NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

Ecological and Physiological Aspects of *Paecilomyces farinosus* (Holm ex S. F. Gray) Brown & Smith, a Potential Biological Control Agent of Spruce Budworm

Sharon Kathleen Harney

A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements
for the Degree of Master of Science at
Concordia University
Montréal, Québec, Canada

January 1989

ISBN 0-315-49085-3

Canada

ABSTRACT

A survey of the microfungal population on leaf litter of a balsam fir forest infested with spruce budworm showed a large population of Paecilomyces farinosus on freshly fallen litter from the ground. Needles captured before hitting the ground showed a progressive increase in the abundance of P. farinosus as the summer progressed, suggesting some movement from the ground to the aerial parts. Fifty-four isolates of P. farinosus, twenty-six isolated from the litter and twenty-eight from naturally infected spruce budworm, were examined for some basic ecological and physiological characteristics. The isolates possessed a wide variety of hydrolytic enzymes, with varying degrees of utilization of chitin, gelatin, gallic acid, cellulose, pectin, and starch. No isolate oxidized humic acid. The isolates had the ability to decompose balsam fir litter and to resist desiccation. The results suggest that P. farinosus is an opportunistic parasite, inhabiting the soil and attacking spruce budworm when present. Although a discriminant analysis showed a separation of the isolates based on their origin, there did not appear to be two genetically distinct populations of P. farinosus.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Paul Widden for the opportunity to explore an area of research which interests me a great deal, as well as for his support, encouragement and ideas. Also many thanks go to Dave Perry of the Canadian Forestry Service who provided access to the study areas, the fungal cultures isolated from spruce budworm, and provided encouragement and information. The technical assistance of Susan Hipkin is greatly appreciated. The support of the members of the lab encouraged me throughout the project and I would specially like to thank Kathy Svatek for listening to me for the past two years, as well as Vilma Scattolin, Brenda Breil, and Isabelle Deslandes. Other thanks go to Suzanne Plante, Ann Greer, Margaret Cooke, Janet Rokas, Chris Boer, and many others in the Biology Department at Concordia. Finally I would like to thank my mother Elizabeth Harney for her support and encouragement and Kevin Harney for help with tedious chores.

TABLE OF CONTENTS

List of Figures

List of Tables

INTRODUCTION

The eastern spruce budworm, Choristoneura fumiferana (Clemens) is a major pest in northeastern North America, causing widespread defoliation of balsam fir (Abies balsamea (L) Mill.) and spruce (Picea spp.) forests (Blais, 1968). Continued defoliation causes two major problems: the creation of unproductive wastelands, and the potential fire hazards these areas create (McRae, 1986). Outbreaks often occur after dry seasons in mature conifer stands (Blais, 1965; 1968; 1982). Populations of spruce budworm increase and decrease in cycles of large amplitude over a period of approximately 30 to 40 years (Blais, 1968; Royama, 1981).

Oviposition in spruce budworm occurs in mid-July with the first instar larvae emerging 10 days later. The first instar larvae, which do not feed, search for crevices in which to spin hibernacula. After moulting they overwinter as second instar larvae until early May. The second instar larvae then disperse to feeding sites, and the third larval instars mine the base of the needles. Defoliation begins with the fourth larval instar feeding on the current year's shoots. Feeding continues into June with the fifth and sixth larval instars causing the majority of defoliation and sometimes feeding on older foliage when necessary. Pupation occurs in mid-July followed by adult emergence 8-10 days

later.

The widespread damage which spruce budworm causes has initiated extensive research for effective control methods. Chemical control, begun in 1952, has been the preferred means of controlling outbreaks of spruce budworm. While chemical control (i.e. insecticides) has had encouraging results, problems have arisen (Smirnoff and Juneau, 1982). In general, the use of chemicals for insect control has become controversial. As well as target pest species developing resistance to chemical pesticides, widespread and indiscriminate use of chemicals has led to serious ecological problems (Hall and Papierok, 1982; Garcia, 1983; Pickett, 1988). These include harm to beneficial flora and fauna, and the accumulation of residues in the environment which can affect wildlife outside the ecosystem of the target pest (Hall and Papierok, 1982). For these reasons, alternate control measures have been sought.

Biological control is the decrease in the average abundance of a pest using natural enemies, and thereby reducing the chance of future outbreaks (Debach, 1974; May and Hassell, 1988). Research on the biological control of spruce budworm has been concentrated on Protozoa, viruses, bacteria, and fungi. Biological control with Protozoa (particularly Nosema fumiferanae) and viruses for control of spruce budworm has not been successful to date, although

there has been some success when Nosema fumiferanae is applied with the bacterium Bacillus thuringiensis (Jones, 1986).

Bacillus thuringiensis (Bt) has been, to date, the most successful microbial agent for control of spruce budworm. Bt has been shown to increase larval mortality and foliage protection, and has been used for control in Ontario and Quebec. Bt is a bacterium which attacks a narrow spectrum of insects with little or no toxicity to vertebrates. Problems with Bt include lack of persistance in the field, and, when compared with chemicals, its high cost of production and application (Morris, 1982).

The use of fungi as biological control agents has attracted considerable attention (Hall and Papierok, 1982). Fungi have long been known to naturally attack insect pests, and while early attempts to control insect pests failed, research involving the potential of fungal control of insects has shown some promise. Many of the failures can be attributed to a lack of knowledge of the biology of the fungi, and the ecology of both the fungus and the pest species (Hall and Papierok, 1982). Fungi as disease-causing agents are unique in that infection occurs by direct penetration of the host's cuticle, and the infective structure does not require ingestion (Hall and Papierok, 1982). Examples of fungi successful in the control of

insects include Verticillium lecanii (Zimmerman) Viegas (aphids and white flies under glasshouse conditions) (Hall and Burges, 1979; Harper, 1987), Beauveria bassiana (Balsamo) Vuillemin [lepidopterous pests and the Colorado potato beetle (Leptinotarsa decemlineata (Say))] (Cantwell et al, 1986; Harper, 1987), and Metarhizium anisopliae (Metschn.) Sorokin (spittlebug in Brazil) (Messias and Azevedo, 1980; Harper, 1987).

Fungi which naturally attack spruce budworm and show potential for control include members of the order Entomophthorales (Zygomycetes) and Hyphomycetes. Members of the Entomophthorales have caused natural epizootics of spruce budworm, and show promise as biological control agents. Entomophthorales penetrate the host during germination of the conidium. After penetration, vegetative growth throughout the larva kills in two to five days. Sporulation on the host produces primary conidia (one to two days) or resting spores (five to six days). Resting spores and primary conidia are the source of further infection of the population. Problems associated with the Entomophthorales include the difficulty of culturing some species on artificial media, fragility of the primary conidia, germination of the resting spores (in some species), and mass production of resting spores (Dunphy and Nolan, 1982; Perry et al, 1982; Dunphy et al, 1985).

Hyphomycetes are anamorphic fungi, a number of which are entomopathogens (Hall and Papierok, 1982). The infective structure is the conidium, which directly penetrates the cuticle and does not need to be ingested (Hall and Papierok, 1982). After successful penetration, vegetative growth occurs throughout the haemocoel as yeast-like hyphal bodies. Toxins are then produced and death of the host results. Following host death mycelial growth invades the host's organs, tissues, and cuticle. Finally, sporulation on the host's surface spreads the infection.

Characteristics of this group which render it advantageous as a biological control agent include its diversity in habitat and environmental requirements, high reproductive capacity, saprophytic ability, and production of toxins which can cause rapid death. Disadvantages include the amount of inoculum needed for increased mortality and unknown long-term effects on non-target organisms.

Genera of Hyphomycetes known to attack spruce budworm include Beauveria, Hirsutella, Metarhizium, Nomuraea, Paecilomyces, and Verticillium. While most members of this group have never been found to naturally cause heavy mortality of spruce budworm (Perry and Whitfield, 1984), Paecilomyces farinosus has recently been associated with a larger percentage of larval death in the field than

previously recorded (Perry, pers. comm.). P. farinosus is an insect pathogen often isolated from the soil but never in great abundance (Samson, 1974; Domsch et al, 1980), and its presence has often been attributed to the presence of dead or moribund insects (Domsch et al, 1980). While studies on P. farinosus in relation to insect pathogenicity are fairly numerous (Samsinakova et al, 1977; Samsinakova and Kalalova, 1978; Kuruvilla and Jacob, 1980; Doberski, 1981a, b; Bajan et al, 1982; Kmitowa, 1982a,b; Mierzejewska, 1982a,b; Agudelo and Falcon, 1983; Varma and Mohanan, 1984; Jones, 1986; Houle et al, 1987; Mohanan and Varma, 1988), few studies have examined the biology and physiology of P. farinosus in relation to its basic ecology and the relative importance of saprophytic ability versus parasitic growth in nature.

Most members of the Hyphomycetes are active soil saprophytes which utilize organic matter in the soil ecosystem (Flanagan and Scarborough, 1974; Domsch et al, 1980; Gochenaur, 1984). Soil saprophytes are generally able to use complex substances, such as cellulose, as well as simple substrates (Deacon, 1984), and they play an important role in the degradation of organic matter present on the forest floor. The role of P. farinosus as a saprophyte in the soil has not yet been studied.

The role of fungi as decomposers in forest ecosystems

is well-documented. Numerous studies have examined fungal populations in deciduous forests (Gochenaur, 1978; Widden, 1979; Kjøller and Struwe, 1980; Kuter, 1986) and coniferous forests, including spruce forests (Hayes, 1965a; Söderström, 1975; Söderström and Bååth, 1978; Widden, 1979) and pine forests (Kendrick and Burges, 1962; Hayes, 1965a, b; Widden and Parkinson, 1973; Söderström and Bååth, 1978; Widden, 1979; Kahlki et al, 1986). While the fungal populations in these soils is well-documented, there have been few studies done on the microfungi of fir forests (Hayes, 1965a; Gourbière, 1974; 1981), and none on balsam fir forests.

There are certain differences between fungi inhabiting deciduous versus coniferous forests (Widden, 1979, 1986a, b, c), as well as among those inhabiting different conifer forests (Hayes, 1965a; Söderström and Bååth, 1978; Widden, 1979, 1986a). While there have been no studies done on the microfungi of balsam fir forests, Gourbière (1981) has found a large percentage of P. farinosus inhabiting silver fir (Abies alba Mill.) needles. P. farinosus is widespread and often isolated from soil and litter (Samson, 1974; Domsch et al, 1980), but has never been found in large numbers until the study on silver fir (Gourbière, 1981).

Paecilomyces farinosus is a member of the fungal taxonomic group the Deuteromycotina. Its members reproduce with assexual spores (or conidia) which germinate into

hyphae and grow along a substrate. Although isolates of P. farinosus do not reproduce sexually in culture, they are probably the conidial states of a member of the Ascomycomycotina, thought to be in the genus Cordyceps. Pacioni and Frizzi (1978) as well as Domsch et. al. (1980) list P. farinosus as the conidial state of Cordyceps memorablis, a rare entomogenous Ascomycete found on insects. While it is generally accepted that members of the genus Paecilomyces are anamorphic fungi with teliomorphs in the genus Cordyceps, P. farinosus itself has a long history of taxonomic controversy. P. farinosus, has, over the years, been placed in the genera Isaria, Spicaria, and Coremium (Brown and Smith, 1957; Samson, 1974; Domsch et al, 1980). Though today it is generally recognized as Paecilomyces farinosus, there is still some doubt as to whether isolates of P. farinosus represent a single species or a group of closely related species due to the apparent variety of morphological differences (Bissett, pers. comm.).

While the population dynamics of many soil microfungi are well-documented, very little is actually known about P. farinosus. This study examined the ecology of P. farinosus as a necessary first step regarding its potential as a biological control agent. Trichoderma and Penicillium, two common genera of soil fungi, are known to successfully survive as saprophytes. Characteristics common to these

genera include active decomposition of litter (enzymes) and good competitive ability (possibly due to the production of antibiotics) (Danielson, 1971; Dennis and Webster, 1971a; Gochenaur, 1984; Widden, 1984; Widden and Hsu, 1987). It is possible that P. farinosus may be naturally abundant as a litter decomposer although studies of deciduous and conifer forests suggest not. However, the ecology and physiology of P. farinosus is not well understood, and such information could help in its development as a biological control agent.

The ability of a fungus to survive in the absence of a host is a major advantage when considering an agent for biological control (Mohamed and Nelson, 1985). If a potential entomopathogenic fungus can survive and reproduce in the system without an insect host, infective inoculum may be present when the pest invades and thus possibly prevent a pest outbreak.

There are three alternatives, which are not mutually exclusive, to describe the possible ecology of P. farinosus. It may be a soil saprophyte which attacks insects opportunistically. Four factors which would support this view are abundance on the litter, a large compliment of hydrolytic enzymes, the ability to decompose litter, and the ability to actively compete with other soil fungi. It may be an entomopathogen which has limited survival as a saprophyte. It would then be expected to be abundant on

spruce budworm and possess the chitinases and proteases necessary to penetrate an insect's cuticle (Samsinakova et al, 1977; St. Leger et al, 1986), and possibly lack saprophytic abilities. A third alternative is that there exist two genetically distinct populations. Evidence which would support this theory would include discovering populations which are consistently found on different hosts, and which show morphological, physiological, and genetic differences.

The presence of an entomopathogen in large numbers in an area where an outbreak of an insect pest has occured is often a sign of a potential biological control agent. The question then arises as to whether P. farinosus is a soil saprophyte and opportunistic parasite, or vice versa, and whether it is a resident microorganism present in balsam fir ecosystems. The purpose of this study was to investigate some basic ecological and physiological properties of P. farinosus:

1. To determine the abundance of P. farinosus in a balsam fir ecosystem in relation to other soil microfungi.
2. To determine the origin of P. farinosus in a balsam fir ecosystem infested with spruce budworm. That is, whether it is invading litter from the soil or present in the canopy and

moving to the soil.

3. To investigate the ecological potential of a range of *P. farinosus* isolates by:
   a) examining growth on and decomposition of balsam fir litter
   b) determining whether *P. farinosus* possesses the hydrolytic enzymes necessary for decomposition.
   c) investigating its ability to survive desiccation.
4. To determine whether, based on physiology, enzymatic abilities or morphology, the isolates fall into two distinct populations depending on the source of the isolates.

METHODS

FIELD STUDY

Study Areas

Descriptions of these sites are taken from Régnière and Lethiecq (Unpublished Report).

