# DETECTION AND ASSESSMENT OF ARMILLARIA IN YOUNG CONIFER PLANTATIONS OF NORTHWESTERN ONTARIO AND NORTHEASTERN CHINA

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A graduate thesis submitted in partial fulfillment
of the requirements of
the Master of Science in Forestry Degree
at Lakehead University

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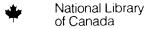
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#### ABSTRACT

Ip, D.W. 1991. Detection and assessment of Armillaria in young plantations of northwestern Ontario and northeastern China. M.Sc.F. thesis, Lakehead University, Thunder Bay, Ontario. xiv + 76 pp. + transparencies. [En,fr,ch]

Keywords: Armillaria trapping; artificial regeneration; disease assessment; disease management; Heilongjiang; Jack Haggerty Forest; Larix laricina; Picea glauca; P. mariana; Pinus koraiensis; plantation management; root disease; root rot distribution; trap bags; trap logs.

Methods for detecting and evaluating Armillaria in plantations were compared in a series of studies. The purposes of the studies were to standardize the Armillaria trapping technique, and to determine if it could be used in practical forest management to monitor and evaluate Armillaria root rot hazard in plantations. Trapping involves burying a removable substrate in the soil for infection by rhizomorphs (RMs). The fungus reacts to the trap by rapidly colonizing the substrate. The distribution of Armillaria is then inferred from the locations of infected traps.

In a study of entrapment methods, spruce (Picea sp.) and poplar (Populus sp.) trap logs were compared with each other and with mesh bags filled with conifer bark. Potato tuber (Solanum tuberosum) traps were unsuccessful. Bark bags were the most successful traps in terms of sensitivity, clarity of infection, and ease of interpretation, but they were more difficult to prepare and install than trap logs. Both species of trap logs detected similar levels of Armillaria prevalence. However, the spruce logs were generally easier to evaluate. Some inconsistencies in detection may be resolved by further refinements in trap preparation.

In a study of young plantations on recent cutovers and one undisturbed, mature spruce stand, estimates of the distribution of Armillaria based on various indicators were compared. Trap logs detected Armillaria in all plots including the mature spruce plot which was mossy and water-logged. The percentages of plot area subjected to Armillaria impact were estimated to be 3-21% using dead trees, 16-54% using residual stand material, and 12-69% using positive trap logs. A comparison of these estimates showed that Armillaria RMs were much more prevalent than was indicated by the dead planted trees. These estimates plus a survey of healthy and infected trees showed that stump presence alone was a poor indicator of potential damage from Armillaria root rot. Mortality surveys were used to augment the trap results. Although current levels of mortality were high (4.8% spruce, 3.6% Larix sp.), it was suggested that the trees may have been predisposed to Armillaria attack by stresses such as root deformity.

To determine the utility of the trapping technique by forest managers unfamiliar with it, the trap bag technique was introduced to a forest management unit in northeastern China. The traps tested in a *Pinus koraiensis* plantation were superior to soil samples for evaluating the presence of viable RMs. Persons with little or no experience in identifying *Armillaria* learned to recognize the fresh, abundant RMs quickly and confidently.

It was concluded that the trap methods described can be used at the management level, but that they should be used in association with sound advice regarding the role of *Armillaria* in overall plantation health.

#### RESUME

Ip, D.W. 1991. La détection et l'évaluation d'Armillaria dans les jeunes plantations au nordouest de l'Ontario et au nord-est de la Chine. Maîtrise en science forestière, Lakehead University, Thunder Bay, Ontario. xiv + 76 pp. + acétates.

Différentes méthodes pour la détection et l'évaluation d'Armillaria furent comparées dans une série d'études. Les objectifs sont les suivants: établir un protocol pour le piègeage de l'armillaire et déterminer si cette méthode est pratique pour l'aménagement forestier en vue de suivre et évaluer le hasard du pourridié aux plantations. Le piègeage consiste à enterrer un substrat temporaire pour l'infection des rhizomorphes (RMs). Le champignon réagit à l'appât par la colonisation rapide du substrat. On peut estimer le territoire de l'armillaire selon la réponse positive de certain appâts.

Les billots d'appât de l'épinette (Picea sp.) et de peuplier (Populus sp.) ont été comparé l'un à l'autre, et avec des sacs à tamis remplis de l'écorce de conifères. Pommes de terre (Solanum tuberosum) comme appât n'a piègé aucun armillaire. Les sacs d'écorce ont été les mieux en démontrant la sensibilité, la clarité de l'infection, ainsi que la facilité de l'évaluation. Par contre, la préparation et l'installation de cette approche était plus difficile que celle des billots d'appât. On pourrait réduire les variations de la détection par billots par le rafinage de la préparation.

Pour l'évaluation de l'impact de l'armillaire, on a basé l'estimation de son territoire par différents indicateurs qui étaient comparés entre jeunes plantations établies sur des coupes récentes, et dans un peuplement naturel d'épinettes matures. Les billots d'appât ont détecté l'armillaire dans toutes les unités expérimentales, même dans un peuplement mature d'épinettes qui était saturé en cau et où la mousse Sphagnum était abondante. Trois indices ont été utilisés pour évaluer l'étendue de la superficie de l'impact de l'armillaire. Selon les arbres morts, le pourcentage de la superficie des unités expérimentales sujettes au présence de l'armillaire était entre 3 et 21%; selon les residuels de la forêt originale, entre 16 et 54%; et selon la réponse positive des appâts, entre 12 et 69%. Une comparaison des valeurs estimées a démontré que la région occupée par les RMs était beaucoup plus grande que la région indiquée par les arbres morts. Ensemble, ces estimations et un suivi des arbres malade et en bonne santé a confirmé que la présence des souches en tant qu'unique indice était un mauvais indicateur du dommage potentiel du pourridié. On a fait des suivies de la mortalité des arbres pour augmenter les résultats des trappes. Le niveau de mortalité était élevé (4.8% épinette, 3.6% Larix sp.), et on a suggéré que peut-être il y avait une prédisposition des arbres vers l'attaque de l'armillaire dû aux pressions tel que la malformation des racines.

Pour déterminer l'utilité de la technique de piègeage par les forestiers débutants, on l'a introduit dans une unité d'aménagement au nord-est de la Chine. Des sacs d'appât enterrés dans une plantation de *Pinus koraiensis* étaient supérieur aux échantillons de sol pour évaluer la présence des RMs vifs. Par cette méthode, les personnes inexpérimentées dans l'identification de l'armillaire pouvaient apprendre rapidement et avec certitude à reconnaître des RMs frais et abondants.

On a conclu qu'on peut utiliser les appâts tel que décrit au niveau d'aménagement forcstier. Cependant, ceux-ci doivent être associés en regard de l'impact de la maladie pour la santé de la plantation.

### 摘 要

对加拿大安大略省函址部积中國東址部的 幼令人工林中蜜环菌(Armillaria)的探測及评价 林学硕士论文 76 頁 安大略省系德贝市雷克治德大学林学院 叶伟杰(17), D. W.)

本研究对人工林中蜜环菌的探测及评价方法进行了比较。其目的五於使捕捉蜜环菌的方法标准心,确走其能否车买陈林业经曹管理中用於监测和评价人工林中爱环菌造成的根质包告。 可用力法是把一个可移动的基质埋於土壤中,使其受菌索的侵梁。 结果表明爱环菌还读中,使其受菌索的侵梁。 结果表明爱环菌还读地工基旗上禁殖生长,从而可从被侵梁的基旗分布情况推断出爱环菌的分布。

把村村国木老旗分别与杨村国木老旗积装 满针叶树皮的网眼袋老物进行此较,从别感性, 低染明显性及评价唯知程度各方面来看,装有针叶树皮的网眼袋子最为成功,但此原木较难, 准备积实效。从两种原木巷旗上, 我们探测出

相似的窟环窗分布。总的来说,杉树圆木叁旗、校客分评价。进一步的准备工作可解决探测中 出现的不一致现象。用马药薯块茎进行楠捉的 方法是不成功的。

车对一处从未受到任何外界治动干扰的特林成熟林分积一处生妆车新近暂戏的人工幼专林进行了研究。对此各种指示物来评价蜜环菌分布情况的方法进行了比较。所有小区,色抬妆满苔藓及水渍的成熟杉木小及中的炸捉圆术上都探测到了爱环菌。受爱坏菌养物的小及面积,以死亡树木衡堂,达3-21%,以林分剩车物衡重,达16-54%,以带爱环菌的原木衡量,达12-69%。通过以上批话比较,表明爱环菌。原的存车较用死亡的入工林树木所显示的情况还要普遍。

上述结果和对健康树木及交被染树木的油重都表明只有树桩的存生一项不足以作为爱坏。 彭根德潜兰包号的指示物。死亡平的湘重常增大了椭块结果。尽管目前死亡平很高(云杉柳,

落叶松3.6%),但作者认为,由於各种不利因 考如极畸形,这种树木光凸受到了爱环菌的侵 袭有关。

为鉴定对上述排版技术不越参的标业经理 们使用此法的有用性,排扱资法被引进到中国 原北部的一个标业经典单位。车一定红松( pinus Aoraiensis)人工标代进得棒投网签试验, 表明此方法优於用上浓煤车来评价治菌疾存至 的方法。对辩认还愿没有经验或经验很少的人 能迅速世常习这种方法,从而肯定世识别新鲜 大重的菌掌。

结果表明呼ば賴投方法就够互经哲中使用,但应该互对有关爱斯固正然于人工标准原中的作用有利分了解的前投不使用。

关疑问: 窟环南排牧; 人工林; 病宫评价; 病宫治理

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#### A DEDICATION AND A PHILOSOPHY

In 1986, I was standing in a plantation holding a red pine which had been killed by Armillaria root rot. A unit manager asked me if Armillaria was a serious problem in that plantation. I said I didn't know. He asked me where the disease was. I said I didn't know. He asked me what I was going to do about it. I said that I would try to find out what he wanted to know.

This thesis is dedicated to all the foresters who came, who saw, who asked. I don't know everything, but when you have a question, I'll try to find the answer.

D. Ip, 1991

If with only a number of diseases, to which certainly just the most important belong, I can arrive at important results for the practical forester, if I can suggest means which can be brought into use against these, then the explanation of the causes and appearances of these diseases will give satisfaction to the educated forester even if practical results cannot immediately be drawn therefrom.

Robert Hartig, 1874 Important diseases of forest trees

You, as forest managers, are responsible for managing the forests under your jurisdiction in the most efficient and productive manner that you can, commensurate with your prescribed management objectives. This requires that you learn and apply new knowledge and technology as it becomes applicable to your situation. Research pathologists are responsible for providing new information and technology. Pest control specialists are responsible for training you in this new technology and helping you to apply it. But you and only you can actually apply new knowledge.

James L. Stewart, 1978 Symposium on dwarf mistletoe control through management

#### 1. GENERAL INTRODUCTION

Armillaria root rot caused by Armillaria mellea (Vahl:Fr.) Kummer sensu lato (= Armillariella mellea (Vahl:Fr.) Karst.) is the world's most widely distributed root disease and is a major disease in northwestern Ontario conifers. As tree-planting programs in Ontario have expanded, the potential for plantation loss to Armillaria has increased. Current methods are inadequate to allow for the detection of this pathogen in the soil. Thus, a means for early detection of the disease potential in plantations is required.

This work focuses on the development of "trapping" methods for detecting Armillaria in cutover forest land. The guiding hypothesis was that the trapping method is a technique with which forest managers can monitor and evaluate Armillaria root disease hazard prior to disease outbreak.

To test this concept, four experimental objectives were established. The results of experiments are reported in independent sections of the thesis, each with its own Introduction, Methods, Results and Discussion sections. The thesis ends with an overall Conclusions section. The objectives are as follows:

Objective 1. Establish sampling methodology including preparation of traps, species to use, plot establishment parameters, implementation and evaluation protocols.

Objective 2. Compare potential traps, viz. short logs (stakes), potato tubers, soil boxes and bark bags.

Objective 3. Estimate impact of Armillaria root disease on young plantations of the Jack Haggerty Forest with information derived from the trapping studies, and compare trap results with estimates based on traditional disease indicators, i.e. dead trees and residual stumps.

Objective 4. Evaluate the usefulness of the trapping method when used by foresters in a practical situation; in this case, in northeastern China, a region ecologically similar to

northwestern Ontario but with a large labor pool and relatively low access to scientific assistance.

Recommendations arising from the hypothesis testing and experimenting are discussed.

#### 1.1. LITERATURE REVIEW

This review is chiefly concerned with Armillaria root disease management. Biological aspects directly pertaining to the detection tests are treated briefly. Comprehensive reviews and classical papers on Armillaria and root disease are listed below:

Hartig's (1874) pioneering studies and descriptions;

Reitsma's (1932) morphology and physiology studies;

Raabe's (1962) host list;

Sokolov's (1964) host list and distribution for the USSR and other countries;

Wargo and Shaw's (1985) general summary for North America;

Anderson and Ullrich (1979), Korhonen (1978) and Watling et al. (1982) for clarification of the Armillaria mellea species complex;

recent reviews by Schönar (1977) and Roll-Hansen (1985);

and the Armillaria root disease handbook (Shaw and Kile, 1991).

The debate over Armillaria nomenclature continues (Kile, 1989; Watling et al., 1982). Therefore, the nomenclature used in this thesis conforms to the recommendations of Wargo and Shaw (1985) and uses the concepts of Anderson and Ullrich (1979). Unless otherwise noted, the name Armillaria mellea refers to A. mellea sensu lato (s.l.). The genus epithet Armillaria italicized will be used when referring to the fungus being trapped in the soil. The disease it causes is referred to as Armillaria root or Armillaria root disease.

#### 1.1.1. BIOLOGY OF ARMILLARIA

The following description is condensed from Sinclair et al. (1987:308-312) and Hartig (1874:12-36). Armillaria mellea is a gilled fungus that fruits for several weeks in the autumn, usually on or near decaying wood and often from rhizomorphs (Armillaria anamorph:

Rhizomorpha fragilis Roth.; main forms: R. subcorticalis and R. subterranea). Signs of the fungus are the groups of honey-colored, ring-stemmed mushrooms; white, mycelial sheets called fans in the cambium; and rhizomorphs (RMs), string-like strands of brown to black branched aggregations of hyphae that grow along roots, under bark, through the soil, or in already dead wood including wood in service.

Armillaria spreads vegetatively by RMs, over distances of several meters, and by hyphal growth through direct root contact. The importance of basidiospores for Armillaria spread and infection is unclear, but is thought to be minor (summarized by Redfern and Filip, 1991). Established genets (genetically discrete units or assemblages, sensu Brasier and Rayner, 1987:384) may persist indefinitely for years, over an area of up to several hundred hectares.

Between and within species, Armillaria activity ranges from saprophytic to opportunistic to pathogenic. Under various silvicultural and edaphic conditions, it attacks numerous plants, woody and non-woody, angiosperm and gymnosperm, native and exotic, juvenile, mature and over-mature, stressed and healthy, managed and wild. Some plants are reported to be symbiotic with Armillaria spp., e.g. Galeola septentrionalis (Hamada, 1939; Terashita and Chuman, 1989) and Gastrodia elata (Zhang and Li, 1980).

Rhizomorphs growing over plant roots produce branches that invade the cambium and lead to death of roots and trees. In conifers, resinosus occurs in response to infection and often coats portions of the bark. Wood decay usually follows mycelial growth in the cambium, and the wood provides energy for further infection. Wood decayed by Armillaria becomes light yellow or white, soft, often stringy in conifers, and marked by black zone lines. Decay is usually restricted to the roots or butt centers until after death. Small trees often die quickly, but large ones may sustain growth loss and decay over many years.

#### 1.1.2. Importance of Biology of Armillaria

The most important biological aspects of Armillaria are the vegetative characteristics.

Armillaria is significantly different from other root parasites in its ability to freely spread via

RMs through soil that is relatively devoid of food material to contact new food substrates (Garrett, 1960). Therefore, its impact is magnified over that of other root diseases which are limited by requirements for sporulation and occurrence of root contact. The ability to kill the cambium and decay xylem constitutes one of the most important characteristics of *Armillaria*, i.e. it can inhabit weakened or dead trees as a decay fungus, and then increase its impact by attacking and killing both healthy and unhealthy trees nearby (Manion, 1981:329).

Basidiomes are commonly used in research to determine Armillaria presence (Intini, 1989; MacKenzie and Shaw, 1977). However, forest managers generally cannot depend on basidiomes to assess the fungus' presence because it fruits so briefly or sometimes not at all, and because of morphological variation (Gibson, 1960; Greig and Strouts, 1983); Laemmlen and Bega, 1974; MacKenzie and Shaw, 1977). It must be emphasized that absence of the mushroom does not indicate absence of the fungus. The presence of mycelial fans in the cambium are a reliable sign, but they are difficult to detect because unless trees are symptomatic (chlorotic, defoliating, reduced leader size, resinous, crown thinning), one does not know which stems to examine (Pawsey, 1973). Even with symptomatic trees, the stem must be cut, causing unnecessary injury. Locating RMs is also difficult because one is uncertain where to look for them (in the soil, on the roots, etc.) and soil-sampling is extremely time-consuming. However, since vegetative propagation by RMs and hyphal contact appears to be far more important than spore propagation (Redfern and Filip, 1991; Rishbeth, 1985), it is logical to use the vegetative state to assess the fungal presence. This approach is supported by the persistence and large area coverage of genets (Anderson et al., 1979; Shaw and Roth, 1976), and the inverse relationship of infection to distance from inoculum sources (Roth and Rolph, 1978).

