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Temporal Distribution of Ectomycorrhizal Fungi and Pollen as a Seasonal Nutrient Source in a Boreal Forest, Canada

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ABSTRACT: Seasonal distribution of ectomycorrhizal associations in various types of forest in a boreal forest in Manitoba, Canada was investigated. Alsohe relationship between ectomycorrhizal growth and pine pollen nutrients was examined. In four different forest stands, ectomycorrhizas tended to be lower in the spring than in the summer and fall samples. In addition, a mature jack pine (*Pinus banksiana*) stand showed higher mycorrhizal activities than a young jack pine stand. Growth of *Suillus brevipes* hyphae wa ts stimulated by additions of pollen representing mean pollen deposition in Mistik Creek study area after 30 and 70 days of growth with dextrose availability. This result suggests that the peak ectomycorrhizal activity is followed by pollen deposition in the study region and therefore, addition of pine and spruce pollen in early or middle of June in the boreal forest can be an important seasonal nutrient source for ectomycorrhizal growth.

Key Words: Boreal forest, Ectomycorrhizal fungi, Pollen nutrient, Pine, Spruce.

INTRODUCTION

The positive relationship between soil organic matter and ectomycorrhizal activity suggests a dependence on soil organic reserves for the ability of a given soil to support abundant ectomycorrhizal associations (Koide *et al.* 1998, Harvey *et al.* 1979). Harvey *et al.* (1976, 1978) have shown that humus and brown cubical decayed wood provide a major substrate for ectomycorrhizas in a Douglas -fir/larch soil in western Montana. They also have shown that decayed wood supports the most ectomycorrhizal activity during the drier period of the growing season (Harvey *et al.* 1978). Soil moisture and ectomycorrhizal activity are strongly related (Worley and Hacskaylo 1959). Muttiah (1972) reported that ectomycorrhizas are relatively drought resistant when compared to nonmycorrhizal roots.

According to Allen (1992), the formation of mycorrhizas represents a special adaptation to surviving in unsuitable conditions and under stress in the habitat. The cold-long winter season of the northwestern Manitoba limits the periods when temperature and moisture favor microbial activity. Recent studies confirm that ectomycorrhizal fungi, associated with most coniferous trees, are able to utilize limiting elements from both the organic and inorganic components of soil (Cairney and Ashford 1991, Pankow *et al.* 1991). Ectomycorrhizas facilitate nutrient procurement of trees growing under limiting conditions and are a critical component of the boreal forest in Manitoba, Canada.

Few studies provide insights into the role of pollen in forest nutrient cycling. Doskey and Ugoagwu (1989) determined that pine pollen is an important source of macronutrients to oligotrophic lakes in northern Wisconsin. Stark (1972) suggested that nutrients found in pollen allow fungi to obtain sufficient energy to complete litter decay, which in turn releases other nutrients needed for tree growth. He specifically reported that pollen was primarily infested by hyphal fungi, not by members of Chytridiales (commonly referred to as zoosporic fungi) even though these organisms are known to degrade pollen. Recent studies (Hutchison and Barron 1997) showed that many lignicolous fungi are capable of degrading pollen. But the studies on pollen as a seasonal nutrient source for ectomycorrhizal fungi growth are rare.

This study was undertaken to add our knowledge of relationships between various types of forest and mycorrhizal associations in a boreal forest in Manitoba, Canada. Specific objectives of the mycorrhizal survey included the following: (i) to measure the levels of mycorrhizal colonization of jack pine (*Pinus banksiana*) and black spruce (*Picea mariana*) in the boreal forest and to compare these to levels found by other studies: (ii) to compare seasonal variation in colonization observed in four forest stands in the boreal forest in northern Manitoba: (iii) to examine the possibility of pine pollen as a seasonal nutrient source for ectomycorrhizal fungal growth.

MATERIALS AND METHODS

Study site

The Mistik Creek is located in west-central Manitoba, Canada. (54°40′N, 101°30′W) (Fig. 1) The climate is sub-humid continental and prevailing winds are from the northwest. The growing season, based on a 4℃ index, lasts from early May to early October. Mean annual precipitation is 484 mm and mean annual temperature is −0.5 ℃. Average land elevation is about 300 m above sea level. The area lies on the Canadian Precambrian Shield. As a result of intensive glaciation, relief is irregular with rocky parallel ridges separating poorly drained depressions and narrow lakes. Drift depositions on the uplands are thin in some places and absent in others where rock barrens of Precambrian granites and gneisses are exposed. Soil fertility is low with a weakly developed podzol profile. The study area includes the northern coniferous section of the boreal forest (Rowe 1972).

