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THE UNIVERSITY OF ALBERTA

THE EPIDEMIOLOGY OF ARMILLARIA ROOT ROT IN WEST CENTRAL ALBERTA

by

HENRY WILLIAM KLEIN-GEBBINCK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

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THE UNIVERSITY OF ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **THE EPIDEMIOLOGY OF ARMILLARIA ROOT ROT IN WEST CENTRAL ALBERTA** submitted by Henry William Klein-Gebbinck in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology.

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(Supervisor)

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Date: March 20, 1989

Abstract

Roots of over 127 lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) were excavated in three stands near Hinton, in west central Alberta. Roots infected with *Armillaria ostoyae* (Romagn.) Herink were identified by the presence of resin impregnated soil on the lesion and mycelial fans under the bark. Infections on the tap root or root collar resulted in tree mortality and were followed by colonization of the entire root system. Lateral root infections typically did not result in tree mortality because mycelial colonization proceeded distally from the point of infection. The pathogen might be capable of spreading from such latent infections, to colonize the entire root system, if the tree were cut in precommercial thinning.

Rhizomorphs were more important than root contacts in initiating infection. Rhizomorphs were present on or near resinous lesions on 108 of 121 infected roots. In contrast, infection occurred on only 13 of 70 roots with no associated rhizomorphs. The rhizomorphs originated from primary inoculum sources more often than from secondary sources. Stumps or debris from the previous stand were implicated as the source of 28 of 40 rhizomorphs. Twenty percent of the rhizomorphs were traced to the roots of young trees which had been killed by the pathogen. Rhizomorphs were capable of infecting seedlings at distances of up to 2.5m from their food base, although 16 of 23 rhizomorphs had grown less than 1.2m before initiating an infection.

Rhizomorphs were attached to the roots or rhizomes of several nonconiferous species. They were attached most frequently to the rhizomes of fireweed (*Epilobium augustifolium* Lam.). Other plants with attached rhizomorphs were *Populus tremuloides* Michx., *Arctostaphylos uva-ursi*, [L.] Spreng., *Salix* sp.L. and *Rosa* sp.L. These roots and rhizomes could play a role in the epidemiology of Armillaria root root by increasing the inoculum

potential of the pathogen at large distances from the initial food base.

Spatial analysis showed that the risk of infection of a given tree was dependant on the health status of its nearest neighbor if that nearest neighbor was within 15cm. This was probably due to the exposure of the two trees to the same rhizomorph system or inoculum source.

Incompatibility analyses were conducted to determine the clonal structure of *A. ostoyae* at two sites. At the first site, nine clones were detected along a 500m belt transect. The largest clone had a diameter of at least 300m, as determined by the compatibility reactions of the 18 sampled isolates. Five of the remaining clones were represented by more than one isolate and ranged from 35 to 90m in diameter. The infected trees were aggregated as determined by the variance-mean ratio of 2.16. There were five clones at the second site. The maximum diameter attained at this site was approximately 75m.

Inoculations studies were conducted to determine if fireweed rhizomes could be a suitable food base for *Armillaria*. Fireweed rhizomes were infected and colonized by *A. mellea* and *A. ostoyae*. Only the former killed fireweed. Both species of *Armillaria* were pathogenic towards pine when aspen segments were used as the food base. However, only *A. mellea* infected lodgepole pine seedlings using fireweed rhizomes as the food base.

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Chapter I. General introduction.

Distribution

Armillaria root rot is found worldwide (Wargo and Shaw, 1985). It has been reported in Australia (Pearce et al., 1986; Podger et al., 1978), New Zealand (MacKenzie and Shaw, 1978), Africa (Leach, 1939; Swift, 1971), Europe (Rishbeth, 1982; Korhonen, 1978, Guillaumin and Berthelay, 1981) and North America (Anderson and Ullrich, 1979; Wargo and Shaw, 1985; Morrison et al., 1985).

The Pathogen

In the past, the name of the root rot pathogen was considered to be *Armillaria mellea* (Vahl ex Fr.) Kummer (Watling et al., 1982). A commonly used syronym was *Armillariella mellea* (Vahl ex Fr.) Karst. However, research in the late 1970's and early 1980's involving taxonomy and genetics showed that *Armillaria mellea* was a complex of several species (Korhonen, 1978; Anderson and Ullrich, 1979; Mallet, 1985; Morrison et al., 1984; Anderson, 1986). Anderson et al., (1980) have reported that similar species occur in North America and Europe. Some of the variation in isozyme and immunological patterns, nuclear DNA-DNA homologies, sporophore morphology, cultural characteristics, symptom expression, host range and pathogenicity was explained by the the delineation of *A. mellea* into several species (Kile and Watling, 1983; Morrison, 1982; Morrison et al., 1984, Jahnke et al., 1987; Lung-Escarmant et al., 1985; Shaw, 1980; Shaw et al., 1981; Rishbeth, 1982, 1985b;

Guillaumin and Berthelay, 1981; Guillaumin and Lung, 1985; Raabe, 1963). A literature review on the different species in Europe has been published (Roll-Hansen, 1985).

By pairing haploid isolates and observing the compatibility reactions, several reproductively isolated groups or biological species have been identified. The intersterility between the different groups is absolute (Anderson and Ullrich, 1979, Anderson et al, 1979). There are eight biological species in North America (Anderson, 1986), five in Europe (Korhonen, 1978), and four in Australia (Kile and Watling, 1983). Taxa, based on morphogical characters of the mushrooms, have been assigned to the European and Australian groups. Species names have been proposed for two of the North American biological species (NABS). The taxa are *A. ostoyae* and *A. bulbosa* for NABS I and VII respectively (Morrison et al, 1984).

Damage

The pathogen may cause severe economic losses, depending on the host attacked. In Australia, *A. luteobubalina* Watling and Kile caused serious losses in karri (*Eucalyptus diversicolor* F. Muell.), jarrah (*E. marginata* Donn. ex Sm), karri-marri (*E. calophylla* Lindl), and other eucalyptus stands (Pearce et al., 1986; Podger et al., 1978). *Armillaria. hinnulae* Kile and Watling has reduced economic returns in eucalyptus stands in Tasmania (Podger et al., 1978). Armillaria root rot was a major cause of mortality in plantations of *Pinus radiata* Don in New Zealand. In 2-year-old plantations, infection by *A. novae-zelandiae* Stevenson and *A. limonea* Stevenson accounted for up to 27% of the mortality, with severity related to species composition of the previous indigenous cover (Shaw and Calderon, 1977). In Kenyan exotic pine plantations, Armillaria root rot was troublesome on sites cleared of certain

forest types (Swift, 1971).

The disease is present in most countries of the northern hemisphere. Losses have been attributed to the disease in the Scandinavian countries (Korhonen, 1978), and Great Britain (Marsh, 1952). Disease centers ranged from a few meters to over 100m in diameter (Rishbeth, 1978b; Durrieu et al., 1985)

In the United States, losses due to Armillaria root rot have been extensive in volume and stand area (Wargo and Shaw, 1985). In some instances, disease incidence was low and was associated with stress (Hofacker et al., 1987; Loomis et al., 1986). However, Armillaria root rot in ponderosa pine (*Pinus ponderosa* Laws.) accounted for an average of 21% mortality in New Mexico (Weiss and Riffle, 1971). In a ponderosa pine forest in Washington, mortality increased from $9m^3$/ha to $24m^3$/ha in 14 years (Shaw et al., 1976). Because the disease centers were enlarging, this forest was not expected to persist as a commercial one. In Washington and Oregon, disease incidence was reported to be increasing, and total annual losses were estimated at over 130 million ft^3 (Loomis et al., 1986). Other conifers, excluding Douglas-fir (*Psuedotsuga menziesii* (Mirb.) Franco), suffered losses in stands less than 30 years old in Montana and Idaho.

In a natural regenerated lodgepole pine stand in Colorado, a survey revealed little loss to the disease over 26 years (Loomis et al., 1986). Initially, annual losses were projected to be 2%. Since 1960, however, annual losses declined to 0.4%. All recent mortality has been in suppressed non-crop trees and mortality has not led to any understocking.

In Canada, the disease occurs on conifers from Newfoundland to British Columbia (Singh, 1981, 1983; Kondo and Moody, 1987; Mallet, 1985; Morrison, 1981). The incidence of *Armillaria* in stands with trees exhibiting red foliage

ranged from less than 5% in jack and red pine in Ontario to 69% in balsam fir in Quebec. In northern Ontario, *Armillaria* was associated with 68% of sampled dead or dying conifer saplings (Whitney and Myren, 1977).

In the Prairie Provinces, mortality is localized but significant in naturally regenerated stands and plantations of conifers (Kondo and Moody, 1987). In eastern Manitoba, mortality ranged from 12.9% to 31.9% in study plots on 9- to 12-year-old red pine plantations (Moody and Cerezke, 1986). Numerous disease centers were active in 30-to 35-year-old red pine plantations in northeastern Manitoba (Moody and Cerezke, 1986).

Mortality in the southern interior of British Columbia was severe in some stands of Douglas-fir with a few disease centers reaching a size of 0.5 ha (Morrison, 1981). There were 0.2-0.3 centers per hectare. In the coastal forests, an average of 10% of 26-to 28-year-old trees were infected with *Armillaria* but the disease was confined to patches of 2-3 trees on average (Morrison, 1981; Johnson et al., 1972). Mortality was insignificant as a result of callusing of the infected roots (Buckland, 1953; Johnson et al., 1972).

In west central Alberta, the disease is common on lodgepole pine and mortality has been as high as 21.1 % (Baranyay and Stevenson, 1964) In a more extensive survey, mortality due to Armillaria root rot was usually less than 1% at most sites (Ives, personal communication). On these latter sites, cumulative tree mortality to 25 years of age was estimated. The cumulative mortality was 9% on high productivity sites but only 1.4% on low productivity sites (Ives, personal communication).

Armillaria root rot may be important in predisposing roots to attack by other pathogens (Barrett, 1970). Heartwood blocks of *Picea sitchensis* (Bong.) Carr. were invaded by *Polyporus schweinitzii* after they had been colonized by *Armillaria* (Barrett, 1970).

The disease is important from a silvicultural perspective. Armillaria root rot may limit the number and rotation lengths in aspen stands (Stanosz and Patton, 1987b). The presence of *Armillaria* in backlog areas could hamper the planting of trees in unstocked or understocked plantations (Muir, 1988).

Dynamics

The pathogen spreads vegetatively or by basidiospores (Wargo and Shaw, 1985, Rishbeth, 1985a). Vegetative spread occurs either by rhizomorphs, mycelial growth across root contacts or by root grafts. The relative importance of different methods of spread may vary with the species of *Armillaria*. In the subtropics, spread is largely through root contacts. In Zimbabwe, the disease was important despite the absence of rhizomorphs, due to an inhibitory agent in the soil (Swift, 1968). Spread of *A. luteobubalina* occurred largely by root contact with infected roots and stumps in eucalyptus plantations in Australia (Pearce et al., 1986; Kile, 1981; Podger et al., 1977). In the northern temperate forests, rhizomorphs and root contacts were important for the spread of *A. mellea* sensu stricto, *A. ostoyae*, *A. bulbosa* and other *Armillaria* species (Shaw, 1980; Rishbeth, 1985a; Morrison, 1981; Redfern, 1973; Kable, 1974; Thomas, 1934). Root contact was inferred to be the main method of spread in peach orchards (Kable, 1974). In apple and blackcurrant orchards in Britain, rhizomorphs were implicated as the main agent of spread (Marsh, 1952). Mycelial spread in the cambium of infected roots of trees, dead or living, was a means by which the fungus expands its territory (Shaw, 1980; Roth et al., 1980; Kable, 1974). On lateral roots of living trees, colonization was distal to the point of infection in conifers (Shaw, 1980, Wargo and Shaw, 1985) and proximal to the point of infection in hardwoods (Wargo and Shaw, 1985,

Kable, 1974, Zeller, 1926).

The pattern of mortality due to *Armillaria* is of three major types. In young plantations of *P. radiata* in New Zealand, there were many patches of dead trees dispersed at random (van der Pas, 1982; MacKenzie and Shaw, 1978) Over a period of 5 years an increase in mortality occurred within patches and a small increase occurred within the gaps. This resulted in an insignificant increase in the total patch area in the plots. Hence, it was concluded that very little secondary spread had occurred. In southwestern Australia, mortality occurred in discontinuous patches in *Eucalyptus* plantations (Pearce et al., 1986). Nevertheless, many lesions were associated with secondary infections (infections caused by inoculum originating from trees of the same, as opposed to the previous generation).

The second pattern occurred when mortality caused the radial expansion of patches. In ponderosa pine stands in the northwestern United States, mortality resulted in expanding disease centers of dead and dying trees (Shaw and Roth, 1976; Shaw et al., 1976). The radial expansion was attributed to secondary spread (Shaw, 1980). This pattern has been observed in Ontario and the interior of southern British Columbia (Huntly et al., 1961; Morrison, 1981). Coalescence of two or more centers has resulted in large areas of timberland becoming unproductive (Wargo and Shaw, 1985; Morrison, 1981; Shaw et al., 1976). In the interior forests of British Columbia, disease centers of 0.2 to 0.3 ha were reported, and up to 95% of the trees were killed within a center (Morrison, 1981). In Winema National Park in Oregon, disease centers 1 to 4 ha in size have been recorded (Filip, 1977). Initially, spread occurred by rhizomorphs and root contact with the primary inoculum (inoculum originating from trees of the previous generation). Subsequently, infections were initiated by transfer of mycelium at points of root contact. Rhizomorphs

from infected or dead trees also played an important role in secondary infection (Kile, 1981; Morrison, 1981; Shaw, 1980; Kable, 1974).