Any action taken to deal with a disease in forest management should be based on the potential impact of the disease (Wargo and Shaw, 1985). Unfortunately, the present confusion over identification and consequently unclear etiology of the various species and strains (Schönar, 1977; Watling et al., 1982) as well as the complexity of identification methods, e.g. nucleic acid analysis, withholds this information from foresters (Watling et al., 1991). Considering the above

lack of agreed upon identity of species, the detection of any Armillaria spp., followed by careful assessment, would be useful for forest managers in the absence of a method that could detect species pathogenic on desired hosts.

#### 1.1.3. Importance of Armillaria in Plantations

Armillaria root rot is a cosmopolitan disease that is especially damaging in artificially regenerated forests (Fedorov and Smoljak, 1989; Raabe, 1962; Singh and Carew, 1971). Root rot damage related to cutover residue is chiefly a concern during the plantation establishment phase (MacKenzie and Shaw, 1977) as pathogen virulence appears to decrease with the deterioration of the food base (dead stumps and roots) (Johnson and Hawksworth, 1977) and increasing resistance of maturing trees (Gibson, 1975; MacKenzie and Shaw, 1977; Morrison et al., 1988). Thirty years ago, economically serious attacks of Armillaria root rot over large areas were not reported for Ontario plantations which were generally established on abandoned farmland, free of dead stump and root food bases (Huntly et al., 1961). It is now known to be the main root disease in Ontario conifers (Whitney, 1978), with an average stem mortality of 1.4% per year in conifer plantations, ranging from 0 to 16% of trees (Whitney, 1988). Armillaria root rot in plantations established on cut-overs elsewhere in the world reduces initial stocking by as much as 50% in the first 10 years of growth (Appendix I).

Shaw and Roth (1978) suggested that the simplest control for Armillaria in plantations is avoidance of high hazard sites. Avoidance of all Armillaria sites is impossible. When it is known to be present, removal of the substrate sources is generally recommended as the most effective treatment (Greig and Strouts, 1983; Pawsey, 1973). Small-scale planting trials may help to determine disease potential before plantation establishment (Gibson, 1975; Wargo and Shaw, 1985).

Detection and removal of diseased trees from infection centers in managed stands should be used for control of Armillaria root rot to reduce plantation losses (Johnson and Hawksworth, 1977; Roth and Rolph, 1978; Roth et al., 1980). Currently, detection requires examination of the individual trees (Shaw, 1980) and residual stumps (Filip, 1989; Roth et al., 1980; Zeglen, 1991)

for signs of the fungus. The latter has proven to be of little use for managers (Roth et al., 1980). Since individual tree examination is prohibitively expensive in management, investigation is usually postponed until the appearance of obvious symptoms such as chlorosis, abnormal foliation, growth decline, and mortality (Intini, 1989). The delay in waiting for these symptoms may be unacceptable as foliar change occurs only when the tree is completely girdled or death is imminent (Baranyay, 1965; Pawsey, 1973), and infection is not always correlated with growth increment (Livingston, 1990; Pronos and Patton, 1977).

#### 1.2. REGIONAL IMPACT

Ontario. The area of crown land planted in Ontario in proportion to the area cut has risen from 13% in 1979 to 33% in 1989, and although the actual area planted each year has fluctuated, there has been a general increase from 21,000 ha in 1963 to 80,000 ha in 1989. With increased planting of cut-over areas, the risks of initial loss to Armillaria are correspondingly increased (Huntly et al., 1961; Shaw, 1980; Shaw and Roth, 1978). For years, Armillaria has been known as a major factor in growth reduction of mature and overmature stands, as well as in natural saplings (Anon., 1970-83; Whitney and Myren, 1978), and is now becoming a great concern in the establishment of numerous, expensive plantations across Ontario (Whitney, 1988). Timber yields would be significantly increased if losses to diseases were reduced considerably (Filip, 1989; Whitney et al., 1983).

China. China has approximately 1.4 x 10<sup>8</sup> ha of fully and lightly stocked forestland, of which about 4% is new plantations (based on Hsiung and Johnson, 1981). China has set goals of having 1.9 x 10<sup>8</sup> ha (about 20% of its land area) forested by the end of the century, and doubling timber output to 1.0 x 10<sup>8</sup> m<sup>3</sup> (Anon., 1984). Some 25% of China's natural forest growing stock is found in the northeast, particularly in Heilongjiang, and a large proportion of China's afforestation efforts, including the Great Green Wall project are concentrated in this region (FAO, 1982). Conifer regeneration is increasing in the northeastern forest region (Zhan et al.,

<sup>&</sup>lt;sup>1</sup> Calculated from Ontario Dep. Lands and Forests Annual Reports 1962-72, and Ontario Ministry of Natural Resources Statistics 1973-89.

1990) which is ecologically similar to Ontario (Burger and Zhao, 1988). Several species of Armillaria appear to be present in all boreal forests (Guillaumin et al., 1989), and it can be expected, as in Ontario, that augmented artificial reforestation efforts will meet with increased risks of mortality due to Armillaria root rot.

The present series of studies reports on a method for monitoring the potential impact of Armillaria root disease in young conifer plantations.

#### 2. ENTRAPMENT OF ARMILLARIA IN YOUNG CONIFER PLANTATIONS

#### 2.1. INTRODUCTION

Forest managers require an effective method of detecting the damage potential of Armillaria root disease before tree symptoms occur. Detection of the vegetative stage seems more appropriate than the fruiting stage since the infection process implicates RMs (Hartig, 1874:14,32), and since fruiting cannot be depended upon (Laemmlen and Bega, 1974). Armillaria presence has been assessed by sampling soil (Hood and Sandberg, 1989; Pronos and Patton, 1977; Wargo, 1988; Wargo et al., 1987), but this method appears to be impractical for forest managers. Zeglen et al. (in prep.) are working on developing a predictive model based on root excavations. Pronos and Patton (1977) suggested that in conifer plantations converted from hardwood sites, foresters could estimate root rot hazard according to herbicide-killed stems. Such an estimate presumes that Armillaria is present. Roth et al. (1980) have attempted to develop a method of identifying infectious stumps that would aid forest managers, but aboveground indicators (age and location of stumps) and nearby dead saplings were inadequate as infection indicators. More importantly, such detection methods require waiting for saplings to become infected. Since symptomatic trees are likely to die (Intini, 1989; Rykowski, 1981), this delayed step may be unacceptable in plantation management. A method is required to detect the current distribution of Armillaria.

The methods of detection investigated herein are referred to as trapping. An Armillaria trap is a selective, removable substrate that is placed in the soil for infection by rhizomorphs. Although the present trapping method is intended as a field management tool, the critical part of the definition is that it is selective. Other definitions of trapping include a lab technique for diagnosing microscopic pathogens (Manion, 1991:297) and spore trapping (Rishbeth, 1970) which were not included in this study.

Successful testing of the trap-log method for examination of Armillaria on a small scale has been reported from a Japanese Prunus sp. plantation (Aoshima and Hayashi, 1981), and a 6-year-old seeded Pinus contorta var. latifolia Engelm. stand in Alberta (Mallet and Hiratsuka,

1985). This method involves planting 70- to 100-cm long hardwood (Quercus and Populus spp.) logs vertically in the soil and leaving them for 4 or 12 months. If Armillaria is in the soil, the logs become colonized by it. Variations of this method are used in many locations (Hood and Sandberg, 1987; Wargo, 1988), and in some cases have been reported to be unsuccessful (Baker et al., in prep.); no standardized, effective methodology for management application is followed.

Wood stakes or fenceposts have been used to study microorganisms associated with decay in wood in contact with soil and to test the efficiency of wood preservatives (Coates and Rayner, 1985; Levy, 1968). Coates and Rayner (1985) isolated over 25 species of fungi including Armillaria species from cut, buried logs.

Infection studies of Armillaria in potatoes (Solanum tuberosum L.) (Thomas, 1934; Garrett, 1956; Gregory, 1985) indicated that potato tubers might be suitable as traps. Following this premise, Wargo (1988) found that colonization of potatoes by Armillaria RMs reflected RM density in the soil. He also used horizontally buried oak stem sections and potato tubers in oak stands to predict RM distribution and quantities. In studies of inoculum vigor in a hardwood stand, new potatoes became colonized in 25 to 30 days and 1-year-old potatoes in 40 to 50 days (P.M. Wargo, pers. comm., Appendix VI).

Soil media bags have been used in soil chemistry and hydrology studies in which various soils were placed under differing conditions (Havas, 1988). Fine-mesh fiberglass bags were used to confine jack pine (Pinus banksiana Lamb.) seedling roots so that all roots could be recovered, in a mycorrhizal field study (Whitney et al., 1972). Shredded fresh white spruce (Picea glauca (Moench) Voss) bark was a suitable medium for the growth of Polyporus tomentosus Fr. (Whitney, 1962; 1965). Extensive growth of Armillaria RMs in conifer bark piles has been observed by the author and others (Appendix II). Hartig (1874:33) used bark pieces with R. subcorticalis to inoculate Pinus sylvestris seedlings.

The objectives of this study were to evaluate several types of traps for usefulness at the forest management level. The characteristics of each trap that would aid or inhibit usefulness for field foresters was particularly emphasized.

#### 2.2. METHODS

#### 2.2.1. Trap Preparation

Three types of traps were tested: 1) trap logs as modified from Aoshima and Hayashi (1981) and Mallett and Hiratsuka (1985); 2) potatoes, as suggested by Wargo (1988; pers. comm., Appendix VI); and 3) trap bags and boxes, developed for this study. Soil samples should be collected to estimate natural RM density but attempts to do so were unsuccessful because of difficulties with soil augering and time requirements.

Trap logs. Trap logs, 30 to 40 cm long and 6 to 10 cm in diameter were cut from local, apparently healthy black spruce (*Picea mariana* (Mill.) B.S.P.) and poplar (*Populus tremuloides* Michx.) trees. All branches were cut flush with the stems. Ends were tapered to a point over a distance of <10 cm. Removing branches usually scarred the bark. Some logs with no branches were lightly scarred with the chainsaw or scalped with an axe to provide similar entrance courts. In May and June, 1989, the logs were hammered to a maximum of 30 cm into the ground. Initial attempts to maintain spacing parallel to tree planting lines failed because of uneven lines, buried wood and rock, standing water, or insufficient soil depth. Final spacing was approximately 1.5 to 2 m between traps locations. The protruding tops were numbered and spray-painted to aid in relocating them and to inhibit spore infection. To compare log species sensitivity to *Armillaria* infection, 92 Po logs were placed adjacent (5-20 cm) to Sb logs. Not all Sb logs were paired with Po logs.

Potatoes. In mid-June, 1989, 24 new potatoes (commercial product of U.S.A.) were hand-planted 8 to 15-cm deep in a 3-year-old larch (*Larix laricina* (Du Roi) K. Koch) plantation where *Armillaria* was actively killing trees. The potato traps were placed adjacent to and inside Plot 2.

Trap boxes. Trap boxes, made of wood and mesh screen, were designed to hold forest soil that had been sifted for RMs. It was expected that growth of RMs into the soil would allow an estimate of rate of spread through the plantation. Boxes were designed in several sizes (1 to

10 dm<sup>3</sup>) and shapes, but were discarded after preliminary tests resulted in difficulties in digging suitably shaped holes, working around obstructions, and sifting the soil.

Trap bags. An acrylic 1.8-mm mesh screen bag was made to hold 1.6 dm<sup>3</sup> of Armillaria -free material in a 20-cm deep column. The bags were filled with freshly peeled spruce (Picea spp.) and fir (Abies spp.) bark in a 4:1 ratio from a mill debarker. Labeled twist-ties were tied to the bag tops and 99 bags were placed in 10-cm wide, 20-cm deep holes dug with a manual soil-core sampler. The bags were covered with soil extracted from the hole but the labels were kept visible. Regular 2-m spacing was attempted initially, but abandoned for the same reasons as for the trap logs. One trap bag was placed for every 3 m<sup>2</sup> of plot area. Assuming that the principal RM horizon is the top 20 cm of soil (Morrison, 1982; Redfern, 1970; Wargo and Shaw, 1985), this arrangement corresponds to 0.25% of the RM horizon by volume.

#### 2.2.2. Site Selection and Plot Layout.

The three types of traps were tested in two plots established at the Jack Haggerty Forest, north of Thunder Bay, Ontario (48° 38'N, 89° 23'W).

Plot 1 was 15 by 20 m and located in a 10-year-old black spruce and white spruce plantation. 93 black spruce (Sb) and 59 poplar (Po) trap logs, and 99 trap bags were placed among the 56 living and 13 dead trees. Plot 2 was 10.5 by 7.5 m in a 3-year-old larch provenance trial. 33 Sb and 33 Po trap logs were placed among 41 living and 7 dead trees.

Locations of the traps, trees, above-ground portions of stumps, slash and large rocks were mapped within ±0.25 m. On Plot 1, loose, dead, woody material, except dead spruce, was removed from the plot to facilitate study. Plot 2 was free of slash.

#### 2.2.3. Field Assessment of Armillaria Impact

After 10 to 12 weeks in the ground, the trap logs were loosened from the soil with an axe and pulled directly out of the ground. Logs and bags were transported to the laboratory in boxes, packed in dried peat moss, and stored outdoors until assessment. After 12 to 14 weeks in the soil, the trap bags were collected and stored outdoors until assessment. Time constraints led

to the extra 2 weeks in the soil for the trap bags. The potatoes were excavated by hand 55 days after burying, and taken to the lab in individual paper bags.

The exterior of each log was examined for RMs and decay on the sharpened end. Notes were made of other fungi, microfauna and symptoms, e.g. decay, wood and bark discoloration, etc. The bark was then carefully peeled or scraped off in layers with a belt-knife. The total visible lengths of attached surface RMs (Rhizomorpha subterranea) and RMs growing in the bark and cambium (Rhizomorpha subcorticalis) were estimated in centimeters. Mycelial appearance and extent of growth were estimated and recorded as follows (see Figure 2.1):

Appearance				
Category 1	Mycelial fans or felts			
Category 2	Mycelial strands - thick aggregations of hyphae			
Category 3	Hyphal strands - single hyphae or thin aggregations of hyphae			
Mycelial class	Extent of fungal growth			
0	No mycelium or hyphal strands			
1-10%	Cambial area or inner bark			
10-25%	surface covered with mycelium			
25- $50%$	on below-ground portion			
>50%	of log.			

The bags were opened and the bark contents gently teased apart. Rhizomorphs were removed and the total length, fresh weight, number of pieces, and number of growing tips measured or counted.

To verify the presence of Armillaria in the plots, all dead trees were pulled up and examined for mycelial fans in or under the bark, or attached RMs. Representative trees were collected for cultural isolation of the fungus. The living trees were non-destructively examined for signs and symptoms of Armillaria infection by examining shoot development, foliage color and root collar appearance.

#### 2.3. RESULTS

#### 2.3.1. Species Identification

The species observed in the present study was A. ostoyae (NABS I). The methods and

results of the cultural studies are given in Chapter 3.

#### 2.3.2. Results of Different Trap Types

#### Trap Logs

On the two plots, 60% of the Sb and 58% of the Po logs were positive for Armillaria, i.e. had mycelial growth and/or subterranean or subcortical RMs in the bark or cambium (Table 2.1). In Plot 1, 60% of both Sb and Po trap logs were positive while in Plot 2, 58% of the Sb and 55% of the Po were positive.

Table 2.1. Positive Armillaria trap logs from a Picea spp. plantation (Plot 1) and a Lariz laricina plantation (Plot 2).

Pο

logs

0

9

1

1

0

4

PLOT 1 PLOT 2  $Po^{d}$ RMs<sup>b</sup>  $\operatorname{Sb}^{\boldsymbol{c}}$ Mycelial SbClassa found? logs logs logs 0 Yes 10 0 7 No 1-10 4 4 4 1-10 Yes 9 4 1 10-25 No 6 0 0 10 - 25Yes 4 4 0 3 > 25No 4 10

28 Yes > 255 4 3 Total positive logs 56 36 19 18 Number of logs placed 93 60 33 33

a. Percentage of log area (intrabark and cambium) occupied by mycelium

Although equivalent proportions of poplar and spruce were positive in each plot, the majority of the spruce traps were 25% or more covered with mycelium, whereas most of the positive poplar logs were 10% or less covered. The total lengths of RMs in logs ranged from 2 to >50 cm.

Of the 92 pairs of traps placed, 36 had both Sb and Po positive and 23 were negative for both. Seventeen Po logs were positive while their paired Sb logs were negative. Conversely, 12 Sb logs were positive while their paired Po logs were negative. Signs of colonization on four pairs were uncertain.

b. RM: rhizomorphs

c. Sb: Picea mariana

d. Po: Populus tremuloides

Although these numbers suggest that Sb is only slightly better than Po, Sb traps were usually more distinctly positive than Po between the pairs in which both species were positive. In 21 of these pairs, Category 1 mycelium appeared more frequently on Sb than Category 2, and Category 2 more so than Category 3. The mycelium in Sb also covered more area than in the Po traps. In another 10 pairs, the signs on each species were equally distinctive. In both species, infection was often associated with scars in the bark or the sharpened end of the trap logs.

The presence of R. subterranea on log surfaces indicated some logs to be positive before peeling off the bark. However, most required peeling and many required 1 to 2 min of peeling to discover mycelium or subcortical RMs (Figure 2.1). The negative logs often required 4 to 5 min of careful peeling to ensure that mycelium between the bark layers was not being missed.

