06/72	Newfoundland
C-771	Picea mariana	12/08/68	Newfoundland
C-807	Tsuga heterophylla	09/82	B.C.
C-808	Populus tremuloides	09/82	Alberta
C-809	Pinus resinosa	04/11/82	Manitoba

Appendix 1. cont.

C-824	Pinus contorta	07/82	Alberta
C-825	Pinus contorta	06/83	Alberta
C-826	Pinus contorta	06/83	Alberta
C-827	Pinus contorta	06/83	Alberta
C-828	Pinus contorta	07/83	Alberta
C-829	Pinus contorta	07/83	Alberta
C-831	Pinus contorta	07/83	Alberta
C-832	Pinus banksiana	22/06/83	Manitoba
C-833	Pinus banksiana	22/06/83	Manitoba
C-834	Pinus resinosa	23/06/83	Manitoba
C-835	Pinus resinosa	23/06/83	Manitoba
C-836	Pinus resinosa	23/06/83	Manitoba
C-859	Pinus contorta	08/83	Alberta
C-860	Basidiocarp	25/04/77	Tasmania
C-861	Basidiocarp	04/77	Tasmania
C-862	Basidiocarp	30/05/77	Tasmania
C-863	Basidiocarp	14/04/77	Tasmania
C-864	Basidiocarp	03/09/77	Finland
C-865	Basidiocarp	19/09/77	Finland
C-866	Picea abies	05/09/79	Norway
C-867	Picea abies	27/09/80	Finland
C-868	Basidiocarp	05/09/74	Finland
C-869	Basidiocarp	28/07/77	West Germany
C-870	Basidiocarp	29/09/79	Norway
C-871	Basidiocarp	23/10/77	Finland
C-872	Basidiocarp	09/74	Finland
C-873	Basidiocarp	10/74	Finland
C-874	Pinus sylvestris	09/74	Finland
C-875	Pinus sylvestris	unknown	Sweden
C-876	Pseudotsuga menziesii	09/83	Alberta
C-877	Cupressus funebris	/58	Kenya

Appendix 1. cont.

C-878	Basidiocarp	09/83	Alberta
C-879	Basidiocarp	11/10/83	Japan
C-880	Basidiocarp	10/83	Japan
C-881	Basidiocarp	11/83	Japan
C-882	Basidiocarp	17/10/83	Japan
C-883	Basidiocarp	05/11/83	Japan
C-884	Basidiocarp	05/11/83	Japan
C-885	Basidiocarp	11/10/83	Japan
C-886	Basidiocarp	10/11/83	Japan
C-887	Basidiocarp	28/10/83	Japan
C-888	Basidiocarp	09/09/83	Japan
C-889	Basidiocarp	09/83	Alberta
C-890	Basidiocarp	09/83	Alberta
C-891	Basidiocarp	09/83	Alberta
C-892	Basidiocarp	09/[illegible]	[illegible]erta
C-893	Basidiocarp	09/83	[illegible]berta
C-894	Basidiocarp	09/83	Alberta
C-895	Basidiocarp	09/83	Alberta
C-896	Basidiocarp	09/83	Alberta
C-897	Basidiocarp	09/83	Alberta
C-898	Basidiocarp	09/83	Alberta
C-899	Picea mariana	05/84	Alberta
C-900	Pinus contorta	06/84	Alberta
C-901	Populus balsamifera	06/84	Alberta
C-902	Abies balsames	06/84	Alberta
C-903	Abies balsmaea	06/84	Alberta
C-904	Pinus banksiana	06/84	Alberta
C-905	Populus tremuloides	06/84	Albetra
C-906	Pinus conorta	06/84	Alberta
C-907	Populus tremuloides	06/84	Alberta
C-908	Pinus contorta	06/84	Alberta

Appendix 1. cont.

C-909	Pinus contorta	06/84	Alberta
C-910	Abies balsamea	06/84	Alberta
C-911	Pinus contorta	06/84	Alberta
C-912	Populus tremuloides	08/84	Alberta
C-913	Pinus contorta	08/84	Alberta
C-915	Abies balsamea	08/84	Alberta
C-916	Betula papyrifera	08/84	Alberta
C-917	Ulmus americana	08/84	Saskatchewan
C-918	Populus tremuloides	09/84	Alberta
C-919	Pinus contorta	09/84	Alberta
C-920	Pinus contorta	09/84	Alberta
C-921	Pinus contorta	09/84	Alberta
C-922	Abies lasiocarpa	09/84	Alberta
C-923	Pinus banksiana	09/84	Alberta
C-924	Betula papyrifera	09/84	Alberta
C-925	Populus tremuloides	09/84	Alberta
C-926	Pinus contorta	09/84	Alberta
C-927	Basidiocarp	10/84	Alberta
C-928	Picea glauca	09/84	Alberta
C-929	Abies lasiocarpa	09/84	Alberta
C-930	Populus balsamifera	09/84	Alberta
C-931	Picea glauca	09/84	Alberta
C-932	Pinus contorta	09/84	Alberta
C-933	Populus balsamea	09/84	Alberta
C-934	Pinus contorta	09/84	Alberta
C-935	Basidiocarp	10/84	B.C.
C-936	Basidiocarp	10/84	B.C.
C-937	Basidiocarp	10/84	B.C.
C-938	P. balsamifera	10/84	Alberta
C-939	A. balsamea	10/84	Alberta