Armagh: This study site is located at the northern limit of the Green Mountains of the Appalachians in Québec (45° 46' N, 70° 39' W) at an altitude of 270m. The dominant tree species is balsam fir (Abies balsamea (L.) Mill.). Other species include red maple (Acer rubrum L.), trembling aspen (Populus tremuloides Michx), white spruce (Picea glauca (Moench) Voss), grey birch (Betula populifolia Marsh), red spruce (Picea rubens Sarg.), and white birch (Betula papyrifera Marsh). The understory is mainly balsam fir, red maple, red spruce, white spruce, and witherod (Viburnum cassinoides L.). Ground cover is scant, with some club mosses and mosses. The soil pH ranges from 3.1 in the humus to 5.2 in the C horizon.

Lac à l'Epaule: This study site is located in Laurentian Provincial Park in Québec (47° 18' N, 71° 12' W) at an altitude of 750m. The dominant cover species is balsam fir, with some white birch, white spruce, and black spruce (Picea mariana (Mill.) B.S.P.). The understory consists mainly of balsam fir and mountain ash (Sorbus americana Marsh). The ground cover consists of club mosses,

ferns, and sedges. The soil pH ranges from 3.5 to 4.35.

These sites have been monitored for spruce budworm dynamics for many years. At both sites 15 $1m^2$ boxes had been set up by the Canadian Forestry Service in Ste. Foy, Québec. These boxes, which were approximately 0.3m high, were monitored for microbial infection of spruce budworm crawling on the ground. Lab-reared, uninfected spruce budworm larvae were placed in these boxes, allowed to crawl around on the ground, and brought back to the laboratory to discover any microbial infection which may have been picked up at these sites.

Sampling and Analysis

The fungi were isolated from freshly fallen balsam fir needles collected from the L_1 layer. Approximately 30 to 40 balsam fir needles were collected from the above boxes using alcohol-sterilized fine forceps. Care was taken to exclude any brown or degraded material. From May to August 1986, balsam fir litter was collected from all the boxes at each site. In 1987, 10 $1m^2$ nylon nets were set up at each site to obtain needles falling from the trees as well as freshly fallen litter from boxes. The nets, mosquito netting with a mesh size of 1mm, were suspended approximately 2m from the forest floor. Due to the amount of work involved with the addition of 10 nets at each site, only 10 boxes were monitored at each site in 1987. Balsam fir needles were

collected every two weeks.

The needles were washed to remove spores and thereby isolate only actively growing fungi (Parkinson and Williams, 1961; Widden and Parkinson, 1973). The needles were washed for 10 two-minute periods (Kendrick and Burges, 1962) using an automatic, multichamber soil-washing apparatus (Bissett and Widden, 1972). The needles were then dried on filter paper in sterile glass petri dishes for 24 hours, cut into 1mm pieces, and plated on Czapek-Dox Agar acidified to pH 4.5 with lactic acid (Widden and Parkinson, 1973; Widden, 1979). Four pieces were plated on each of 25 plates for each sample and incubated at room temperature.

In 1986, all the fungi growing on the plates were identified and recorded. In 1987 the addition of the suspended nets made it logistically impossible to identify every fungus isolated and forced a change in procedure. The number of fungi which developed from each piece of litter was recorded, as well as the occurence of Paecilomyces farinosus, Trichoderma spp., Penicillium spp., Cladosporium spp., Phoma spp., Epicoccum purpurescens, Aureobasidium pullulans, and a dark rust-colored sterile fungus (A349).

LABORATORY ANALYSIS OF PAECILOMYCES FARINOSUS ISOLATES

Isolates

Paecilomyces farinosus isolates PF1 to PF25 and PF54

were obtained from balsam fir needles collected during the sampling period. Isolates PF26 to PF53 were isolated from eastern spruce budworm larvae (Choristoneura fumiferana) by the Canadian Forestry Service in Ste. Foy, Québec. The P. farinosus isolates were maintained on 1% Malt Extract Agar (MEA) slants at 4°C and were periodically recultured to maintain viable cultures.

Enzyme Hydrolysis

The basal medium used in all enzyme studies was based on that of Aaronson (1970) as described in Kjøller and Struwe (1980):

K_2HPO_4	0.1g
$MgSO_4.H_2O$	0.05g
NaCl	0.05g
$CaCl_2$	0.01g
$FeCl_3$	0.005g
Water to	100ml

All 54 isolates of P. farinosus were examined for hydrolysis of each enzyme after a two week incubation period at 25°C. Morphological characteristics (presence of synnemata and exudate, sporulation, colony color and diameter) were examined at 7 and 14 days. All media were sterilized using an autoclave.

Chitin

Crustacean chitin was purified according to Skujins et al (1965). Technical chitin was dissolved in concentrated

HCl and left at 4°C until a syrupy liquid was formed. The liquid was then filtered through glass wool and the filtrate poured into a 50% aqueous ethanol solution while stirring vigorously. The precipitate was washed with distilled water several times to obtain a pH of approximately 4. The precipitate was washed using a centrifuge at 5000 rpm for five to ten 10 minute periods.

The chitin agar was prepared by adding the washed precipitate (approximately 10g chitin in 100ml water) to 900 ml of the basal medium. A clear zone around the colony indicated chitin degradation (Kjøller and Struwe, 1980). Chitin degradation was not measured as the clearing zones around the colonies were very small (1mm or less) and often very hard to observe. Chitin hydrolysis was rated as 0 (negative), 1 (medium), 2 (good), and 3 (very good).

Gelatin

Gelatin agar was prepared by adding gelatin to the basal medium for a 0.4% gelatin agar (Kjøller and Struwe, 1980). A clear zone around the colony indicated proteolytic activity. The clearing zone was measured (in cm) to determine the degree of hydrolysis.

Pectin

Pectin agar was prepared using 0.5% apple pectin in the

basal medium with agar (Hankin and Anagnostakis, 1975; Kjøller and Struwe, 1980). Cellophane (dialysis tubing) was placed on the agar and the isolates inoculated onto the cellophane. After two weeks, the cellophane and fungi were removed, and the plates flooded with a 1% cetavlon solution (cetyltrimethylammonium bro- mide) (Hankin and Anagnostakis, 1975; Kjøller and Struwe, 1980). A clear zone indicated pectin degradation as the cetavlon solution precipitates intact pectin in the media (Hankin and Anagnostakis, 1975; Kjøller and Struwe, 1980). Pectin degradation was not measured as the clearing zone was difficult to detect and often very irregular in shape. Pectin hydrolysis was recorded as 0 (negative), 1 (good), and 2 (very good).

Cellulose

Cellulose agar was prepared by plating a 1% microcrystalline cellulose (Sigma Cell Type 20) agar solution onto solid basal medium agar. Cellulose degradation was indicated by a clear zone around the colony and hydrolysis was recorded as 0 (negative), 1 (good), and 2 (very good). Cellulose degradation was very hard to detect and the clearing zone very small (1mm or less) and therefore not measured.

Starch

Amylolytic activity was measured using the ability to degrade starch as an indicator (Hankin and Anagnostakis, 1975; Kjøller and Struwe, 1980). Starch agar was prepared using 0.5% starch in the basal medium and agar. Amylolytic activity was detected after flooding the plates with an iodine solution. A yellow zone around the colony indicated starch degradation; otherwise, the starch reacts with the iodine to turn the medium blue. Starch degradation was not measured as the clearing zones were very irregular and did not follow colony diameter. Amylase activity was rated on a scale of 0 (negative) to 4 (excellent).

Gallic Acid and Humic Acid

The ability to hydrolyze humic acid and gallic acid (as well as cellulose) is often used to detect the ability of a fungus to degrade lignin (Flanagan and Scarborough, 1974). Gallic acid agar was prepared using 0.5% gallic acid and adjusted to a pH of approximately 4 with NaOH. A positive reaction occurs when a brown zone develops around the colony from the extracellular p- diphenol oxidases activity oxidizing gallic acid into a polymerized compound (Kjøller and Struwe, 1980). The zone around the colony was measured to determine the degree of activity.

Humic acid agar was prepared using humic acid pellets

dissolved in NaOH. The 0.1% humic acid agar was acidified to pH 5.5. Decolorization of the humic acid around the colony indicates oxidation activity (Hurst et al, 1962; Flanagan and Scarborough, 1974).

Resistance to Desiccation

The 54 isolates of P. farinosus were tested for the ability to withstand desiccation. The isolates, in replicates of 5, were inoculated on cellophane (Park, 1982) and placed on 0.5% gelatin agar. The plates were incubated at 25°C for one week. The cellophane and cultures were then removed, placed into plastic petri dishes and incubated on a laboratory bench for 7, 14, 21, or 28 days (Park, 1982). After the drying period the colony diameter was measured. The cellophane and isolates were reinoculated onto 0.5% gelatin plates, incubated for one week at 25°C, and the colony diameters measured. When measuring colony diameters after the drying period, care was taken to measure only newly-grown mycellium and not fresh growth from surviving spores.

As a control, Trichoderma harzianum and Penicillium brevicompactum, soil fungi known to be susceptible to desiccation (Park, 1982), were tested using the above method on 2% MEA.

Decomposition

The ability to decompose fir litter was examined by placing 2.000g of balsam fir needles, obtained from the L_1 horizon and freeze-dried, into 50ml Erlenmeyer flasks with 10ml of distilled water. The flasks were sterilized and the *P. farinosus* isolates inoculated into the flasks. The inocula consisted of 0.5mm disks from 1 week old cultures (grown on gelatin agar at 25°C). The flasks were incubated at 25°C for three months. The growth of the isolates in the flasks was scored from 1 (flat) to 5 (floccose), and the needles removed, dried, and weighed. Control flasks were also set up in the same manner but without any fungus. Three replicates were made in each case.

Production of Synnemata

Each isolate (in replicates of two) was cultured on Sabouraud's medium, and allowed to grow for four to five weeks. The production of synnemata was then rated from 0 (negative) to 5 (excellent).

Statistical Analysis

The analysis consisted of descriptive statistics and analysis of variance. To determine differences in fungal populations between sites, year, origin, and study date, analysis of variance was done. Discriminant analysis was done to determine whether isolates, based on morphological

and physiological characteristics separated into two groups according to source. The statistics were performed on the Concordia University mainframe computer using the SPSS subprograms condescriptive, anova, and discriminant (Nie et al, 1975).

Simple statistics were done for the dehydration and decomposition studies using the SPSS subprogram condescriptive (Nie et al, 1975). These data were combined with the data from the enzyme hydrolysis study and synnemata production and discriminant analysis performed using the SPSS subprogram discriminant (Nie et al, 1975), in an attempt to discover consistant differences and any major trends between isolates from spruce budworm and the litter.

RESULTS

FIELD STUDY

Paecilomyces farinosus was abundant when compared with other deciduous and conifer litter studies. The most common groups of fungi found during the study in 1986 are listed in Table 1. The fungi found more frequently at Armagh were Aureobasidium pullulans, Cladosporium spp., Mucor spp., Penicillium spp., P. thomii, sterile fungi, Trichoderma spp., and T. koningii. Fungi more frequently found at Lac à l'Epaule, were Paecilomyces farinosus, Phoma spp., and the sterile fungus A349. There was no significant effect of site on the occurence of Epicoccum purpurescens. There was a significant seasonal effect on the abundance of all genera except E. purpurescens and Mucor spp. Cladosporium spp. and P. farinosus were less abundant in early summer, increasing until August, and then decreasing. Trichoderma spp., T. koningii, and Phoma spp. were abundant in the early season, decreasing with time, and increasing at the end of the season. A. pullulans, P. thomii, and the sterile fungus A349, had two peaks, at the very early sampling and in late June-early July. Penicillium spp. were abundant in the early season and generally decreased as the season progressed. The sterile fungi population tended to increase as the season progressed.

Table 2 lists the genera of fungi isolated and

TABLE 1. Mean percentage of litter particles colonized by each of the common genera in 1986.

Taxon	Site		Level of Significance of ANOVA	
	Armagh	Lac à l'Epaule	Effect of Location	Effect of Time
Aureobasidium pullulans (de Bary) Arnaud	8.32	5.07	**	***
Cladosporium spp.	5.53	4.13	***	***
Epicoccum purpurescens Ehrenb ex. Schlecht	0.18	0.04	ns	ns
Mucor spp.	4.07	1.16	***	ns
Paecilomyces farinosus Brown and Smith	9.75	18.20	***	***
Penicillium spp.	12.43	4.50	***	***
P. thomii Maire	4.46	0.77	***	***
Phoma spp.	4.87	37.72	***	***
sterile fungi	21.02	17.29	***	***
sterile A349	8.96	12.37	***	***
Trichoderma spp.	12.52	3.16	***	***
T. koningii Oud. aggr.	7.58	1.26	***	***

** Significant at P<0.01
*** Significant at P<0.001
ns Not Significant

TABLE 2: Mean percentage of litter particles colonized by each of the common genera in 1987, isolated from the boxes.

Taxon	Armagh	Lac à l'Epaule	Level of Significance of ANOVA: Effect of Location	Level of Significance of ANOVA: Effect of Time
A. pullulans	7.53	4.13	*	***
Cladosporium spp.	1.49	0.46	***	ns
E. purpurescens	3.56	4.19	ns	ns
P. farinosus	3.39	17.44	***	ns
Penicillium spp.	5.45	5.40	ns	***
Phoma spp.	5.80	10.79	***	***
sterile A349	8.56	11.38	ns	***
Trichoderma spp.	10.83	12.18	ns	**

* Significant at $p<0.05$

** Significant at $p<0.01$

*** Significant at $p<0.001$

ns Not Significant

identified from the boxes on the ground in 1987. The fungi most frequently isolated from balsam fir needles at Armagh were A. pullulans and Cladosporium spp. At Lac à l'Epaule, the most frequently isolated fungi from the needles on the ground were P. farinosus and Phoma spp. There was no significant site effect on the occurence of E. purpurescens, Penicillium spp., Trichoderma spp., and the sterile fungus A349. There was a significant seasonal effect for all taxa except Cladosporium spp., E. purpurescens, and P. farinosus. A. pullulans had two peaks, at the very early sampling period and in late June, early July. Phoma spp. and Penicillium had low abundance in early May, increased in mid-season, and decreased late in the season. The sterile fungus A349 was abundant in the early season with a gradual decrease as the season progressed. Trichoderma spp. showed two peaks, one early and one late in the season.