#### Trap Bags

Armillaria RMs were found in 72% of the trap bags (Table 2.2). The RMs grew freely through the acrylic mesh and onto the bark inside the bags, while plant roots did not. Total lengths per bag ranged from 0.03 to 7.1 m. Virtually all were R. subterranea even though many were penetrating or tightly attached to pieces of wood or bark. The number of RMs growing into and out of the bags was not measured, but when many broken ends were observed outside the bag, growth was generally profuse inside and on the bag.

Table 2.2. Lengths of Armillaria rhizomorphs (RM) found in trap bags.

RM content (m)	No. of bags
0	28
0.01 - < 0.50	16
0.50 - < 1.0	14
1.0 - < 2.5	14
>2.5	27

In trying to accurately measure the weight of RMs, several attempts to remove all bark and wood while preserving the RM material proved very difficult or impossible. Therefore, the fresh weights of the RMs were not measured. Actual diameter of RMs did not appear to vary between bags. The number of RM growing tips appeared to be slightly related to total RM

length. RMs with total lengths <1 m generally had less than 30 growing tips while those with >1 m generally had more than 30 tips. The RMs in traps with the greatest total lengths (>5 m) did not necessarily have the most growing tips.

The trap bags with >0.50 m of RMs were determined to be positive within seconds of opening the bags. RMs were found in most of the other positive bags in less than 1 minute. Those without RMs were thoroughly sifted in about 1 minute.

On Plot 1, where both logs and bags were tested, one or two positive trap logs occurred at 67 trap locations; 61 of these locations were within 1 m of a positive trap bag and all were within 1.7 m of a positive trap bag (Figure 2.2). The bags indicated the presence of Armillaria everywhere that the combined Po and Sb logs did. In addition, five more places at least 2 m from any positive trap log were positive.

#### Potatoes

The potatoes showed no signs of Armillaria infection. Based on these results, it was decided not to attempt a larger test.

#### 2.3.3. Dead Planted Trees

All plots were located in the vicinity of dead trees. In Plot 1, there were 13 dead spruces, including two sets of doubly planted trees, as of May 1989. Four of them had been killed by brush saw cuts. One died during the study bringing the total to 14 (Figure 2.3). There were seven dead larch ramets in Plot 2. Although five infected living trees were detected in Plot 2, none of them died during the 1989 field season. Armillaria was found in the roots of seven of the ten dead spruce and five of the seven dead larch. Cause of death in four spruces was brush saw damage (roots not examined) and could not be determined in three spruce and two larch.

#### 2.3.4. Relationship of Positive Traps to Residual Slash and Dead Trees

The distribution pattern of positive traps in both plots showed no apparent relationship to the distribution of residual material, tree pockets, or dead planted trees, nor was there any apparent relationship between residual material and dead planted trees. Positive traps occurred within less than 2.0 m of every dead tree in both plots except for two in Plot 2. The locations of these two dead trees, which had died and had disappeared by the time of the survey, were marked by survey pins in the south-east corner of the plot. There were 24 residual hardwood, softwood and unidentified stumps in Plot 1 and 22 in Plot 2. The stumps were not examined for Armillaria infection.

Four living spruce trees had resinosus at the root collar but otherwise showed little indication of possible root infection. Differences in leader length and crown form were present only in one spruce tree. This tree, upon excavation, had extensive mycelial growth in the roots and root collar (Figure 2.3).

#### 2.4. DISCUSSION

#### 2.4.1. Different Trap Types

#### Trap logs

The present study supports and adds to Mallett and Hiratsuka's (1985) statement that the trap-log method can be effectively used to detect *Armillaria* RMs in soil. Both poplar and spruce logs became infected. Although about the same proportion of Po and Sb traps were positive, the positive Sb traps were qualitatively more successful, i.e. the fungal signs were clearer and more readily discovered.

Colonization of the traps in 10 weeks suggested that a site evaluation can be completed in one field season. Poplar logs may be just as suitable as Sb logs if left longer, perhaps 12 months. However, there may be variations in host preference of the particular Armillaria species. In a preliminary study (Appendix II) comparing Po logs with Sb logs over a 12 month period, the Sb were colonized within 14 weeks while Po logs required 35 weeks to begin showing signs of infection and 52 weeks to reach infection levels similar to that in the Sb traps. In another test (Appendix III), 18 of 20 Po trap logs were infected in a Pinus resinosa Ait. plantation after 12 months.

It is unclear whether the quality of infection reflects the level of inoculum potential as opposed to ecological influences. Possibly, the extent of mycelium development is related to aeration and plant substances in the log cambium (Garraway et al., 1991). Microsite conditions affecting RM abundance may explain the infection of only one of each log in 29 Po/Sb pairs. However, these traps represent a high proportion (42%) of the paired log locations at which Armillaria was found. More consistent results might be obtained by making the entrance courts into the logs more uniform, or by placing the test logs closer together.

#### Trap bags

The trap bags with spruce/fir bark were superior to the trap logs because of 1) higher sensitivity, 72% of bags versus 60% of logs positive; 2) clearer signs of Armillaria; and 3) easier interpretation, due to uniformity. In addition, it required much less time to evaluate the trap bags than the trap logs. The bag design is simple and could easily be prepared commercially. The higher sensitivity of the trap bags may have been due in part to the high susceptibility of fir bark to colonization by A. ostoyae (P.M. Wargo, pers. comm.). Shredded or chipped bark, which is easily obtainable, should be tested from a number of species.

Armillaria may have been present but undetected in 28% of the locations. Given more time, more bags may have become infected. Armillaria developed relatively slowly in spruce logs (Appendix II) suggesting that the 2 extra weeks would not have been a significant differentiating factor between the bags and logs. However, Sokolov (1964) concluded that optimum growth temperatures for A. mellea could be used in growth assessment and disease control. For example, A. mellea grows well in surface roots and tree butts for only 2.5 to 3 months a year in Leningrad District versus 8 to 9 months per year in Auckland, New Zealand. Therefore, lengths and dates of interment should be tested for effect on trap sensitivity.

The main drawback to the trap bag method lies in digging the holes. This is reasonably easy in sandy, stone-free soils, but when the soil is tightly compacted, has a thick vegetation mat, or has a high rock content, making the holes can be extremely difficult. Various soil core samplers are available (Jackson, 1987; Karahashi et al., 1987; Loveday, n.d.:154-163; Stanosz

and Patton, 1991) that may be better than the standard sampler used in this study.

#### Potatoes

The potato traps did not become colonized with Armillaria after 55 days in the soil, nor did they sprout or become infected with other pathogens. Gregory's (1985) experiments with potatoes and woody inocula resulted in infections, although with confounding variability. The trap tests should be repeated considering Wargo's (1988; pers. comm., Appendix VI) success in northeastern U.S.A. Factors that may influence the infection level are the disease resistance of the potato strain, the species of Armillaria, whether or not the potatoes were treated with fungicide, and the duration of exposure at this latitude.

#### 2.4.2. Field Assessment of Armillaria Impact

The potential for tree infection by Armillaria can be estimated from the presence of the fungus. A tree planted at the same location as a trap is located would seem to have the same likelihood of being contacted by Armillaria. The trap method for detecting Armillaria will provide foresters with a tool for estimating how widespread Armillaria is in a given site before extensive mortality occurs.

The trap bag method appears to be more indicative of Armillaria presence than soil RM density since the RMs elongate rapidly and branch prolifically upon entering the bark bags, making them easier to detect than the shorter, less branched fragments in the soil. The correlation of trap results with soil RM density was not measured in this test. In other tests (Chap. 4; Appendix IV), positive trap distribution generally reflected soil RM occurrence but not density. Stanosz and Patton (1991) used trench and soil core samples to measure RMs by weight in Populus spp. stands. However, Falck (1924) believes that it is very difficult to draw conclusions about Armillaria based on soil samples. Twery et al. (1990) reasoned that RM length was more relevant than weight since a few thick RMs might affect overall weight disproportionately to inoculum potential. The same line of reasoning was adopted in the present study.

It is now necessary to relate trap results to future tree infection and mortality, and species of Armillaria. As a primary pathogen, Armillaria would be considered a danger to all trees wherever it occurs. When acting as a facultative parasite, i.e. chiefly saprophytic but capable of parasitism under certain conditions, it would pose no threat to healthy, stress-free trees. However, young trees are more likely than mature trees to succumb to stress agents such as insect attack and drought, and, consequently, are more likely to succumb to Armillaria root rot.

## 3. IMPACT OF ARMILLARIA ROOT ROT IN YOUNG PLANTATIONS OF THE JACK HAGGERTY FOREST

#### 3.1. INTRODUCTION

Armillaria root rot is the main root disease of natural conifer forests in northwestern Ontario (Whitney, 1978; in prep.) and has been found killing conifers in 49 "high-value" plantations across northern Ontario (Whitney, 1988). It is a major concern in the reforestation of cut-overs worldwide (Appendix I).

Identification of the biological species of isolates greatly helps in evaluating the importance of Armillaria in causing disease (Wargo and Shaw, 1985). According to Dumas (1988), the known biological species of Armillaria in northern Ontario mixed wood forests are North American Biological Species (NABS) I (A. ostoyae (Romagn.) Herink = A. obscura (Secretan) Herink), NABS III (A. calvescens Bérubé et Dessureault), NABS V (A. sinapina Bérubé et Dessureault) and NABS VII (A. gallica Marxm. = A. bulbosa (Barla) Kile et Watling). Based on basidiome isolations, NABS I was the most common species (83% of collections) and NABS V the second most common (13%). NABS III and VII occurred rarely.

The Jack Haggerty Forest (48°38'N, 89°23'W) is a 1000-ha forest maintained for educational and research purposes some 36 km north of Thunder Bay, Ontario (Figure 3.1). It is typical boreal forest (Rowe, 1972) originating from fire or cutting, 40 to 100 years ago, on shallow to moderately deep, sandy soil with moderate rock content (Hawkins and Pickard, 1986). Approximately 470 ha of the forest are classified as productive for growing trees (Finstad, 1982). In the past 19 years, approximately 90 ha, representing some 20% of this productive forest area, have been harvested and replanted. Although numerous common tree diseases including Armillaria root rot have been collected by students annually from the Forest, to date no systematic survey of diseases or their impact on the Forest has been conducted.

Assessment of Armillaria root disease hazard is commonly based on current mortality (Whitney, 1988) or potential food bases, such as stumps and residual material (Klein-Gebbinck

et al., 1991; van der Pas, 1981). The rate of disease spread is also important in estimating inoculum potential (van der Plank, 1963:275). The following studies indicate the approximate range of spread or impact estimates. Morrison et al. (1988) noted that lethal RM infections usually occur within 30 cm of the RM food base. Shaw (1974) found the average distances between symptomatic and healthy trees in stands of mean DBH 13, 23 and 28 cm to be 4.5, 5.5 and 7.5 m, respectively. MacKenzie and Shaw (1977) used a radius of 3.5 m to mark a 'circle of influence' enclosing most of the mortality around Beilschmiedia tawa stumps. In dense stands of natural Pinus ponderosa Laws. with many old-growth stumps, Armillaria root rot spread in intermittent waves at average rates of about 3 feet (about 1 m) per year (Roth et al., 1977). Armillaria spread rates have been calculated at 1.0 m/yr in a Washington P. ponderosa forest (Shaw and Roth, 1976), 0.8 to 1.3 m/yr in a Prunus persica (L.) Batsch orchard in New South Wales (Kable, 1974), 1.1 to 1.5 m/yr in various English situations (Rishbeth, 1968), and 5.2 m/yr in a Rhodesian Pinus elliottii forest where, interestingly, RMs were not formed (Swift, 1968).

The objectives of the present study were to detect Armillaria root disease in young plantations of the Jack Haggerty Forest using traps and mortality surveys, to compare the trap results with estimates of disease distribution and potential impact based on traditional disease indicators, i.e. dead saplings and residual material, and to determine the species of Armillaria involved.

#### 3.2. METHODS

#### 3.2.1. Study Area and Plot Establishment

The presence of Armillaria was based on trap log results and surveys of dead saplings. Four study plots were located in 3- to 10-year-old plantations and one in a 90- to 100-year-old black spruce stand (Figure 3.1). Plot selection within plantations was arbitrary, although the plots were placed at least 10 m inside each plantation edge. At all locations, some trees showed symptoms of root disease.

Plot 1 was in a black and white spruce plantation, planted in 1979 in Cutover 7801 (Block

1.3N). Hardwood competition was removed with brush saws in 1983 and 1988 to release the planted spruce. In June, 1989, as part of the present study, mortality was determined on three temporary survey plots located in the released (free-to-grow) area of the plantation.

Plots 2 and 3 were in blocks I09 and I06 of a 3-year-old larch provenance trial, planted in 1986 in Cutover 8502 (Block 1.1S). The trial site was partially root raked before plantation establishment, and the plantation was generally maintained with herbicide and hand tools. In addition to the plots placed in these two blocks, locations of the trees in all 28 blocks, present and missing, were mapped to determine patterns of mortality (Figure 3.2). Approximately 30% of the dead saplings were excavated and examined for signs of Armillaria.

Plot 4 was in Cutover 8103 (Block 1.11N) in a 1982 black spruce plantation that became dominated by naturally-seeded jack pine. Overall black spruce mortality was estimated from random transect tallies.

Plot 5, located in a 90- to 100-year-old black spruce stand across a road from the larch plantation in Block 1.1S, was the only non-plantation site. It was selected to determine if trap logs could be used in a mature stand to detect the presence and activity of Armillaria prior to harvesting. Unlike the plantations where the soil was sparsely covered, this stand was carpeted with thick Sphagnum moss, and the water table was visible until late summer. Plots 4 and 5 were untended.

For each plot, all saplings, stumps, traps and rock outcrops were mapped during the summer of 1989. Individual tree health was determined visually. Severely chlorotic or stunted trees were called "unhealthy"; slightly chlorotic or light green were counted as healthy. A multiple planting was counted as one stem, and was called "infected" if consisting of living and Armillaria -killed trees. Representative dead saplings were collected for cultural isolation of Armillaria.

#### 3.2.2. Trap Establishment

The trap log technique was used in each plot to detect Armillaria. Several types of traps

were tested in two of the plots. The Armillaria evaluation was based on black spruce trap logs only. Detailed descriptions of the trap-log preparation and assessment methods were given in Chapter 2. The trap logs were pounded into the soil in all plots except Plot 5 where they were sometimes driven into deep Sphagnum moss.

#### 3.2.3. Cultural Isolations and Identification

Isolation of Armillaria into pure culture was attempted by placing spores, pieces of basidiome, mycelial fans from the traps, RMs, and woodchips from infected saplings on agar media in test tubes and petri dishes. Five isolations were attempted from each source. Because of high rates of contamination by non-basidiomycetes, and various growth rates, different media preparations were tried (Table 3.1). No experimental design was used to determine if any one media was better than another.

Table 3.1. Agar media preparations used for study of Armillaria spp. in culture.

Medium	References
1.25% malt extract agar (MEA)	(Nobles, 1948)
1.5% MEA	(Davidson, Campbell and Blaisdell, 1938)
3.0% MEA	(Dumas, 1988)
1.25% MEA + 25 ppm Benomyl	(after Hunt and Cobb, 1971)
3.0% MEA + 500 ppm 100% ethanol	(after Weinhold, 1963)
1.5% potato dextrose agar (PDA)	
2.0% PDA	
3.9% PDA	
3.9% PDA + .006% orthophenylphenol (OPP)	(Russell, 1956; Whitney et al., 1978)
3.9% PDA + .006% OPP, adjusted to pH 3.6 w	ith 25% lactic acid
4.3% PDA adjusted to pH 3.6	
3.0% MEA + $2.0%$ D-glucose + $0.5%$ peptone	(Adams, 1974)
3.0% MEA + 2.0% D-glucose + 0.5% peptone w	with .003% OPP, adjusted to pH 5.2
3.0% MEA + $2.0%$ D-glucose + $0.5%$ peptone w	vith .006% OPP
3.0% MEA + 2.0% D-glucose + 0.5% peptone w	vith .006% OPP, adjusted to pH 5.0

Isolates were identified by pairing usually diploid cultures with known haploid tester strains as described by Morrison et al., (1985). A positive mating is indicated by a distinct change in the amount of aerial haploid mycelium (Guillaumin et al., 1991; Kile, 1983).

Rhizomorphs were prepared for culturing according to Adams (1974). They were washed in running tap water at 12°C for 6 to 8 hours, treated in 1% or 6% NaOCl for 5 to 10 min, and rinsed briefly (2 to 3 minutes) in sterile water. Aseptically cut pieces were placed on agar media in petri dishes and test tubes.

Petri dishes were sealed with ethanol-rinsed paraffin film, and tubes and dishes were placed at 22 to 26 °C in the dark.

#### 3.3. RESULTS

### 3.3.1. Trap Results

Table 3.2 gives the characteristics and trap establishment description for each plot. The experiment was originally intended to have a ratio of one trap log for every planted tree. This intention failed because of difficulties in determining original planting patterns.

Table 3.2. Conifer mortality in Jack Haggerty Forest plots.

Plot No.	Plot Size (m)	Tree Species <sup>a</sup>	Age <sup>b</sup> (yrs)	No. of Living Trees	No. of Dead Trees	No. of Trap Logs
1	15.0x20.0	Sb,Sw	10	55	13	93
<b>2</b>	10.5x7.5	Ĺ	3	41	7	33
3	10.5x7.5	L	3	31	17	35
4	19.2x17.5	Sb	7	8	0	96
		$P_{j}(v)$	<8	159	3	
5	17.2x10.8	Sb	90-100	31	34	59

a Sb: Picea mariana; Sw: P. glauca; L: Larix laricina; Pj: Pinus banksiana (volunteer).

b Age does not include nursery growth.