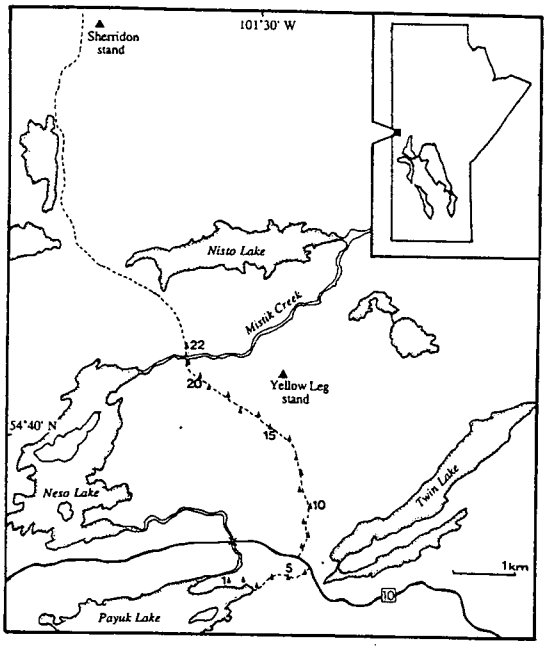

Fig. 1. Location of the study area (Mistik Creek) in west-central Manitoba, Canada. Sites are designated by numbers : Site 5=mature jack pine stand; Site 6=mature black spruce stand; Site 13=black spruce stand in bog; Site 22 =jack pine seedling stand.

Vegetation and site description

Descriptions of four collectiong sites are in Table 1. Within the area, black spruce (*Picea mariana* [Mill.] BSP) is the dominant tree in poorly drained lowlands. Jack pine (*Pinus banksiana* Lamb.) stands are common on well-drained, sandy sites and on upland, rock outcrops. Mixed stands of white spruce (*Picea glauca* [Moench] Voss), balsam fir (*Abies balsamea* [L.] Mill.), Trembling aspen (*Populus tremuloides* Michx) and balsam poplar (*P. balsamifera* L.) occur under more favorable soil conditions. Trees are 5~18 m high with a mean diameter of 11.2 cm at breast height. Mean age of mature jack pine trees is 65 yrs (n=49) and that of jack pine seedling is 5 yrs (n=20). Especially Site 13 is characterized by open and wet habitats and presence of tamarack (*Larix larcina* [Du Roi] K. Koch).

Measure of ectomycorrhizal fungal activity

Sampling for ectomycorrhizas was conducted at four sites in the study area. Soil samples consisted of cylindrical cores 10 cm (diameter) × 12.5 cm (depth) taken randomly at study site 5 (a mature jack pine stand), site 22 (a jack pine seedling stand), site 6 (a mature black spruce stand), and site 13 (a black spruce stand in bog site).

On each of the collecting dates, 26 May, 18 June, 22 August, 10 October 1993 and 9 July, 31 July, 28 August 1994, ten soil cores were gathered for each site. Upon return to the laboratory, woody

Table 1. Site descriptions of four sites where soil samples were collected for ectomycorrhizal activities in boreal Manitoba, Canada. (* Most common tree species at each site; +next common tree species). JP = jack pine (*Pinus banksiana*), BS = black spruce (*Picea mariana*).

Site	Canopy trees	Tall trees and others	Forest floor	Canopy height	Forest soil
Site 5 (Mature JP)	**Pinus banksiana* +*Picea mariana*	*Alnus crispa*	Grasses, lichens, open	12~18 m	On sandy plain, dry
Site 6 (Mature BS)	**Picea mariana* +*Populus tremuloides*	*Alnus crispa* *Cornus canadensis*	Fallen trees and herbs	15~18 m	On the lake plain, dry
Site 13 (BS in Bog)	**Picea mariana* +*Larix laricina*	*Alnus crispa* *Betula papyrifera*	Mosses, open	5~8 m	Open bog, wet
Site 22 (JP seedling)	**Pinus banksiana*	*Alnus crispa*	Open	1 m	On the sandy hill, dry

materials, mineral, and humus aggregates in the soil were removed before wet sieving through a 2-mm mesh for 5 minutes. Subsequent to cleaning, root samples were cut into 3~5 cm long segments and fixed in 50% ethanol. Visual assessment, at 12 to 25 times magnification, of the number of active ectomycorrhizas in a sample of 50 root tips was made from ten cores for each site and date (Criteria used in identifying active ectomycorrhizal tips are described by Harvey *et al.* 1976). Total ectomycorrhizas for each of the collecting dates is expressed as the mean percentage of active mycorrhizas from ten cores at each site. Values for each core were scored as number of finds (active mycorrhizas) divided by number of starts (50 root fragments surveyed).