Table 3 shows the genera of fungi isolated and identified from the nets in 1987. The most common genera of fungi found at Armagh were A. pullulans, Phoma spp., the sterile fungus A349, and Cladosporium spp. At Lac à l'Epaule the most common genera were Phoma spp., the sterile fungus A349, P. farinosus, and A. pullulans. Of the fungi isolated from the balsam fir needles from the nets, A. pullulans and Cladosporium spp. were more often found at Armagh. The fungi from the nets associated with Lac à

TABLE 3: Mean percentage of litter particles colonized by each of the common genera in 1987, isolated from the nets.

Taxon	SITE		Level of Significance of ANOVA		
	Armagh	Lac à l'Epaule	Effect of Location	Effect of Time	Effect of Origin
A. pullulans	20.48	3.05	***	ns	***
Cladosporium spp.	3.59	1.08	***	***	***
E. purpurescens	0.20	0.34	ns	ns	***
P. farinosus	1.49	4.95	***	***	***
Penicillium spp.	1.30	1.14	ns	***	***
Phoma spp.	15.91	18.28	ns	***	***
Sterile A349	9.82	15.18	***	***	***
Trichoderma spp.	0.51	0.57	ns	***	***

* Significant at $p<0.05$

** Significant at $p<0.01$

*** Significant at $p<0.001$

ns Not Significant

l'Epaule included P. farinosus and the sterile fungus A349. There was no significant difference in abundance between sites for E. purpurescens, Penicillium spp., Phoma spp., and Trichoderma spp. There was a significant effect of time of isolation for all genera except A. pullulans and E. purpurescens. P. farinosus, Cladosporium spp., and the sterile fungus A349 were more abundant late in the season. Penicillium spp. and Phoma spp. had their lowest abundance at the beginning and end of the season, with a peak in mid-season, and a decrease at the end. Trichoderma spp were low in abundance all season with a peak in late July-early August.

There was a significant difference for all identified genera of fungi isolated from balsam fir needles between those from the ground versus the nets (Table 3). A significant effect of year of isolation occured for A. pullulans, Cladosporium spp., E. purpurescens, P. farinosus, Phoma spp., and Trichoderma spp, where there was none for the sterile fungus A349.

The frequency of P. farinosus was generally higher at Lac à l'Epaule than Armagh, from the balsam fir needles from both the suspended nets and the boxes on the ground (Figures 1, 2, and 3). The frequency of P. farinosus isolated from the L_1 layer (Figures 1, 2, and 3) showed a trend (although not significantly significant) towards increasing frequency

Figure 1: Percentage of Litter (95% Confidence Limits) Colonized by P. farinosus at Armagh and Lac à l'Epaule in 1986.

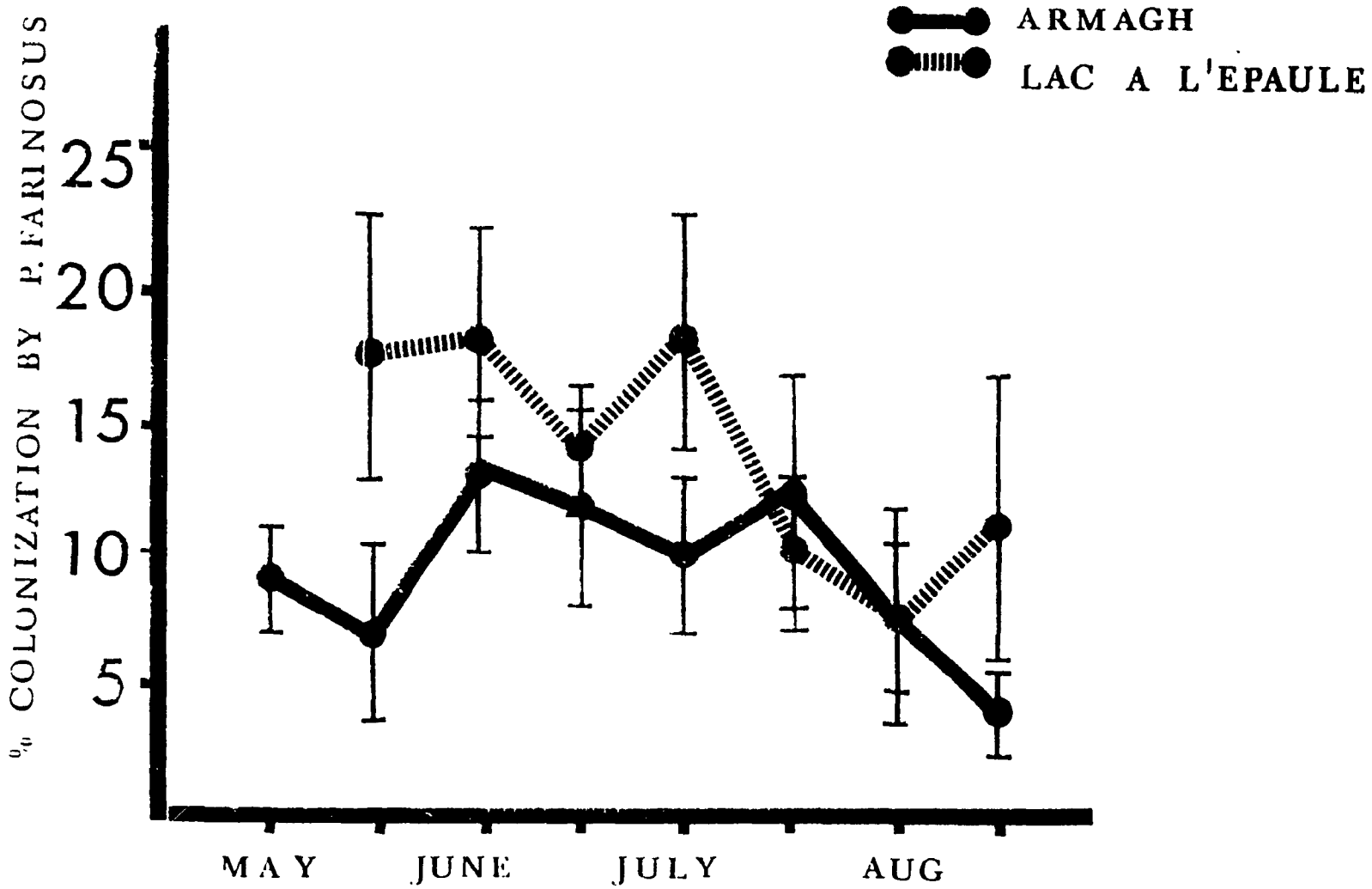
ARMAGH
LAC A L'EPAULE
% COLONIZATION BY P. FARINOSUS
25
20
15
10
5
MAY
JUNE
JULY
AUG

Figure 2: Percentage of Needles (95% Confidence Limits) Colonized by P. farinosus from the Ground and Nets at Armagh in 1987.

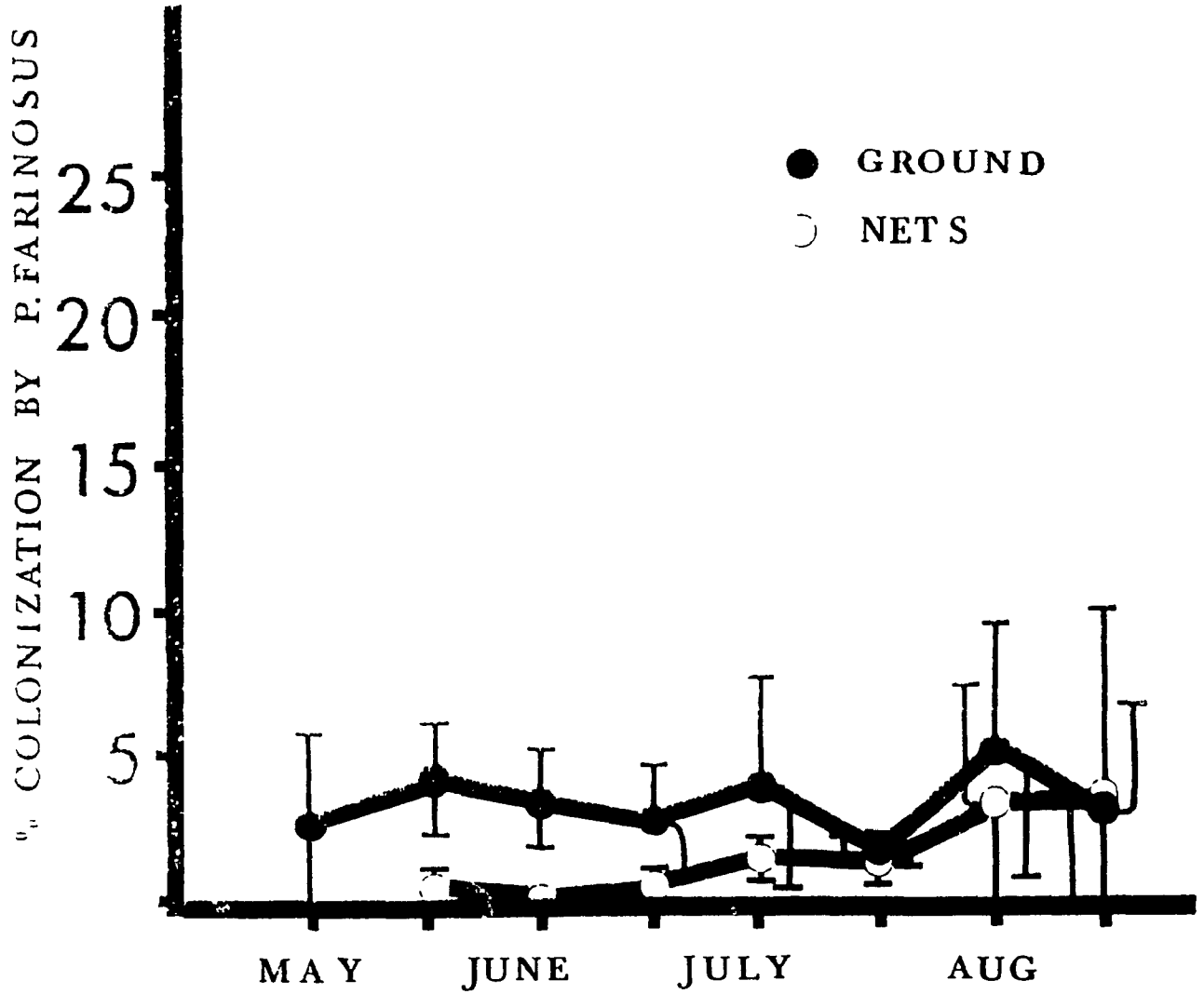
% COLONIZATION BY P. FARINOSUS
25
20
15
10
5
GROUND
NETS
MAY
JUNE
JULY
AUG

Figure 3: Percentage of Needles (95% Confidence Limits) Colonized by P. farinosus from the Ground and Nets at Lac à l'Epaule in 1987.

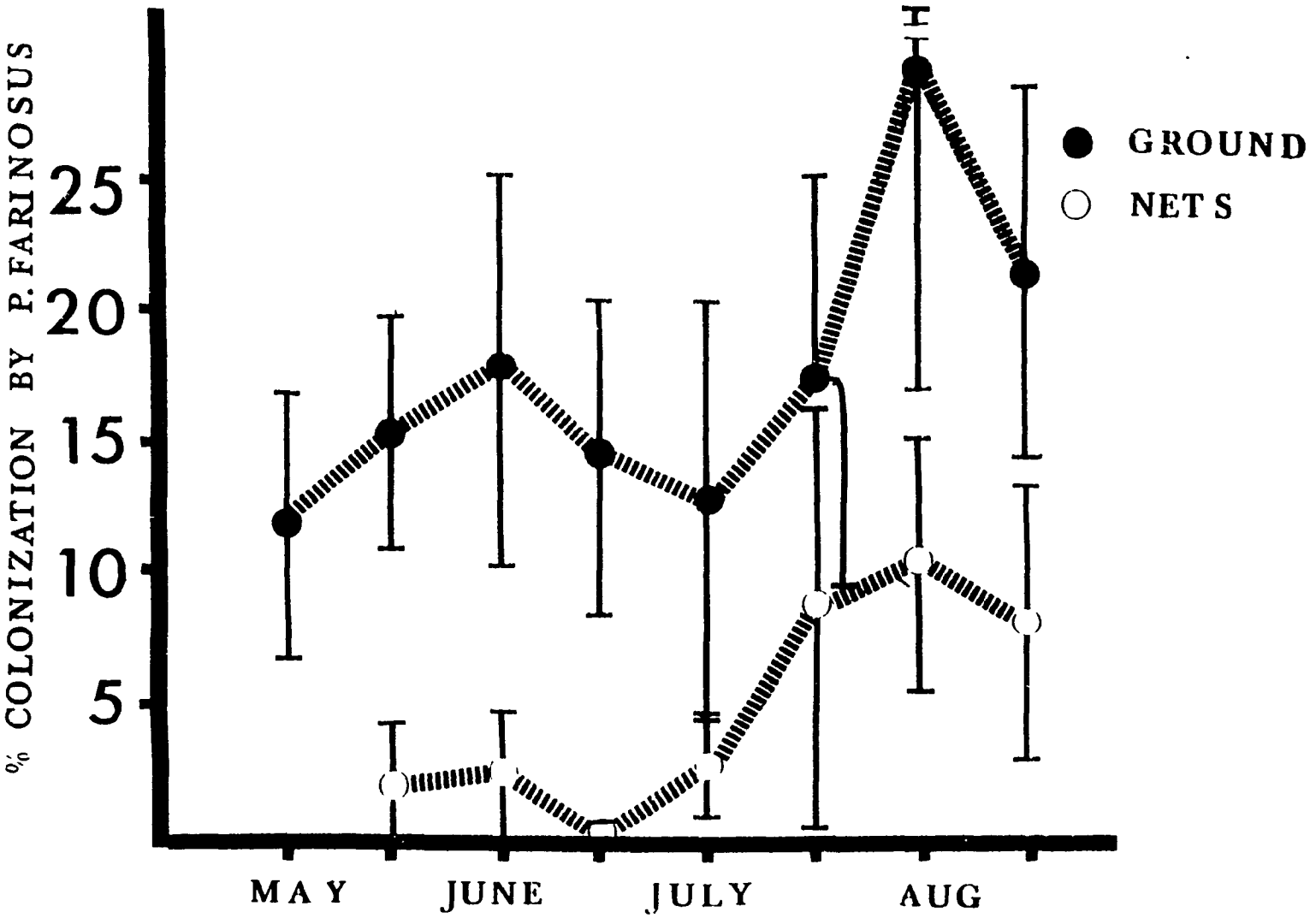
% COLONIZATION BY P. FARINOSUS
25
20
15
10
5
MAY
JUNE
JULY
AUG
GROUND
NETS

TABLE 4: Results of substrate utilization by *P. farinosus* isolates.