Armillaria was found in 15% to 59% of the traps (Table 3.3) indicating its general presence in all plantations. There was a higher occurrence of positive traps in Plots 1, 2 and 3 than in Plots 4 and 5. Some traps had only RMs or minimal mycelium (1-10% log area); others had both RMs and extensive mycelial development (Table 3.3, Figure 2.1). There was no pattern in the frequency of occurrence of mycelium classes or RMs.

Generally, foresters do not examine all dead saplings and residual material to verify the presence of Armillaria. They must rely on a few indicators, and then estimate the potential impact to the site. The potential areas of impact estimated from the distributions of living and dead saplings, residual material, and positive traps are shown in Figures 3.3 to 3.8 (envelope) and summarized in Table 3.4. Residual material is defined as stumps, roots and stems of trees from the previous stand, which could be Armillaria food bases. To estimate the area of impact, an arbitrary radius of 1 m around each indicator was used. This corresponds to a well-

Table 3.3. Number of positive black spruce trap logs according to quality of

Armillaria infection from plots in conifer stands and plantations.

Mycelium class <sup>a</sup>	RMs <sup>b</sup> found?	1 10-yr-old spruce	2 3-yr-old larch	Plot <sup>c</sup> 3 3-yr-old larch	4 7-yr-old spruce/ jack pine	5 90- 100- year-old spruce
0	Yes	6	0	1	2	2
1-10	No	4	4	2	4	5
1-10	Yes	4	1	2	1	4
10-25	No	6	0	3	<b>2</b>	2
10-25	Yes	4	0	0	0	0
>25	No	3	10	6	4	1
>25	$\mathbf{Yes}$	28	4	2	1	1
Total Positive	Traps	55	19	16	14	15
(Percent of No	-	(59)	(58)	(46)	(15)	(25)
Ùncertain	- /	• •	4	. ,	16	$2^{'}$
No. of traps pl	aced	93	33	35	96	59
^						

<sup>&</sup>lt;sup>a</sup>Percentage of intrabark or cambial area occupied by mycelium.

established spread rate of 1 m/yr, and because the majority of saplings occurred within 2 m of a visible potential inoculum base, i.e. stump, slash, or dead sapling. The trap log area and the residual area each overlapped about 50% of the dead sapling area. However, about 34% of the residual material area overlapped only 39% of the positive trap log area.

Table 3.4. Plot area subject to Armillaria impact, as estimated by various indicators.

Plot No. <sup>a</sup>	Plot Area	Are	Area (m <sup>2</sup> ) <sup>b</sup> of Impact		Area (m <sup>2</sup> ) <sup>b</sup> of Impact Area (m <sup>2</sup> ) of Overlap between			verlap
	(m <sup>2</sup> )	Dead Saplings <sup>c</sup>	Residual Material <sup>d</sup>	Positive Trap Logs	Positive Trap logs and Dead Saplings	Positive Trap logs and Residual	Dead Saplings and Residual Material	
1	300	27	128	206	19	80	10	
2	184	21	49	35	12	23	17	
3	184	39	29	30	16	13	15	
4	336	9	180	42	1	23	5	
5	186		93	44	-	23		
Totals	1190	96	479	417	48	162	47	

See text for description of plots.

bRMs: rhizomorphs

<sup>&</sup>lt;sup>c</sup>See text and Table 3.2 for description of plots.

bArea of impact was set at a radius of 1 m from the indicator (see Figures 3.3-3.8).

<sup>&</sup>lt;sup>c</sup>Planted saplings that have died and are still present.

dResidual stumps, dead standing trees and slash; only slash in close contact with the ground, i.e. potential inoculum food base, was tallied.

# 3.3.2. Spruce Plantation Survey

In the spruce plantation where Plot 1 was located, 6.1% of the saplings were dead, including 1.3% that were dead, and 3.4% were severely chlorotic (Table 3.5). Based on an initial spacing of 2.4 by 2.4 m (Finstad, 1982), there appears to have been a 15% decrease in original stocking, exclusive of current mortality. This apparent decrease may be due to failure at the time of planting, or silvicultural problems, such as multiple plantings and injury from hardwood removal activities.

Table 3.5. Mortality in a 10-year-old Picea mariana and P. glauca plantation. a

Survey	Area	Number of saplings					
$Plot^{\mathbf{a}}$	$(m^2)^b$	Healthy	Infected/	Infected	Living	Dead	Total
			Unhealthy	Dead	Cut	-Cut	
1	1610	234	3	7	3	<b>2</b>	249
2	1910	186	7	10	0	2	205
3	1920	284	17	21	1	6	329
Total	5440	704	27	38	4	10	783
% of Total		90	3.4	4.8	0.5	1.3	100

<sup>&</sup>lt;sup>a</sup>Method: Three temporary plots were tallied in June, 1989; see text for definitions. <sup>b</sup>Estimated total released (free-to-grow) area: 33 700 m<sup>2</sup>.

It appeared that most of the dead saplings counted in 1989 were killed in 1988 or 1989. Thus, Armillaria was still active in this plantation after 10 years. Armillaria had attacked apparently healthy saplings, many of which were multiple plants or may have been damaged. During hardwood removal, 1.8% of the saplings still present, including one fifth of the dead saplings, had been wounded by cutting equipment (Table 3.5); many had been cut through the lower stem. The importance of the disease impact relative to the impact from poor planting and tending damage was unclear. Armillaria infections might have been lower with better silvicultural treatment.

### 3.3.3. Larch Plantation Survey

In the larch plantation, 24% of the total 5762 planted ramets<sup>2</sup> were dead (including missing trees) and another 6.5% were symptomatic of Armillaria by November, 1989 (Appendix V).

<sup>&</sup>lt;sup>2</sup> Ramets are clonally produced individuals. For the present site, roots were induced in a greenhouse before planting.

Cumulative mortality within the blocks ranged from 10 to 58% (Figure 3.2). The differences in mortality between provenances and between replicate blocks at the time of survey were not significant (F-distribution, P=0.226 and P=0.105 respectively). The rate of cumulative mortality increase with multiplication (van der Plank, 1963:21) among the larch trees from 1988 to 1989 was 0.27 and from 1988 to 1990 was 0.22 (Appendix VII).

The root systems of 69 of 233 dead ramets representing 16 blocks were examined for signs of Armillaria. Well-developed, prolific Armillaria fans and/or rhizomorphs were found in the roots of 61 trees. Dead ramets with a root collar diameter of >1 cm and half of those <1 cm in diameter had been killed by Armillaria. From above-ground symptoms - chlorosis, resinosus, foliage wilt - 19 unexcavated trees appeared to have died in 1989 from Armillaria root rot. Some saplings showed profuse basal resinosus indicating a vigorous response to infection.

Of the 340 replacement trees planted in 1987, 163 had shed by June 1989; the cause of death in most replacement trees was undetermined. Many had disappeared, and many of the remainder showed little leader growth. Of eight of these trees that were examined, four died of Armillaria root disease. Absence of signs does not mean the fungus was never present; they might have disappeared from the small root systems after the tree died.

Geographically, the mortality appeared heaviest along the southern edge of the study site, bordering on an uncut mixed wood stand. There were no radiating patterns typical of root disease pockets (Figure 3.2). The mortality does not appear to be related to provenances, although Provenance 12 (Big Trout Lake) was the only one to have less than the average 24% mortality in all four replicate blocks.

# 3.3.4. Jack Pine/Spruce Site

In the jack pine/spruce site in Block 1.1N (Plot 4), random line tallies run in the northern-most part of the cutover (3 ha), indicated that current spruce stocking was less than 40%, assuming 2- by 2-m initial spacing. The remaining 17-18 ha were not surveyed as the extent of the plantation was unclear. No dead or symptomatic spruce saplings were found along

the tally lines. All the spruce were much smaller than the naturally established jack pine. For unknown reasons, most of the planted spruce died and disappeared, presumably soon after planting.

# 3.3.5. Root Deformity

Root form was examined on most of the dead plot saplings (Figure 3.9). Moderate to severe root deformity occurred in 60% of the dead larch trees. Seven of the eight dead spruce trees from Plot 1 had slight to moderate root deformity and one was a multiple plant. The three dead jack pines (seeded) from the Block 1.1N cutover had tightly bunched roots, probably resulting from the shallow soil. All three were killed by Armillaria root disease.

# 3.3.6. Results of Cultural Isolations and Species Identification

In addition to the positive traps and infected saplings, the presence of Armillaria sp. was confirmed on Plot 2 in the larch provenance trial by the collection of 12 basidiomes on or within 10 m of the plot. Basidiomes were not found in any other plot. A total of 1195 isolations were attempted from 265 sources (Table 3.6).

Table 3.6. Isolations of Armillaria from various sources in the Jack Haggerty Forest.

Source	Plots	No. of Sources	Successful Isolations
Basidiomes	1	12	7
Trees	1,2,3,4	22	13
Sb traps	All	84	46
Po traps	1,2	34	17
Bark trap bags	1	4	0
Total positive sources			83

Armillaria isolates were obtained from 32% of the sources. From 49 isolates crossed with haploid testers, 41 showed positive matings with NABS I, one was positive with NABS VII, and seven were discarded because of uncertainty or lack of clarity. The NABS VII specimen from a Sb trap log was contaminated with *Penicillium* sp. which may have affected the mating test. It appeared that the test was inconclusive, based on the appearance of mycelium and RMs found in the trap log, and the fact that this Sb trap log was paired with a Po trap from which A.

ostoyae was confirmed.

Using the black demarcation indicator line (Adams, 1974), preliminary crossings of field isolates with each other indicated that several Armillaria genets may be present at each site.

# 3.4. DISCUSSION

This study evaluates Armillaria presence in conifer stands. The potential for Armillaria impact on reforested areas of the Jack Haggerty Forest is discussed from the perspective of a field forester who would have only survey information and general observations, such as the presence or absence of food bases. Other factors such as potential infection from root contact (Morrison et al., 1988), probability of stump infection (Shaw, 1981), and size of residual stump systems (Roth et al., 1977) are not discussed here since they are inestimable without further intensive survey work.

# 3.4.1. Armillaria and Tree Mortality in the Spruce and Jack Pine Stands

Armillaria root rot was present in various levels at sampled locations in the Jack Haggerty Forest. The positive traps in the uncut spruce stand (Plot 5) showed that Armillaria was capable of immediately infecting trap logs even in the often water-logged mossy conditions, a condition in which Armillaria is rarely noted (Redfern and Filip, 1991). It can be expected that disturbance from cutting the stand will stimulate RM production (Shaw and Roth, 1976; Stanosz and Patton, 1991) throughout the site, as has occurred in the larch plantation across the road.

MacKenzie and Shaw (1977) reported a 16% Armillaria kill in a 27-month-old Pinus radiata plantation in New Zealand. They suggested that an additional 38% of trees, which were infected, would die and that the level of infection would undoubtedly rise. In Ontario, Whitney et al. (1989) found that 58% (average) of symptomless trees that surrounded A. obscura-infected symptomatic trees were also infected.

In the jack pine/spruce site (Plot 4), it appears that the spruce has not become established because it is not as well suited to the site as the jack pine, rather than because of the Armillaria

which is present. The current low level of Armillaria -kill among the jack pine is likely an average removal of inherently weaker trees.

#### 3.4.2. Armillaria in the Larch Provenance Study

Armillaria was present unevenly throughout the entire larch family test site and was responsible for 88% of the 1989 mortality, or 3.6% of the plantation. Based on the 1989 survey of the whole plantation, the cumulative mortality of 24% (all causes) could be expected to rise by at least 6%, i.e. those unhealthy in 1989, and probably more. A 1990 survey (G. O'Reilly and R.E. Farmer, pers. comm.), which showed the cumulative mortality to be 27% for the whole plantation, supported this conclusion. Although the selection of trees for examination was arbitrary rather than systematic, it can be concluded that most mortality of trees greater than 1-cm in diameter was due in part to Armillaria. Also, it is important to note the absence of other mortality agents in this plantation. Although Armillaria has not been determined to be the principal stress factor, it was responsible for the ultimate death of many of the trees.

Larch was selected for this provenance test because it generally grows quickly and has few serious pests. Although various species of larch are known to be attacked by Armillaria (Nobles, 1948; Ono, 1970), its susceptibility to damage and potential for loss due to root rot in forest regeneration is not well-known (Howse, 1983). Greig and Strouts (1983) list Larix as likely being resistant enough to infection to ensure planting success in hazardous areas. Armillaria has been reported killing mature Japanese larch in a British Columbia plantation (Molnar, 1962). Larix occidentalis Nutt. had higher mortality due to A. ostoyae than did Pseudotsuga menziesii (Mirb.) Franco, Pinus contorta, and Picea engelmannii Parry in unraked plantations in British Columbia but developed greater resistance after 20 years (Morrison et al., 1988). Zalasky (1958) reported Armillaria associated with the decline of large L. laricina trees in Saskatchewan. No reports of Armillaria on L. laricina in Ontario were found.

With these conflicting reports, it would be wrong to conclude that the disease impact will not decrease as it normally does after the first 5 to 15 years of cutover planting (Beveridge, 1973; Morrison et al., 1988; Sinclair et al., 1987:308). MacKenzie (1987) observed that about one

in three of 270 Pinus radiata D. Don infected by Armillaria in 1976 was uninfected in 1985. The resinosus in the larch trees indicated that while infection was continuing, some trees were resisting attack while others were overcome. Most of the remaining 177 replants that have not developed new shoots will likely die regardless of the presence of Armillaria, because of competition stress (contributing) from weeds and their vigorous neighbors. Many of these replants were included in the 6% of trees called "unhealthy" as they were often stunted, overgrown with weeds, or generally declining.

# 3.4.3. Armillaria Impact According to Trap Results

The trap log area of impact overlapped about 50% of the dead sapling area and 34% of the residual material area. These comparisons revealed that Armillaria was much more prolific than was indicated by the dead saplings and than could be inferred from the residual material. Other studies also have shown high levels of Armillaria infection in non-symptomatic trees (Rykowski, 1981; Whitney et al., 1989). Furthermore, the small overlap between the residual material and positive trap log areas suggested that very little residual material was a source of inoculum. Shaw (1981) found Armillaria in only 18% of thinned Tsuga heterophylla (Raf.) Sarg. and Picea sitchensis (Bong.) Carr. stumps in southeastern Alaska. The positive traps theoretically give a more reliable estimate of the viable fungus distribution than the simple presence of stumps gives since the Armillaria in the traps is living. If positive stumps alone were required to estimate areas of impact (Shaw, 1981), all buried material would have to be examined. Such an examination is beyond the scope of most forest managers' resources.

The estimated areas of impact are dependent upon trap density and selected radius of impact. Therefore, the calculated values should only be used as general guides. It is interesting to note that the spruce and larch plantations had the most mortality, and their plots had almost 60% of the traps positive, while the jack pine/spruce site had the lowest current mortality, and, in its plot, only 16% of the traps were positive. However, replicate test plots are needed to accurately correlate levels of trap infection with current and future mortality. Twery et al. (1990) found that RM abundance in defoliated mixed oak stands was correlated with

distance to dead saplings and stumps (p<0.01, r<sup>2</sup>=0.94), but could not infer anything about the relationship. Soil sample RMs are often used as a measure of disease potential, but some workers feel that *Armillaria* distribution cannot be easily inferred from soil samples (Falck, 1924). In this study, it was not determined whether the rate of infection in the traps reflected the RM density in the soil. In a previous test by the author (Appendix IV), and in a similar test using trap bags (Chap. 4), positive trap distribution generally reflected soil RM occurrence.

# 3.4.4. Factors Affecting Infection

Excepting tree genotype, factors that might have affected Armillaria infection are root form, soil moisture, nearness to inoculum sources, site preparation activities, and species of Armillaria.

Root form. Root deformity was associated with many of the killed saplings. Livingston (1990) showed a close association between lethal Armillaria infections and root deformity in planted spruce. He suggests that container stock is predisposed to root aggregation which increases susceptibility to infection followed by spread within the planted seedling. Ouellette et al. (1971) found root rots (including Armillaria) associated with self-strangled roots of planted white spruce that died. Buckland (1953) noted that all Armillaria -killed Pseudotsuga menziesii in a 10-year-old plantation had malformed roots. Rykowski (1981) found that 75% of diseased trees and 90% of dead trees in a Scots pine plantation had root deformity. He suggested that the planting problems had accelerated the course of Armillaria impact. A study of the larch and spruce root systems should be done to determine if poor root form is predisposing the trees to root rot attack.

Soil moisture. Root rot in natural stands, mostly 30 to 150 years of age, tends to be more severe on upland sites with low moisture regimes than on lower, wetter sites (Whitney, 1976). Soil moisture was not measured in this study. However, among the four plantations in 1989, disease incidence was highest at the larch site, which had standing water in many places during the first half of each summer since establishment (G. O'Reilly, pers. comm.), and lowest at the jack pine site which was noticeably drier and less lush. Also, Armillaria was present in

the saturated moss layer of the mature spruce stand where standing water was present in 1989 until late summer. In Japan, severe disease incidence in larch was associated with a high or perched water table (Kawada et al., 1962). Soil moisture may affect the decay of wood in older living trees differently than it affects the killing of young trees by root rot. The relationship of Armillaria root rot with soil moisture is worth investigating.