Morrison (1981) observed a third pattern in the coastal forests of British Columbia. Small patches of one to five dead trees were present in stands between 5 and 10 years of age. Trees older than 25 years were rarely killed. Total mortality, due to *Armillaria*, was usually below 1% of the stand. Excavations showed that these trees usually were infected mainly by rhizomorphs and more rarely by root contact. Radially expanding disease centers were not common in the coastal forests.

Logistics of infection

The rate of spread has been estimated by several researchers. Direct observations have provided estimates of 0.8 - 3.2 m/year in peach orchards (Kable, 1974) and 1.0 m/year in open fields (Redfern, 1973). Using the logarithmic model to fit mortality in *Pinus elliotii* Engelm. over a period of 8 years, Swift (1971) estimated an apparent infection rate of 1.333 to 1.605. The apparent infection rate declined in subsequent years. In ponderosa pine, the diameter of a disease center was estimated to expand by up to 2 m / year in the Pacific Northwest (Shaw, 1980; Shaw and Roth, 1976).

Inoculum

Incidence of the disease has been associated with stumps of the previous generation. (Pearce et al.,; 1986; Rishbeth, 1985a). As the distance from an infected stump increased, there was a corresponding decrease in the proportions of trees killed by *Armillaria* (Pearce et al., 1986; MacKenzie and

Shaw, 1977; Podger et al., 1978). In *Pinus radiata* plantations, peaks in mortality occurred at different times depending on the distance of the trees from the stumps (Roth et al., 1979). The authors questioned whether the rate of radial expansion would be constant or whether the the rate would be different outside the periphery of the stump root system. If little or no mortality were to occur outside of the periphery of the stump root system, it would be appropriate to conclude that secondary spread is not important.

The distance over which rhizomorphs can cause infection appeared to be related to inoculum potential (Garrett, 1956). There was a distance from inoculum beyond which the rhizomorphs were unable to infect potato tubers, and this limit depended on the initial weight of the inoculum segment.

It is likely that most rhizomorphs originate from lateral roots rather than from stumps *per se*. Assuming that inoculum potential varies with distance from the food base, disease incidence should vary with distance from colonized lateral roots. Thus, the spatial distribution of the lateral roots could be important in determining the spatial and temporal distribution of disease incidence.

Stumps

In computer simulations, the inoculum present in a stand at inventory assessment is very important because of the potential for further mortality (Namee et al., 1987). This is probably of more importance in stands where the disease occurs as radially expanding disease centers.

In areas where agricultural lands have reverted back to forests, the primary inocula are probably basidiospores which have infected thinned stumps or stumps after clearcutting (Rishbeth, 1978b; Shaw, 1981; Swift, 1971).

Stumps and their lateral roots, however, do serve as the primary food base which initiates or maintains the disease center in the next stand. Once a stump becomes colonized, it may continue to be infectious for many years (Roth et al., 1980). For example, some stumps contained viable inoculum for 30-35 years after harvest in Washington Pathogen survival is thought to depend on the diameter of the stump (Shaw et al., 1985), which determines total stump biomass.

The ability of a colonized stump to be an efficient food base appears to be related to the stump type: hardwood or coniferous. Hardwood stumps were the primary source of inoculum in a number of coniferous plantations (Pronos and Patton, 1977; Redfern, 1975; Rishbeth, 1972; MacKenzie and Shaw, 1977). In one survey in Ontario, 224 chlorotic saplings were excavated to determine the inoculum source (Whitney and Smith, 1983). The primary inocula, which accounted for 46.6 % of the infections, were stumps and debris of black spruce (*Picea mariana* [Mill.] B.S.P.), white spruce (*P. glauca* [Moench] Voss), jack pine (*Pinus banksiana* Lamb.) and aspen. Conifer stumps were source of the inoculum in other stands (Roth et al., 1980; Shaw, 1980; Filip, 1979).

More rhizomorphs were produced from hardwood inocula than from conifer inocula (Rishbeth, 1972; Redfern, 1975, 1970; Benjamin and Newhook, 1984a; Guillaumin and Lung, 1985). Rhizomorph dry weight production on sycamore (*Acer pseudoplatanus* L.) segments was almost twice that produced on red spruce (*Picea rubens* Sarg.) segments for 3 out of 4 isolates (Redfern, 1975) and the pathogenicity of the pathogen on sycamore segments was greater or equal to that on spruce segments. In another study, there was no interaction between inoculum type (*Quercus pendunculata* Ehrh. and pine) and biological species (*A. ostoyae* and *A. mellea*) (Guillaumin and Lung, 1985).

Generally, there were no differences in colonization between nonconiferous and coniferous wood segments inoculated with *A. novae-zelandiae* or *A. limonea* if all of the wood segments were considered together (Benjamin and Newhook, 1984a). However, if only completely colonized wood segments were considered, the hardwoods supported a greater amount of rhizomorphs than the conifers. The researchers mentioned that the degree of colonization, time of incubation and segment size could be important factors in determining rhizomorph production.

Infection of inoculated trunk sections of European beech (*Fagus sylvatica* L.) and Norway spruce (*Picea abies* [L.] Karst.) decreased with time following felling because of primary saprophytes (Gramss, 1983). The entire cambial cylinder of both species was colonized after inoculation after felling. However, twelve months after felling, colonization had decreased to 17% of the cambial cylinder of beech and 0% of the cambial cylinder of spruce.

More recently, another classification scheme has been used in a root rot simulator: heartwood versus nonheartwood species (Shaw et al., 1985). Heartwood-producing species include Douglas-fir pines, western redcedar (*Thuja plicata* Donn) and western larch whereas some nonheartwood-forming species are true firs (*Abies* spp Mill.), hemlock (*Tsuga* spp. [Endl.] Carr) and spruce.

In some cases, some stumps appeared to be associated with diseased trees even though the stumps appeared to be colonized by other wood rotting fungi such as *Heterobasidion annosum* (Fr.) Bref. (Filip, 1979). However, excavations revealed that infected saplings were in contact with the lateral roots of the same stumps that were colonized by *Armillaria*. This was attributed to quiescent lesions on the lateral roots that became active and colonized the lateral root after the tree was harvested. *Armillaria* was considered to be a

good competitor because of its ability to produce rhizomorphs and its presence in the cambium of the root system (Morrison and Johnson, 1977). These characteristics give *Armillaria* a distinct advantage over its competitors (Morrison and Johnson, 1977; Garret, 1960).

Sites

A factor to consider in the epidemiology of the disease is the site or environment of the host and pathogen. Ono (1970) regarded soil conditions as the most important factor influencing the incidence of *A. mellea* on Japanese larch (*Larix leptolepis* Sieb. and Zucc.). Other researchers have stressed the importance of pH, moisture, nutrients, organic matter, texture, and compaction, on the growth of the fungus in other host species.

Environmental conditions interact with the host to affect its vigor. Armillaria root rot has been reported to occur on suppressed trees (Huntley, 1961; Buckland, 1953; Shields and Hobbs, 1979), vigorous trees (Patton and Riker, 1959; Pronos and Patton, 1977), and both suppressed and vigorous trees of the same species (Singh, 1983). Armillaria-decayed trees were significantly less vigorous than nondecayed trees which occurred on sites with higher pH, total N, exchangeable calcium, cation exchange capacity and phosphorus levels (Shields and Hobbs, 1979). Redfern (1978) suggested that vigor does not play an important role in disease development.

Infection occurred on the roots of vigorous roots of Douglas-fir but were contained by resin production and callus formation (Buckland, 1953). With the onset of stress, the fungus was able to advance from the localized lesion. In west central Alberta, disease incidence was greater on sites that were most favorable for lodgepole pine growth (Ives, personal

communication). It is conceivable that the greater incidence on the better sites may have been due to a larger number of roots contacting inoculum on these sites. Also, mortality attributed to *Armillaria* at these sites could have been the result of density dependent stress.

Whitney (1978, 1984) reported that Armillaria root rot was more prevalent on sites with course textured soils than on sites with fine textured soils. Rhizomorphs were more prevalent in loam soils than in clay-loam soils in spruce forests in Newfoundland (Singh, 1981). Rhizomorphs were not produced from woody inocula in sand (Garrett, 1956). In a mixture of soil and sand, rhizomorphs were produced, but not as many were produced in soil alone.

Soil texture affects soil moisture by affecting drainage. Overall, disease incidence and rhizomorph growth were positively correlated with moisture levels (Whitney, 1978, 1984; Morrison, 1976; Singh, 1981). Inocula in a soil of unknown type were viable and produced approximately the same amount of rhizomorphs at 40% field capacity as at 80% and 100% field capacity (Garrett, 1956).

The response of rhizomorph growth to moisture was correlated with oxygen availability (Morrison, 1976). Oxygen concentration was observed to have profound effects on rhizomorph growth and morphology (Smith and Griffin, 1971; Rishbeth, 1978a). Rhizomorphs originating under lower oxygen partial pressures flattened, became plaque-like, and grew more slowly (Smith and Griffin, 1971). Moisture and its effects on oxygen concentration determined the vertical distribution of the rhizomorphs (Morrison, 1976). Rhizomorphs were more abundant in the upper 10 cm of the soil profile in moist soils and in the lower horizons on drier sites. Rhizomorphs rarely were found below a soil depth of 30 cm (Singh, 1981; Morrison, 1976; Redfern, 1973).

The upper limit of rhizomorph development was determined by the moisture level in drier years (Morrison, 1976).

The vertical distribution of rhizomorphs could be related to the organic matter in a particular horizon. Organic matter tends to decrease with soil depth (Morrison, 1982; Redfern, 1973). The production of rhizomorphs from woody inocula was related to the amount of organic matter in the soil (Gramss, 1983; Morrison, 1982; Redfern, 1973). Leachate from humus or nutrient solution stimulated rhizomorph production (Morrison, 1982). Rhizomorph branching was profuse in soils with high levels of organic matter (Redfern, 1973).

Organic matter content may be related to the growth of other microorganisms which may stimulate the production of rhizomorphs. *Aureobasidium pullulans* (de Bary) Arnaud produced ethanol which stimulated rhizomorph production (Pentland, 1967). Organic matter content could also affect the presence of saprophytes in the soil which could invade freshly killed roots and limit colonization of the wood by *Armillaria* (Garret, 1960). For example, it is possible that other fungi could replace *A. bulbosa* and *A. ostoyae* (Thompson and Boddy, 1983).

Rhizomorph production is affected by pH (Wargo et al., 1987; Redfern, 1970, Morrison, 1974). Rhizomorphs were more abundant in alkaline soils (Redfern, 1970). However, root rot incidence in a number of host species was greater in a sandy soil at pH 4.9 than at pH 7.5. Overall, the acidic soil had a disease incidence of 43.3% whereas the alkaline soil had a disease incidence of 29.6%. Differences in disease incidence on loam soils with different pH values were not significantly different. In Newfoundland, disease incidence in conifers was higher in soils with low pH (Singh, 1983). On malt agar, the maximum growth rate for several isolates from western white pine occurred at

initial medium pH values of 4.5 and 5.5 (Benton and Ellrich, 1941).

The response to soil acidity may be related to the growth habit of the rhizomorphs. Isolates with monopodially branching rhizomorphs produced at least as much of rhizomorph dry matter in alkaline soils (pH 7.6) as in acidic soils (pH 4.4) (Morrison, 1974). In contrast, rhizomorph dry matter production was greater in acidic soils for isolates with dichotomous branching rhizomorphs. Rishbeth (1982) reported that *A. ostoyae* did not kill pines planted in alkaline soils, but in acidic soils the trees were attacked and usually killed.

Rhizomorph production was lower in soils from high elevation montane forests than from a low elevation hardwood forest type (Wargo et al., 1987). Forest type, lead and manganese concentration, and pH accounted for 29% of the variation in rhizomorph occurrence.

Temperature influences mycelial growth and rhizomorph development (Bliss, 1946; Redfern, 1973; Rishbeth, 1968, 1978a). Colonization of tree stem segments was optimum at 25 ° (Rishbeth, 1968). In culture media, isolates did not grow at 33 C (Rishbeth, 1968). Rhizomorph production by most isolates from different parts of the world was optimum at 15-20 C (Rishbeth, 1978a). Minimum temperature for rhizomorph production was 5 to 10 C. No rhizomorphs were produced from wood inocula at temperatures greater than 30 C (Rishbeth, 1968). Diurnally fluctuating temperatures did not affect rhizomorph growth (Rishbeth, 1978a). However, if the soil temperature was raised from 20 to 30 C and maintained at the latter temperature, rhizomorph growth ceased after 2 days. Returning the temperature to 20 C did not result in growth of the rhizomorphs until 11 days later. This growth was associated with the appearance of new rhizomorphs.

The number of branches in rhizomorph systems produced from *Quercus*

sp. and *Salix* sp. segments was greater at higher temperature (Redfern, 1973). At 25 C there were 167 branches on 31 systems whereas at 15 C there were 60 branches on 19 systems. Each rhizomorph system had the same mean dry weight for both temperatures.

In inoculation experiments, infection of several hosts occurred at temperatures in the range of 7 to 25 C (Bliss, 1946). However, the optimum temperature range for infection was related to the host species. For peach *Prunus persica*, [L.] Batsch), pepper tree (*Shinus molle* L.), "geranium" (*Pelargonium hortorum* Bailey), and apricot (*P. armeniaca* L.), the optimum range was 15 to 25 C. In contrast, the optimum temperature was 10 to 18 C for infection of sweet orange (*Ciitrus sinensis* [L.] Osbeck), sour orange (*C. aurantium* L.) and rose (*Rosa* sp. L.). Resistance to infection was correlated with temperatures favorable for root growth (Bliss, 1946).

Light may be an important factor (Redfern, 1978). Shade had little effect on mortality in *Tsuga heterophylla* (Raf.) Sarg, But in the shade, mortality in *Quercus robar* L. increased to 22 % from 2% in the light.