	Rating of Utilization					Clearing Zone (cm)	
Isolate (PF)	Starch[1]	Chitin[2]	Pectin[3]	Humic Acid	Cellulose[3]	Gelatin	Gallic Acid
1	2	2	0	0	2	5.30	3.00
2	2	1	0	0	2	5.80	3.70
3	1	1	2	0	2	5.70	3.00
4	2	2	0	0	2	5.20	2.10
5	4	2	0	0	2	6.40	2.00
6	2	2	1	0	2	6.00	2.80
7	3	1	2	0	2	6.30	3.20
8	1	2	2	0	2	6.20	3.60
9	1	2	2	0	2	6.00	3.10
10	3	2	2	0	2	6.20	1.80
11	3	2	1	0	2	6.40	2.10
12	1	2	1	0	2	8.70	0.00
13	3	2	1	0	2	8.70	2.60
14	2	2	1	0	2	5.50	1.30
15	1	2	1	0	2	5.50	1.80
16	0	1	1	0	1	8.70	2.95

Table 4 (continued):

Isolate	Starch	Chitin	Pectin	Humic Acid	Cellulose	Gelatin	Gallic Acid
17	3	1	2	0	1	5.30	2.50
18	2	2	1	0	2	5.80	2.80
19	1	0	0	0	2	8.70	2.70
20	2	2	1	0	2	5.80	2.20
21	2	2	1	0	2	6.20	3.00
22	1	1	0	0	2	8.70	3.00
23	3	2	0	0	2	6.00	3.00
24	1	2	2	0	2	8.70	1.10
25	3	1	1	0	1	8.70	3.20
26	1	2	0	0	2	8.70	0.00
27	2	2	1	0	1	5.70	3.00
28	1	2	2	0	2	6.10	3.70
29	2	1	1	0	2	6.00	4.00
30	2	2	1	0	2	8.70	3.70
31	2	2	2	0	2	6.80	2.70
32	1	2	0	0	2	5.50	2.90
33	2	2	2	0	1	5.50	2.50
34	3	1	2	0	2	6.50	3.60
35	2	0	2	0	2	4.60	3.10
36	2	2	0	0	2	6.60	2.80
37	3	1	1	0	2	7.10	2.80
38	3	3	2	0	2	6.30	1.80

TABLE 4 (Continued):

Isolate	Starch	Chitin	Pectin	Humic Acid	Cellulose	Gelatin	Gallic Acid
39	1	2	2	0	2	6.30	2.90
40	3	3	2	0	1	5.80	3.40
41	3	3	2	0	0	7.00	3.10
42	3	2	2	0	1	8.20	2.40
43	3	3	2	0	1	6.60	2.60
45	2	3	2	0	2	6.20	3.30
46	3	3	2	0	0	6.00	2.20
47	3	2	2	0	1	6.60	3.50
48	3	3	3	0	2	6.40	2.60
49	3	2	2	0	1	6.00	3.00
50	3	3	2	0	1	6.70	3.30
51	2	3	1	0	2	6.50	2.50
52	2	2	2	0	2	6.40	2.60
53	2	2	2	0	2	6.50	3.10
54	3	3	2	0	2	6.30	3.20

[1] 0=none, 1=poor, 2=good, 3=very good, 4=excellent

[2] 0=none, 1=good, 2=very good, 3=excellent

[3] 0=none, 1=good, 2=very good

until August, and then a decrease by the end of August, although the frequency at Armagh in 1987 was low. The frequency of P. farinosus from suspended nets (Figures 2 and 3) was low at the beginning of the isolation period, with a slight increase at the end of the sampling period (which was significant). Again, the frequency of P. farinosus appeared to be lower at Armagh.

LABORATORY ANALYSIS OF PAECILOMYCES FARINOSUS ISOLATES

Enzyme Hydrolysis

Substrate utilization by the 54 isolates of P. farinosus is shown in Table 4. Starch, chitin, pectin, humic acid, and cellulose utilization were scored (or rated) along a scale from zero to a maximum which reflected their highest utilization. The actual clearing zones of gelatin and gallic acid were measured. The rate of utilization of starch ranged from 0 (no utilization) to 4 (excellent) with no apparent differences between isolates from the litter (PF1 to PF25, and PF54) or from spruce budworm (PF26 to PF53). Chitin and cellulose utilization were, in general, very good or there was no utilization at all. Again there were no apparent differences in utilization between isolates from the litter or spruce budworm. Pectin utilization had the greatest variation of utilization, with 81.5% of the isolates showing degradation of pectin. Again there

appeared to be no significant diffence in utilization between isolates from the litter or spruce budworm. None of the isolates showed utilization of humic acid. All isolates utilized gelatin, with different clearing zone widths, depending on colony diameter. There appeared to be no significant difference between isolates from the litter and spruce budworm. The majority of isolates (96.3%) showed oxidation of gallic acid, with no significant difference between isolates.

Colony Characteristics

Table 5 shows growth characteristics (synnemata production, surface and reverse colony color) of P. farinosus isolates. Synnemata production varied from none to covering the colony. Some isolates only produced 1 or 2 synnemata after a 5 week period. The production of synnemata did not appear to be correlated with the origin of the isolate. Colony color again varied between isolates, from white flat colonies to deep orange floccose colonies. Although there appeared to be a trend toward distinguishable colony morphologies, there were no significant differences between isolates of different origin.

Decomposition and Resistance to Desiccation

Growth rates and percent decomposition of balsam fir needles are shown in Table 6. Growth rates on gelatin (the

TABLE 5: Growth Characteristics of *P. farinosus* Isolates.

	SCORE		
		Colony Color[2]	
Isolate (PF)	Synnemata[1] Production	Surface	Reverse
1	5	5	4
2	0	1	4
3	1	4	5
4	0	1	1
5	1	4	2
6	4	4	4
7	1	1	4
8	0	1	2
9	1	4	4
10	5	4	5
11	0	4	3
12	0	4	3
13	2	4	3
14	0	4	5
15	0	4	2
16	0	1	3
17	0	4	3
18	5	5	5
19	3	4	5

Table 5 (continued):

Isolate (PF)	Synnemata Production	Colony Color	
		Surface	Reverse
20	0	4	1
21	5	5	4
22	0	4	5
23	5	4	4
24	0	4	2
25	2	4	4
26	0	1	1
27	1	4	5
28	2	4	4
29	0	4	5
30	0	4	4
31	0	4	4
32	0	2	4
33	0	4	4
34	0	4	5
35	5	5	4
36	0	4	4
37	0	4	3
38	0	1	3
39	5	4	4
40	5	5	5

TABLE 5 (Continued):

Isolate (PF)	Synnemata Production	Colony Color Surface	Colony Color Reverse
41	0	4	4
42	3	3	5
43	2	4	5
44	1	4	4
45	0	3	4
46	3	4	4
47	1	2	4
48	3	4	4
49	5	4	4
50	4	4	4
51	0	4	4
52	0	4	2
53	1	4	4
54	5	4	3

[1] 0=none, 2=1 or 2, .2=few, 3=some, 4=lots, 5=covered

[2] 1=white, 2=yellow-white, 3=deep yellow, 4=orange-yellow, 5=deep orange

preferred medium) ranged from 1.07mm/day to 3.50mm/day. Growth scored on balsam fir needles in Erlenmeyer flasks ranged from flat growth over the surface of the needles (1) to fluffy, floccose growth throughout the flask (5). Percent decomposition of balsam fir needles varied among isolates. Growth rates after several periods of dehydration are also shown. These results varied with no apparent trend in growth rate; although in the majority of cases, growth rate after dehydration was lower than growth rate without a drying period. As a control, Trichoderma harzianum and Penicillium brevicompactum were examined for resistance to desiccation. Both T. harzianum and P. brevicompactum showed no new hyphal growth after 1 to 2 weeks of dehydration, although spore production did occur in both species.

Discriminant Analysis

Table 7 shows a discriminant analysis of isolates from the litter and from spruce budworm larvae using source of isolate as the discriminator variable. The isolates could weakly be separated into two groups (see Figure 4). The characteristics which appeared to separate the isolates into two groups (litter isolates and spruce budworm isolates) were growth rate on gelatin, cellulose and chitin, pectin utilization, synnemata production, and percentage growth after four weeks of drying (Table 7). The characteristics associated with isolates from spruce budworm were higher

TABLE 6: Growth Rates of P. farinosus Isolates.

Isolate	Growth Rate On Gelatin (mm\day)	Growth[1] On Balsam Fir Needles	Percent Decomposition of Balsam Fir Needles	Growth Rate After Dehydration (mm\day) Week 1	2	3	4
1	2.79	3	2.112	2.29	0.94	0.65	0.79
2	2.71	4	2.077	2.11	1.23	1.27	0.78
3	1.93	2	1.918	1.55	0.62	1.28	1.00
4	2.14	2	1.848	1.29	0.92	0.82	1.88
5	2.29	1	1.936	1.60	0.88	1.42	1.19
6	2.18	2	2.429	1.55	1.75	1.43	2.33
7	2.54	4	2.306	1.20	1.50	1.42	0.80
8	2.00	1	3.625	0.96	0.54	1.29	0.30
9	2.21	3	2.869	1.02	1.17	1.21	0.57
10	2.21	2	3.837	0.94	1.80	1.14	0.63
11	1.07	2	2.200	1.16	0.76	1.24	1.17
12	2.50	4	2.235	1.74	0.01	0.65	0.19
13	1.71	2	3.590	1.74	1.08	1.50	1.31
14	1.64	3	3.608	1.72	0.27	1.27	1.14
15	1.64	3	2.658	1.20	0.68	0.59	0.64
16	2.64	3	4.951	1.24	1.54	1.26	1.61
17	2.21	2	4.881	1.17	1.80	2.06	1.30

Table 6 (continued):

Isolate (PF)	Growth Rate On Gelatin (mm\day)	Growth On Balsam Fir Needles	Percent Decomposition of Balsam Fir Needles	Growth Rate After Dehydration (mm\day) Week 1	2	3	4
18	2.00	3	4.446	1.33	1.97	1.79	1.90
19	2.43	3	3.347	1.86	1.73	1.63	1.87
20	2.14	4	4.027	1.73	2.03	1.30	1.37
21	1.93	3	3.940	1.17	1.74	1.81	1.99
22	2.43	3	3.853	1.71	2.06	1.44	1.74
23	2.29	2	3.679	1.29	1.46	1.34	1.24
24	1.57	1	4.027	1.09	1.46	1.67	1.71
25	2.86	3	5.248	2.37	2.74	1.86	1.83
26	2.75	1	5.492	2.91	2.79	2.51	1.90
27	2.07	4	4.010	1.47	1.76	1.66	1.06
28	2.64	3	4.358	1.93	1.81	1.60	1.17
29	2.50	3	3.957	2.13	1.94	1.99	2.06
30	2.50	1	4.480	2.24	1.89	2.11	2.64
31	2.29	3	3.029	2.57	2.51	2.46	2.53
32	2.50	5	3.773	2.57	2.31	2.23	2.34
33	2.14	1	3.224	1.86	1.91	1.57	1.69
34	2.43	1	3.082	2.29	1.74	1.74	1.83
35	1.29	.5	2.550	2.00	2.06	2.19	2.29
36	3.07	2	3.330	2.37	2.29	2.01	2.29

TABLE 6 (Continued):

Isolate (PF)	Growth Rate On Gelati- (mm\day)	Growth On Balsam Fir Needles	Percent Decomposition of Balsam Fir Needles	Growth Rate After Dehydration (mm\day) Week 1	2	3	4
37	3.50	2	3.666	1.27	1.64	1.51	2.20
38	2.57	2	2.763	1.70	1.61	1.90	1.86
39	3.14	2	3.294	2.90	1.71	1.47	2.31
40	2.64	4	2.692	2.47	2.77	1.83	1.89
41	2.86	1	4.003	1.26	0.63	0.37	0.17
42	3.29	2	6.111	1.94	1.90	1.47	1.40
43	2.79	4	2.710	1.17	1.83	1.56	1.34
44	3.14	4	3.170	0.60	1.46	1.37	0.34
45	3.00	3	2.958	1.70	1.43	1.80	1.91
46	3.07	2	4.167	1.69	1.37	1.43	1.41
47	3.00	2	4.372	1.64	1.47	1.24	1.47
48	3.21	2	4.320	1.79	1.21	1.27	1.31
49	2.21	4	4.133	1.91	1.89	1.49	1.26
50	3.43	2	3.364	1.90	2.03	1.86	2.03
51	3.36	3	4.372	2.03	2.03	2.09	1.90
52	3.07	3	3.757	1.23	1.70	1.09	1.97
53	2.93	2	3.911	1.47	1.93	1.63	1.83
54	3.20	4	4.833	1.46	2.50	2.20	2.50

[1] 1=poor, 2=poor-medium, 3=medium, 4=medium-good, 5=very good

growth rates on gelatin, better pectin utilization, higher growth rates on chitin, and greater percentage growth after four weeks of dehydration. Factors which correlated with litter isolates were higher growth rates on cellulose and synnemata production. The main factors which appeared to distinguish the two groups were growth rates on gelatin (spruce budworm) and growth rates on cellulose (litter). Other growth characteristics (i.e. from chitin utilization to percent decomposition) did not appear to be strongly correlated with either group.