Nearness to inoculum source. The highest disease incidence in the larch study occurred in blocks adjacent to the still standing forest. Major infection sources concentrated in this stand may be invading the site. It is generally believed that Armillaria infections in reforested sites originate in residual stumps, etc. (Filip, 1979; Shaw and Roth, 1976; Twery et al., 1990), and for this reason, some potential impact and inoculum studies are concerned with stumps (MacKenzie and Shaw, 1977; Wargo and Shaw, 1985). This approach seems to be more useful on tolerant hardwood cutovers (oak, maple, etc.) than on conifer cutovers (Redfern, 1970; but cf. Klein-Gebbinck et al., 1991). Shaw (1989) reported frequent occurrence of Armillaria spp. in stumps and root systems of older Tsuga heterophylla, T. mertensiana (Bong.) Carr. and Picea sitchensis in Alaska, without corresponding infection in nearby young trees. At the larch site, 37% of the dead trees (n=362) occurred within 2 m of visible stump material, whereas 63% were more than 2 m away. Furthermore, 57% of the living trees (n=489) occurred within 2 m of stumps on the same plots. Although there is likely a strong relationship between buried (unseen) material and larch mortality in the present situation, a forester would be unable to assess hazard to the living trees based on proximity to visible stumps without extensive surveys of individual stump size, species and actual infection.

Site preparation activities. Stump removal has been recommended as a means of reducing Armillaria root disease inoculum hazards on sites with high root disease potential, and has been successfully demonstrated several times (Johnson and Thompson, 1975; Morrison et al., 1988; Roth and Rolph, 1978). However, recent attempts to reduce Armillaria hazard in northwestern Ontario through root raking have resulted in apparently elevated incidence of attack (Canadian Pacific Forest Products Ltd., Unpub. data; Whitney to Palmer, in litt.,

Appendix VI). Although many factors other than root-raking, notably site selection, have contributed to the fungus' activity levels in these sites, the root-raking likely caused some redistribution, and consequently stimulation of Armillaria mycelium in infected residue. The specific areas of the larch site that were root-raked in December, 1985 are unknown. However, this activity may have contributed to the high incidence of Armillaria infections recorded during this study.

Species of Armillaria. Only one species of Armillaria was positively identified from the Jack Haggerty Forest, A. ostoyae or NABS I. This corresponds with Dumas' (1988) findings that NABS I is the most prevalent species in the Ontario mixed wood boreal forest. Its virulence is generally moderate or high towards young conifers (Gregory et al., 1991), and can therefore be expected to attack apparently healthy as well as unstressed trees.

# 3.4.5. Disease Increase Rate

Based on a linear increase (without multiplication) in disease rates, van der Pas (1981) concluded that mortality in planted trees was associated with initial inoculum rather than tree-to-tree spread. Swift (1972), calculating with multiplication, concluded that infection had spread from tree to tree in 8-year-old *Pinus taeda*. Hood *et al.* (1991) advocate caution in drawing conclusions from this statistical method. There were no typical concentric patterns of root disease spread (foci) evident in either the larch or spruce plantations, but early mortality in the spruce site may have shown such patterns. The disease increase rates calculated for the larch in Section 3.3.3 (with multiplication) are insufficient to indicate any trend in the disease progression except for lower mortality in the third year than in the second. In this young plantation, tree-to-tree root disease spread would be enhanced by the close spacing (1.5 m) and ease of infection in deformed roots, but might be limited by food base (small tree size) and block layout. The rates are not necessarily a reflection of root disease conditions as the actual cause of death in trees prior to 1989 was not investigated in this study. Furthermore, since the plantation was only 4 ha, the infectable population was very small, i.e. finite (Manion, 1991:350). With 27% of the trees dead as of November, 1990, it can be expected that there will soon be a leveling-off

phase as the number of living trees decreases.

# 3.4.6. Host Stress vs. Pathogen Virulence

The future disease impact on the larch plantation is difficult to predict without better knowledge of host susceptibility. Root diseases are governed by complex interactions that are difficult to analyze (Hansen and Goheen, 1989). Two predominant concepts concerning Armillaria impact focus on stress-induced changes in host susceptibility (Wargo and Harrington, 1991), and pathogen virulence (Gregory et al., 1991). Some scenarios that a forester could face in the present situation are briefly outlined here, using these two concepts.

According to Manion's (1991:330-334) decline spiral theory, trees are subjected to predisposing, inciting and contributing stresses. In the Jack Haggerty Forest, predisposing stresses might include host provenance (out of natural range), root deformation, and microsite conditions, e.g. soil moisture. Inciting factors are events such as drought or early/late frosts that healthy trees can generally withstand while unhealthy trees cannot. Armillaria root rot can be considered as a contributing stress factor. It could be present as latent infections, presumably invading only when the host is subjected to other stresses. In this role, it would act as a scavenger, removing the weakest members of the population (Kile et al., 1991). If this is the case in the present situation, the trees that die from root rot would presumably die anyway, before maturity.

If no predisposing stress can be identified, it might be concluded that the present pathogen is highly virulent. The abundant stumps in the larch site and the absence of discrete infection centers (Hansen and Goheen, 1989) indicate that the root rot attack has probably arisen from widespread inoculum bases. We might hope that disease incidence would decrease as these stumps decompose (Stanosz and Patton, 1991). However, if this is a primary pathogen attack, and if the root systems in the adjacent standing forest are a major source of inoculum, the Armillaria impact will not end with decay of the residual stumps, and it can be expected that there will be further significant losses.

# 4. ARMILLARIA TRAPPING IN A PINUS KORAIENSIS PLANTATION IN NORTHEASTERN CHINA

#### 4.1. INTRODUCTION

Armillaria root disease is a worldwide disease of natural forests and forest plantations (Guillaumin et al., 1989; Kile and Watling, 1988; Mohammed et al., 1989). Its impact in natural forests has been well studied (Wargo and Shaw, 1985), and explanations of the damage are being refined with the recognition of the intersterile species of Armillaria (Korhonen, 1978). The role of Armillaria in plantations is less clear than in natural forests because of the artificial conditions and the generally short history of large-scale plantations in most countries.

Armillaria mellea has been listed as being an important tree pathogen in 13 provinces of China, generally those north of the Yangtze River (Liu, 1982). It has recently been reported as a major pathogen of Korean pine (*Pinus koraiensis* Sieb. et Zucc.) in plantations of northeastern China (Ju, 1982; Zhang et al., 1989) and in the Republic of Korea (Lee et al., 1987). Nordin (1985) noted that Armillaria caused a significant root and butt rot of *Pinus*, *Picea*, *Betula* and *Populus* at the Langxiang Forestry Bureau in Heilongjiang (47°N, 129°E).

Conifer plantations are being established at the rate of 4.5 million has per year in China (WRI, 1989: Table 18.1). As these forestation projects continue, Armillaria root rot is expected to continue to play an important part. Therefore, it is of great importance to protect the investment of time, money, trees and labor against loss to agents such as Armillaria root rot. Improved knowledge of the distribution of Armillaria in regeneration sites is required.

One evaluation method useful in forest management is called Armillaria trapping. Trapping involves placing a nutrient source, such as logs or bark on which this fungus grows freely, in a forest soil. Such food bases become colonized by Armillaria if it is present in the soil. Traps are then removed, and the distribution of Armillaria is inferred from the traps showing signs such as mycelial fans or rhizomorphs (RM).

Some testing of trap logs was reported for a Prunus sp. orchard in Japan (Aoshima and

Hayashi, 1981), and a natural conifer stand in Alberta (Mallett and Hiratsuka, 1985). Some refinements and variations were tried in Ontario conifer plantations (see Chap. 2).

The trapping method of detecting Armillaria in soil does not require elaborate equipment or a high level of technical specialization. Therefore, the method might be applicable in Chinese plantations where Armillaria occurs but has not been extensively surveyed; where disease control is carried out at the field management level (forestry bureaus) rather than by specialist institutes; and where labor is generally inexpensive compared with technological solutions such as with machinery and chemicals.

This paper reports on the use of the trap bag method as an experimental tool for detecting Armillaria in a forest plantation, and assesses its practicability for forest management in northeastern China.

#### 4.2. METHODS

Three study plots, 18 by 20 m each, were selected in a 25-year-old *P. koraiensis* plantation in Taiping Management Unit, Xinglong Forest Bureau, Heilongjiang (46°30'N, 128°30'E) on which trap bags would be installed. The plantation was on the southeastern aspect of a deep sand hill. The trees were approximately 9 to 11 m tall. Stand maintenance included pruning, but no fertilization, irrigation, thinning or pesticide application had been done.

Nylon screen trap bags were made to hold a 1.6 dm<sup>3</sup> column of bark, 20 cm long. Holes in which the trap bags were later placed, were dug 20-cm deep with a custom-made steel soil-core sampler, 7.5-cm in diameter. Each 1.6 dm<sup>3</sup> of soil was kept for RM assessment. The trap bags were filled with pieces of bark collected from Korean pine and *Larix gmelinii* Rupr. logs at the Taiping Unit sawmill. The logs had been cut up to 1 year before. The filled traps were soaked in rainwater or spring water the night before placing in the soil to ensure uniformly high moisture content in the bark.

In mid-July, 1990, 72 traps were placed in the freshly drilled holes just below the litter layer in each plot at 2- by 2-m spacing, and covered with litter. The locations of the traps,

trees, and stumps were mapped to the nearest 0.25 m. After 10 weeks (October), the traps were removed and opened at the site. The negative traps were disposed of at the site. Positive traps (with visible RMs on or between the bark pieces) were carried to the lab for further examination. All visible RMs were removed from the bark, and lengths were measured to the nearest centimeter if less than 1 m, to the nearest 10 cm if between 1 and 2.5 m, and to the nearest 50 cm if greater than 2.5 m. Soil core samples were sifted by hand, and the RM lengths recorded similarly. Representative macroscopic RM identifications were confirmed in the laboratory by microscopic examination.

#### 4.3. RESULTS

The plots were selected to represent three stand densities (Table 4.1, Figure 4.1a) in an area where mycelial fans and RMs were found on dead trees.

> Table 4.1. Description of plots in a 25-year-old Pinus koraiensis

	plantation in the Taiping Forest Management Unit.					
$\mathbf{Plot}^{\mathbf{a}}$	Living	$\mathbf{Dead}$	Current	Percent	Number	
	Trees	Trees	Density	Estimated	$\mathbf{of}$	
			(No./ha)	Cumulative	Residual	
				Mortality <sup>D</sup>	Stumps	
1	98	21	2700	39	40	
<b>2</b>	68	4	1900	<b>57</b>	22	
3	30	6	800	82	9	

Plot 1 was in an almost pure pine stand with no understory (Figure 4.2). Plot 3 was on a site dominated by Betula platyphylla in the canopy and on which there were thick grass and shrub layers (Figure 4.3). Plot 2 was intermediate between Plots 1 and 3 in stand composition and structure. This plot was split into two subplots because of plantation discontinuity.

Rhizomorphs were found in 48% of the 213 trap bags (Table 4.2). Three traps were spoiled. Total lengths ranged from 0.01 m to approximately 5 m. Most RMs were monopodially branched (Figure 4.4). Some very short pieces were unbranched. Both subterranean and subcortical RMs were found (Figure 4.5). and were easily identified macroscopically. Total lengths of RMs in the 99 positive soil samples ranged from 0.01 to 0.15 m. Identification of RMs from

a Each plot was 18 by 20 m.
b Total mortality assumes original stocking of 4400 trees/ha.

the soil cores was much more difficult than from the trap bags due to the short length, brittleness, and low moisture content of RMs; also to the presence of plant roots, litter and hyphae of other fungi in the soil with which RMs could be confused. Examination by microscope and staining was often necessary (Cairney et al., 1988).

Table 4.2. Armillaria rhizomorphs (RM) in trap bags after

10	) weeks in a <i>Pinus koraiensis</i>	plantation.
Category	RM content (m)	No. of bags
0	0	110
1	0.01 - < 0.50	42
2	0.50 - < 1.0	20
3	1.0 - < 2.5	32
4	>2.5	9
N.A.		3

The lengths of RMs in trap bags were unrelated to the presence, absence or lengths of RMs in the soil samples. The average RM length in the traps where no soil RMs were found was 1.10 m (n=46, S.D. 1.04) and in traps coinciding with positive soil samples was 0.96 m (n=56, S.D. 1.10).

The distribution of positive traps within the plots is shown in Figure 4.1b. There were no distinctive patterns with respect to total RM length and distribution, but some features were notable. The traps with >1.0 m of RMs seemed to be in closer association with other positive traps than did those with <0.5 m, suggesting a possible relationship between the extent of RM growth in the trap bag and the spread of the fungus in the soil. Disease pockets did not appear to be closely related to the lengths of RMs in traps. While this suggestion is not supported by the RMs found in the soil cores (Figure 4.1c), it is not negated by the data. The soil-core RMs never exceeded 15 cm in length, and did not have a similar variation to those in the traps making correlation determinations difficult.

The distribution of Armillaria according to the RMs found in the soil samples is shown in Figure 4.1c. The presence of RMs in the traps does not appear to be closely related to the incidence of RMs in the respective soil cores. Only 49 of the 103 positive traps coincided with soil cores having RMs. However, a comparison of Figures 4.1b and 4.1c indicates that the gen-

eral presence of Armillaria RMs in the site is reflected by the distribution of positive traps. Both methods show a high occurrence of RMs (>50% of traps positive) in sections of Plots 1 and 2a, and a moderate (40%) occurrence in Plot 3. They also both indicate 6-m wide areas of low occurrence (<20%) in the center of Plot 1 and in one corner of Plot 3.

#### 4.4. DISCUSSION

The primary objectives of the work were to test the effectiveness of the trap bag method for detecting *Armillaria* that is inconspicuous in the forest, and to determine the practicality of using trap bags in regular forest management.

This study has conclusively shown the superiority of the trap bags over soil core samples for detecting Armillaria RMs. In the 60% of the positive traps that had >0.50 m of RMs, determination of Armillaria presence took only a few seconds (Figures 4.6 and 4.7). Even in traps with <0.50 m of RMs, the RMs were usually visible after opening the bag and shaking the bark loose. Persons with little or no experience in identifying RMs learned to recognize the fresh abundant RMs in the trap bags rapidly and confidently. The traps with <0.10 m of RMs required careful examination, but even the short lengths of RMs were more easily recognized (Figure 4.8) than the samples from the soil cores. Identification of soil core RMs required experience in recognizing hyphae, wood fibers, RM cortex, plant roots and bark as well as interpreting staining results. The soil cores required at least 1 min to be sifted by hand and, often, verification in the lab required another 5 to 20 min of work per sample, plus packaging and transporting time.

The initiation of RM growth is closely tied to nutrient availability (Garrett, 1953). The growth of RMs in traps where none were found in the soil indicated that the bark can stimulate unseen Armillaria fragments that may be semi-dormant to a highly active state. There may have been other semi-dormant viable RMs that were not activated during the 10-week interment period. The timing of the trap setting needs to be standardized by determining if a different start date or a longer period of interment can increase the number of positive traps.

Comparing Plot 3 in Figures 4.1a and 4.1b indicated some interrelationship between RM lengths and the current distribution of trees. However, the nature of this relationship was not investigated. Multivariate analysis and multiple regressions may elucidate these relationships but such work would require many more sample plots.

The exact lengths of RMs are probably not related to Armillaria spread patterns in the forest. However, general categories of lengths may be related. Mallett and Hiratsuka (1985) suggested that intra-positive trap association represented territorial patterns of the fungus. The relationship of inoculum potential to RM intensity in the traps (Table 4.2) should be investigated, i.e. are Category 3 and 4 sites more hazardous (higher expected mortality) than Category 1 and 2 sites? In the present study, it is sufficient to note that there is generally a low incidence of RMs in stand openings >6 m wide where there are no trees.

Chlorotic or dead trees may indicate the presence of Armillaria (Intini, 1989; Whitney, 1988). However, they often do not reflect the actual level of infection (Roth et al., 1980; Rykowski, 1981; Whitney et al., 1989). This study provides further evidence that dead trees do not accurately indicate the presence of Armillaria in the soil. While 71% of the dead trees occurred less than 2 m from positive traps, 58% of the positive traps were more than 2.5 m from any dead trees. This lack of an obvious relationship between the positive traps and the dead trees may be in part due to the ecological behavior of this species of Armillaria, as well as the single point-in-time observation.

The biological species of Armillaria encountered here has not yet been determined. However, all branched RMs had the monopodial branching pattern, suggesting that this is not a highly pathogenic species (Morrison, 1989). Most of the dead trees were understory or suppressed trees, suggesting that the present Armillaria sp. was acting as a saprophyte or a secondary pathogen. The relationship of the present species to actual tree infection should be confirmed.

It may be possible to study the relationship of Armillaria to changes in composition and tree distribution in a stand by using the trapping technique periodically during a rotation. The

natural thinning thus far has resulted in very uneven distributions. In Plots 2a and 3, fast-growing species that invade the openings increase the competitive stress on the pines. The role of Armillaria in changing the species composition at this site is unclear, but it may be similar to that of Phellinus weirii (Murr.) R.L. Gilbertson in Oregon forests (McCauley and Cook, 1980). There, vegetation diversity increased as mortality due to the fungus increased. Armillaria root rot is not thought to be a serious problem in the Taiping Management Unit (Y.Q. Miao, pers. comm.). Although the estimated mortality levels in Table 4.1 seem high, the current densities in Plots 1 and 2 were not low for a young korean pine plantation (Shim et al., 1985). It is more important, at present, to examine the distribution of the remaining trees. Good management entails removing diseased trees that could lead to further infection and that are also occupying growing space that could otherwise be used by healthy trees (Boyce, 1961:513).

In some areas, trap logs may be easier and more economical to use than trap bags. The trap bag method may be impractical for regular forest management when using the present prescription. Although it is simple, it is labor-intensive. The four major labor requirements are making the trap bags, collecting and preparing the bark, transporting the filled traps, and coring the soil.