Differences in pathogenicity among *Armillaria* species

Raabe (1962) compiled a comprehensive host list which showed the wide diversity of species attacked by *Armillaria*. Pathogenicity tests of different isolates from different hosts and locations gave similar (Raabe, 1967) and dissimilar results (Raabe, 1967; Shaw, 1977) for the different isolates. Morrison (1982b) reported that isolates with different rhizomorph growth habits differed in esterase isozyme patterns and showed differences in pathogenicity to 3-year-old conifer seedlings. It is possible that some of the isolates were different biological species.

Armillaria bulbosa was not highly pathogenic. It was found only on trees that were weakened by suppression or other causes. *A. mellea* was more aggressive than *A. ostoyae* on hardwoods whereas *A. ostoyae* was more aggressive on conifers (Guillaumin and Lung, 1985; Guillaumin and Berthelay, 1981; Rishbeth, 1982, 1985b). *A. mellea* had a more extensive host range than *A. ostoyae* (Rishbeth, 1982, 1985a) In west central Alberta, *A. ostoyae* was more common on infected and dead lodgepole pine than North American Biological Species (NABS) V even though the latter was more pathogenic on this host (Mallet and Hiratsuka, 1988; Mallet, 1985). These results may not be applicable over the lifetime of coniferous stands as age of the hosts may affect the apparent pathogenicity of the pathogen (Rishbeth, 1982).

In southeastern Australia, the distribution of various *Armillaria* species was different (Kile and Watling, 1983). *Armillaria fumosa* sp. nov. was most common in localized wet or poorly drained areas within a dry sclerophyll forest. *Armillaria novae-zelandiae* and *A. hinnulea* occurred in the wet forests. However, the former was in the cool temperature rainforest and mixed forest whereas the latter was largely found in the mixed forest and the wet sclerophyll forest.

In British Columbia, however, the same species were present in both coastal and interior forests (Morrison et al., 1985). It was concluded that the differences in disease impact between the two locations were due to variation in pathogenicity among the isolates within species. However, some of the environmental factors listed above could be interacting with biological species. Further experimentation is required to determine the interaction of the different species of *Armillaria* with different environmental factors.

Host factors

Stand age appears to be important in determining pathogenicity (Whitney, 1985; Morrison, 1981). In a peach (*P. persica* [L.] Batsch) orchard, the disease incidence was 2% for trees less than 15 years of age and 60% for older trees (Marsh, 1952). Decayed trees of grand fir (*Abies grandis* [Doug.] Lindl.) and Douglas-fir were slightly older than trees without decay (Shields and Hobbs, 1979). In conifers, the opposite trend was true in some locations (Rishbeth, 1982; Morrison, 1981). Age effects may be related to resin production (Rishbeth, 1982; Gibbs, 1970). In 5-year-old Corsican pines (*Pinus nigra* var. *maritima* [Aiton] Melville), the ratio of root area infected to resin production was 8.5 time greater for roots infected with *A. mellea* than for roots infected by *A. ostoyae*. The mean area of bark infected with *A. mellea* was one-tenth of the area infected by *A. ostoyae*. Thus, it appeared that resistance decreased the number of infections. Simulations using disease resistance were shown to have reduced mortality by a factor of 15 (McNamee et al., 1987).

The size of the disease centers was correlated with stand age in ponderosa pine (Shaw, 1980). It reflected the effect of the horizontal extension of infected lateral roots and root closure on the development of new secondary infections (Roth et al., 1977, 1980; Roth and Rolph, 1978; McNamee et al., 1987). Root contacts with other roots were more common in older stands (Reynolds and Bloomberg, 1982).

Using a simulation model, it was determined that the stand density could be important in the epidemiology of the disease. Increasing tree density in the model resulted resulted in a higher spread rate and a larger number of centers after clearcutting (McNamee et al., 1987). The density of saplings around the stump was positively correlated with disease incidence in

Eucalyptus species (Pearce et al., 1986).

Other factors

Stress induced by insects or other pathogens may affect the incidence and severity of Armillaria root rot. This was observed in balsam fir (*Abies balsamea* [L.] Mill.) plantations in Newfoundland attacked by aphids [*Adelges piceae* Ratz.] (Hudak and Wells, 1974) and oak borer (*Agrilus bilineatus* Weber) infested oak (*Quercus* spp L.) plantations in the eastern States (Dunbar and Stephens, 1975). The presence of *Armillaria* mycelial fans could be attributed to colonization of the root system as a result of tree death. A needle blight (caused by *Dothiostroma pini* Hulbary) of *P. radiata* behaved synergistically with Armillaria root rot to reduce the growth of infected trees (Shaw and Toes, 1977).

Silvicultural practises have been associated with increases in disease severity. Precommercial thinning was implicated in the increased incidence of *Armillaria* in red cedar (Koenigs, 1969) and eucalyptus plantations (Edgar et al., 1976). Armillaria root rot was the major cause of mortality in thinned lodgepole pine plots in west central Alberta (Johnstone, 1981). In contrast, Armillaria root rot was not associated with thinned ponderosa pine stands in Oregon (Johnson and Thompson, 1975).

Severed rhizomorphs produced several branches, 20 to 30 cm in length, from the cut ends (Redfern 1973). These rhizomorphs had sufficient inoculum potential to infect and kill two of 10 larch (*Larix* sp.) trees. It thus seems possible that scarifying clearcut stands could produce the same effect.

Maintaining genetic variation in fungi

Genetic variation is maintained in fungi by several mechanisms. The main mechanisms are mutation, sexual recombination, parasexual recombination, and cytoplasmic inheritance (Agrios, 1978).

New characters are formed when a mutation results in a nonlethal change of the sequence of nucleotides within a gene. The mutagenic effect may be the result of insertion, deletion, inversion or alteration of a base (Kesser and Kuenen, 1967).

Sexual and parasexual recombination maintain variation by rearranging existing variation (including nonlethal mutations) to form new genotypes. The parasexual cycle is an important method to create new recombinations in fungi for which no sexual recombination exists. The steps in the cycle are formation of heterokaryons and the formation of a diploid nucleus, mitotic recombination, and finally, occasional haploidization (Kesser and Kuener, 1967).

Cytoplasmic inheritance is the acquisition of extrachromosomal genetic material through the cytoplasm. Self-replicating extrachromosomal DNAs are referred to as episomes, transfer factors, or plasmids (Frobisher et al, 1974). Plasmids have been implicated in physiological changes such as tolerance to toxic compounds (Agrios, 1978). Virulence may be affected by the presence of plasmids. Weakly pathogenic isolates of *Rhizoctonia solani* Kuhn contained plasmids whereas pathogenic isolates did not (Hashiba et al, 1984).

Vegetative mycelium population structure

The association of dead trees with stumps could be the result of

basidiospore infections arising from the fruiting bodies on or near stumps. Shaw (1981) reported that the basidiospores were capable of surviving a harsh winter[illegible]laska. It is possible that spores could percolate down the stem, lodge between bark scales, and initiate mycelial growth. Mycelial growth of *A. luteobubalina* has been observed in the bark of stems of eucalyptus (Marks et al., 1976). Swift (1965) reported that *A. mellea* was capable of utilizing suberin as a substrate, allowing the pathogen to colonize bark. The fungus could then spread to the cambium and colonize the roots.

Stumps are important sites for infection by basidiospores (Rishbeth, 1982). Direct evidence for the role of basidiospores was provided by inoculating freshly cut stumps with a spore suspension. Two years later, the stumps were monitored for infection by cutting a 3-5 cm disks from the stumps and incubating then in a saturated atmosphere for 2 weeks. Stumps of several species were infected, although in low proportions. However, there were no controls to exclude the possibility that the apparent infections had resulted from vegetative spread from latent infections. Inoculations were not successful when spore suspensions were applied to large pruning wounds (Rishbeth, 1982).

Observations from spore traps indicated that basidiospores of the fungus were present and were viable as indicated by infection of disks of *P. elliotii* under laboratory conditions (Swift, 1971). Swift (1971) inoculated stumps of indigenous species, freshly cut discs, and roots of *P. elliotii* in Zimbabwe and incubated them under a variety of field conditions. No infections were observed, perhaps because of the types of wood used.

Indirect evidence for the role of basidiospores was obtained in first rotation stands established on agricultural land (Rishbeth, 1978b) In plantations of oak, beech, or birch (*Betula verrucosa* Ehrh.), *Armillaria* foci

were located by searching for rhizomorphs. These foci were associated with stumps resulting from thinning. The foci were not the result of vegetative spread because isolates from different foci were incompatible. Rishbeth concluded that these stumps had been infected by basidiospores.

However, vegetative spread is more important than spread by basidiospores in the epidemiology of the fungus. Research into the distribution of compatibility factors, and diploid mycelial pairings have provided evidence of the importance of vegetative spread (Adams, 1974; Anderson et al., 1979; Ullrich and Anderson, 1978; Shaw and Roth, 1976; Korhonen, 1978; Kile, 1983; Pearce et al., 1986; Berthelay and Guillaumin, 1985).

In a given plantation or forest stand, the vegetative mycelia may be of different species or different clones of the same species. A number of methods have been used to determine whether two isolates were of the or different biological species (Korhonen, 1978). Haploid-haploid pairings resulted in both isolates remaining fluffy if isolates were from different biological species; otherwise the pairings resulted in the formation of diploid, crustose mycelium, providing that the correct combination of incompatibility alleles was present (Ullrich and Anderson, 1978; Korhonen, 1978). Haploid-diploid pairings resulted in the haploid isolate becoming diploid if the isolates were from the same biological species, but remaining fluffy if they were not (Korhonen, 1978; Kile 1983). A black line formed between pairs of diploid isolates of different species, but no such line formed between isolates of the same species (Mallet, 1985).

Somatic incompatibility has been employed by several researchers to determine the mycelial population structure in the Ascomycetes and the Basidiomycetes. Ascomycetes included *Leucocytospora kunzei* (Sacc.) Urban (Proffer and Hart, 1988) and *Ceratocystis ulmi* (Buis) Moreau (Brasier, 1986).

Several Basidiomycete fungi on which the technique has been used were *Phellinus weirii* (Murr.) Gilb. (Childs, 1963, Hansen, 1979), *Fomes canjanduri* Karst. (Adams and Roth, 1967), *Peniphora rufa* [Fr.:Fr.] Boidin (Chamuris and Falk, 1987) and *Heterobasidium annosum* (Chase and Ullrich, 1983; Stenlid, 1985). Stenlid (1985) concluded that while somatic incompatibility, incompatibility factors, and isozyme patterns produced the same results, the first technique was the easiest to use.

To identify different genotypes or clones within the same species, pairings of several haploids or pairings of diploid mycelia may be used (Ullrich and Anderson, 1978; Shaw and Roth, 1976; Berthelay and Guillaumin, 1985; Kile, 1983). The use of haploids allows the investigator to determine the alleles of the compatibility factors (Berthelay and Guillaumin, 1985; Ullrich and Anderson, 1979), but the method is very tedious.

The method using diploid mycelia is less tedious and any source of diploid mycelium - rhizomorph, mushroom or infected tissue - can be used. An isolate is simply paired with another to determine if the pair of isolates are genetically similar or not. A line of demarcation between the isolates on agar indicates the pairs are representatives of different clones (Adams, 1974; Shaw and Roth, 1976; Korhonen, 1978; Kile, 1983).

A problem to consider is the misclassification of isolates that arise from different clones that are siblings. If compatibility factors determine the confrontation behavior, siblings will behave as though they are representing the same clone (Korhonen, 1978; Kile, 1983). Fifty percent of the sibling pairings from three different parental stocks of *A. ostoyae* and 68% of the sibling pairings from a fourth parent were compatible. Pairings between mycelia of parent and daughter cultures resulted in some lines of demarcations (Korhonen, 1978). Similar results were observed for *A.*

luteobubalina (Kile, 1983). In contrast, Kile (1983) reported that 10% of the pairings of outbred isolates which differed by one or two mating alleles were compatible. This suggests that the compatibility in diploid mycelia is under polygenic control (Brasier, 1984).

The somatic incompatibility technique has been used to determine clone distribution and size in several *Armillaria* species including *A. mellea* (Adams, 1974; Shaw and Roth, 1976), *A. luteobubalina* (Kile, 1983; Pearce et al., 1986), *A. hinnulea* (Kile, 1986) and *A. nova-zelandiae*. (Benjamin and Newhook, 1984b). Korhonen (1978) used the incompatibility factors and somatic incompatibility to determine the clonal structure of several species of the *A. mellea* complex, including *A. ostoyae*. The two techniques gave similar results and suggested that both methods were suitable for determining the population structure of the pathogen.

The numbers and sizes of *Armillaria* clones appear to vary with species and location. Numerous clones of *A. mellea*, each occupying up to 50m, were identified in Vermont (Ullrich and Anderson, 1978). Similar clone sizes were reported for *A. hinnulea* in wet sclerophyll eucalypt forests in Tasmania (Kile, 1986) and *A. mellea* in oak plantations in Britain (Rishbeth, 1978b).

Larger clones have been reported by other authors. Studies in Finland indicated that clones of *A. mellea* sensu stricto, *A. ostoyae*, and *A. bulbosa* attained a size of 120-150 m in diameter (Korhonen, 1978). Areas up to 34,000 m^2 were found in eucalypt forests in southeastern Australia (Kile, 1983).

Objectives of thesis

Little is known about the epidemiology of Armillaria root rot in juvenile lodgepole pine stands in west central Alberta. The objectives of this study were

to 1. compare the relative importance of spread by rhizomorphs and spread by root contact, 2. determine the relative importance of stumps from the previous generation and infected roots of trees in the current generation as inoculum sources, 3. compare the pattern of tree mortality with the distribution of clones in a stand, and 4. determine the potential for a herb, *Epilobium augustifolium* Lam., which is abundant in west central Alberta, to be a food base for the fungus.