TABLE 7: Discriminant Analysis of P. farinosus isolates with the Disciminating Variable being Origin of Isolation

Characteristic	a*	r**
Diameter on Gelatin	.50685	.65334
Diameter on Cellulose	-.53094	-.59604
Pectin Utilization	.45346	.43230
Synnemata Production	-.40297	-.07455
Diameter on Chitin	.30707	.21442
Percent Growth After 4 Weeks Dehydration	.23910	.25297
Chitin Utilization		.36072
Reverse Colony Color		-.23723
Starch Utilization		.21652
Cellulose Utilization		-.21098
Gelatin Clearing Zone		-.19493
Diameter on Gallic Acid		-.16066
Gallic Acid Clearing Zone		-.15639
Diameter on Humic Acid		-.11592
Diameter on Starch		.10614
Surface Colony Color		.07920
Percent Decomposition		.02826

* a=standardized canonical discriminant function coefficient
** r=correlation of the variable with the discriminant function (structure coefficient)

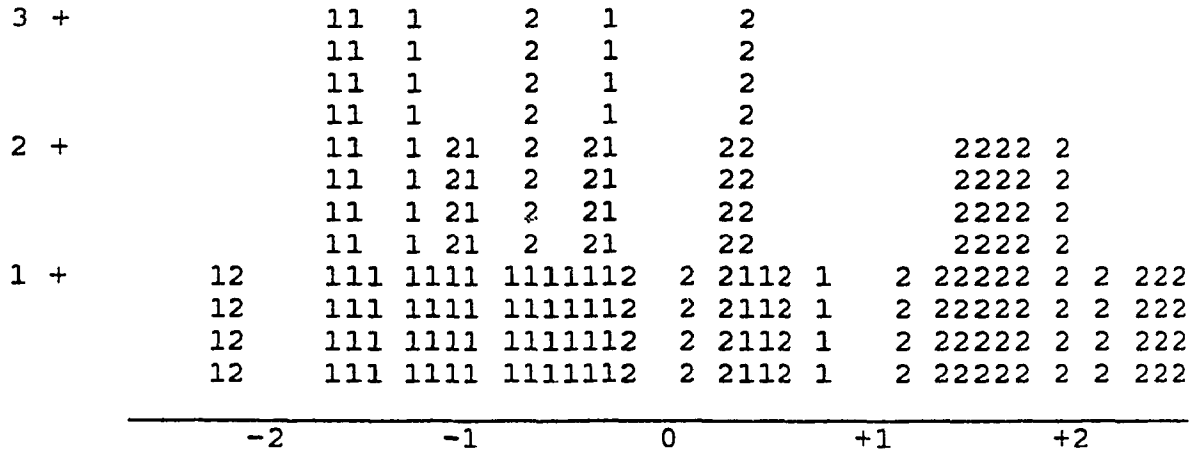

FIGURE 4: Position of P. farinosus Isolates along Discriminant Function, using Source of Isolation as Discriminator Variable.

DISCUSSION

The results from the survey of isolates from the balsam fir needles in L_1 layer (see Tables 1 and 2) generally show a microfungal community typical of those found in conifer forests with one exception. Aureobasidium pullulans, Cladosporium spp., Mucor spp., Penicillium spp, Phoma spp., and Trichoderma spp. are regularly isolated from forest soil and litter studies, often in abundance (Domsch et al, 1980). E. purpurescens, while often isolated from litter, is rarely isolated in abundance (Domsch et al, 1980). The one exception is P. farinosus, which is widespread and often isolated from the soil and litter, but rarely in abundance (Samson, 1974; Domsch et al, 1980), its presence generally being attributed to dead or moribund insects (Domsch et al, 1980). Previous to this study, P. farinosus has been isolated in abundance in only one field study of leaf litter. Gourbière (1981) isolated a high percentage of P. farinosus in a study on the microfungal population in a silver fir forest in France. This high abundance of P. farinosus is unusual, and it is not known whether it is naturally abundant in fir forests or abundant due to a large population of insects. Gourbière (1981) does not mention the presence of an insect outbreak, which suggests that in silver fir forests, P. farinosus may be naturally abundant.

This study took place in an area infested with spruce

budworm. In both Armagh and Lac à l'Epaule, the population of spruce budworm was on the decline after several years of intense infestation (Perry, pers comm). The spruce budworm population at Armagh was low, particularly in 1987, and consistantly lower than the population at Lac à l'Epaule. The Canadian Forestry Service in Ste. Foy, Québec, as part of an ongoing study on spruce budworm dynamics, monitored disease incidence in spruce budworm larvae. In 1986, infection of spruce budworm with P. farinosus was higher than previously recorded at both Armagh and Lac à l'Epaule. In 1987, very few spruce budworm were present at Armagh. At Lac à l'Epaule, though the spruce budworm population was fairly low, the infection rate of spruce budworm with P. farinosus was again higher than normal. This again raises the question of whether P. farinosus is abundant in response to the presence of spruce budworm or if it is naturally abundant in fir forests.

The abundance of P. farinosus was consistently higher at Lac à l'Epaule which could reflect the higher population levels of spruce budworm at this site. The spruce budworm populations were higher at Lac à l'Epaule than Armagh for both years during this survey. As the site at Lac à l'Epaule is a pure balsam fir stand and the site at Armagh is a mixed balsam fir-spruce stand, P. farinosus may be naturally more abundant at Lac à l'Epaule. This is unlikely,

however, as care was taken that only balsam fir needles were examined. The abundance of P. farinosus is possibly due to the presence of the spruce budworm, as indicated by the drop in abundance of P. farinosus at Armagh when very few spruce budworm were present. In 1986, when population levels of spruce budworm were higher at Armagh than in 1987, P. farinosus was also more abundant. In 1987, with extremely low levels of spruce budworm at Armagh, the abundance of P. farinosus was lower, which suggests that the presence of spruce budworm may be a factor in the abundance of P. farinosus.

The pattern exhibited by P. farinosus (in relation to spruce budworm) during this study is similar to that of a predator-prey relationship. Unfortunately it is unknown whether P. farinosus was present in such abundance in previous years. Coupled oscillations associated with predator-prey cycles normally begin with an increase in the prey (i.e. spruce budworm) followed later (in this case possibly years) by an increase in the predator (i.e. P. farinosus) (Anderson and May, 1980; May, 1983; Emlen, 1984). As the predator population increases, that of the prey decreases, followed by several oscillations (Anderson and May, 1980; May, 1983; Emlen, 1984). It is possible that P. farinosus, while present in these ecosystems, was low in abundance prior to the spruce budworm outbreak. As the

spruce budworm population grew, the P. farinosus reacted to this large population and started to increase. It would follow that after the decrease of spruce budworm in the area, the abundance of P. farinosus would also decrease, as suggested by the data from Armagh. In 1986, with the spruce budworm population on the decline, there was still a fairly high percentage of P. farinosus. In 1987, with extremely few spruce budworm in the system, the abundance of P. farinosus decreased further, possibly following a predator-prey isocline.

The time of isolation (Figures 1, 2, and 3) may be a factor in the abundance of P. farinosus. The frequency of isolation of P. farinosus from the needles on the ground in May was low and increased as the season progressed until the end of the summer, although this was not statistically significant. In August, P. farinosus began to decrease in abundance. This pattern, or trend, while never statistically significant, appeared in both 1986 and 1987 (Figures 1, 2, and 3) at both Armagh and Lac à l'Epaule. It could be suggested to follow the pattern of spruce budworm infestation. When the spruce budworm first appear in the spring, P. farinosus, while present in both the canopy and the soil, is in low abundance, and with the development of spruce budworm larvae, increases until the larvae have metamorphosed and few insects are left. With the departure

of the majority of the insects as adults, the level of P. farinosus appears to slightly decrease, and possibly remains stable until a new insect outbreak occurs, stimulating another increase in the fungus. The results from the 1986 study, when the level of spruce budworm at both sites was higher, particularly shows this trend of increase at the beginning of the season and decrease at the end of the season. The Lac à l'Epaule study site is approximately two weeks later in season than Armagh, and if the data for Lac à l'Epaule on Figure 1 were shifted two weeks later, the same peaks and depressions would appear at approximately the same time as Armagh, although this is not statistically significant. Although the season is later at Lac à l'Epaule, the spruce budworm life cycle ends at approximately the same time, with the sixth larval instar occuring in July (Perry, pers comm). The larvae emerge later at Lac à l'Epaule but the life cycle is shorter and both populations reach the adult stage at approximately the same time.

The question of where the P. farinosus is coming from also arises. In 1986, only freshly fallen litter picked up from the ground was examined for the presence of P. farinosus. While it was known that there was an outbreak of spruce budworm in both areas and that a large percentage of death was due to P. farinosus, it was unknown whether P.

farinosus was a natural inhabitant of the litter or if it was present in such abundance due to the spruce budworm. In 1987, an attempt was made to determine the origin of P. farinosus. Nets were set up to capture freshly fallen balsam fir needles before they hit the ground.

There appears to be movement of P. farinosus from the soil to the canopy. At the beginning of the sampling period, very little P. farinosus was isolated from the needles in the nets, while it was fairly abundant on the ground litter. As the season progressed, the abundance of P. farinosus on the needles from the trees increased. This suggests movement of P. farinosus from the ground upwards, rather than downward from the trees. Over time, as P. farinosus is more abundant on the ground, more conidia may be produced and carried upward by wind dispersal, establishing themselves in the canopy of the trees. It may also be suggested that the increase of P. farinosus on these needles is due to the movement of spruce budworm. The second to fourth larval instars of spruce budworm often disperse short distances within the infested area (Perry, pers comm). They sometimes crawl around the floor of the forest and climb either the trees from which they have dropped or nearby trees (Perry, pers comm). It is possible that P. farinosus reaches the tree tops during the dispersal of the larvae. A final possiblility may concern upward

movement due to other insects present in the ecosystem.

The presence of P. farinosus in the tree tops would be beneficial in terms of biological control. It is possible that the spruce budworm larvae which disperse along the ground and back into the trees may carry P. farinosus to the tree tops. Once in the trees, it appears that P. farinosus spreads to the rest of the spruce budworm population. If P. farinosus is an efficient entomopathogen of spruce budworm and can survive in the tops of the trees, it may be a potential means of controlling spruce budworm.

The abundance of P. farinosus in this ecosystem and the high degree of natural disease incidence of spruce budworm due to P. farinosus (Perry, pers comm) appear to indicate that P. farinosus may have potential use as a biological control agent. While studies on the pathogenicity and effectiveness of P. farinosus as a biological control agent were available, (Samsinakova et al, 1977; Samsinakova and Kalalova, 1978; Kuruvilla and Jacob, 1980; Doberski, 1981 a, b; Bajan et al, 1982; Kmitowa, 1982a, b; Agudelo and Falcon, 1983; Varma and Mohanan, 1984; Jones, 1986; Houle et al, 1987; Mohanan and Varma, 1988) there was no information on its general biology, physiology, and ecology. In order for a biological control agent to be effective, both its biology and ecology, as those of the target pest, must be well understood (Hall and Papierok, 1982). Too often, biological

control of pests with fungi has been initiated without this information and failed, leading many researchers to discount the potential of fungi as biological control agents (Hall and Papierok, 1982). P. farinosus has been shown to naturally attack spruce budworm (Perry, pers comm). As this is usually the first step in recognizing a potential biological control agent (Harper, 1987), it is essential to gather more information about the fungus.

It is unknown whether P. farinosus is primarily an insect pathogen, or an opportunistic pathogen, i.e. inhabiting soil and litter and attacking insects when they are present. An obligate insect pathogen would only be found if the host (i.e. insect) were present (Kendrick, 1985). Opportunistic pathogens are normally saprophytic, but can act as parasites when conditions are favorable (Kendrick, 1985).

If P. farinosus can actively survive and reproduce as a saprophyte, this ability would be a considerable advantage in its use as a biological control agent. P. farinosus as a saprophyte and opportunistic entomopathogen could actively grow in the ecosystem and therefore be present during outbreaks of spruce budworm. In this manner, P. farinosus may act as a preventive measure against spruce budworm. Many researchers hold the opinion that preventive measures may be an essential component in the control of insect

outbreaks. By preventing large outbreaks of insect pests, both expensive crop loss and the belated use of costly chemical and biological control methods may be avoided. A live pathogen present in strategic areas of an outbreak may be the ultimate control measure. Many control measures (both chemical and biological) used today do not remain active in the ecosystem after original insect control, therefore further outbreaks are not prevented (Barfield and Stimac, 1980).

An active saprophyte needs to be able to degrade organic matter and actively compete with other soil microorganisms, often achieved with extracellular antibiotics which inhibit potential competitors (Dennis and Webster, 1971a, b; Tronsmo and Dennis, 1978). Organic matter present in soil ecosystems, normally from plant remains, includes cellulose, lignin, and possibly pectin (Hankin and Anagnostakis, 1975). Thus an ability to use these substances often indicates saprophytic ability (Gochenaur, 1984). In general, both isolates from the litter and from spruce budworm larvae showed utilization of both cellulose and pectin, with no observable difference between individual isolates. The ability to use cellulose is often a characteristic of secondary saprophytes (Deacon, 1984), which normally inhabit the soil and/or litter. Cellulose is a polysaccharide which comprises a large part of plant cell

walls (Kendrick, 1985) along with hemicellulose (up to 70%) (Deacon, 1984), and therefore is often present in abundance in soil ecosystems. The ability of the majority of P. farinosus isolates (96.3%) to utilize cellulose may indicate that P. farinosus can survive as an active saprophyte.

Pectinases dissolve the cement which holds plant cells together (Kendrick, 1985), and are often associated with plant parasitic fungi (Dahm et al, 1987; Strzelczyk et al, 1987). Together with cellulases, pectinases can break down live plant material (Kendrick, 1985). Although not all P. farinosus isolates showed the ability to utilize pectin, the majority (81.5%) did. While this does not neccessarily indicate saprophytic ability, it does appear that P. farinosus can utilize a large proportion of plant polysaccharides. This may be an asset if P. farinosus is to survive and remain active in the soil.

The ability to utilize humic acid and gallic acid, as well as cellulose, was often in the past used to represent the ability to degrade lignin (Flanagan and Scarborough, 1974), although this is not a valid indication of lignin degradation. Lignin is a complex substance and few organisms can degrade a lignin polymer (Deacon, 1984). Often the microorganisms which can break down lignin perform only partial degradation (Deacon, 1984). The P. farinosus isolates were examined for the ability to breakdown humic

acid and gallic acid to help determine the range of their enzymatic abilities. The majority of P. farinosus isolates utilized cellulose and gallic acid, but no isolate utilized humic acid. It seems extremely unlikely that P. farinosus could break down lignin, although the ability to oxidize gallic acid is surprising. It may be that in the absence of any other substrate, P. farinosus may be able to perform partial lignin degradation, to obtain energy, although this seems unlikely.

Starch is an 'accessible' carbon source often used by primary saprophytes, often called sugar fungi (Deacon, 1984). Primary saprophytes normally survive in the trees before the litter falls to the ground, where they are ususally outcompeted by secondary saprophytes. The majority of P. farinosus isolates (98.1%) utilized starch, although the ability varied among individual isolates.

The ability to degrade whole balsam fir needles would most probably be necessary to survive in a balsam fir ecosystem. The degradation of balsam fir needles alone, without any other saprophytic soil microorganism, would also indicate active saprophytic ability. Balsam fir needles, as any other complex compound, are difficult to break down, and therefore difficult to obtain energy from. P. farinosus isolates were shown to be able to degrade balsam fir needles without the presence of other soil microorganisms. The

percentage decomposition ranged from 1.85% to a high of 6.11% over a period of three months, with an average percent decomposition of 3.55%. Although the decomposition rate is slow and the percent decomposition not very large, it does appear that P. farinosus can utilize balsam fir needles as a substrate and derive energy from their breakdown. The growth on the balsam fir needles was abundant after three weeks to one month, which seems to indicate that P. farinosus can grow healthily on the needles. There does not appear to be a significant difference between the isolates from spruce budworm and litter. Both groups utilized the balsam fir needles to approximately the same degree, with variation among individual isolates.