Making the trap bags would be relatively cheap if done on a large scale, and commercial production would reduce the work for foresters wanting to use them. The collection and preparation of the bark was laborious and inefficient in this test. If chipped bark from a mill could be used (Chap. 2), a supply of bark could be stockpiled with which bags would be easily filled at convenient times. The only difficulty then would be transportation. During the present study, the 216 filled traps, weighing approximately 80 kg, had to be carried manually from the field station to the plot site, approximately 1.5 km. Unless mechanized transportation is readily available, this amount of work seems unreasonable, particularly as the distance and number of traps increase.

Finally, any method of soil sampling requires a lot of time and effort in procurement and analysis (Stanosz and Patton, 1991). Digging the trap holes with a soil-core sampler is particu-

larly difficult in heavy soils. At our study site, coring the soil was only moderately difficult because the soil was sandy and generally free from obstructions. However, both the work and the trap layout can be hampered by the presence of buried rocks, roots, etc. (Chap. 2 and 3). Trap holes made with a shovel would probably not be suitable because sizes would vary. Also, close contact between the soil and the trap, which is necessary for efficient trapping, might not be ensured.

If the problems of bark acquisition and transportation can be overcome, the difficulty of soil coring may be acceptable. Because of these problems, the trap bag method may not yet be feasible for general use, but could be considered for disease monitoring on high-value sites, e.g. seed orchards and provenance tests.

#### 5. GENERAL CONCLUSIONS

Armillaria trapping can be defined as placing a selective, removable substrate in the soil for infection by viable rhizomorphs. If the fungus is present at the trap site, it rapidly colonizes the trap substrate. Within one growing season, the traps can be removed from the soil, and the distribution of Armillaria can then be inferred from the pattern of positive (infected) traps.

The guiding hypothesis in this series of studies was that the technique of Armillaria trapping could be used in forest management to monitor and evaluate Armillaria root rot hazard before the disease appeared at serious levels.

# 5.1. ARMILLARIA TRAPPING METHODS

Trap logs and bags prepared according to the methods given were found effective in detecting Armillaria where aboveground indicators were absent - fruiting bodies; stumps and slash with identifiable RMs, mycelium or decay; or infected trees. It appears that spruce trap logs are preferable to poplar for certainty of identification. However, results indicated that the Armillaria distribution can be determined by either species of trap log. Additional refinement of the preparation of logs can be expected to improve consistency of results.

The work supports the conclusions of Mallett and Hiratsuka (1985) that the trap log method can be used to determine *Armillaria* distribution. It also provides refined prescriptions of trap log preparation and interpretation. For example, smaller logs, 7-cm diameter by 40-cm long can be used, rather than logs 10 cm by 100 cm.

Spacing between traps should probably vary according to site conditions. For broad application, it appears that 1 by 1-m spacing (Mallett and Hiratsuka, 1985) is unnecessarily intensive while 5 by 5-m spacing (Aoshima and Hayashi, 1981) may be too spread out except for general detection. The range of 1.5 to 2 m used between traps in the present study is suited to typical plantation spacing and Armillaria spread rates. The forest manager must decide whether the amount of area covered warrants the extra work required.

Bark bags appeared to be superior to logs and potato tubers as traps. Although they

require more preparation and placement work than trap logs, they are more uniform, more sensitive, and the results are easier to interpret. Also, using standing trees to make trap logs might conflict with other management objectives, particularly if large numbers of traps are required. Further testing should be done to compare other bark and log species, species of *Armillaria*, and optimal duration of emplacement.

# 5.2. IMPACT OF ARMILLARIA AT THE JACK HAGGERTY FOREST

The use of Armillaria traps in conjunction with mortality surveys at the Jack Haggerty Forest has shown that Armillaria was present and active in all plantations examined. Use of the traps has shown that the area likely to continue being affected by Armillaria was not wholly inferable from traditional indicators i.e. residual stumps and dead planted trees. This supports other workers' conclusions (e.g. Whitney et al., 1989). The positive traps in the mature uncut stand at the Forest proved that viable inoculum was present there also. It can be expected that harvesting the stand will stimulate RM activity, and that subsequently planted trees may be attacked similarly to those in the larch plantation less than 50 m away.

It is not recommended to discourage planting in cutovers just because Armillaria is present, such as in the three cutovers examined in the Jack Haggerty Forest. However, preventive silvicultural treatment (stump removal) may be prohibitively expensive or even ineffective. Some tree species are generally more resistant to root rot than others but, at present, it is not possible to recommend certain species as being resistant since the preferred reforestation species, spruce, larch and jack pine, were all attacked to some extent.

The study has revealed that much of the mortality at the Forest was associated with poor root form. Roots deformed during seedling and ramet production or establishment may lead to self-girdling, wounds, poor stability and inhibited nutrient absorption, all factors which may increase the likelihood of pathogen infection.

It has been amply demonstrated that root deformation leads to declining vigor and subsequent infection (Boyce, 1961:515-516; Buckland, 1953; Livingston, 1990; Rykowski, 1981). The

association of fatal Armillaria attack with root deformation could be experimentally investigated using the trap technique to select a site with uniform RM distribution.

# 5.3. ARMILLARIA TRAPPING IN A PINE PLANTATION IN NORTHEASTERN CHINA

The study in China showed that trap bag RMs were easily identified by workers inexperienced in the assessment of Armillaria root rot, whereas RMs from soil cores were difficult to identify, even for experienced workers. The trap bag method proved successful in determining the presence of viable Armillaria RMs throughout a plantation where only a few dead trees showed signs of the disease. To make the results directly useful to foresters, the relationship between tree mortality and results from the traps over time should now be investigated.

The trap method has potential for study of Armillaria spread patterns. Comparing current stand conditions with Armillaria distribution may elucidate the ecological role of this fungus in conifer plantations of northeastern China. This should be investigated further.

It is unclear just how important Armillaria root rot is in Chinese forests generally. This technique should be applicable in the management of high-value plantations and in determining disease distribution in China and other countries. Several problems concerning labor requirements need to be solved before the method is applicable in general forest management in China.

# 5.4. APPLICATION OF ARMILLARIA TRAPPING IN FOREST MANAGEMENT

The role of forest pathologists in enhancing plantation health is one of cooperation with forest managers. Pathologists should be available for consultation before plantation establishment, and should be able to demonstrate the value of good management objectives such as avoidance of high hazard areas, ensuring healthy root development, maintaining tree vigor, and adherence to species - site criteria.

It is the author's opinion that while further research is necessary to protect forests against losses to disease, enough research results are available to improve planted tree survival under current conditions. Studies based on basidiomes and tree mortality have already shown that

Armillaria is present in virtually all temperate and boreal forest ecosystems. The trap technique may be used to precisely determine the prevalence of this fungus. Such information could then be used to manage plantations more effectively beginning at the time of plantation establishment.

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# APPENDIX I

# PLANTATION LOSSES DUE TO ARMILLARIA ROOT ROT FOR VARIOUS CONIFERS AND AGES

The following mortality levels have been reported in plantations affected by Armillaria in various countries around the world. An extensive reference list is given in Hood et al. (1991:Table 9.1).

Species	Mortality	Plantation Age	Investigators
Pinus resinosa	27%		Anon., 1970-1983 (FIDS)
P. radiata	40-50% (cumulative, 78-96% of which might be Armillaria)	5	Beveridge, 1973
P. resinosa; P. strobus P. resinosa	29%; 55% 28%; 26%	21; 31 14; 18	Huntly et al., 1961
Abies balsamea	9% in 10th year	10	Livingston et al., 1982
P. radiata	16% (+28% infection)	2	Mackenzie and Shaw, 197
Larix leptolepis	204 trees/ha	2	Ono, 1970 (37 plantations)
P. resinosa	12-37% (cumulative)	10	Pronos and Patton, 1977
P. sylvestris	3-16.5%	2-5	Rykowski, 1981
P. radiata(?)	24-38%	2.5	Roth et al., 1979
Abies balsamea Picea glauca Pinus resinosa	4.8% 3.0% 3.3%	10 10 18	Singh and Carew, 1971
Pinus ponderosa	21% (cumulative) 9% (current annual)	<b>6</b> 8	Weiss and Riffle, 1971
Picea glauca P. mariana Pinus banksiana P. resinosa	1.4% (current annual) 1.5% 0.5% 2%	6-20 7-20 6-21 6-21	Whitney, 1988

# APPENDIX II

# PRELIMINARY TRAP LOG TEST: LOG SPECIES COMPARISON

The following project was carried out between July 29, 1987 and July 20, 1988 at the Canadian Forestry Service Insectory, Sault Ste. Marie, Ontario.

Approximately 7.5 m<sup>3</sup> of conifer bark chips from a roadside pile where *Armillaria* rhizomorphs had been found were transported from Cochrane, Ontario to Sault Ste. Marie, Ontario. The bark was deposited on two sheets of plastic in an open field, in 4.3 by 4.3 by 0.3 m piles. Rhizomorphs were distributed evenly and profusely throughout the bark piles. Twelve black spruce (Sb) and 12 poplar (Po) trap logs were placed alternately in each pile at 0.8 m intervals.

Three times during the study period, one pile was watered with approximately 350 L of simulated acid rain at pH 2.6 and the other pile was watered with pH 5.6 water.

Two trap logs of each species were pulled from each pile after 14, 35, 39, 46 and 52 weeks. All the Sb logs pulled out at 14 weeks were colonized by *Armillaria* as were all those that were pulled out later. None of the Po logs were colonized after 14 weeks. After 39 weeks, half were colonized, and all were colonized by the 52nd week. No differences in infection were noted between the two pH levels (ANOVA for pH, n=12: Sb P=0.066; Po P=0.770).

#### APPENDIX III

#### PRELIMINARY TRAP LOG TEST: POPLAR LOGS IN A RED PINE PLANTATION

In August 1986, a test of the trap log method for detecting Armillaria in soil was conducted in a 7-year-old red pine (Pinus resinosa) plantation, about 50 km northeast of Sudbury, Ontario (46°42'N, 80°26'W). Armillaria rhizomorphs (RMs) were known to be present at this site.

Twenty 60-cm long poplar (*Populus grandidentata* Michx.) stakes were cut and sharpened at one end. Two 3-cm strips of bark, 30 cm long, were shaved from each stake so that each log had approximately 120 cm in length of exposed phloem - cambium. The logs were driven 20 to 30 cm into the ground at 1-m intervals in a 3- by 4-m grid. In July, 1987, all the trap logs were excavated or carefully pulled out. Mycelium and RM lengths were measured. After surface signs were noted, all the bark was removed and cambial or intrabark signs were noted. Measurements were made on below-ground areas only as no signs were seen above ground level.

Mycelium and RMs were present on 18 and 14 logs, respectively. Mycelium or RMs were visible on the surface of 15 of the logs and uncertainly apparent on two others. The two logs that were uncertainly identified in the field were found to have mycelial fans on 6% and 42% of the cambial surfaces and no RMs. Of the three logs with no infection apparent in the field, none had RMs attached and one had mycelial fans over 1% of its surface. The other 15 logs had an average of 180 cm of attached RMs and 42% of the surface area occupied by mycelium.

The intensity of mycelial development coincided approximately with the proliferation of RMs. Five of the nine logs with >40% of the cambium covered with mycelium were five of the six logs with >2 m total length of attached RMs (highly infected). The six traps with 0-11% mycelial development had 0-11 cm of RMs attached (lightly infected). Each highly infected trap was within 1.5 m of another highly infected trap. The lightly infected logs were similarly grouped together.

There were ten red pines in and around the plot. In August 1986, one was dead with

Armillaria. By July 1987, another had died with Armillaria infection. The traps nearest to these two trees were in the least infected corners of the plot. The other eight trees all had RMs in the soil around their roots and five had RMs attached. The root collars on six of these eight trees were 50 to 100% covered with resin and/or lesions. There were no above-ground symptoms of infection except in the tree that died.

Two 5-dm<sup>3</sup> sifted soil samples from the plot had 35 cm and 10 cm of RMs/dm<sup>3</sup> of soil. No RM relationships could be discerned with soil horizons, soil moisture content, or proximity to positive trap logs or infected trees.

The presence of RMs throughout the plantation, the positive results on most of the trap logs, and the proximity of the dead pines to the least infected logs suggested that the lack of correlation between trap log infection and tree infection were due to *Armillaria* host suitability. This conclusion is supported by the colonization of fresh spruce logs by *Armillaria* in a bark pile after 14 weeks while fresh poplar logs remained uninfected (Appendix II).

#### APPENDIX IV

# PRELIMINARY TRAP LOG TEST: SPRUCE LOGS IN A JACK PINE PLANTATION

#### INTRODUCTION AND METHODS

In November, 1987, a modified Armillaria trap log test was initiated in a 7-year-old jack pine plantation some 30 km north of Sault Ste. Marie, Ontario (46°52'N, 83°57'W). The purpose of the test was to investigate the relationship of soil RM density to trap log colonization by Armillaria.

Ninety black spruce trap logs, freshly cut from living trees, were set in a 13- by 25-m plot at approximately 1.7- by 1.7-m spacing. There were 90 living and 8 dead jack pines on the plot. Eighteen 5- by 5-cm spruce lumber stakes were also placed at 1.7-m spacing.

The trap logs and lumber stakes were removed in August, 1988. To estimate Armillaria rhizomorph (RM) density, soil samples, approximately 2.5 dm<sup>3</sup> each, were dug at locations between the trap logs. Trap logs were assessed by measuring RMs growing on or in the bark, and mycelium growing in the bark or cambium. Locations of the jack pine, residual stumps and positive trap logs and soil samples were mapped to examine their interrelationships. Soil RM density was measured according to Wargo et al., (1987).

### RESULTS AND DISCUSSION

A total of 175 dm<sup>3</sup> of soil from the 325 m<sup>2</sup> plot was sifted by hand. RMs were found in 41 of the 74 soil samples. The average RM density in the samples with RMs present was 10.2 cm of RM/dm<sup>3</sup> of soil (S.D. 10.2, maximum 30.9).

Thirty-three of the 90 trap logs were positive, i.e. had Armillaria mycelium and/or RMs in the bark. The distance relationships between positive and negative trap logs and the positive and negative soil samples, respectively, are summarized in Table A4.

There were 47 residual stumps on the plot, of which five had above-ground surface areas  $>0.3~\text{m}^2$ . With respect to stump presence only, there did not appear to be a relationship with RM density or trap log success. Stump species, quality of the stumps, and presence of *Armillaria* 

Table A4. Average distances between trap logs and soil sample locations with and without Armillaria present.

Between	n	Mean distance	Standard deviation	Maximum distance
Any trap + nearest soil sample	90	0.9	0.4	2.7
Positive trap + nearest positive soil sample	33	1.5	0.8	3.6
Positive soil sample + nearest positive trap	41	1.6	0.7	2.9
Negative trap + nearest negative soil sample	57	1.4	0.7	2.7
Negative soil sample + nearest negative trap	33	1.0	0.6	2.7
Negative trap + nearest positive soil sample	5 <b>7</b>	1.4	0.8	3.5

in the stumps were not investigated. Armillaria was found at varying levels, both near to and away from stumps of all sizes.

Positive soil samples were collected within 1.1 m of seven of the eight dead saplings. Average distance from each dead sapling to the nearest positive trap was 1.7 m (S.D. 0.9, maximum 3.3 m).

The numbers and distribution of positive trap logs generally reflected the Armillaria distribution according to soil samples with RMs. Armillaria was present within an average of 1.5 m of each positive trap, but also within an average of 1.4 m of each negative trap. These values indicate that the positive traps were not any more likely to reflect soil RMs nearby than an absence of RMs. However, determining the relationship of positive traps to Armillaria inoculum in the soil was complicated by several factors. (1) The sample locations were offset from the traps. (2) The pattern of sample locations was irregular, i.e. traps were not equidistant from the soil sample locations. (3) The viability of the soil sample RMs was undetermined whereas the Armillaria in the traps was obviously viable. (4) The soil samples were irregular sizes, and were all larger in size than the space occupied by the trap logs, which would influence the chances of finding RMs in them.

Wargo et al. (1987) and Hood and Sandberg (1987) regard RMs from soil samples as being representative of the true distribution. Falck (1924) believes that Armillaria distribution is difficult to determine based on RMs from soil samples. This author believes that disease potential estimated from the simple presence of RMs in the soil may be misleading since RM viability may be uncertain (Hood and Sandberg, 1987).

Mathematical analyses of the relationships between soil sample RMs, trap success, stump distribution, dead sapling distribution, and site would help to clarify the usefulness of these various indicators for assessing Armillaria importance (Bruhn et al., 1989; van der Pas, 1981). However, many more replicate plots would be necessary for a useful analysis. In future investigations, soil inoculum viability should be determined (Hood and Sandberg, 1987; Johnson and Hawksworth, 1977). Also, soil samples should be uniform in size and collected from the locations where the traps are set.