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Chapter II. Spread of *Armillaria ostoyae* in young stands of lodgepole pine in west central Alberta.

INTRODUCTION

Armillaria root rot is a destructive disease in many parts of the world (Wargo and Shaw, 1985). In Canada, the disease is present in all regions of the country (Singh, 1981; Mallet and Hiratsuka, 1987; Morrison, 1981).

Spread is largely by rhizomorphs and root contact (Wargo and Shaw, 1985) but the relative importance of these two modes of spread varies. In the tropics, increase in disease incidence was attributed to root contact with the inoculum sources: roots of stumps and diseased trees (Swift, 1968). No rhizomorphs were found in plantations of *Pinus elliotii* in Zimbabwe. In eucalyptus plantations in Australia, root to root spread was believed to be the main method of spread (Pearce et al., 1986). In these plantations, most rhizomorphs attained a length of 4 to 8 cm. In the Pacific Northwest, rhizomorphs were found to be the most important agent for infection of ponderosa pine (Shaw, 1980).

In British Columbia, two patterns of mortality were present, depending on location (Morrison 1981). In coastal forests, the disease was largely present as a butt rot and the disease incidence was low. Fungal spread occurred largely by rhizomorphs. However, in the interior forests, Armillaria root rot often produced disease centers in which mortality was extensive, leading to unstocked or understocked openings. In trees, aged 10 years or less, infections were initiated by rhizomorphs. Subsequent spread occurred by mycelial transfer at root contact points.

Rhizomorphs may arise from primary sources (roots and stumps of the previous stand) or secondary sources (roots of infected trees in the current stand). Primary inoculum sources in the form of decomposed logs and

decaying stumps were the inoculum source for 26.2% and 20.4% of the infections, respectively, in 7- to 17-year-old stands of black spruce (*Picea mariana* [Mill.] BSP.), white spruce (*P. glauca* [Moench] Voss) and jack pine (*Pinus banksiana* Lamb) (Whitney and Smith, 1983). In contrast, secondary inoculum was thought to be important in causing the expansion of disease centers (Roth and Kolph, 1978; Shaw, 1980). For example, Shaw (1980) found a significant relationship between stem diameter and the distance to asymptomatic, infected trees lying outside the periphery of the center. This implied that secondary inoculum was important in the epidemiology of Armillaria root rot of *Pinus ponderosa* Laws. in Washington.

Lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) accounts for 20% and 40% of the annual timber harvested in British Columbia and Alberta, respectively (Kennedy, 1985). Although lodgepole pine is quite susceptible to Armillaria root rot (Morrison, 1981), little is known of the epidemiology of this disease in juvenile lodgepole pine stands.

The objective of this study was to determine the inoculum source and method of spread by *Armillaria ostoyae* (Romagn.) Herink in juvenile lodgepole pine stands in West Central Alberta

MATERIALS AND METHODS

Site description. Lodgepole pines, 7- to-18-years-old, were excavated at three sites located within 40 km of Hinton, Alberta in 1986 and 1987. All sites had been scarified and allowed to regenerate to lodgepole pine.

The first site was a Brunisolic Gray Luvisol with a sandy silt to clay loam texture, pH 5.6-5.9, imperfectly to moderately well drained. The soil at the second site was a Gleyed Eluviated Eutric Brunisol. It was comprised largely of a loamy sand and was slightly acidic to neutral (pH 6.5 - 7.0) throughout the profile. The land was moderately stony and moderately well drained. The soil

at the last site was classified as a weakly developed Brunisolic Gray Luvisol. The texture was sandy clay to clay loam and the pH increased from 6.0 to 7.0 with increasing depth in the soil profile. The soil had imperfect to moderate drainage. Trees were excavated at the first site in 1986 and at the second and third sites in 1987.

Excavation of trees. Trees were meticulously excavated using garden hand tools. First, superficial debris, herbs, grasses and duff were removed using a garden rake. A circle of diameter 0.5 - 1.0 m in diameter was cut with a shovel around each tree sampled at the first site. The tree and its root ball, together with attached soil, were removed. The soil was then carefully removed from the roots with a jackknife. The trees were excavated in this way because the soil structure was blocky and excessive rhizomorph breakage tended to occur when soil was removed from the roots. If any rhizomorphs were found, their original positions were noted and they were traced to the inoculum source. Rhizomorphs were assumed to be penetrating (as opposed to emerging from) the root if the rhizomorph branches converged as one moved away from the lesion. Infection was also assumed to have occurred if there were any signs of rhizomorph anchoring such as the presence of one or two short rhizomorph branches (Thomas,1934) on the surface of the lesion. In 1987, trees within 15 to 45 cm of symptomatic trees were excavated to obtain an estimate of the proportion of infections that could be attributed to primary inoculum sources. The granular structure of the soil at sites 2 and 3 permitted direct excavation of the trees using the jackknife to loosen and remove the soil from the roots. All rhizomorphs were traced to their source.

Spatial Analysis. Nearest neighbor analysis (Pielou, 1961) was used to determine whether there was an association between stumps and diseased trees and to determine if pines near infected trees were at a greater risk of being

attacked than pines near healthy trees. This method was chosen because it avoids problems caused by overlapping of lateral roots of stumps (van der Pas, 1981). On a 100 m by 50 m plot, ten equidistant transects were marked perpendicular to the long axis. Along each transect, ten equidistant points 5 m apart were marked. The tree nearest to each point was selected and its health status was recorded. The distance from this tree to its nearest stump was also recorded. From the tree selected initially, the nearest healthy neighbor was selected. From this second tree the distance to its nearest neighbor and its health status were recorded. This was repeated for the nearest diseased tree to the tree selected initially. The nearest stump data was analyzed to compare the distance between stumps and healthy trees with the distance between stumps and diseased trees.

For the nearest neighbor data, the distances were categorized into three distance classes. Within each distance class, a X^2 test of independence was performed on a 2 by 2 data table of the health status of the nearest neighbors to trees with known health status.

Identification of *Armillaria* species. The most common species of *Armillaria* on lodgepole pine in this area of Alberta was determined to be *A. ostoyae* (Mallet, 1985; Mallet and Hiratsuka, 1988). A known *A. ostoyae* diploid tester was paired with several isolates obtained from infected lodgepole pine at site 1. Confrontations between different species of *Armillaria* result in the formation of a black line between them (Mallet, 1985) whereas no black lines are formed between isolates from the same species.

RESULTS

Root collar and taproot infections. Resin impregnated soil was attached to the taproot and/or the root collar of 80 of the 83 diseased trees which were

and/or the taproot of all 83 trees. The remaining three trees which were dead and had mycelial fans in the cambial areas of the root, did not display any resinosis on the tap root nor on the root collar. However, on one of these trees, a lateral root did have a resin impregnated lesion. Eighty two out of 88 trees with resin impregnated lesions had above ground symptoms. The six healthy-appearing trees had resinosis on the taproot and/or the root collar, and were infected with the pathogen as indicated by the presence of mycelial fans.

On all dead trees with tap root and/or root collar infections, all of the lateral roots appeared to be colonized to some degree. On trees with light-to-yellow green foliage, only 40 to 60 percent of the root collar was colonized and colonization of the lateral roots rarely extended more than 20 cm from the root collar. Dead trees with red to brown needles were girdled by mycelial fans. Roots of trees with bright red needles were found to be entirely colonized with white to creamy white mycelium. Rather compressed rhizomorphs and pseudosclerotial plates were evident under the bark of the lateral roots of two partially defoliated trees with dark reddish brown needles.

On the lateral roots of the healthy trees, colonization was distal to the point of infection (Fig. II-1). In most cases, the entire distal portion of these roots was colonized. On a few lateral roots, there was evidence of colonization proximal to the lateral root lesion. This however occurred on lateral roots which also had a proximal lesion on the lateral roots.

Lesions were associated with rhizomorphs. Infection of 108 of 121 infected roots (including both tap and lateral roots) was associated with the presence of rhizomorphs either attached to the root, embedded in the resin-soil matrix or within 10 to 20 cm of the lesion. In one excavation, one young rhizomorph was found to have initiated infections on four different lateral roots. At each infection point, there was a small amount of resin exuding from

the root at the point where the rhizomorph was anchored.

Of 70 tap and lateral roots with no associated rhizomorphs, only 13 were infected, as indicated by the presence of resinosis. These 13 roots may have been infected following direct contact with the remains of the lateral roots of stumps or other debris which were found nearby. Some of this debris displayed a yellow stringy rot or zone lines typical of Armillaria root rot. On the other hand, roots often were in direct contact with colonized debris or infected roots without being infected (Fig. II-2). In 1987, mortality was observed in three of 17 cases where roots of previously infected trees had made contact with infected trees.

The distances traversed by the rhizomorphs, from source to infection point, are summarized in Fig. II-3. Approximately 73% of the rhizomorphs grew for at least 30 cm from the source to the point of infection. Three rhizomorphs grew at least 2.0 m before infection occurred. Rhizomorph branching accounted for more than one tree becoming infected by the same rhizomorph system. This was found on three occasions, with the greatest observed distance between two diseased trees being 80 cm.

Primary vs. secondary inoculum. In small compact clumps of trees, all of which had presumably had originated from a single cone, it was not uncommon to find all of the members showing aerial symptoms. Mycelium was present on adjacent roots of adjacent trees. Extreme resinosis prevented determination of whether the fungus had spread from one tree to another by root grafts, but the absence of root grafts between adjacent healthy suggests that spread of the pathogen via root grafts was not occurring.

Rhizomorphs were very fragile and susceptible to breakage. As a result, the source of inoculum was almost impossible to trace in 1986 because rainy

made the soil more friable and rhizomorphs were more easily excavated and traced to the inoculum source. In 28 of 40 cases where the inoculum source was found (Table II-1), primary inoculum was implicated. Only eight of the rhizomorphs arose from colonized roots of dead juvenile trees.

The distal one meter of one lateral root from a needleless sapling was covered by flat rhizomorphs and pseudosclerotia. The rhizomorph had extended about 50 cm through the soil and produced a network of several branches, two of which led to infections. These pseudosclerotia were found in several colonized lateral roots of trees that had been dead for some time. In one case, the intact plate was removed and split open, revealing almost complete decay.

Other species attacked. Occasionally, rhizomorphs were attached to the roots or rhizomes of other species, especially fireweed (*Epilobium augustifolium* Lam.; Table II-2). Some of these rhizomorphs were also attached to pine. In all cases, the roots of these other plants were infected and colonized as indicated by the presence of mycelial fans and/or rhizomorphs. Because these roots were found after the excavation was well underway, the effects on the forbs and grasses were not determined. In the case of the shrubs, no aerial symptoms were evident.

Spatial analysis. The disease incidence in the plot was approximately 18 per cent based on the number of diseased trees that were nearest neighbors to the quadrat points. Dead trees appeared to be clumped, with cone siblings forming many of these clumps. Often a cone was firmly attached to stems or roots between several adjacent trees. The clumps appeared to be dispersed randomly through the stand and gave no real clear indication of the existence of discrete disease centers.

The average distance from diseased trees to their nearest stump (1.32 m

based on 19 trees) was not significantly different than the average distance from healthy trees to their nearest stumps (1.25 m based on 75 trees).

As the distance between two nearest neighbors increased, there was a decreasing risk of infection for neighbors associated with infected trees (Table II-3). For nearest neighbors in the distance class 0.00-0.15m, the risk of the nearest neighbor of a diseased tree being infected was significantly greater than that of the nearest neighbor of a healthy tree and was sixty five per cent. Nearly 30% of the trees within 0.15-0.45 m of an infected tree were infected whereas only 7% of the trees within this distance of a healthy tree was infected. These percentages however were not significantly different. For nearest neighbors in the last distance class, the risk of infection was the same for the neighbors of both the healthy and diseased trees. If a tree was healthy, the probability of its nearest neighbor being infected was approximately 10 per cent in the three distance categories.

***Armillaria* species identified.** All isolates tested were determined to be *A. ostoyae*.

DISCUSSION

In three lodgepole pine stands, with trees aged 7-18 years, the major source of inoculum was debris or stumps from the previous stand. Only 22% of the identified inocula sources were roots of infected trees of the same stand.

Rhizomorphs were far more important than root contacts in initiating infections. In ponderosa pine stands, most infections occurred within centimeters of the inoculum source (Shaw, 1980). In contrast, rhizomorphs on these three sites often grew considerable distances before infecting their host. The ability of rhizomorphs to cause infection after having grown for a meter

Lateral root infections did not result in tree mortality because subsequent growth of the fungus was distal to the point of infection. Similar results have been reported with other pine species. Trees killed by *Armillaria* had infections at the tap root or root collar. Examination of dying trees revealed that progressive colonization of the tap root or root collar was accompanied by progressive development of foliar symptoms. Once the tree had died, the lateral roots became almost completely colonized, and in some cases, rhizomorphs and pseudosclerotial plates were produced. These dead trees were not as important an inoculum source as the stumps and debris from the previous generation perhaps because the roots of small trees tend to dry out or decay more rapidly than the roots of larger trees.

Precommercial thinning could be playing an important role in the epidemiology of *Armillaria* root rot. Viable mycelium in lesions on the lateral roots might be able to colonize the whole root system of thinned trees, thereby increasing inoculum potential and tree mortality.

Research on the effects of thinning on Armillaria root has been scanty and the results have been mixed. In New Zealand, thinning was found to decrease the apparent infection rate of *A. novae-zelandiae* in *Pinus radiata* D. Don. (van der Pas, 1981). In those experiments, however, the entire sapling, including most of the roots, was removed at thinning. Similarly, mortality due to *Armillaria* in thinned plots of ponderosa pine in Oregon was less than in untreated stands (Johnson and Thompson, 1975). In thinning trials of lodgepole pine in west central Alberta, mortality, which was largely due to *Armillaria*, varied from 3% to 44% in the different plots, but did not differ among the thinning treatments (Johnstone, 1981). Koenigs (1969) found that the number of western red cedar (*Thuja plicata* D. Don.) trees attacked by *A.*

controls. Although most of the roots we encountered were quite small, and thus would not be expected to support survival of *Armillaria* for long, the existence of pseudosclerotia suggests that *Armillaria* might be able to persist for some time.