The ability of P. farinosus isolates to utilize such a wide variety of substances, from simple to complex (excluding humic acid) indicates that P. farinosus should be able to survive as an active saprophyte. It appears that, with such a large complement of enzymes, P. farinosus may be a very versatile saprophyte in a soil/litter ecosystem. Without any information about competitive ability, it can only be stated that P. farinosus does appear to have the necessary enzyme apparatus to survive successfully as a saprophyte.

P. farinosus is known to attack a wide variety of insects, including the Colorado potato beetle (Campbell et

al, 1986), the brown planthopper (Inch et al, 1986), gypsy moth eggs (Dunbar et al, 1972; Carroll, 1987), and the elm bark beetle (Doberski, 1981a, b), as well as several other insect pests (Balakrishnan and Nene, 1980; Kuruvilla and Jacob, 1980; Bajan and Fedorka, 1982; Mierzejewska, 1982a, b; Agudelo and Falcon, 1983; Varma and Mohanan, 1984; Mohanan and Varma, 1988; Pendland et al, 1988).

A successful entomopathogen needs to possess the necessary enzyme apparatus to penetrate an insect's cuticle (Samsinakova et al, 1977; Hall and Papierok, 1982; St. Leger et al, 1986). The insect's cuticle contains protein, chitin, lipids and phenolic compounds (Hall and Papierok, 1982). The penetration of the host's cuticle, which can form a successful barrier, is often the most critical step in the infection (Pendland and Boucias, 1986). In general, it has been found that the amount of secreted enzymes, particularly proteases and chitinases, affects the degree of intensity of the infection (Samsinakova et al, 1977; St. Leger et al, 1986). Quantitative studies of chitinases from P. farinosus have involved isolates from insects, particularly the Colorado beetle (Samsinakova et al, 1977), but none from other sources such as the soil or litter.

A major component of an insect's cuticle is chitin; therefore, chitinases are perhaps the most important enzymes necessary for penetration. Chitin is difficult to degrade

and few saprophytic fungi possess chitinases (Gochenaur, 1984). The majority of P. farinosus isolates (96.3%), however, were able to utilize chitin as a carbon source.

The second important group of enzymes necessary to penetrate the cuticle are proteases. When chitinases are present, the ability to penetrate the cuticle is dependent on proteases (Samsinakova et al, 1977; St. Leger et al, 1986). All P. farinosus isolates used gelatin (a protein) to some degree. Of all the substrates tested, the growth rate and the rate of utilization of gelatin was greatest. Again, there appeared to be no difference between isolates from the litter and spruce budworm in the utilization of gelatin. In combination with their utilization of chitin, the use of gelatin indicates that the P. farinosus isolates have the necessary enzymes to penetrate the cuticle of insects.

While it is accepted that entomopathogenic fungi attack insects through the cuticle, there is no field evidence that this is the primary mode of infection in the field. Laboratory studies have shown that the conidia of anamorphic entomopathogenic fungi are capable of penetrating the cuticle (Samsinakova et al, 1977; Pekrul and Grula, 1979; Balakrishnan and Nene, 1980; St. Leger et al, 1986), and in these cases, chitinases and proteases together are of primary importance. It may also be suggested that under

field conditions, insects could be infected through ingestion of the conidia. Most fungi are not extremely sensitive to acid conditions, which would be encountered in an insect's stomach. If this were the case, proteases would become the primary enzymes neccessary for successful insect infection. Evidence has shown that Metarhizium anisopliae, while capable of penetrating an insects' cuticle, showed increased pathogenic activity when ingested by the pales weevil (Hylobius pales), although the conidia did not penetrate the intestine (Schabel, 1976). Further laboratory and field studies should be initiated to determine the degree of pathogenicity of cuticular versus internal infection and the actual modes of infection in the field.

As isolates from both spruce budworm and litter were used in this study, morphological characteristics were examined to determine whether any consistent differences existed between these groups of isolates. P. farinosus is known to possess certain typical characteristics: a distinct yellow or orange colony color, the presence of synnemata, and sometimes exudate. If there are actually two different populations (or possibly species) present in the ecosystem, it may be possible that two distinct morphologies are present. All 54 isolates were examined for the ability to produce synnemata, and colony color (on both the surface and reverse of the colonies). The production of exudate, also a

characteristic sometimes associated with P. farinosus, is not included in the results, as its presence was not consistant for any one isolate on any medium.

The production of synnemata is often associated with fungal insect pathogens. Synnemata are aerial, multihyphal structures in which the apices of the component hyphae advance together and ultimately produce spores (Watkinson, 1979). The production of synnemata, as they are associated with entomopathogens, is thought to decrease with repeated subculturing on artificial media (Brown and Smith, 1957; Samson et al, 1980), as in some cases, pathogenicity is also thought to decrease (Daoust and Roberts, 1982; Samsinakova and Kalaova, 1983; Goettel, 1987b). The P. farinosus isolates were tested for the production of synnemata to determine any possible differences between isolates from different origins. If the assumption that synnemata are produced primarily by insect pathogens and disappear in the absence of an insect host is true then the synnemata would be more prevalent in the isolates from spruce budworm than from litter isolates. No observable difference between these two groups was seen, although there was variation in the type and number of synnemata produced. The synnemata produced varied from one or two, to many which completely covered the colony. In fact, of the 52% of the isolates which do produce synnemata, 54% were litter isolates and 50%

were spruce budworm isolates. The production of synnemata in P. farinosus, therefore, does not appear to be correlated with insect isolates.

There appeared to be three fairly distinct colony colors in these isolates of P. farinosus, although they do not appear to be associated with any particular group. Colony color (and morphology) varied between isolates, from white, flat colonies, through yellow colonies (neither flat nor floccose), to deep, vibrant orange colonies, which tended to be dense and floccose (and normally produced synnemata which completely covered the colony). Although these characteristics are hard to quantify, the fact that each isolate consistently showed the same morphological characteristics appears to indicate that, while there is genetic diversity, these characteristics cannot be associated with any one group of isolates. These characteristics cannot be used to distinguish between isolates from spruce budworm and the litter.

As mentioned previously, the ability to survive in the tops of the balsam fir trees would be a very good asset when considering a fungus for biological control of spruce budworm. If P. farinosus can survive in these conditions, it may be present to infect spruce budworm directly from the trees, and migration of the larvae on the ground would not be necessary to spread the infection to other members of the

spruce budworm population. Fungi which typically inhabit the phylloplane are dark-spored and resistant to desiccation (Park, 1982). Phylloplane fungi need to be able to withstand ultraviolet radiation from the sun (Park, 1982). Secondary saprophytes, typical members of the soil mycoflora, are normally not dark in color. They are usually green (for example, Trichodermas and Penicillia), or hyaline such as (Verticillium and Acremonium). P. farinosus is normally considered to be a hyaline fungus, and therefore it would not be expected to be able to survive in the tree tops. Typically, hyaline fungi dry out when exposed to ultraviolet radiation (Park, 1982).

P. farinosus isolates, when tested for resistance to desiccation for periods of one to four weeks, showed varying degrees of ability to resist dehydration. Only hyphal growth was actually measured, as fungal spores can often survive for varying periods of time while the colony dries out and sporulate when favorable conditions appear, with new colonies developing from the sporelings (as occured with the control fungi T. harzianum and P. brevicompactum). The ability of P. farinosus to withstand desiccation is surprising, and could possibly be a big advantage in the control of spruce budworm (Zimmerman, 1982). If control measures were taken with P. farinosus being sprayed in the trees to control spruce budworm outbreaks, it apppears that

the fungus could withstand desiccation by the sun and actively grow in the trees. It may then be possible to infect the second larval instars when they emerge in the spring, thus preventing any damage to young balsam fir and spruce foliage.

The hypothesis that there might be two genetically distinct populations of P. farinosus, one found in the litter and one on parasitized spruce budworm, did not appear to be supported by the data. A discriminant analysis of all the data was performed to determine if the isolates could be classified into two groups based on source of isolation (Table 7, Figure 4). Surprisingly, there did appear to be a trend in separating the two populations into two groups (Figure 4), although there was much overlap between the groups. A factor analysis, while not presented here, confirmed the trend for the isolates to separate into two groups based on the source of isolates. While most characteristics did not differ between groups, some characteristics appeared to be associated with one particular group. The growth rate of the isolates on gelatin after a two week period appeared to be the 'ultimate' characteristic which separated the two groups. A large colony diameter on gelatin was primarily associated with isolates from spruce budworm. This is not unexpected as a characteristic of insect pathogens is the production of

proteases, which aid in penetrating the insect cuticle. Gelatin is a protein which all isolates utilized and on which all grew well. Somewhat surprising is that the growth rate on gelatin and not the utilization was the characteristic which correlated with spruce budworm isolates. The utilization of gelatin did not strongly correlate with either group.

The factor which most strongly correlated with litter isolates was colony diameter on cellulose. This again is not unexpected as the possession of cellulases is typically associated with soil saprophytes (Deacon, 1984). Cellulases aid in breaking down plant cells and are essential for the survival of soil saprophytes. Again, cellulose utilization does not appear to be strongly correlated with either group, which is surprising. Cellulases would not be essential to an insect pathogen as there is no cellulose in the insect cuticle. Litter isolates and spruce budworm isolates express characters which would be associated with their mode of survival. Utilization of these substrates would also be expected to be correlated with a population's way of life. The utilization studies on these enzymes (particularly that of cellulose) were not strictly quantitative, and further studies would be of interest to determine quantitative use of these substrates.

While growth on gelatin and cellulose appears to be the

factor which most strongly categorizes the two groups, other factors do appear to be correlated with either group. Pectin utilization, growth diameter on chitin, and resistance to desiccation were primarily associated with the spruce budworm isolates. Correlations between isolates from spruce budworm and growth on chitin and resistance to desiccation, while not strongly correlated, are not unexpected. As mentioned previously, chitin is a major component of an insect's cuticle, and an entomopathogen would be expected to successfully survive on chitin. Resistance to desiccation could also be associated with an entomopathogen which attacks insects which live in the tops of trees. Pectin utilization would typically not be associated with an insect pathogen. Pectinases are normally associated with plant pathogens (Dahm et al, 1987; Strzelczyk et al, 1987) as pectin is the cement which holds plant cells together. The only possible suggestion which comes to mind is the association of survival in live plants or trees, and pectins. Possibly, the possession of pectinases may aid in survival when no insect host is present in the trees. The fungi may then turn to alternate energy sources and utilize living plant material, although this does not seem likely. The possession of pectinases in these isolates is somewhat surprising, as is the correlation between pectinases and isolates from spruce budworm larvae.

In general, the results appear to indicate that P. farinosus is probably a soil saprophyte and opportunistic parasite, surviving and reproducing on litter and attacking insects when present. It can decompose balsam fir litter and it possesses the necessary enzymes to survive as a saprophyte. The abundance of P. farinosus isolated from the L_1 layer suggests that it is a natural inhabitant of balsam fir forests and may increase in response to a large population of spruce budworm (or possibly of other insects). Its ability to attack spruce budworm can lead to a high incidence of insect mortality when infestation occurs. It may be carried up into the trees by migrating insect larvae where it may attack the rest of the population. It has the basic characteristics necessary to attack and possibly control outbreaks of spruce budworm. Its ability to survive as a saprophyte may be an asset when considering its potential as a biological control agent.

The results from the discriminant analysis indicate that there are subtle differences between populations of P. farinosus from litter and insects. While further in-depth investigation would aid in identifying the presence of genetically different strains, we cannot make this conclusion based on the present study. The only statement that can be made is that isolates from spruce budworm and from litter show some varying characteristics. There

appears to be a wide degree of variation among isolates of P. farinosus. The isolates from spruce budworm may constitute a 'typical' example of P. farinosus which are more opportunistic than that of the litter isolates. That is, the isolates obtained from the spruce budworm were 'better' at attacking the insect and, therefore, were ecologically selected (i.e. outcompeted) over litter isolates.

Further information is needed on these isolates with respect to their competitive ability, pathogenicity, and enzyme complement, i.e. with gel electrophoresis. This would aid in determining whether there are two distinct populations and possibly different species or strains. Differences in pathogenicity between isolates is very probable and has been observed in other studies involving P. farinosus (Samsinakova et al, 1977; Kmitowa, 1982a; Jones, 1986) as well as other entomopathogenic fungi (Samsinakova et al, 1977; Daoust and Roberts, 1982; Samsinakova and Kalalova, 1983; Soares et al, 1985; Romana and Fargues, 1987). In order to implement a biological control program with P. farinosus, the right combination of characteristics, one of which is a high degree of pathogenicity, needs to be identified in one isolate. Others factors would include good saprophytic ability if the organism is intended to remain in the ecosystem as a preventive measure, and

tolerance to any chemicals (i.e. herbicides, pesticides) which may be used in control of other organisms or in integrated control of the target pest.

The evidence from this study appears to indicate that P. farinosus may be a possible control agent for spruce budworm. Pathogenicity tests from other studies on the Colorado beetle (Samsinakova et al, 1977; Bajan et al, 1982) and spruce budworm (Jones, 1986; Perry, pers comm) indicate that under favorable conditions, P. farinosus can cause high degrees of mortality. Factors which may affect the pathogenicity of P. farinosus are temperature (Jones, 1986), humidity (Doberski, 1981; Mierzejewska, 1982b), and the presence of herbicides (Bajan and Kmitowa, 1982). When conditions are favorable, P. farinosus may be able to successfully control spruce budworm. Previous studies have found that P. farinosus has a fairly wide range of tolerance to temperature when attacking spruce budworm (Jones, 1986). It appears that, of nine genera of entomogenous Hyphomycete fungi examined, P. farinosus (in terms of temperature tolerance and pathogenicity) showed one of the highest potentials for spruce budworm control (Jones, 1986). The potential of P. farinosus as a biological control agent of spruce budworm as well as other insects should be further explored.