### APPENDIX V

Summary of mortality in larch family trial by replicate and provenance. a

Rep.	Prov.	Living	Dead	Unhealthy	Total	%Mortality <sup>b</sup>
1	1	178	62	32	240	26
1	5	146	94	31	240	39
1	6	165	75	27	240	31
1	7	188	52	25	240	22
1	9	211	29	31	240	12
1	10	139	101	20	240	42
1	12	186	39	4	225	17
2	1	147	93	19	240	39
2	5	189	51	24	240	21
2	6	183	49	10	$\boldsymbol{232}$	21
<b>2</b>	7	174	65	11	239	27
2	9	185	42	6	227	19
2	10	204	23	9	227	10
2	12	160	40	13	200	20
3	1	165	64	13	229	28
3	5	181	37	12	218	17
3	6	171	28	3	199	14
3	7	200	21	15	221	10
3	9	189	36	9	225	16
3	10	135	23	7	158	15
3	12	138	26	6	164	16
4	1	120	45	11	165	27
4	5	83	117	7	200	59
4	6	97	36	<b>2</b>	133	27
4	7	145	32	5	177	18
4	9	167	<b>57</b>	12	224	25
4	10	59	26	2	85	31
4	12	79	15	6	94	16
Totals		4384	1378	372	5762	24

aSee Figure 3.2 for replicate and provenance layout.
bCumulative to November, 1989. Cause of mortality from 1986-88 was not determined; Armillaria caused at least 88% of the 1989 mortality.

Mean m	ortality	(M) by prove	enance (P	') as a perc	entage of	trees pla	nted.
P	1	5	6	7	9	10	12
M	30	33	23	19	18	24	18

Significance of mortality associated with provenance and replicate block.

Source	D.F.	S.S.	M.S.	Test Statistic	Reference Distribution	P
Provenance	6	962.0	160.3	1.752	F <sub>6.10</sub>	0.2256
Replicate	3	639.8	213.3	2.331	${}^{ m F}_{6,18} \ {}^{ m 6,18}_{3,18}$	0.1050
Error	18	1647.4	91.5		3,10	
Total	27	3249.3				

#### APPENDIX VI

### PERSONAL COMMUNICATION, in litt.

Philip M. Wargo Northeastern Forest Experiment Station USDA Forest Service 51 Mill Pond Road Hamden, CT 06514

David Ip
School of Forestry
Lakehead University
Thunder Bay, Ontario
Canada P7B 5E1

June 7, 1989

Dear David,

Our results with potato tubers last summer verified the previous summer's experience, i.e. potatoes can be used as a trap substrate for an inoculum potential measure of Armillaria in forest stands. We did find, however, that new (this season's) potatoes work best. They are more readily colonized in a shorter period of time than last years tubers (25-30 days vs. 40 to 50 days), they rot less, and they don't sprout which results in immediate deterioration of the tuber. That creates a minor problem in getting new potatoes. We had some shipped from our southern states until the new crop was available here.

So far we have only tested the tubers in hardwood stands. We will use them this summer to estimate inoculum in some Christmas tree plantations recently established on former mixed hardwood conifer sites.

I appreciate your concern using only one substrate. We also used two in our studies: potatoes and oak sampling stakes. Our soils were extremely rocky and we used an irregular grid. That did not seem to cause us too much trouble but we were mostly measuring inoculum vigor, not density or frequency of occurrence.

Hope your work goes well and please feel free to call if you want to discuss any of this.

Sincerely yours,

(signed) Philip M. Wargo Research Plant Pathologist

### PERSONAL COMMUNICATION (Memorandum extract)

R.D. Whitney, Forest Pathologist Great Lakes Forestry Centre P.O. Box 490 Sault Ste. Marie, Ontario P6A 5M7

Ms. Lynn Palmer
Ontario Tree Improvement Council Coordinating Officer
School of Forestry
Lakehead University
Thunder Bay, Ontario P7B 5E1

September 11, 1989

Armillaria Root Rot in Ontario Seed Orchards - A Status Report, September 9, 1989

In May 1988, excessive killing by Armillaria root rot was noted in black spruce seed orchards at Goody Lake South (Sioux Lookout) and Ferguson Township (Ignace). Accumulated mortality amounted to 12 to 15% of trees at GLS and 15 to 20% at FT. Precise figures were not available because of removal of some dead trees prior to inspection. Some dead trees at GLS were not infected by Armillaria sp. and appeared to have been killed by something else. Most dead and chlorotic trees examined at FT contained Armillaria root rot, and appeared to be ultimately killed by this fungus. The levels of infection and tree killing by this root disease seemed excessive compared with those found in routine reforestation black spruce plantations in Ontario (Whitney, 1988). Accumulated initial mortality of 13% was found, however, in a 10-year-old black spruce plantation at Oly Lake, near Longlac, Ontario, and the annual mortality due to this root rot averaged 4.8% per year in a plantation near Kennedy Creek in Wawa district. The annual rate of mortality at GLS and FT have been difficult to establish due to removal of dead trees and because root rot was not responsible for all tree deaths, necessitating root examination for diagnosis, which was not done as dead trees were removed.

R.D. Whitney

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### APPENDIX VII

## DISEASE INCREASE RATES IN THE LARCH PLANTATION, 1987-1990

Disease increase rate =

 $\log_e({\rm Year}~2~{\rm mortality/survival})~{\rm -log}_e({\rm Year}~1~{\rm mortality/survival})$ 

Number of years between Year 1 and Year 2

A correction factor was applied since the infectable population of trees was finite. The 1987 data could not be used because replacement planting was done that year, and thus the plantation was considered to have 0% mortality. (Source: R.E. Farmer and G. O'Reilly, School of Forestry, Lakehead University; Method: Manion, 1991:350; van der Plank, 1963).

Plot	1987-88	1988-89	1989-90	1988-89	1988-90
Number	Mortality	Mortality	Mortality	Cumulative	Cumulative
	Rate	$\mathbf{Rate}$	Rate	Mortality	Mortality
				Rate	Rate
I 01	-1.52	-1.24	-1.14	0.36	0.24
I 05	-0.62	-2.56	-1.42	0.17	0.11
I 06	-1.26	-1.18	-1.03	0.42	0.28
I 07	-1.73	-1.21	0	0.34	0.32
I 09	-2.59	-0.16	0	0.69	0.57
I 10	-0.94	-1.37	-1.58	0.41	0.25
I 12	-1.52	-3.08	1.12	-0.07	0.06
П 01	-0.49	-2.45	-0.95	*	*
II 05	-1.52	-2.38	-0.70	-1.43	-0.62
П 06	-1.32	*	*	*	*
II 07	-1.10	-2.38	*	-3.50	-1.75
II 09	-1.58	-2.31	*	-3.50	-0.90
II 10	-2.20	-1.28	-0.42	-1.28	-0.37
II 12	-1.82	-0.94	-0.72	-0.77	-0.19
III 01	-0.94	-2.23	0.86	-2.53	-0.52
III 05	-1.90	-1.58	0	-1.28	-0.34
III 06	-2.09	-1.09	-0.71	-1.38	-0.43
III 07	-2.44	-1.03	0.30	-2.15	-0.25
III 09	-2.44	0	0.63	-0.14	0.56
III 10	-1.90	-1.99	1.58	-2.00	-0.09
Ш 12	-1.99	-1.90	-0.70	-1.90	-0.74
IV 01	-1.20	-2.68	0.95	-2.68	-0.69
IV 05	0.08	-3.97	0	-3.02	-1.33
IV 06	-1.99	0.09	-0.68	0.09	0.30
IV 07	-1.82	-1.66	0	-1.66	-0.47
IV 09	-1.82	0.08	-2.86	0.23	0.15
IV 10	-1.15	*	*	-2.74	-1.37
IV 12	-2.09	-0.66	-0.43	-0.35	0.05
Overall		-1.38	-0.25	0.27	0.22

<sup>\*</sup> Not calculable.





Figure 2.3. Planted spruce tree with Armillaria mycelium in the roots.

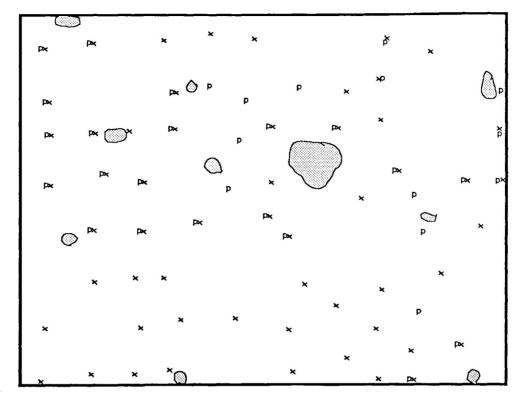


Figure 2.2a Distribution of postive trap logs in Plot 1. x: black spruce; p: poplar

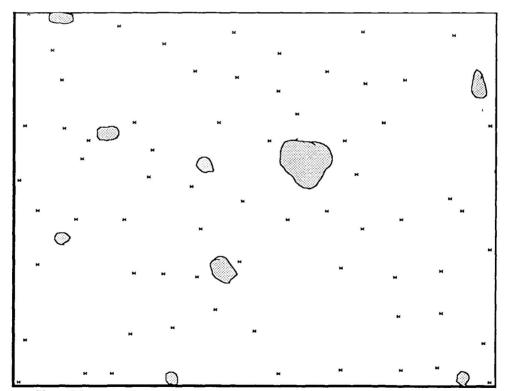


Figure 2.2. b. Distribution of positive trap bags in Plot 1. The distribution of positive trap bags was just as dense or even denser than that of the trap logs.

Figure 2.2. Plot 1. Distribution of positive <u>Armillaria</u> trap logs and trap bags.

Logs and bags both indicated that virtually the whole plot was occupied by Armillaria.

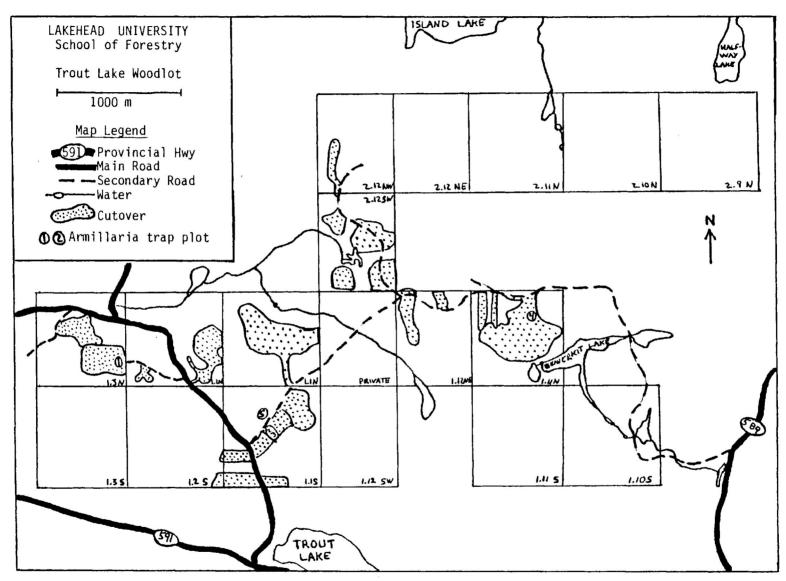


Figure 3.1. Lakehead University Woodlot (Jack Haggerty Forest) showing approximate locations and sizes of forest areas cut between 1972 and 1989, and locations of Armillaria trap plots. (Drawn by D.W. Ip according to previous maps by C.R. Birston (1979), R. Pickard (1982), and a GIS map by R. Pickard (1989).)

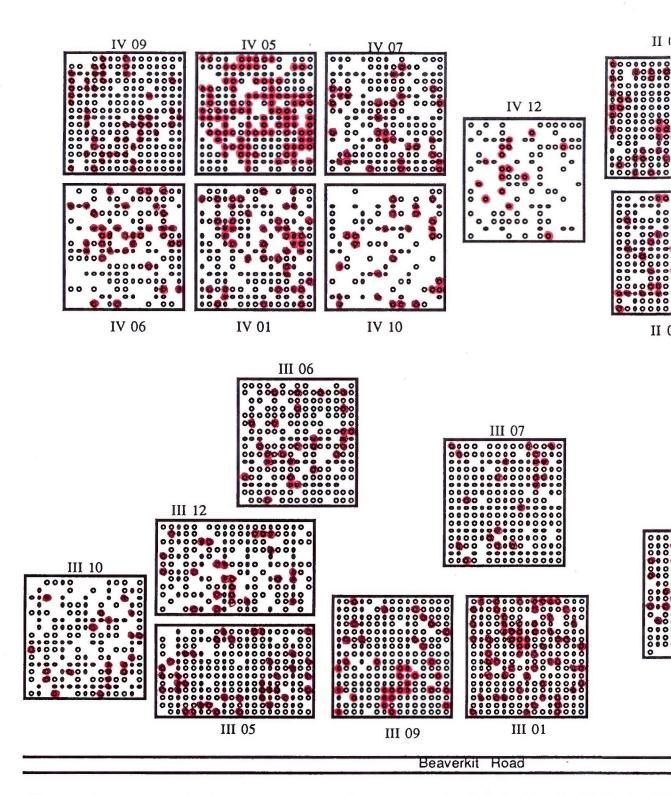
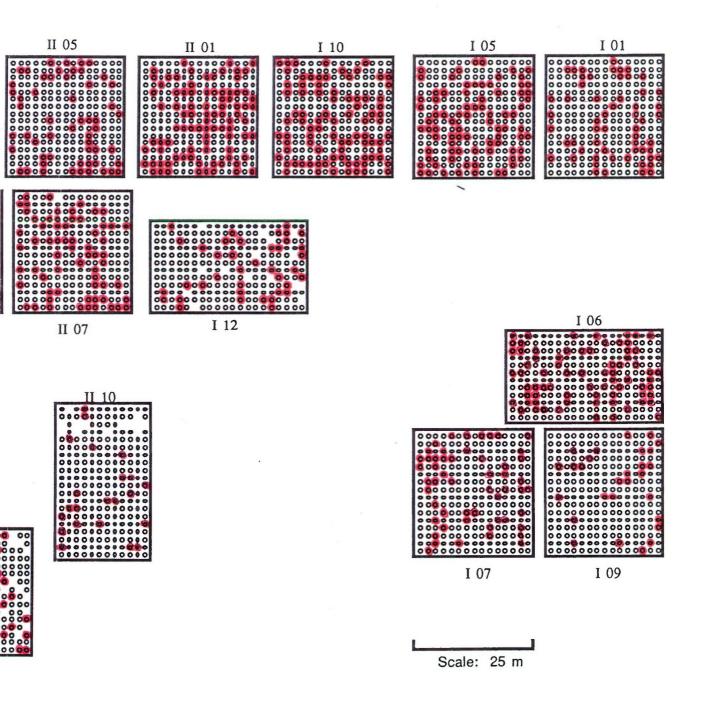


Figure 3.2. Cumulative mortality in a 3-year-old Larix laricina far 1378 were dead (red) after 3 years. There were no radiating patter appeared to be random among replicate blocks (I,II,III,IV) and provided test; there were insufficient trees to fill every block. (So R.E. Farmer, School of Forestry, Lakehead University.)



Island Lake Road 300 m ->

rial at the Jack Haggerty Forest. 5762 trees were planted in 1986; mortality, typical of root disease in natural forests. Mortality (01,05,06,07,09,10,12). The trial was designed as a randomized 1989 Mortality survey by D.W. Ip, corroborated by G. O'Reilly and

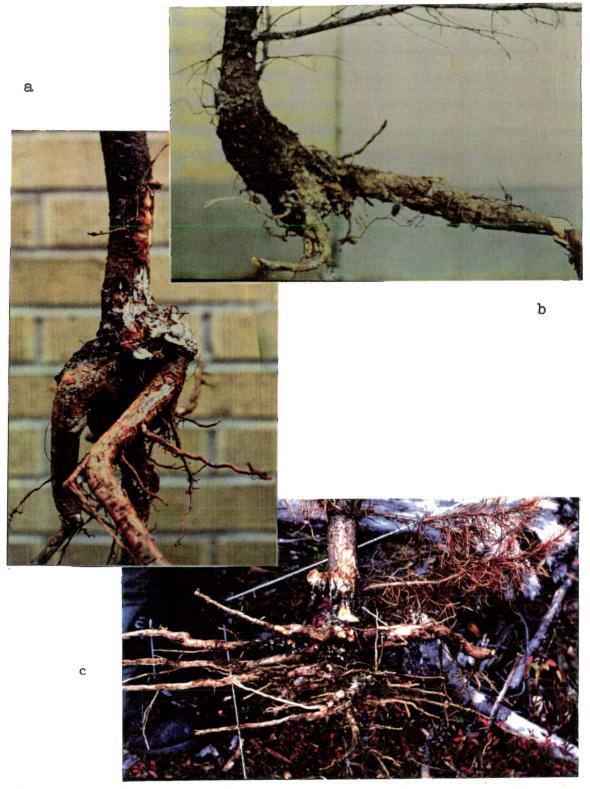


Figure 3.9. Root deformation in young conifers killed by Armillaria.

a. 4-year-old Larix laricina planted ramet. b. 12-year-old Picea glauca planted sapling. c. 7-year-old Pinus banksiana natural seedling.

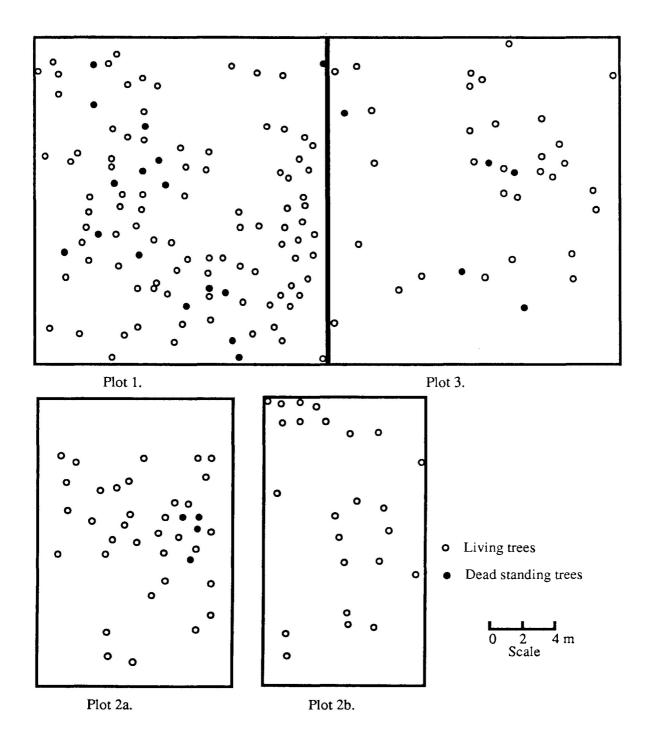


Figure 4.1a. Locations of surviving *Pinus koraiensis* in plots of a 25-year-old plantation. Plots were chosen to represent different current densities of pines. Plot 1: 2700 trees/ha; Plot 2: 1900 trees/ha; Plot 3: 800 trees/ha.