There was no significant difference between the mean healthy tree to nearest stump distance and the mean diseased tree to nearest stump distance. This result contrasts with those obtained by MacKenzie and Shaw (1977) and van der Pas (1981) who found that the proportion of dead trees tended to decrease with increasing distance from stumps. Our results were somewhat surprising because in a small sample of 20 stumps, all appeared to be colonized. It may be that a combination of a high frequency of stump infection, extensive colonization of the lateral roots of stumps, and the long distance traversed by rhizomorphs, resulted in most trees having an equal risk of infection regardless of their distance from a stump.

Similarly, the probability of a tree being infected depended on the health status of its nearest neighbor only if that nearest neighbor was within 0.15 m of that tree. Trees within 0.15 m of each other often had arisen from the same cone and the death of one tree was often accompanied by the death of another from the same cone, either from inoculum from the same source or because of short distance secondary spread of the fungus. In contrast, if trees were greater than 0.15 m apart, the likelihood of a tree becoming infected was independent of the health status of its nearest neighbor.

The nearest neighbor results could be biased because of a nonrandom sample. Dependence arises when a given plant has a high probability of beings its nearest neigbor's nearest neighbor. Because of this dependence the test statistic will not follow a chi-square distribution and may result in

may be overcome by deriving the critical values of the test statistic emperically (Meagher and Burdick, 1980). However, this approach would require mapping all trees in the sampled area.

Large clump are more likely to be sample than small clumps. This, however, should have no effect on the risk that a tree becomes infected given that its nearest neighbor is diseased.

The presence of rhizomorphs on a variety of non-tree species suggests that these may also serve as an inoculum source. Rhizomorphs also were reported to be present on the clumps of a graminaceous species, toetoe (*Cortaderia fulvida* [Buchanan] in New Zealand (Shaw et al., 1976). Trees in close proximity to the dead toetoe clumps were more frequently infected than pines with no grass clumps within 60 cm. This suggested that the grass may serve as a minor inoculum source of *Armillaria*. Studies are underway to test the hypothesis that *E augustifolium* may be an inoculum reservoir of *Armillaria*.

In conclusion this study has shown that for juvenile stands of lodgepole pine in Alberta: 1. Primary inoculum is more important than secondary inoculum, 2. Rhizomorphs more commonly initiate infections than root contacts, 3. Tap root and root collar infections kill trees whereas lateral root infections do not, 4. There is no association between dead trees and stumps or between dead trees and the health of trees located more than 0.15 m away, 5. Non-tree hosts, especially fireweed, may play a role in the epidemiology of this disease.

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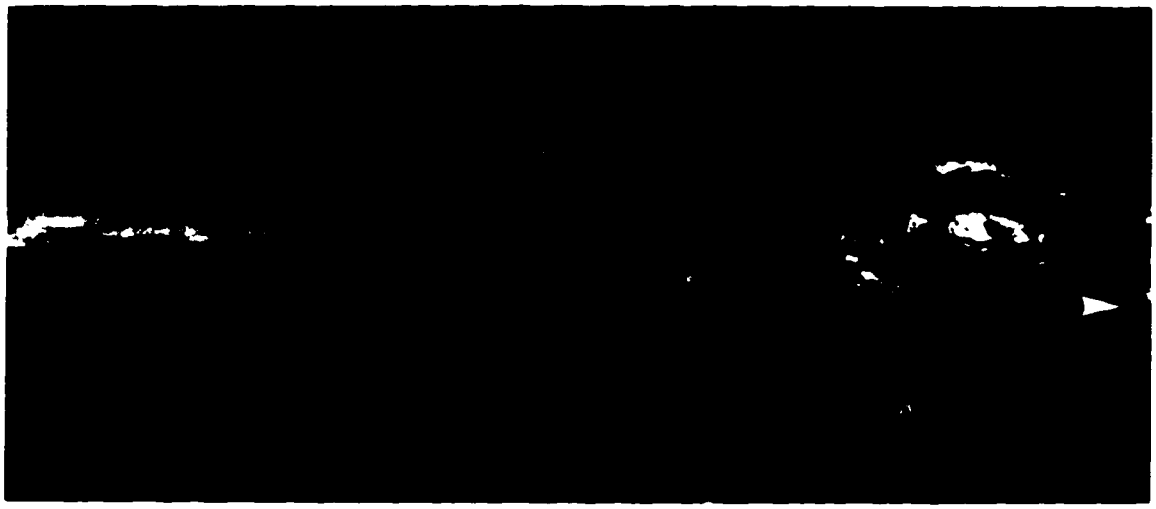

Fig. II-1 Lateral root from a healthy tree displaying colonization distal (as indicated by the direction of the arrow) to the lesion.

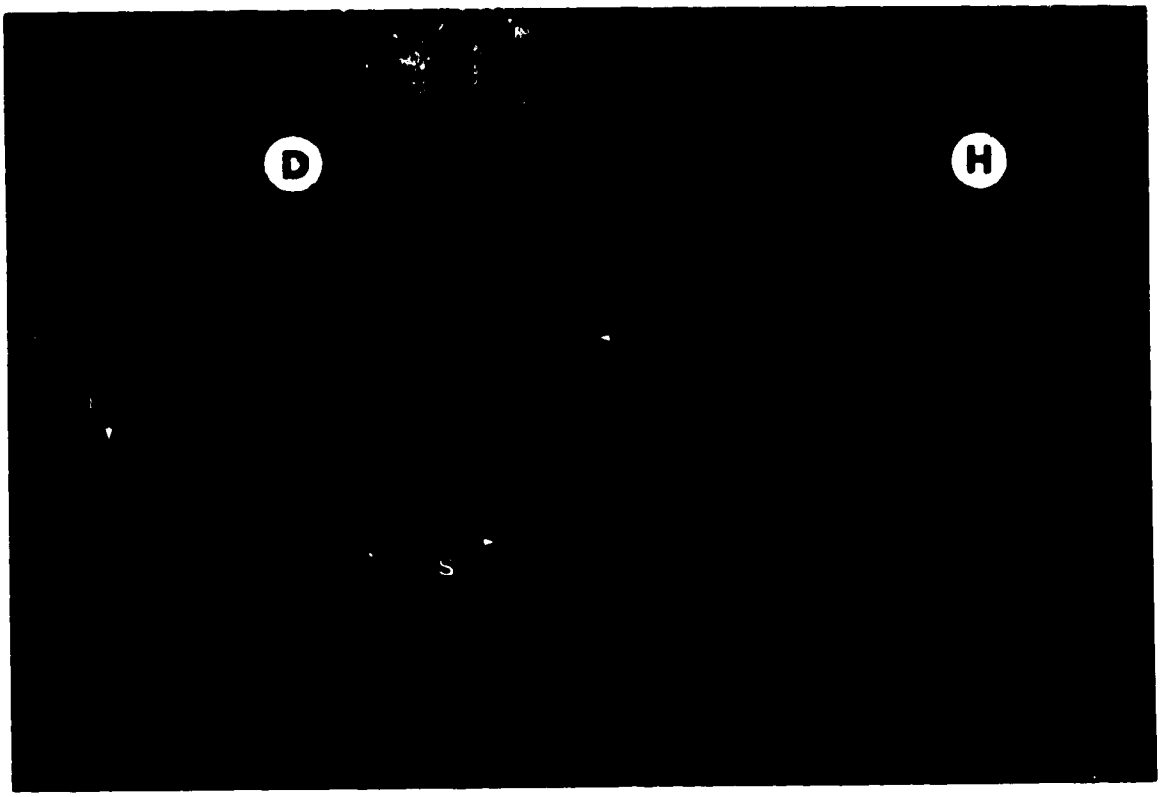

Fig. II-2. Root contact with the inoculum. Note the lack of resinosis on lateral roots at the points of contact with the stump lateral root (s) and infected root (r) of the dead tree (D). Healthy tree (H).

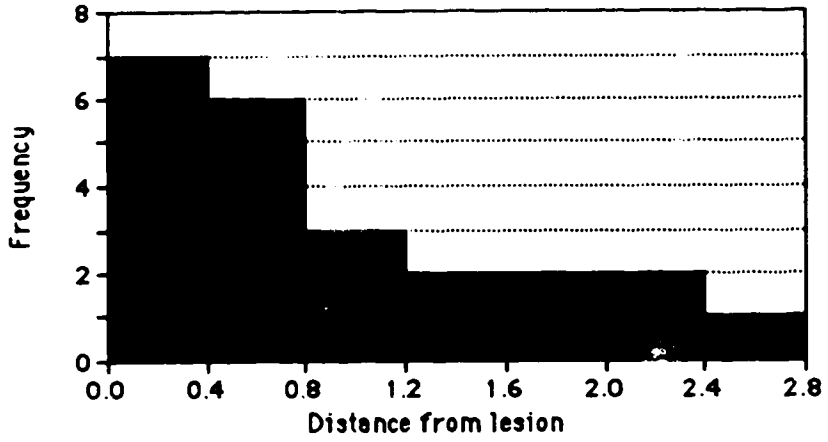

Fig. II-3. Distance of rhizomorph growth from inoculum source to infected root at sites 2 and 3 in 1987.

Table II-1. Origin of rhizomorphs found on or near roots in excavations at sites 2 and 3 in 1987.

Source	Frequency
Primary	
Stump	19
Debris	3
Stump or Debris *	6
Secondary (Infected roots of juvenile trees)	8
Uncertain **	4
Not found	12

* Nonintact rhizomorphs, on or near an infected root, arising from stump or debris colonized by *Armillaria*.

** Stump and infected roots found in vicinity where rhizomorph was broken.

Table II-2. Occurrence of rhizomorphs attached to the roots or rhizomes of species other than lodgepole pine.

Species	Common name	Number of infected plants
Populus tremuloides Michx.	Aspen poplar	1
Arctostaphylos uva-ursi (L.) Spreng.	Bear berry	1
Salix sp. L.	Willow	1
Rosa sp. L.	Wild rose	2
Graminaceous species	Grasses	4
Epilobium augustifolium Lam.	Fireweed	11

Table II-3. Distribution of healthy or diseased nearest neighbors to trees with a particular health status.

Distance to nearest neighbor	Base tree	Nearest neighbor: Diseased	Nearest neighbor: Healthy	X^2 #
0.00 - 0.15	Healthy	2	20	15.84 **
	Diseased	17	9	
0.15 - 0.45	Healthy	1	13	2.58 ns
	Diseased	7	17	
0.45 +	Healthy	8	57	< 0.10 ns
	Diseased	4	29	

\# Calculated as for a test of independence.

** Significant at P = 0.05.

ns Nonsignificant at P = 0.05.

Chapter III. Mycelial population structure of *Armillaria ostoyae* and spatial pattern of infected trees in west-central Alberta.

INTRODUCTION

Armillaria root rot is one of the most important diseases of conifers in North America (Wargo and Shaw, 1985). *Armillaria ostoyae* (Romagn.) Herink has been implicated in the death of many lodgepole pines (*Pinus contorta* var. *latifolia* Engelm.) in west central Alberta (Mallet and Hiratsuka, 1988). The spread of the pathogen is mostly by rhizomorphs or root contact. Basidiospores generally are considered to be less important in the epidemiology of this disease, although not insignificant on the time scale of forest stands (Rishbeth, 1985).

Compatibility studies between haploid mycelia of *A. ostoyae* have been used to determine the clonal population structure in an isolated region (Berthelay and Guillaumin, 1985). Clonal patterns can be determined by pairing diploid isolates on agar (Adams, 1974; Korhonen, 1978; Shaw and Roth, 1976; Wargo and Shaw, 1985) or wood substrates (Hood and Morrison, 1984). Dissimilar genotypes are detected by the failure of the paired isolates to grow together. Complete intermingling of the mycelia indicates that the paired isolates are clones.

In ponderosa pine (*Pinus ponderosa* Laws) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) stands, large disease centers have occurred (Shaw et al., 1975; Morrison, 1981). These unstocked or understocked openings were up to 0.5 ha or more in size and presumably resulted from ow vegetative spread of *Armillaria* from an old stump. In Australia, Armillaria root rot in eucalyptus (*Eucalyptus diversicolor* F. Muell.) plantations was distributed as discontinuous patches of diseased trees and infected stumps (Pearce et al.,

1986). In west central Alberta, small scattered groups of *Armillaria*-killed lodgepole pines frequently are seen in juvenile stands. In a single 10 m by 10 m plot, eight areas of *Armillaria* colonization were delineated (Mallet and Hiratsuka, 1985).

The objective of this study was to determine if the small patches of dead juvenile lodgepole pines in west central Alberta (Mallet and Hiratsuka, 1985), are separate infection centers associated with different clones of the pathogen.

MATERIALS AND METHODS

Clones in West Central Alberta. Two sites near Hinton, Alberta were chosen. One hundred trees, 7 to 15 years old, were sampled from each site.

At site one, the trees were sampled along a 500 m by 10 m transect and at site 2, the trees were sampled from a Y shaped transect 10 m wide (Fig. III-2). Patches of of one or more dead trees were selected along the transect. An average distance of 5 m separated the patches. One tree was selected from each patch and one to three lateral roots, 10 to 20 cm long, were removed and examined for colonization. The root pieces were placed into paper bags, taken to the laboratory within 2 days, and stored at 4 C.