Studies with other species of entomopathogenic

Hyphomycetes have showed a promising future for insect control with fungi. (Hall and Burges, 1979; Hall and Papierok, 1982; Sweeney, 1982; Mohamed and Nelson, 1985; Harper and Huang, 1986; Goettel, 1987a). Field trials with Beauveria, Metarhizium, Tolypocladium, Verticillium, Culcinomyces, and Hirsutella have shown that with the necessary ecological information, successful control is possible (Hall and Burges, 1979; Hall and Papierok, 1982; Sweeney, 1982; Campbell et al, 1985; Mohamed and Nelson, 1985; Gardner and Pillai, 1987b). When conditions are favorable, outstanding results can occur, as in the control of aphids and whiteflies in greenhouses (Hall and Papierok, 1982). V. lecanii has shown excellent control of these pests in conditions where the humidity is high (as in glasshouses) (Hall and Burges, 1979). Knowledge of the ecology of both the fungus and the host, as well as favorable conditions under which infection can occur, are obviously necessary before control measures are implemented. There are many insect-attacking fungi, but most are not considered potential biological control agents, as conditions for infection are not feasibly manipulated (as in Coelomomyces which attacks mosquitoes) (Hall and Papierok, 1982).

While P. farinosus has the basic qualities associated with insect control, further information is necessary before

field trials and potential control programs are initiated. Studies on Metarhizium, Beauveria, Tolypocladium, and Culcinomyces, have concerned their enzyme complements, the production and pathogenicity of blastospores (Smith and Grula, 1981; Bidochka et al, 1987), mass production and storage (Campbell et al, 1978; Campbell et al, 1983; Goettel, 1987c), and field trials (Campbell et al, 1985; Goettel, 1987a). Many researchers feel that the ultimate insect control method with Hyphomycete fungi is with blastospores. Blastospores are asexual spores produced in liquid culture and are generally easier to obtain in large quantities (Bidochka et al, 1987) than are conidia. Blastospores are generally as pathogenic to insects as conidia (Gardner and Pillai, 1987a). Other researchers feel that the fragile nature of the blastospore in the field discounts their possible effectiveness as a control measure (Goettel, 1987a, b; Thomas et al, 1987). Conidia, while often more difficult to produce, usually are more resistant to the environment. The production and pathogenicity of blastospores by P. farinosus should be further investigated before its use in insect control is considered.

The number of conidia needed in a suspension for effective control of insects is normally very high (approximately 10^7 conidia/ml or more) (Cantwell et al, 1986; Gardner and Pillai, 1987b; Goettel, 1987a).

Inexpensive methods of producing such high concentrations of spores are currently being sought for a number of fungi (Thomas et al, 1987), and, once a satisfactory isolate or strain of P. farinosus is identified as a potential control agent, should be sought for P. farinosus. The production of conidia themselves is often subject to very difficult culture conditions and may be very slow (Samson, 1982; Goettel, 1987c; Thomas et al, 1987). The formulation of a suitable medium for abundant, fast production of conidia is a necessary first step in mass production (Samson, 1982). Once conidia are produced, there is the added problem of storage of these products (Samson, 1982; Goettel, 1987a, b). In addition to space considerations, problems arise also due to the virulence of the conidia (Samson, 1982). Most conidia produced at present have a shelf-life (i.e. viability) of approximately six months to 2 years maximum (Goettel, 1987c). This decreases their value in terms of mass production and cost efficiency. Means of prolonging shelf-life for a more cost efficient process are necessary to increase interest in fungi as biological control agents.

Field trials with anamorphic fungi have proven that these fungi have potential for insect control (Sweeney, 1982; Mohamed and Nelson, 1985). Although there have been many failures (Sweeney, 1982; Goettel, 1987c), the successes indicate a potentially good future. Under controlled

conditions, where environmental factors can be manipulated, fungi have proven to be successful control agents (Hall and Burges, 1979). There has also been some success in field trials where environmental factors could not be manipulated, but where the ecology of both the host and fungus was well understood, as was the environment in which the fungus was being tested (Sweeney, 1982; Campbell et al, 1985, Goettel, 1987a). Application of fungal conidia in the field is under investigation (Storey and Gardner, 1986). At present, the conidial suspension is normally applied with sprays or through irrigation systems. This may impede control as it appears that the fungus does not always reach the host, although the irrigation systems have proven to overcome part of this problem (Storey et al, 1987).

When considering a fungus for biological control, it's toxicity to life other than the target pest must be examined. While most Hyphomycetes are nontoxic to vertebrates, there must exist concrete evidence which addresses possible problems. Part of the reason why biological control programs have been initiated is the growing concern over side-effects of chemicals (Mishra et al, 1987). Many chemicals have the potential to seriously harm the environment and life besides that of the target pest (Mishra et al, 1987). While some species of Paecilomyces have been shown to infect man and other

vertebrates, P. farinosus has never been isolated from vertebrates or shown any potential to infect life other than insects, although this has not been intensively studied. Paecilomyces fumosoroseus, an entomopathogen of several pests, has been shown to be non-toxic to birds (Wasti et al, 1980). This is another aspect of P. farinosus which needs to be examined.

Possibly the greatest potential for using fungi as biological control agents is in integrated control. Integrated control programs have been initiated in some areas with a good degree of success. Integrated control with fungi would involve the fungus itself along with another form of control. Although this other form is usually chemical, it may also be another biological control agent, or alternately the environment may be manipulated in some way. The fungus could be used as a preventive measure once other methods of control have decreased the pest population, in this manner complete control may be possible.

In summary, the results indicate that P. farinosus is an active soil saprophyte, surviving in the soil and acting as an opportunistic parasite on spruce budworm. When large numbers of spruce budworm appear in these systems, P. farinosus may respond with its own increase in abundance, parasitising spruce budworm with a possible potential for a high rate of mortality. The possibility that there are two

genetically different populations of P. farinosus is still not answered. The evidence suggests a physiologically versatile, genetically diverse population of P. farinosus, from which some selection takes place during the process of colonization of litter, or parasitization of the budworm. Studies on the genetics (using gel electrophoresis) of the enzyme systems of these isolates would help determine their genetic similarities or differences. Further information on pathogenicity and application of P. farinosus is necessary to determine its full potential for insect control.

CONCLUSION

P. farinosus is a secondary soil saprophyte which is a natural member of the microfungal community in a balsam fir forest. The results from the study on the saprophytic ability show that it possesses a large number of hydrolytic enzymes necessary for survival in the soil, and that it can actively degrade balsam fir needles. Its isolation in such high abundance from balsam fir needles also indicates that it is a natural inhabitant of balsam fir forests, although whether this was due to an outbreak of spruce budworm in the study areas is unknown. Data on the soil microfungi in previous years is unavailable, and future monitoring would help form a conclusion. Results obtained from the Canadian Forestry Service in Ste. Foy, Québec, indicate that its abundance is probably due to the presence of spruce budworm. The infection of spruce budworm with P. farinosus in previous years was very low, with a sharp increase during the years of this study, suggesting that P. farinosus increased in abundance in response to the budworm.

It also appears that P. farinosus has the necessary basic characteristics of a successful insect pathogen. The possession of both proteases and chitinases, necessary for the penetration of insect cuticle, was found in the majority of isolates tested. The ability to resist desiccation also indicates a successful potential biological control agent

for spruce budworm, as this would allow the fungus to survive in tree tops and directly infect the insects. The infection of the insect by the fungus probably occurs on the ground, while some larvae migrate into other trees and carry the spores of the fungus. This is indicated by the results from the study on the abundance of P. farinosus on needles dropping from the trees before hitting the ground.

The evidence in favor of two genetically distinct ecotypes of P. farinosus, one from the litter and one from spruce budworm, is weak; however, growth rate on gelatin (a protein) is correlated with isolates from spruce budworm and growth rate on cellulose with isolates from the litter. Further information about the genetics of these isolates is needed. The potential of P. farinosus as a biological control agent needs to be further explored. Other studies involving the pathogenicity of P. farinosus have shown that high insect mortality can occur. This study indicates that the ecology of P. farinosus is an advantage when considering this fungus for biological control. The ability of P. farinosus to survive in the absence of an insect host would be beneficial in a biological control program.

REFERENCES

Aaronson, S. 1970. Experimental Microbial Ecology. Academic Press, New York.

Agudelo, F. and Falcon, L. 1983. Mass production, infectivity, and field application studies with the entomogenous fungus Paecilomyces farinosus. J. Invertebr. Pathol. 42:124-132.

Anderson, R. and May, R. 1980. Infectious diseases and population cycles of forest insects. Science 210:658-661.

Bajan, C. and Fedorko, A. 1982. The course of diminishing numbers of entomopathogenic fungi introduced to soil. Pol. ecol. Stud. 8:453-471.

Bajan, C., Fedorka, A., and Kmitowa, K. 1982. Factors responsible for effectiveness of microbial control of plant pests as exemplified by Colorado beetle (Leptinotarsa decemlineata Say). Pol. ecol. Stud. 8:363-407.

Bajan, C., and Kmitowa, K. 1982. The effect of herbicides: Simazin 50, Avadex and Antyperz on four species of entomopathogenic fungi. Pol. ecol. Stud. 8:489-497.

Balakrishran, S. and Nene, Y. 1980. A note on the mode of penetration of the fungus Paecilomyces farinosus (Dickson Ex. Fries) Brown and Smith into the whitefly

Bemisia tabaci Gennadius. Science and Culture 46:231-232.

Barfield, C. and Stimac, J. 1980. Pest Management: An entomological approach. BioScience 30:683-689.

Bidochka, M., Pfeifer, T., and Khachatourians, G. 1987. Development of the entomopathogenic fungus Beauveria bassiana in liquid cultures. Mycopathologia 99:77-83.

Bissett, J. and Widden, P. 1972. An automatic, multichamber soil-washing apparatus for removing fungal spores from soil. Can. J. Micro. 18:1399-1404.

Blais, J. 1965. Spruce budworm outbreaks in the past three centuries in the Laurentide Park, Québec. For. Sci. 11:130-138.

Blais, J. 1968. Regional variation in susceptability of eastern North American forests to budworm attack based on history of outbreaks. For. Chron. 4:17-23.

Blais, J. 1985. The ecology of the eastern spruce budworm: A review and discussion. In: Recent Advances in Spruce Budworm Research: Proceedings of the CANUSA Spruce Budworms Research Symposium (eds. C. Sanders, R. Stark, E. Mullins, and J. Murphy). Minister of Supply and Services Canada, Ottawa, Canada.

Brown, A. and Smith, G. 1957. The genus Paecilomyces Bainier and its perfect stage Byssochlamys Westling. Trans. Br. mycol. Soc. 40:17-89.

Campbell, R., Anderson, T., Semel, M., and Roberts, D. 1985. Management of the Colorado potato beetle using the entomogenous fungus Beauveria bassiana. American Potato Journal 62:29-37.

Campbell, R., Barnes, G., Cartwright, B., and Eikenbary, R. 1983. Growth and sporulation of Beauveria bassiana and Metarhizium anisopliae in a basal medium containing various carbohydrate sources. J. Invertebr. Pathol. 41:117-121.

Campbell, R., Perring, T., Barnes, G., Eikenbary, R., and Gentry, C. 1978. Growth and sporulation of Beauveria bassiana and Metarrhizium anisopliae on media containing various amino acids. J. Invertebr. Pathol. 31:289-295.

Cantwell, G., Cantelo, W., and Schroder, R. 1986. Effect of Beauveria bassiana on underground stages of the Colorado potato beetle, Leptinotarsa decemlineata (Coleoptera: Chrysomelidae). Great Lakes Entom. 19:81-84.

Carroll, G. 1987. Fungi isolated from gypsy moth egg-masses. Mycotaxon 29:299-305.

Dahm, H., Strzelczyk, E., and Majewska, L. 1987. Cellulolytic and pectolytic activity of mycorrhizal fungi, bacteria and actinomycetes associated with the roots of Pinus sylvestris. Pedobiologia 30:73-80.

Danielson, R. 1971. The ecology and physiology of Trichoderma in forest soils. PhD Thesis. Raleigh, N.C.

Daoust, R. and Roberts, D. 1982. Virulence of natural and insect-passaged strains of Metarhizium anisopliae to mosquito larvae. J. Invertebr. Pathol. 40:107-117.

Debach, P. 1974. Biological Control by Natural Enemies. Cambridge University Press, Great Britain.

Deacon, J. 1984. Introduction to Modern Mycology. 2nd ed. Blackwell Scientific Publications, England.

Dennis, R. and Webster, J. 1971a. Antagonistic properties of species-groups of Trichoderma. I Production of non-volatile antibiotics. Trans. Br. mycol. Soc. 57:25-39.

Dennis, R. and Webster, J. 1971b. Antagonistic properties of species-groups of Trichoderma. II Production of volatile antibiotics. Trans. Br. mycol. Soc. 57:41-48.

Doberski, J. 1981a. Comparative laboratory studies on three fungal pathogens of the elm bark beetle Scolytus scolytus: Pathogenicity of Beauveria bassiana, Metarhizium anisopliae, and Paecilomyces farinosus to larvae and adults of S.scolytus. J. Invertebr. Pathol. 37:188-194.

Doberski, J. 1981b. Comparative laboratory studies on three fungal pathogens of the elm bark beetle Scolytus scolytus: Effect of temperature and humidity on

infection by Beauveria bassiana, Metarhizium anisopliae, and Paecilomyces farinosus. J. Invertebr. Pathol., 37:195-200.

Domsch, K., Gams, W., and Anderson, T. 1980. Compendium of Soil Fungi. Vol. 1. Academic Press, New York.

Dunbar, D., Weseloh, R., and Walton, G. 1972. A fungus observed on egg clusters of the gypsy moth, Porthetria dispar (Lepidoptera: Lymantriidae). Ann. Entom. Soc. Am. 65:1419-1421.

Dunphy, G., Chadwick, J., and Nolan, R. 1985. Influence of physical factors and selected media on the growth of Entomophthora egressa protoplasts isolated from spruce budworm larvae. Mycologia 77:887-893.

Dunphy, G. and Nolan, R. 1982. Simplified growth media for Entomphthora egressa protoplasts. Can. J. Microbiol. 28:815-821.

Emlen, J. 1984. Population Biology: The Coevolution of Population Dynamics and Behavior. MacMillan Publishing Company, New York.

Flanagan, P. and Scarborough, A. 1974. Physiological groups of decomposer fungi on Tundra plant remains. In: Soil Organisms and Decomposition in Tundra. (eds. A. Holding, O. Heal, S. MacLean, and P. Flanagan). Tundra Biome Steering Committee, Stockholm, Sweden.

Garcia, R. 1983. Mosquito management: Ecological

approaches. Environmental Management 7:73-78.

Gardner, J., and Pillai, J. 1987a. Tolypocladium cylindrosporum (Deuteromycotina: Moniliales), a fungal pathogen of the mosquito Aedes australis: II. Methods of spore propagation and storage. Mycopathologia 97:77-82.