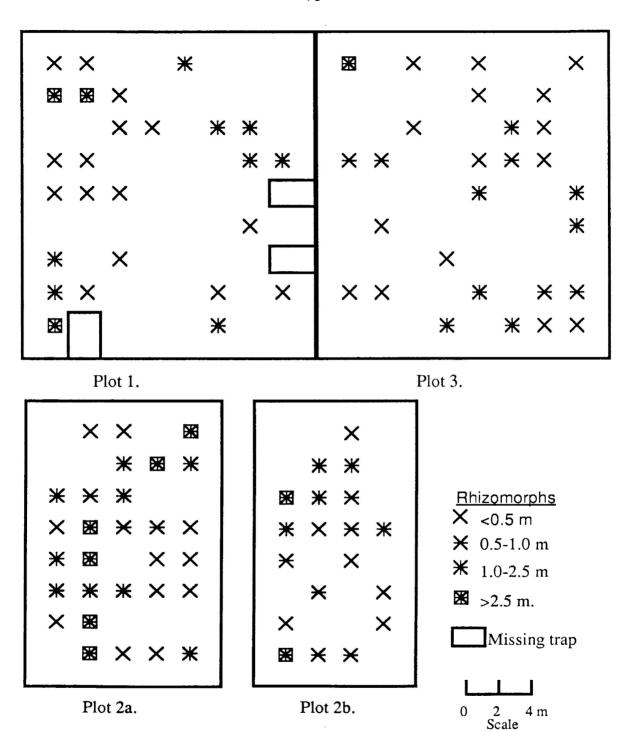


Figure 4.1b. Locations of positive *Armillaria* rhizomorph traps in a *Pinus koraiensis* plantation. Plot 1: Almost pure pine. Plot 2: Mainly pine with some hardwoods. Plot 3: Pine mixed with volunteer hardwoods dominating the canopy.

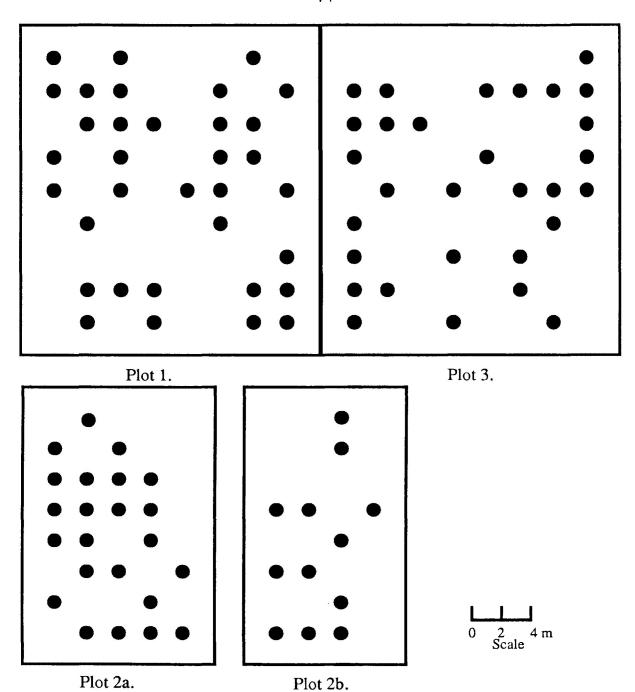


Figure 4.1c. Locations of soil samples with *Armillaria* rhizomorphs in a *Pinus koraiensis* plantation. Maximum rhizomorph length was 15 cm in 1.6 L sample.



Figure 4.2. Armillaria trap Plot 1 in an almost pure pine section of a 25-year-old Pinus koraiensis plantation. Herb layer was sparse; there was no shrub layer.

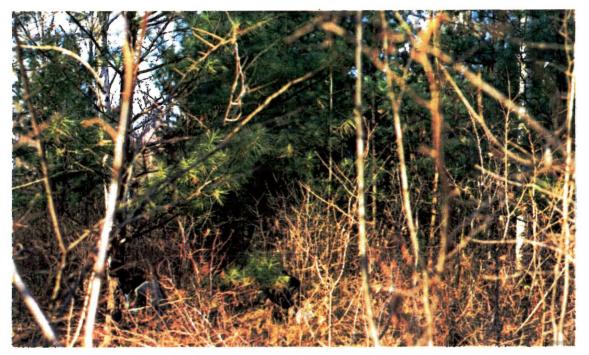


Figure 4.3. Armillaria trap Plot 3 in a mixed species section of a 25-year-old Pinus koraiensis plantation. Fast-growing hardwoods were growing vigorously in openings left by pines that had died. Herb and grass layer was thick; the pines were small and infrequent.

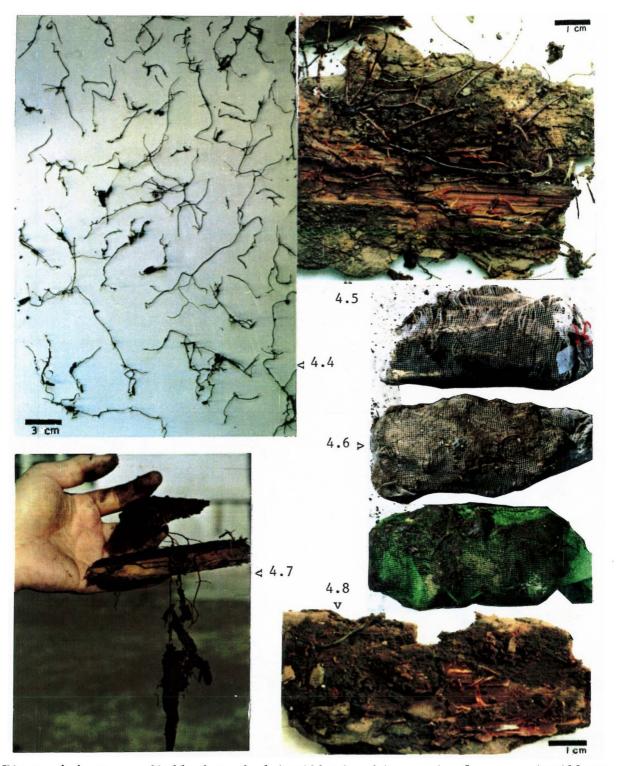
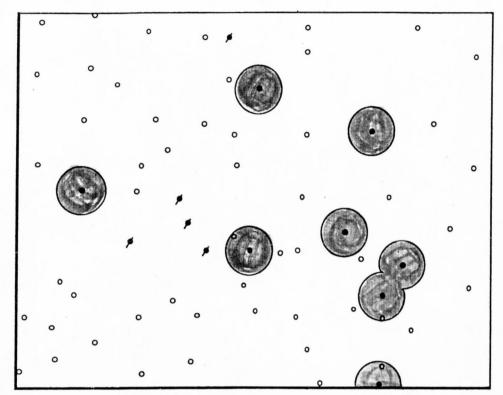
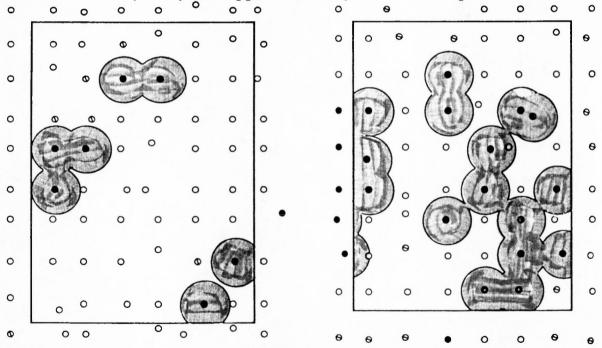


Figure 4.4. Monopodially branched <u>Armillaria</u> rhizomorphs from one <u>Armillaria</u> trap bag. Fig. 4.5. Subcortical and subterranean RMs attached to bark from a <u>Armillaria</u> trap bag. Fig. 4.6. <u>Armillaria</u> trap bags showing RMs growing on the outside. Fig. 4.7. <u>Armillaria</u> RMs from a trap bag. These strong, fresh and abundant RMs are readily distinguished from plant roots or litter. Fig. 4.8. A 2-cm long piece of <u>Armillaria</u> RM. Only one RM was found in this trap bag, but it was still easy to identify compared with RMs of similar lengths from soil cores.

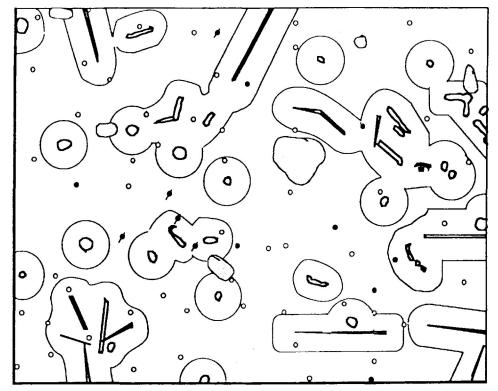


a. Plot 1. Distribution of 12- and 13-year-old white spruce trees in a 10-year-old plantation. The dead trees indicate a hazard area (blue) of approximately 1% of the plot.

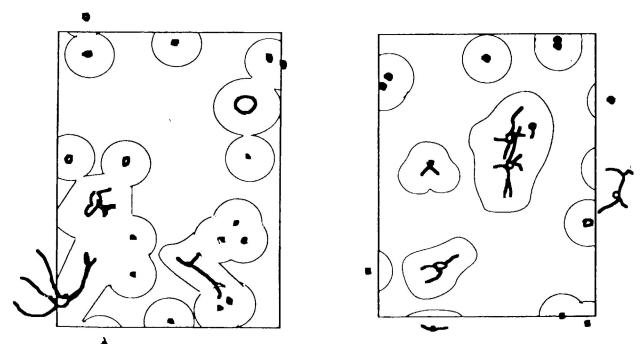


**b** and **c**. Plots 2 and 3. Distribution of healthy, unhealthy and dead larch ramets in a 4-year-old provenance trial. The hazard area (blue) according to the distribution of dead trees appeared to be about 10 and 25 % of the plots.

Figure 3.3. Dead trees and associated disease distribution (blue areas) in Plots 1, 2 and 3.

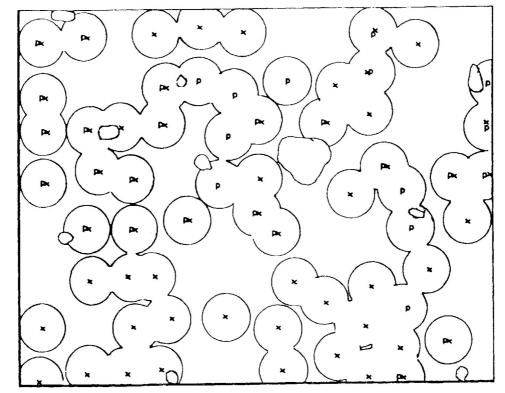


a. Plot 1 in a spruce plantation on a formerly mixed hardwood - conifer site.

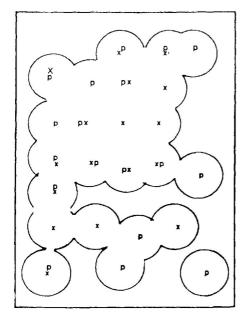


**b** and **c**. Plots 2 and 3 in a larch plantation on a formerly spruce-dominated site. The pattern of dead planted trees related very poorly to residual root systems and stumps. At this stage of the plantation development, it did not appear that avoiding slash would have increased tree survival.

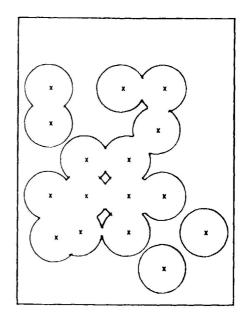
Figure 3.4. Residual material and associated disease distribution in Plots 1, 2 and 3.



**a.** Plot 1. Distribution of *Armillaria* was almost ubiquitous according to positive spruce and poplar trap logs.



b. Plot 2. Distribution of positive spruce and poplar trap logs indicated that Armillaria existed throughout the plot. Pockets of dead and unhealthy trees still present were encircled by the positive trap area.



c. Plot 3. Armillaria distribution appeared lower here than in Plot 2 according to the trap results, even though more trees had died in Plot 3. The differences in unhealthy trees may be important to investigate.

Figure 3.5. Positive Armillaria traps and associated disease distribution in trap Plots 1, 2 and 3.

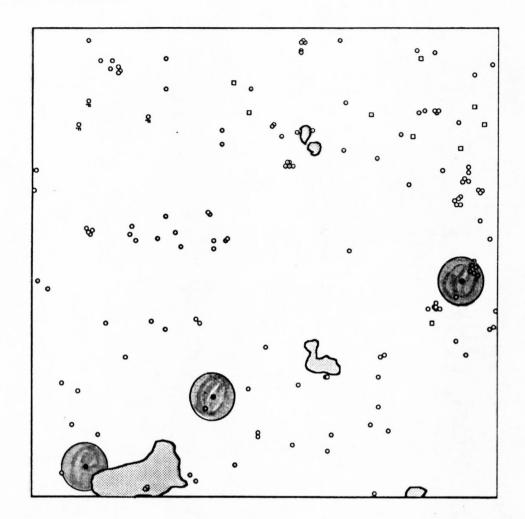
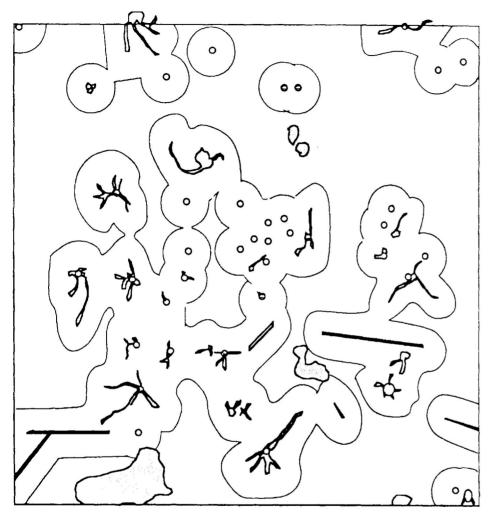
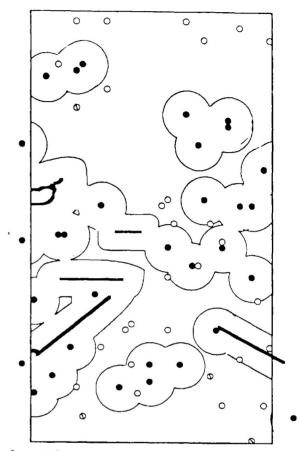


Figure 3.6. Dead naturally seeded trees and associated disease distribution (blue) in Plot 4. All planted spruce trees appeared healthy, but their occurrence was insufficient to determine original stocking or planting locations. Three dead jack pines were found, all having Armillaria in their roots.

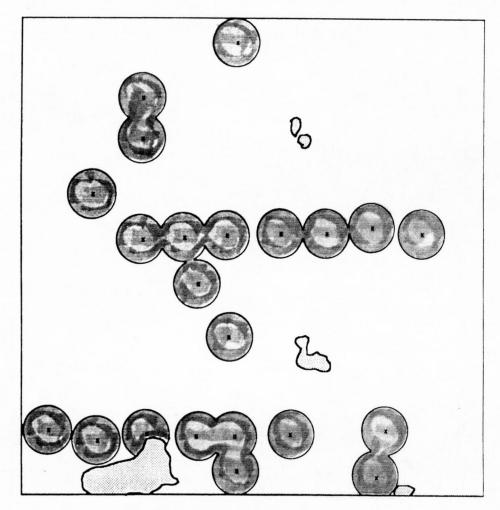


**a.** Plot 4. Spruce plantation on a former jack pine site. Most of the area estimated to be subject to root disease impact was clustered in one area. Based on this estimate, one might expect the clear areas to be safe for planted trees.

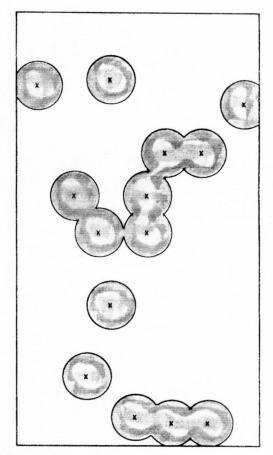


b. Plot 5. distribution of stumps and living and dead trees in an undisturbed 90-to 100-year-old spruce stand. The method of estimating area of disease impact in association with this material indicated a clustered infection area in the center of the plot.

Figure 3.7. Dead seeded trees, residual material and associated disease distribution in Plots 4 and 5.



**a.** Plot 4. Much less area was indicated to be impacted according to the traps than according to the stumps, etc. The pattern of distribution also suggested some inter-trap association.



**b.** Plot 5. The impact area was much less than that indicated by the stumps, etc., although most of it overlapped the stump area.

Figure 3.8. Positive Armillaria traps and associated disease distribution in Plots 4 and 5.