Within 1 week after sampling, isolations were attempted by cutting and removing a 1 to 2 cm square of bark and plating a small amount of the exposed mycelium and wood onto 3% malt agar (Difco) amended with 1 ml per liter of 25% lactic acid. A total of six mycelial transfers were made for each root. For those roots which did not yield *Armillaria*, isolations were repeated 3 weeks later. The isolates were transferred to vials containing malt agar which were used to start cultures for the pairing experiments.

Pairing Experiments. Forty-eight isolates were chosen randomly from each site in Hinton and ordered according to their position along the transect. The isolates were separated into groups of three and these were paired in all combinations.

Agar plugs were cut with a No. 2 cork borer from the edge of 3-week-old colonies and plated 1 to 3 mm apart on 3% malt agar in plastic petri dishes. Four pairings for each combination were made except for pairings of the same isolate for which two were made. The petri dishes were placed in plastic bags and incubated in the dark at room temperature. Three weeks later, the pairings were judged to be compatible if the colonies had completely merged.

For groups in which some isolates were compatible, one isolate of each clone was chosen at random. These chosen isolates were again placed in groups of three and paired in all combinations. This was continued until there were 12 or less isolates remaining for the two sites. These final sets of isolates were paired in all combinations.

Any pairings in which the compatibility was not clear were repeated. Pairings were placed on a 5 cm segment of an autoclaved wooden popcicle sticks (Lewiscraft, Toronto, Ontario) that had been placed on the surface of 1.5% water agar in plastic petri dishes. The plates, placed into plastic bags, were incubated in the dark at room temperature for 12 weeks.

Biological species determination. Four isolates from each site were paired with an *A. ostoyae* tester and observed for the presence of a black line. Absence of a black line indicates that the isolates are representatives of the same biological species (Mallet, 1985).

Spatial pattern - Quadrat analysis. The distribution of infected trees at site 1 was determined by counting the number of healthy and infected trees in

m) long was established over the last 350 m of the transect that had been used to collect samples for clonal analysis.

The area occupied by the separate clones could not be determined precisely because the locations of the isolates were not mapped and because the transect cannot be assumed to have passed through the center of each clone. Nevertheless, estimates of the minimum clonal diameter were obtained by multiplying the number of isolates between the first and last position of a clone by the average distance between isolates.

RESULTS

Biological species. The isolates were representatives of *A. ostoyae* as determined by absence of a black line between the isolates and the tester. No black lines were observed for any of the compatibility pairings.

Isolate pairings. On agar plates a mycelial line formed between incompatible isolates with a zone of sparse growth on either side of the line. In some cases rhizomorphs growing into an incompatible isolate underwent a browning reaction (Fig. III-1a). Little or no rhizomorph discoloration occurred if the isolates were compatible (Fig. III-1b).

For pairings which did not give clear results, compatibility was determined by making pairings on popcicle sticks (Fig. III-2). Compatible pairings were characterized by a continuous brown discoloration on the surface of the stick between the two isolates (Fig. III-2a). A brown line along the periphery of the popcicle stick segment or a brown discoloration were evident on the bottom (Fig. III-2b). Mycelium, with or without rhizomorphs, was evident on the undersurface. Pairings lacking a zone of discoloration on the surface between the two isolates were categorized as incompatible.

Only one isolate was found to give inconsistent results. On malt agar the

complete intermingling of the mycelia and fusion of the pseudosclerotia. However, the colors of the pseudosclerotia from the two colonies were different shades of brown and the isolates produced different amounts of exudate. On popcicle sticks, the mycelia fused, and no differences of coloration were evident.

Clones in West Central Alberta. All of the isolates tested were diploid, as indicated by the crustose mycelium.

Site 1. Nine clones were detected along the 500 m transect (Fig. III-3). The largest clone, designated as clone A, was comprised of 18 isolates and spanned approximately 300 m. This group was discontinuous and found in two areas of the transect separated by two other clones. Clones B, C, E, F, and G were found to span the transect by approximately 55, 70, 90, 35 and 35 m respectively. Clones D, H and I were represented by one isolate each.

Site 2. Four large clones were present on the arms of the transect and two different clones were present on the tail of the transect (Fig. III-4). Clone U was the largest and was represented by 15 isolates, nine of which were on one arm and six on the other.

Quadrat Analysis. The mean number of saplings per quadrat was 3.96 with a variance of 14.91. The mean disease incidence was 11 percent with a standard deviation of 2.24 percent. Mortality was aggregated; the variance-mean ratio was 2.16 which was significantly different from 1.0 ($P < 0.01$). There appears to be a second level of aggregation at which several quadrats with diseased trees are adjacent to one another (Fig. III-4).

DISCUSSION

The somatic incompatibility technique has been used to determine clone distribution and their size in several *Armillaria* species. These include *A*

et al., 1986), *A. hinnulea* (Kile, 1986) and *A. nova-zelandiae* (Benjamin and Newhook, 1984).

Korhonen (1978) found that 68 of 132 different pairings of synthetic diploid siblings of species A resulted in an incompatible response. Kile (1983) reported that 44% of pairings of inbred synthetics of *A. luteobubalina*, were incompatible whereas 10% of the pairings of outbred synthetics were compatible. Applying the risk attached to the inbreds to the current investigation, one would expect to find less than the observed number of pairings to grow together. In other words, one should expect to find more clones in the same area if these clones are siblings. The 2 subgroups of isolates in group A could be two genotypes.

The largest estimated clone size of 300 m (Fig. III - 3) in this study is much larger than that found in Vermont where the maximum size was 50 m (Ullrich and Anderson, 1978) but is smaller than the maximum clone size of 450 m found in the Pacific Northwest (Shaw and Roth, 1976). However, our transect may not have bisected the clone and thus 300 m may be an underestimate.

In a $10m^2$ plot, several small patches of one to six dead trees were delimited (Mallet and Hiratsuka, 1985). A similar pattern was observed here (Fig. III-4). Some of the dead trees in such patches were from the same cone. Others presumably were not as they were several cm apart. It is clear that each of these small patches were not caused by different genotypes of the fungus. In a total distance of over 1 km, only 15 clones were found, and some were quite large. The minimum diameter of these clones was much larger than the diameter of the patches and the length of most interpatch distances.

Because the exact locations of the sampled dead trees were not mapped, it

However, the spatial pattern in Fig. III-4 indicates that no clear disease centers, such as have been reported in older and mature stands are apparent.

In stands such as these, there presumably would be clusters of stumps colonized by the same fungus. However, because infected roots extend from stumps and because rhizomorphs extend from infected roots, the periphery of the clones cannot be determined by examining the pattern of juvenile mortality.

In conclusion, among juvenile lodgepole pine in west central Alberta, 1. the pattern of diseased juvenile trees is aggregated. 2. The aggregates of dead trees are dispersed and give rise to small delineated areas. 3. Single clones were responsible for the death of a number of discontinuous patches of trees. 4. Vegetative spread by mycelium through roots and by rhizomorphs results in fairly large clones.

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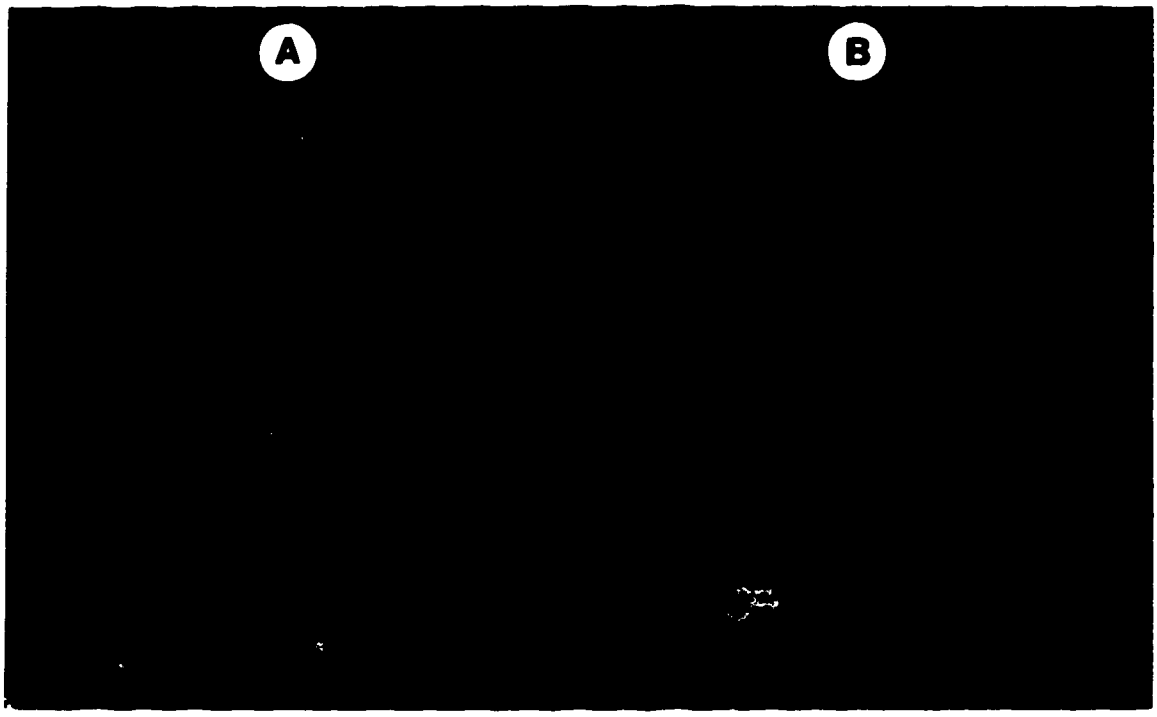


Fig. III-1. Behavior of rhizomorphs in compatible and incompatible reactions. A. Rhizomorphs from compatible isolates are not discolored. B. Rhizomorphs from incompatible isolates displaying discoloration in the reaction zone.

Fig. III-2. Compatible and incompatible pairings on wooden popcicle sticks. A-C. Compatible pairings. The growth is continuous on the upper surface (A) and on the lower (B-C) surface. D - F. Incompatible pairings. Note the zone of demarcation on the upper (D) and lower (E - F) surface.

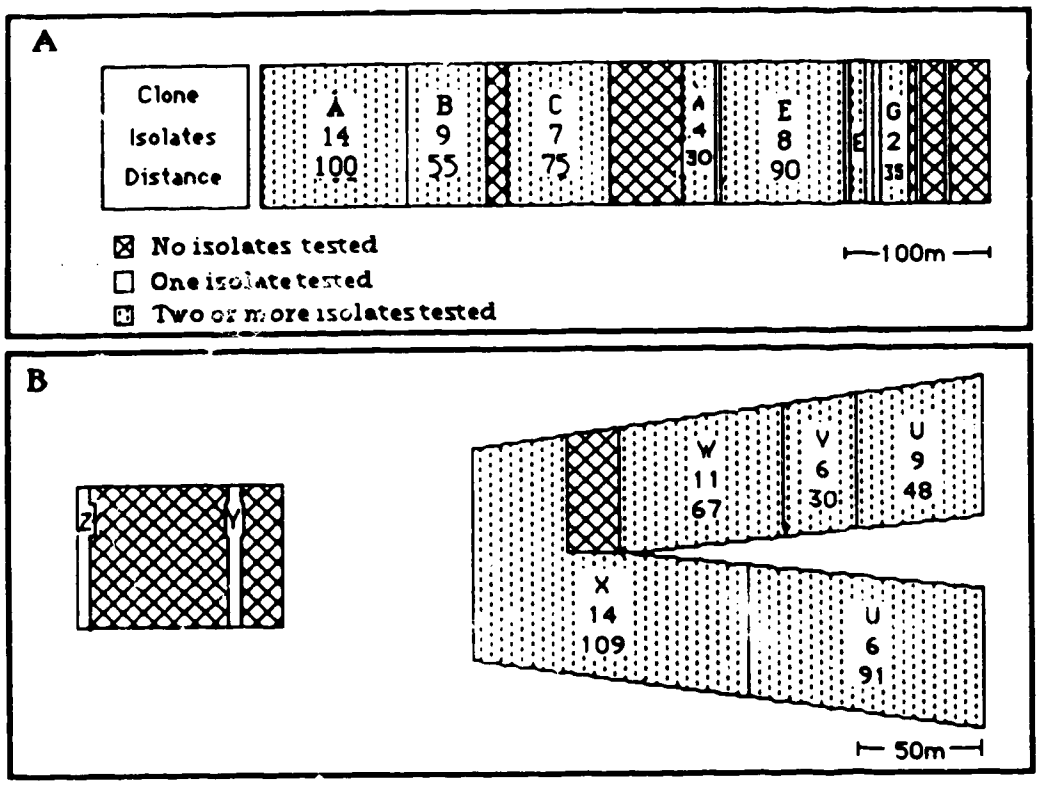
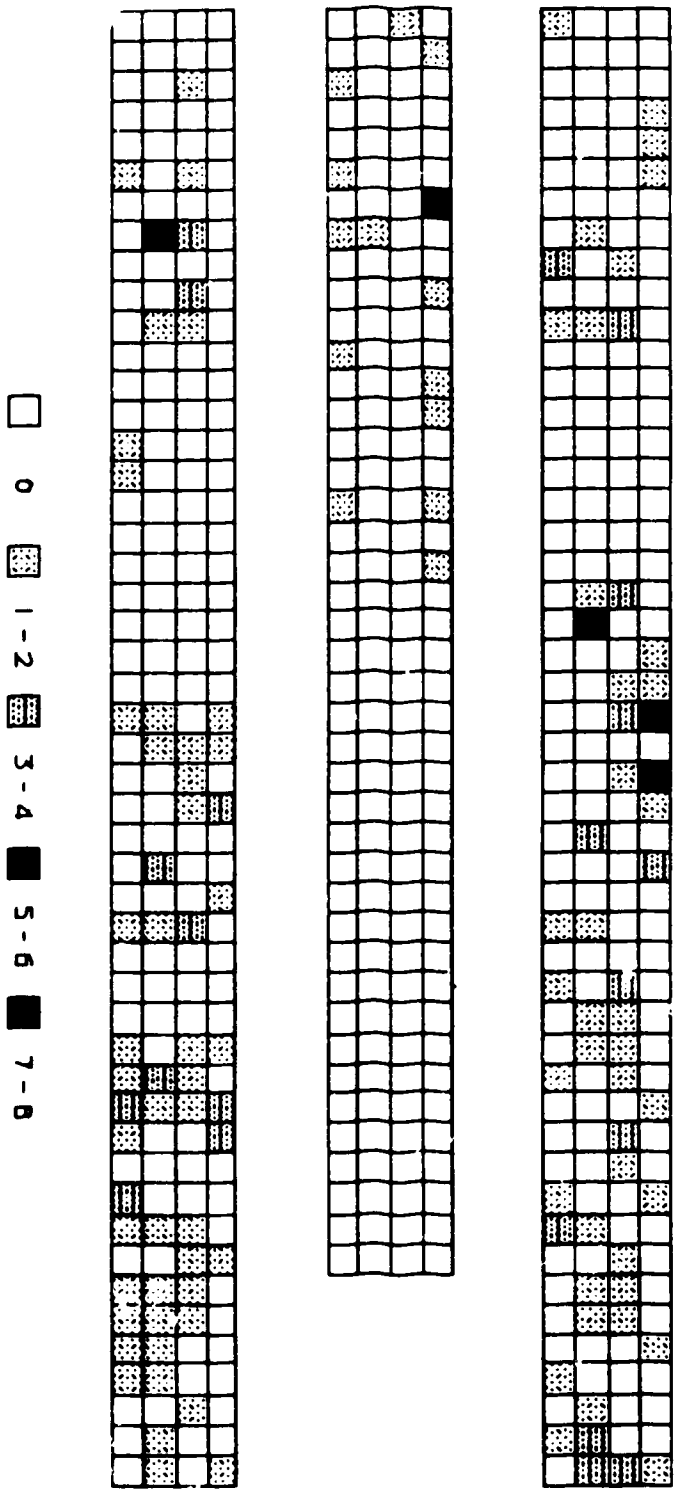