Gardner, J., and Pillai, J. 1987b. Tolypocladium cylindrosporum (Deuteromycotina: Moniliales), a fungal pathogen of the mosquito Aedes australis: III. Field trials against two mosquito species. Mycopathologia 97:83-88.

Gochenaur, S. 1978. Fungi of a Long Island oak-birch forest I. Community organization and seasonal occurence of the opportunistic decomposers of the A horizon. Mycologia 70:975-994.

Gochenaur, S. 1984. Fungi of a Long Island oak-birch forest II. Population dynamics and hydrolase pattern for soil Penicillia. Mycologia 76:218-231.

Goettel, M. 1987a. Preliminary field trials with the entomopathogenic Hyphomycete Tolypocladium cylindrosporum in central Alberta. J. Amer. Mosq. Control Assoc. 3:239-245.

Goettel, M. 1987b. Serial in vivo passage of the entomopathogenic Hyphomycete Tolypocladium cylindrosporum in mosquitoes. Can. Entom. 119:599-601.

Goettel, M. 1987c. Conidial viability of the mosquito pathogenic Hyphomycete Tolypocladium cylindrosporum, following prolonged storage at -20°C. J. Invertebr. Pathol. 50:327-329.

Gourbière, F. 1974. Les champignons microscopiques liés aux aiguilles de sapin (Abies alba Mill.) 2. Variations saisonières de la microflore des aiguilles tombantes. Bull. Soc. Mycol. Fr. 90:325-333.

Gourbière, F. 1981. Champignons des aiguilles de sapin (Abies alba Mill.) 7. Microflores basophiles. Bull. Soc. Mycol. Fr. 97:81-89.

Hall, R. and Burges, H. 1979. Control of aphids in glasshouses with the fungus Verticillium lecanii. Ann. Appl. Biol. 93:235-245.

Hall, R. and Papierok, B. 1982. Fungi as biological control agents of arthropods of agricultural and medical importance. Parasitology 84:205-240.

Hankin, L. and Anagnostakis, S. 1975. The use of solid media for detection of enzyme production by fungi. Mycologia 67:597-607.

Harper, A. and Huang, H. 1986. Evaluation of the entomophagous fungus Verticillium lecanii (Moniliales: Moniliaceae) as a control agent for insects. Environ. Entomol. 15:281-284.

Harper, J. 1987. Present and future status of microbial

control of arthropods. Crop Protection 6:117-122.

Hayes, A. 1965a. Studies on the decomposition of coniferous leaf litter II. Changes in external features and succession of microfungi. J. Soil Sci. 16:242-257.

Hayes, A. 1965b. Some microfungi from Scots pine litter. Trans. Br. mycol. Soc. 48:179-185.

Houle, C., Hartmann, G., and Wasti, S. 1987. Infectivity of eight species of entomogenous fungi to the larvae of elm bark beetle, Scolytus multistriatus (Marsham). J. New York Entomol. Soc. 95:14-18.

Hurst, H., Burges, A., and Latter, P. 1962. Some aspects of the biochemistry of humic acid decomposition by fungi. Phytochemistry 1:227-231.

Inch, J., Humphreys, A., Trinci, P., and Gillespie, A. 1986. Growth and blastospore formation by Paecilomyces fumosoroseus, a pathogen of brown planthopper (Nilaparvata lugens). Trans. Br. mycol. Soc. 87:215-222.

Jones, B. 1986. Effects of temperature on development and infectivity of anamorphic fungi with potential for control of the eastern spruce budworm. MSc Thesis. University of Waterloo.

Kahlki, R., Kloidt, M., and Lysek, G. 1986. Phylloplane inhabiting fungi of pine needles (Pinus sylvestris L.) in Berlin (West). Nova Hedwigia 42:597-601.

Kendrick, B. 1985. The Fifth Kingdom. Mycologue Publications. Waterloo, Ontario, Canada.

Kendrick, W. and Burges, A. 1962. Biological aspects of the decay of Pinus sylvestris leaf litter. Nova Hedwigia 4:313-342.

Kjøller, A., and Struwe, S. 1980. Microfungi of decomposing red alder leaves and their substrate utilization. Soil Biol. Biochem. 12:425-431.

Kmitowa, K. 1982a. Characteristics of strains of Paecilomyces farinosus (Dicks) Brown et Smith. Pol. ecol. Stud. 8:419-431.

Kmitowa, K. 1982b. Preliminary studies on the effect of passaging through the host on pathogenicity of entomopathogenic fungi. Pol. ecol. Stud. 8:433-441.

Kuruvilla, S. and Jacob, A. 1980. Pathogenicity of the entomogenous fungus Paecilomyces farinosus (Dickson Ex. Fries) to several insect pests. Entomon. 5:175-176.

Kuter, G. 1986. Microfungal populations associated with the decompostion of sugar maple leaf litter. Mycologia 78:114-126.

May, R. 1983. Parasitic infections as regulators of animal populations. American Scientist 71:36-45.

May, R. and Hassell, M. 1988. Population dynamics and biological control. Phil. Trans. R. Soc. Lond. B. 318:129-169.

McRae, D. 1986. Prescribed burning for stand conversion in budworm-killed balsam fir: An Ontario case history. For. Chron. 96-100.

Messias, C. and Azevedo, J. 1980. Parasexuality in the Deuteromycete Metarhizium anisopliae. Trans Br. mycol. Soc. 75:473-477.

Mierzejewska, E. 1982a. Mixed infection of Acantnoscelides obtectus Say with entomopathogenic fungi. Pol. ecol. Stud. 8:443-447.

Mierzejewska, E. 1982b. Germination of spores of entomopathogenic fungi in various habitats. Pol. ecol. Stud. 8:449-452.

Mishra, S., Keller, J., Miller, J., Heisey, R., Nair, M., and Putnam, A. 1987. Insecticidal and nematicidal properties of microbial metabolites. J. Indust. Microbiol. 2:267-276.

Mohamed, A. and Nelson, F. 1985. Pathogenicity of Metarhizium anisopliae var. anisopliae to mosquito larvae. J. Entomol. Sci. 20:398-404.

Mohanan, C. and Varma, R. 1988. Paecilomyces farinosus, a potential biocontrol agent of some lepidopterous pests in India. Trans. Br. mycol. Soc. 90:119-122.

Morris, O. 1982. Report of the 1981 Cooperative Bacillus thuringiensis (B.T.) spray trials. Minister of Supply and Services, Canada. Report FPM-X-58.

Nie, N., Hadlai-Hull, C., Jenkins, J., Steinbrenner, J., and Bent, D. 1975. Statistical Package for the Social Sciences, 2nd ed. McGraw-Hill, Toronto.

Pacioni, G. and Frizzi, G. 1978. Paecilomyces farinosus, the conidial state of Cordyceps memorabilis. Can. J. Bot. 56:391-394.

Park, D. 1982. Phylloplane fungi: Tolerence of hyphal tips to drying. Trans. Br. mycol. Soc. 79:174-178.

Parkinson, D. and Williams, S. 1961. A method for isolating fungi from soil microhabitats. Plant and Soil 13:347-355.

Pekrul, S. and Grula, E. 1979. Mode of infection of the corn earworm (Heliothis zea) by Beauveria bassiana as revealed by scanning electron microscopy. J. Invertebr. Pathol. 34:238-247.

Pendland, J. and Boucias, D. 1986. Lectin binding characteristics of several entomogenous Hyphomycetes: Possible relationship to insect haemaglutinins. Mycologia 78:818-824.

Pendland, J., Heath, M., and Boucias, D. 1988. Function of a galactose binding lectin from Spodoptera exigua larval haemolymph: Opsonization of blastospores from entomogenous Hyphomycetes. J. Insect Physiol. 34:533-540.

Perry, D., Tyrrel, D., and DeLyzer, A. 1982. The mode of

germination of Zoophthora radicans zygospores. Mycologia 74:549-554.

Perry, D. and Whitfield, G. 1984. The interelationships between microbial entomopathogens and insect hosts: A system study approach with particular reference to the Entomophthorales and the eastern spruce budworm. In: Invertebrate-microbial Interactions (eds. Anderson,J., Raynor, A., and Walton, D.). Cambridge University Press, Cambridge, England.

Pickett, J. 1988. Integrating use of beneficial organisms with chemical crop protection. Phil. Trans. R. Soc. Lond. B 318:203-211.

Régnière, J. and Lethiecq, J.L. Comparative description of the physical environment and vegetaton of five sites used by the Canadian Forestry Service in the study of spruce budworm population dynamics. Unpublished Report.

Romana, C. and Fargues, J. 1987. Sensibilite des larves hemathophage, Rhodnius prolixus (Triatominae) aux Hyphomycetes entomopathogenes. Entomophaga 32:167-179.

Royama, T. 1981. Fundamental concepts and methodology for the analysis of animal population dynamics, with particular reference to univoltine species. Ecol. Mono. 51:473-493.

Samsinakova, A., Bajan, C., Kaloava, S., Kmitowa, K., and

Wojciechowska, M. 1977. The effect of some entomopathogenic fungi on the Colorado beetle and their enzyme activity. Bulletin de L'Academie Polonaise des Sciences. Series des sciences biologiques. Cl. II Vol. 25:521-526.

Samsinakova, A and Kalalova, S. 1978. The fungus Paecilomyces farinosus Br. et Smith (Deuteromycetes). Z. ang. Ent. 8:68-75.

Samsinakova, A and Kalalova, S. 1983. The influence of a single-spore isolate and repeated subculturing on the pathogenicity of conidia of the entomophagous fungus Beauveria bassiana. J. Invertebr. Pathol. 42:156-161.

Samson, R. 1974. Paecilomyces and some allied Hyphomycetes. Studies in Mycology 6:1-119.

Samson, R. 1982. Laboratory culture and maintenance of entomopathogenic fungi. In: Invertebrate Pathology and Microbial Control. Proc. 3rd. Colloq. Invertebr. Pathol., Brighton, England.

Samson, R., McCoy, C., and O'Donnell, K. 1980. Taxonomy of the acarine parasite Hirsutella thompsonii. Mycologia 72:359-377.

Schabel, H. 1976. Oral infection of Hylobius pales by Metarhizium anisopliae. J. Invertebr. Pathol. 27:377-383.

Skujins, J., Potgieter, H., and Alexander, M. 1965.

Dissolution of fungal cell walls by a Streptomycete chitinase and B-(1-3) glucanase. Arch. Biochem. and Biophys. 111:358-364.

Smirnoff, W. and Juneau, A. 1982. Physical analysis of the dispersion of Bacillus thuringiensis against spruce budworm. Department of Supply and Services, Canada. Information Report LAU-X-55.

Soares, G., Riba, G., Caudal, A., and Vincent, J. 1985. Comparative studies of eleven isolates of the fungal entomopathogen Tolypocladium cylindrosporum and two isolates of Tolypocladium extinguens. J. Invertebr. Pathol. 46:115-120.

Söderström, B. 1975. Vertical distribution of microfungi in a spruce forest soil in the south of Sweden. Trans. Br. mycol. Soc. 65:419-425.

Söderström, B. and Bååth, E. 1978. Soil microfungi in three Swedish coniferous forests. Holarctic Ecology 1:62-72.

St. Leger, R., Charnley, A., and Cooper, R. 1986. Cuticle-degrading enzymes of entomopathogenic fungi: Mechanisms of interaction between pathogen enzymes and insect cuticle. J. Invertebr. Pathol. 47:295-302.

Storey, G. and Gardner, W. 1986. Sensitivity of the entomogenous fungus Beauveria bassiana to selected plant growth regulators and spray additives. Appl.

Environ. Microbiol. 52:1-3.

Storey, G., Gardner, W., Hamm, J., and Young, R. 1987. Recovery of Beauveria bassiana propagules from soil following application of formulated conidia through an overhead irrigation system. J. Entomol. Sci. 22:355-357.

Strzelczyk, E., Dahm, H., and Kowalski, S. 1987. The influence of temperature on cellulolytic and pectolytic activity of Cylindrocarpon destructans (Zins.) Scholt. J. Phytopathology 120:97-103.

Sweeney, A. 1982. Field evaluation of fungal pathogens of mosquito larvae, with particular reference to Culcinomyces. In: Invertebrate pathology and microbial control. Proc. 3rd. Int. Colloq. Invertebr. Pathol., Brighton, England.

Thomas, K., Khachatourians, G., and Ingledew, W. 1987. Production and properties of Beauveria bassiana conidia cultivated in submerged culture. Can. J. Microbiol. 33:12-20.

Tronsmo, A. and Dennis, C. 1978. Effect of temperature on antagonistic properties of Trichoderma species. Trans. Br. mycol. Soc. 71:469-474.

Varma, R. and Mohanan, C. 1984. Paecilomyces farinosus, a potential biological control agent for major pests of Ailanthus in Kerala, India. In: British Crop

Protection Conference: Pests and Diseases. The Lavenham Press Limited, Lavenham, Suffolk, England.

Wasti, S., Hartmann, G., and Rousseau, A. Gypsy moth mycoses by two species of entomogenous fungi and an assessment of their avian toxicity. Parasitology. 80:419-424.

Watkinson, S. 1979. Growth of rhizomorphs, mycelial strands, coremia and sclerotia. In: Fungal Walls and Hyphal Growth (ed. J. Burnett and A. Trinci). London. Cambridge University Press.

Widden, P. 1979. Fungal populations from forest soils in southern Québec. Can. J. Bot. 57:1324-1331.

Widden, P. 1984. The effects of temperature on competition for spruce needles among sympatric species of Trichoderma. Mycologia 76:873-883.

Widden, P. 1986a. Microfungal community structure from forest soils in southern Québec, using discriminant function and factor analysis. Can. J. Bot. 64:1402-1412.

Widden, P. 1986b. Seasonality of forest soil microfungi in southern Québec. Can. J. Bot. 64:1413-1423.

Widden, P. 1986c. Functional relationships between Québec forest soil microfungi and their environment. Can. J. Bot. 64:1424-1432.

Widden, P. and Hsu, D. 1987. Competition between

Trichoderma species: Effects of temperature and litter type. Soil Biol. Biochem. 19:89-93.

Widden, P. and Parkinson, D. 1973. Fungi from Canadian coniferous forest soils. Can. J. Bot. 51:2275-2290.

Zimmerman, G. 1982. Effect of high temperatures and artificial sunlight on the viability of conidia of *Metarhizium anisopliae*. J. Invertebr. Pathol. 40:36-40.