Fig. III-3. Sampling scheme and mycelial population structure at two sites near Hinton, Alberta. Scale indicates length along the transect only. **A**. Site 1. 100 isolates were sampled from a 500 m transect. A subsample of 49 isolates was selected for genotype determination. Clones D, F, G, F, H, and I each were represented by a single isolate. **B**. Site 2. The two arms were 200 m long and were 50 m apart at the open end. The tail on the left was 75 m long. Fifty four diseased trees were sampled from the upper arm, thirty - four from the lower arm, and twelve were from the tail. A subsample of 48 isolates was used to determine the population structure.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Chapter IV. Infection of lodgepole pine by *Armillaria* using infected fireweed rhizomes as inoculum

INTRODUCTION

Armillaria root rot is a devastating disease of many trees and shrubs (Wargo and Shaw, 1985). It occurs on several herbaceous crops including asparagus (*Asparagus officinales* L.), sugar beet (*Beta vulgaris* L.), rutabaga (*Brassica napobrassica* Mill.), pigeon pea (*Cajunus cajan* Millsp.), carrot (*Daucus carota* var. *sativa* DC.), strawberry (*Fragaria* sp. L.), lupine (*Lupinus* sp. L.), alfalfa (*Medicago sativa* L.), and tulip (*Tulipa* sp. L.), as well as on *Cortaderia sellodana* Aschers. and Graebn. (Raabe, 1962).

In New Zealand, a grass known as toetoe (*Cortaderia fulvida* [Buchanan] Zotov) was associated with a few infected trees of *Pinus radiata* D. Don. (Shaw et al., 1976). Upon excavating a few specimens it was discovered that *Armillaria* mycelial growth was present in the clumps. It was speculated that toetoe played a role, albeit small, in the epidemiology of the disease.

In excavations near Hinton in west central Alberta, rhizomorphs were found attached to the rhizomes of fireweed (*Epilobium augustifolium* Lam.). The objective of this study was to determine if *Armillaria* was able to infect fireweed in the laboratory and secondly, if root rot of pine seedlings could be induced by using colonized fireweed rhizomes as inocula.

MATERIALS AND METHODS

Inoculum preparation. Segments of trembling aspen (*Populus tremuloides* Michx.) 10 cm long and 1.5 cm to 2.0 cm in diameter were autoclaved with 50 mL distilled water in covered 250 mL glass jars at 121 C and 15 psi for one hour. After cooling, the liquid was decanted and the segments left overnight.

Approximately 25 ml of 3% malt extract agar was added to the jars which then were autoclaved for an additional 20 minutes. Trembling aspen was used because it had been shown to be a satisfactory food base for inoculation studies (Mallet and Hiratsuka, 1988; Mallet, 1985).

One isolate, 86-254, was obtained near Hinton, Alberta. It was shown to be *Armillaria ostoyae* in pairings using a diploid mycelium tester. An *Armillaria mellea* s. str. isolate, C-736, which had been recovered from *Fraxinus excelsior* L. in England, also was used because preliminary inoculations of fireweed with this isolate resulted in infection of 4 of 8 rhizomes. The isolates were grown on 3% malt agar in plastic petri dishes for 3 weeks in the dark. The colonies were cut into six pieces and three pieces were placed equidistantly on the agar in the jars containing aspen segments. The jars were covered with metal lids and sealed with parafilm to prevent moisture loss. They were incubated in the dark at room temperature for 5 months.

Inoculation of Fireweed. Rhizomes of fireweed were collected along a roadside near Devon, Alberta in September, 1987. Only healthy-appearing rhizomes with pinkish buds were selected. To prevent breakage, the rhizomes were placed on a layer of moist sandy soil in a plastic tray and covered with the same soil. Additional layers of rhizomes and soil were added. The container was covered with a plastic bag and placed in the cold room for 6 weeks to break bud dormancy.

In a preliminary experiment, it was determined that inoculating the plants following shoot emergence was disruptive and resulted in chlorosis and shoot death. Hence inoculation of the fireweed was performed at the time of planting. Five hundred mL plastic containers were half filled with pasteurized soil mix (3 field loam: 2 peat: 2 sand). One rhizome per pot was gently pressed

into the soil with the buds facing upwards. An inoculum segment was placed vertically into the soil in contact with the rhizome. Then the rhizome and inoculum segment were covered with soil. Twenty four rhizomes were inoculated with each isolate and twenty four noninoculated fireweed plants served as controls.

The potted plants were placed in a greenhouse maintained at a temperature of 20 C and photoperiod of 14 hours. Supplemental light was provided by high pressure sodium vapor lamps (400 W) with an intensity of 425 $\mu E m^{-2} s^{-1}$. To prevent seed production, flower buds were removed periodically.

Each month after inoculation, one inoculated plant per isolate was removed and examined for infection. This was repeated for 5 months until the first infected plant was found. Rhizomes were removed when shoot chlorosis was evident, and examined for mycelial fans. Due to a mealy bug infestation which began eight months after planting, the experiment was terminated at that time. All remaining fireweed were excavated and classified as infected or uninfected based on the presence of mycelial fans. Inoculum segments also were examined. The pathogen was classified as nonviable if there was no evidence of mycelium beneath the bark. Infected fireweed plants were used as inocula.

Isolations were made on rhizomes from four symptomatic plants inoculated with *A. mellea*.

Inoculation of pines. One-year-old pine seedlings growing in 30 mL Rootrainers© (Spencer/Lemaire Ltd) were used. The trees were transplanted into larger 400 mL Rootrainers© containing soil (3 field loam: 2 peat: 2 sand) and placed in the greenhouse maintained at the above conditions.

The seedlings were inoculated as rhizomes with mycelial fans became

available. To inoculate seedlings, the Rootrainers© booklet was opened, soil was gently removed from one side of the tap root and one infected rhizome, pruned to a length of 4-6 cm, was placed in contact with the exposed root and covered gently with moist soil. The book was closed and the inoculated seedling was watered. Three noninoculated rhizomes were placed in contact with pine seedlings as controls. Eight additional seedlings were inoculated with aspen segments, four with each isolate. Symptomatic seedlings were removed and observed for mycelial fans in the cambial tissue of the roots

RESULTS

Symptoms on fireweed. The shoots of noninoculated fireweed plants remained healthy and their attached rhizomes had no discoloration (Fig 1). Foliage of some shoots from infected rhizomes displayed a purple-yellow discoloration. At the base of these shoots, at the point of attachment to the rhizome, necrosis and mycelial fans were evident and in some cases had extended upward to the soil line (Fig. 1). Mycelial fans were present beneath the epidermis of healthy appearing appendages attached to infected rhizomes (Fig. 2). The centers of infected rhizomes had a soft rot, yellow to brown in color. A few rhizomorphs were attached to the surface of the rhizomes. *A. mellea* was re-isolated from the infected tissues. Pairing one of these isolates with the original, C-736, resulted in a compatible reaction.

Infection incidence on fireweed. One of the twenty four uninoculated control plants had died by the day of harvest. Neither discoloration nor mycelial fans were evident and only bacteria were recovered from tissues plated onto malt agar.

Armillaria ostoyae did not survive in 15 of the 24 aspen segments. Four of the remaining rhizomes were infected although there were no foliar

symptoms on these plants. In contrast, *A. mellea* survived in 16 of 24 inoculum segments. Fourteen plants were infected and 12 had developed chlorosis before the end of the 8 month period.

Mortality in lodgepole pine seedlings. One of three seedlings grown with an uninoculated rhizome died. A number of the pine seedlings used in this experiment had been infected by *Endocronartium harkensii* prior to inoculation, although none showed symptoms at that time. A gall was present on this dead seedling. There was no evidence of any root infection. Moisture stress may have contributed to mortality of this seedling because when the root was removed the soil was dry whereas the cells in other Rootrainers© were moist.

Eleven weeks after inoculation, mortality occurred in one of four pine seedlings inoculated with aspen segments containing *A. ostoyae* The dead seedling also had a gall rust infection on the lower stem but mycelial fans were evident when the bark of the root was removed. None of the four inoculated lodgepole seedlings were infected by this isolate when a colonized fireweed rhizome was used as the inoculum.

In contrast, root rot incidence was severe in pine seedlings inoculated with *A. mellea*-infested aspen segments or fireweed rhizome segments. All inoculations with aspen segments and 6 of 14 rhizome segments resulted in mortality. Resinosis and mycelial fans were evident on the roots of all of the dead seedlings. Time to mortality of the pine seedlings was 7 to 14 weeks for pines inoculated with aspen segments and 9 to 22 weeks for pine seedlings inoculated with rhizome segments. Three of the rhizome-inoculated pine seedlings also had galls on the lower stems but they did not shorten the time to mortality. Seedling death had occurred in 10 to 22 weeks regardless of whether galls were present.

DISCUSSION

The two species of *Armillaria* used were able to colonize the aspen segments. However, the survival of *A. mellea* was better than the survival of *A ostoyae* on colonized segments placed in soil and more pine seedlings were killed by *A. mellea* when aspen was used as the substrate. Similar results were obtained elsewhere (Mugala, personal communication). These results could be attributed to differences in the degree of substrate colonization or it may be possible that the two species of *Armillaria* react in dissimilar ways to the soil used.

Armillaria mellea killed both fireweed and pine. The fungus successfully colonized fireweed and used it as a food base to infect almost 50% of the pine seedlings. *Armillaria ostoyae* was able to infect fireweed, but killed pine only when the aspen segment was the food base. No inoculations with *A. ostoyae*-colonized rhizomes resulted in pine seedling mortality but this may be because only four seedlings were inoculated. The latter species may still be capable of using fireweed rhizomes as a food base.

The results are somewhat confounded by the presence of galls on a number of the seedlings. It is certain, however, that stress associated with such galls was not a prerequisite for infection regardless of the inoculum used. Pine seedlings without galls were infected, trees with galls were alive at the time of infection (as indicated by the presence of resinous lesions) and trees with galls died no more quickly than trees without galls.

Fireweed could play a role in the epidemiology of Armillaria root rot. Rhizomorphs were found to be attached to rhizomes of fireweed in excavations of infected lodgepole pine saplings. The fungus may be able use the rhizome as a food base to increase inoculum potential. Subsequently the pathogen could infect lodgepole pine roots through contacts with an infected rhizome

Fire would not be expected to eradicate the pathogen from the stand but it could reduce the population. The fungus survived temperatures up to 36 C for seven days but growth was delayed at temperatures above 27 C (Munnecke et al., 1981). Temperatures attained during a fire vary with soil depth and type of fire. During a Douglas-fir slash burn, there was a temperature gradient of 432 C at 0.6 cm below the soil surface to 62 C at 12.7 cm (Neal et al., 1965). However, temperatures in the range of 30-40 C are not uncommon in the upper soil profile (Ahlgren and Ahlgren, 1960). The high temperatures could stress the pathogen and make it vulnerable to its antagonists such as *Trichoderma* spp. (Munnecke et al., 1976, 1981). If the pathogen could exploit another host, it could possibly escape the effects of the antagonist.

Fireweed is a common pioneer of following fire or other disturbances such as windthrow and logging (Dyrness, 1973; Weaver, 1951; Budd and Best, 1964). It is a common herb in burned areas and along roadsides in England (Martin, 1982). The herb has been reported in North America and Europe in coniferous forests (Dyrness, 1973; Durrieu et al., 1985). It is possible that fireweed could be infected by other members of *A. mellea* complex. Fireweed then could serve as a reservoir until the pines or other tree hosts become established on the site.

In conclusion, 1. fireweed is a potential host for *Armillaria*, and 2. under laboratory conditions, the fungus *Armillaria mellea* infected lodgepole pine using fireweed as the substrate. This could have significant implications in the epidemiology and ecology of the pathogen.

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Fig. IV-1. Symptoms on fireweed rhizome inoculated with *Armillaria*. Note the extensive necrosis of the infected rhizome (A) and mycelial fans (indicated by the arrow). Noninoculated rhizome (B)

Fig. IV-2. *Armillaria* mycelial fans under the epidermis on a healthy appearing fireweed rhizome.

Chapter V. General Discussion

The infection process begins with rhizomorphs which were shown to be more important than root contacts for the spread of *Armillaria ostoyae* in west central Alberta (Table II-1). Rhizomorphs grow through the soil and eventually contact and infect a root.

The extent of colonization after infection depends on root type. Tap root infections are lethal (Chapter II; Shaw, 1980) and are followed by the colonization of the entire root system. In contrast, lateral root infections are not lethal. Through the production of resin, proximal colonization is prevented or slowed by occlusion and/or fungistatic properties of the resin (Rishbeth, 1985; Wargo and Shaw, 1985). Colonization is distal to the point of infection. However, when pines with lateral root infections are killed by stem girdling by rodents, western gall rust or other agents, the entire root system may become colonized.

A single rhizomorph may branch and infect several roots. Rhizomorphs are able to grow through the soil, sometimes for distances greater than 2 m from the inoculum source (Fig. II-3). A high inoculum potential may not be maintained as the distance from the food base increases (Garrett, 1956). However, large rhizomorphs often have their interiors filled with a compact mass of mycelium (Stanosz et al., 1987; Motta, 1969). The pathogen could draw on the biomass present in this tissue for further rhizomorph growth and infection (Stanosz et al., 1987). Indeed, large severed rhizomorphs were reported to produce rhizomorph branches and subsequently infect roots of *Larix* sp. (Redfern, 1973).

On a number of occasions, rhizomorphs were attached to the roots of other species (Table II-2) which were infected and occasionally well

colonized. This suggests that the rhizomorphs could increase in inoculum potential by using these species as a food base. A single isolate of *A. mellea* was able to colonize firewee rhizomes and spread from these rhizomes to lodgepole pine seedlings.

After a tree is killed, the mycelium spreads through the root system and pseudosclerotia and rhizomorphs may be produced. The pathogen may then spread, by rhizomorphs or root contact, to nearby ees. Among clumps of trees that presumably had arisen from the same cone or more trees often were cted (Table II-3). These saplings could have been infected by the same rhizomorph or rhizomorph system or by direct mycelial transfer fr an appressed colonized root. Root grafts were not observed in this study.

In young lodgepole pine stands in west central Alberta, however, secondary infections were not as importan as infections initiated by rhizomorphs arising from primary so ces (Table II-1). In contrast, other researchers have stressed the importance of secondary infections by rhizomorphs and root to root contact (Kable, 1974; Morrison, 1981; Pearce et al., 1986). The relative importance of secondary spread could be a function of the size of the roots (Wargo and Shaw, 1985). Size is probably related to food reserves and moisture retention. Longevity may be increased by the production of pseudosclerotia plates around the wood although pseudosclerotia plates did not decrease the rate of moisture loss from blocks of beech wood (Lopez-Real and Swift, 1975).

Secondary spread could occur in a number of ways. First, rhizomorphs could emerge and grow from the the distal portions of lateral roots. Secondly latent infections on lateral roots could be reactivated following tree mortality. The pathogen could colonize most of the root system of the dead tree and produce rhizomorphs which could infect other roots. Although this occurred

infrequently in young pine, it might still enable the fungus to spread over a larger area because rhizomorphs may arise from any point on colonized roots. Thirdly, root contact may play an important role in older stands. In Douglas-fir, the number of root grafts increased with age because of larger root diameter (Reynolds and Bloomberg, 1982).

Certain silvicultural practices may increase the incidence of Armillaria root rot. Because tree death may result in reactivation of primary infections and colonization of the entire root system, shorter rotations could lead to an increase in the amount of inoculum in the stand. In aspen stands in Ontario and Minnesota, the number and length of rotations could be limited by the presence of Armillaria root rot (Shaw and Patton, 1987). Similarly, thinning infected juvenile stands would be expected to result in an increase in disease incidence. The ability of severed rhizomorphs to produce branches (Redfern, 1973), suggests that scarification may increase the probability of a rhizomorph encountering a root.

In west central Alberta, mortality due to Armillaria root rot in stands over 25 years old has not often been reported. This could be attributed to increasing resistance to infection as the stand matures, perhaps because of increasing resin production with age (Gibbs, 1971). Compartmentalization may be playing a role in resistance to the disease as has been observed in *Eucalyptus* species (Pearce et al., 1986). In the coastal forests of British Columbia, of 1320 15-to 17-year-old Douglas-fir trees displaying root rot symptoms, 25% were classified as healthy 11 years later, apparently due to callusing of lesions (Johnson et al., 1972). A physical mechanism could be playing a role; as the roots increase in diameter, the soil around the roots could be compressed forming a barrier to further rhizomorph penetration. Another possibility is that the disease does occur in older stands, but has not frequently

been reported.

There was no association between diseased trees and distances to their nearest stumps in contrast to what has been reported with other *Armillaria* systems (MacKenzie and Shaw, 1977; van der Pas, 1981; Pearce et al., 1986). The majority of infections were initiated by rhizomorphs which had arisen from stumps or their laterals (Table 1). Given that lateral roots extend some distance from stumps and rhizomorphs could grow some distance from lateral roots, this lack of association is not surprising.

Clumps of saplings often occur because several seeds may germinate and develop from a single cone. If one sapling is infected, the other trees in the same clump may be at risk from the same rhizomorph system or from tree-to-tree spread. This is less likely to occur if saplings are further apart and thus it is not surprising that the probability of infection is independent of the health status of a nearest neighbor if that nearest neighbor is more than 0.15 m away.

If older lodgepole pine are resistant, then the pattern of mortality suggests that *Armillaria* could function as a natural thinning agent in some cases. In stands which were regenerated after a fire, *Armillaria* did not decrease the density to a level where understocking occurred (Johnstone, 1981).

The work involving the mycelial population structure indicated that a number of clones of *Armillaria* were present on the site. This in turn suggests that basidiospores are important in the epidemiology of the disease. It is possible that some of these genotypes are the result of mutations. But if these are incompatible with the underlying genotype in a particular area, they may be excluded from further colonization of the substrate. This is suggested from the pairings on popcicle sticks. Incompatible pairings resulted in delimitation

of the two isolates (Fig. III-2 D-F).

There is a risk that isolates which are not clones may be compatible (and thus incorrectly judged to be clones) if they are siblings. Forty-four percent of pairings of *A. lutescubalina* inbred synthetic diploids were compatible and thus appeared to be clones (Bruhn, 1983). It is unlikely that such misclassifications were occurring in this study. Consider clone A in Fig III-3A. There were 14 isolates in this clone. If the probability of two siblings appearing to be from the same clone is 0.5, the probability of obtaining 14 compatible isolates, if the isolates were siblings and not clones, is approximately one in a hundred thousand. Further evidence for the reliability of this method was provided by Pearce et al., (1986) who tested the compatibility of isolates which reasonably could be assumed to be clones because they were from isolated, discrete and expanding disease centers. Isolates from 10 of eleven disease centers were compatible. Thus it would appear that the compatibility test is a reliable method for determining if two isolates are clones.

Somatic incompatibility could be used to verify that trees with lateral root infections result in colonization of the root system. Following artificial inoculation of a lateral root, the sapling could be thinned. Colonization of the root system could be monitored and the genotype of the spreading mycelium could be determinec by isolating the fungus and pairing it with a culture used for inoculation. As has been suggested elsewhere (Proffer and Hart, 1988), using somatic incompatibility as a genetic marker could be extended to other fungal pathosystems in which background contamination poses a problem.

A potential biological control strategy could use somatic and mating compatibility to achieve control with a destructive cytoplasmic factor (Caten, 1972; Proffer and Hart, 1988). One possible way is to use virus-like agents to

limit mycelial growth such as has been done with hypovirulence in *Endothia parasitica* (Murr.) And. (Jaynes and Elliston, 1980. Anagnostakis and Day, 1979). Although vegetative incompatibility may limit the use of hypovirulence for biological control (Caten, 1972), it may be possible to overcome this difficulty by making use of the Buller phenomenon. If a mixture of ds-RNA-containing basidiospores with different compatibility factors were applied to infected stumps, the likelihood of transferring the virus-like agent would be increased.

An interesting question is whether the Buller phenomenon occurs in nature because it is a possible mechanism whereby the population may achieve genetic variation. For example, insects could transfer basidiospores from the basidiocarps to infected stumps or trees. The resulting haploid mycelium could then mate with the diploid creating a new genotype.

In conclusion this study has shown that for juvenile stands of lodgepole pine in west central Alberta: 1. Primary inoculum is more important than secondary inoculum, 2. Rhizomorphs more commonly initiate infections than root contacts, 3. Tap root and root collar infections kill trees whereas lateral root infections do not, 4. There is no association between dead trees and snags or between dead trees and the health of trees located more than 0.15m away, 5. The mycelial population consists of several genotypes some of which occupy rather large areas of more than 100m in diameter. The area enclosed by a particular clone consists of discontinuous patches of dead trees. 6. Non-tree hosts, especially fireweed, may play a role in the epidemiology of this disease.

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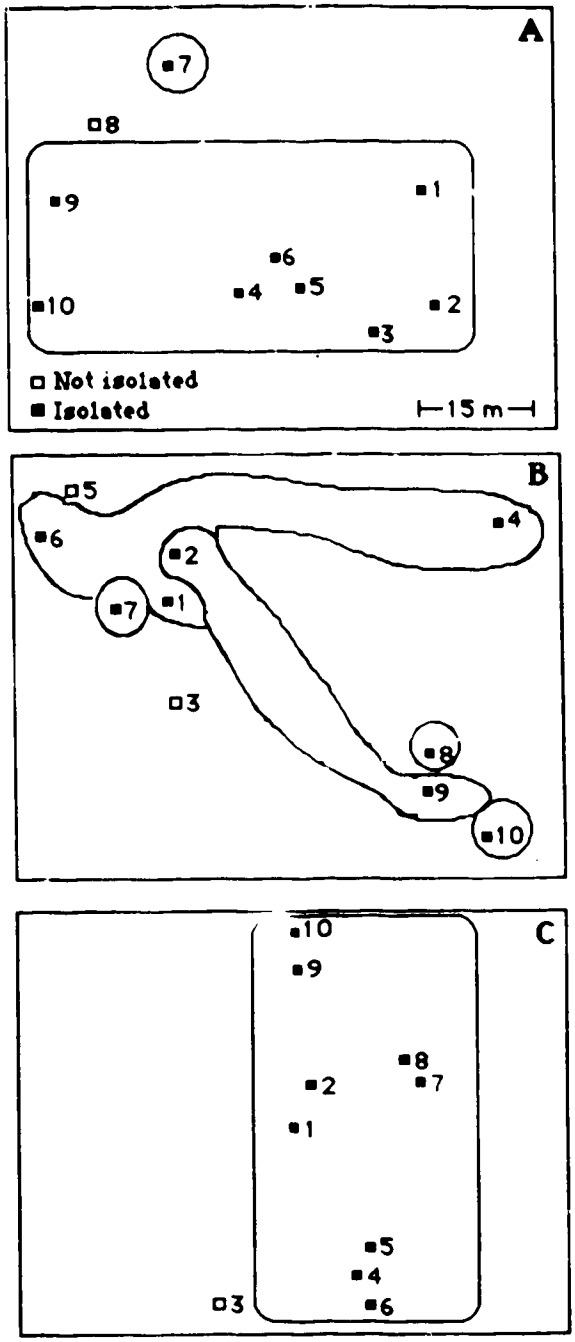
Appendix 1. Genotypes of *Armillaria ostoyae* isolates from dead red pine.

Fig. A1-1. Mycelial genotypes in three disease centers in a red pine plantation in Belair Provincial Forest, Manitoba. Samples of bark with mycelial fans were collected from dead trees with their approximate positions shown on the map. Enclosed areas indicate the same mycelial genotype as determined by the pairings of diploid mycelium. The isolates were representatives of *Armillaria ostoyae* (determined by Dr. Y. Hiratsuka).


A
7
8
9
1
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7
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Appendix 2. Glossary

Aggressiveness: a measure of the rate at which a virulent isolate produces a given amount of disease.

Apparent infection rate: The proportionality constant of the logistic equation; measures the speed of an epidemic.

Backlog area: An unstocked area scheduled for rehabilitation treatment.

Biological species: Reproductively isolated groups as determined by compatibility of haploid or diploid mycelial testers.

Colonization: The growth and spread of mycelium in the root.

Disease center: A localized area in a stand consisting of dead and diseased trees with or without healthy trees.

Disease focus: The site of a local concentration of diseased trees around the source of primary inoculum.

Diploid siblings: Isolates formed as a result of mating of compatible haploid mycelia which arise from the same basidiocarp.

Food base: Source of nutrition for the developing rhizomorph; stumps, debris, and/or infected roots.

Infection: The establishment of the pathogen within the host plant.

Infection center: See disease center.

Inoculum potential: The energy that is available for infection; reflects the combined action of all factors in infection.

Latent infection: Lesions that are quiescent and that may become active as a result of changes in the environment or health status.

Pathogenicity: The ability of a organism to cause disease.

Primary inoculum: Inoculum originating from stumps or debris of trees from the previous generation.

Pseudoscleroríal plate: Sheets of fungal mycelium which enclose a mass of substrate and mycelium; the zone lines observed in decayed wood.

Released plots: Plots in which trees have been thinned.

Resinosis: The flow of resin in response to mechanical injury or infection.

Secondary inoculum: Inoculum originating from trees of the same, as opposed to the previous generation.