Jack pine pollen collection

Male cones were collected, prior to anthesis, from the Mistik Creek study area in June 1993. Picked cones were placed on tin foil in the laboratory and dried for three days at room temperature. Shed pollen was subsequently gathered and separated from cone debris by sieving the grains through a 53 μm mesh. Once cleaned, pollen was stored in plastic bags and held at room temperature.

Isolation and axenic culture of hyphae

Suillus brevipes (Pk.) Kuntz was collected from site 5. Culture of this ectomycorrhizal fungus was obtained by plating excised stipe segments (~5 mm cubed) on Melin Norkrans (MMN) medium as modofied by Marx (1969). Bacterial contamination was eliminated by adding antibiotics (0.2 g penicillium G and 0.2 g streptomycin sulphate per liter to the medium). The isolate was subcultured and maintained in tubes at 4℃ on MMN slants free of antibiotics.

***Suillus brevipes* hyphal growth experiment**

Standard fungal explants were prepared by removing 6 mm plugs from the margin of an actively growing colony developed at 20℃ on a sugar agar medium containing 15 g agar, 10 g dextrose and antibiotics per liter. Prior to inoculation with fungal plugs, ten replicates of each pollen treatment: i) control, without pollen : ii) level 1, at 1.56 mg of pollen per plate; iii) level 2, at 6.25 mg (mean pollen deposition mass / unit area of research site); and iv) level 3, at 25 mg (4× mean pollen deposition); and v) level 4, at 100 mg (16× mean pollen deposition), were prepared by dusting freshly collected pine pollen onto the surface of water agar (15 g agar/liter and antibiotics) or the sugar agar, described above, in plastic petri plates. The plates were sealed with strips of paraffin film and inoculated at 20 ℃ during the day (16 hrs) and 15℃ during the night (8 hrs). Fungal growth was expressed as hyphal area (cm^2) measured after 15, 30 and 70 days.

RESULTS

Temporal pattern of ectomycorrhizas

In four different forest stands, ectomycorrhizas tended to be lower in the spring (May-June) than in the summer (July-August) and fall (October) samples (Table 2). Seasonal pattern of ectomycorrhizas in the jack pine stand indicated a trend of summer high numbers, and spring and fall lower numbers. Mycorrhizas on black spruce roots (in mixed leaf litter) were higher in summer and fall than in spring. Those of jack pine seedling roots were lower in mycorrhizas in general and showed high members in summer samples. Spruce roots in bog site showed a similar pattern to a mature spruce site but lower in numbers and early decline in numbers. Generally, peak activity of ectomycorrhizal fungi is in the middle to end of each growing season in spruce and pine stands. Mycorrhizal activities showed higher in a mature jack pine stand than a young jack pine stand.

Table 2. Seasonal variations of active ectomycorrhizas in four different forest stands in boreal Manitoba, Canada (n=5). JP=jack pine (*Pinus banksiana*), BS = black spruce (*Picea mariana*). Values are mean percentages of active mycorrhizal tips±1SD. Dashes (-) indicate that data were not collected.

Forest stand	May 26, '93	June 18, '93	Aug. 22, '93	Oct. 10, '93	July 9, '94	July 31, '94	Aug. 28, '94
Site 5 (Mature JP)	55.2± 8.6	62.8± 8.9	68.4±12.0	58.4± 6.7	78.0±10.9	77.6± 8.8	78.4± 7.6
Site 22 (JP seedling)	33.2± 8.2	36.8±10.3	54.4±11.6	37.2±11.4	53.6±13.6	66.8± 9.0	66.4±12.2
Site 6 (Mature BS)	64.8±13.1	71.8± 8.4	80.8± 8.6	78.8±10.5	73.6±8.1	71.2±11.6	80.0±11.9
Site 13 (BS in bog)	-	-	-	-	74.8±8.4	61.2±11.7	60.4±10.9

Suillus brevipes hyphal growth experiment

Growth of *Suillus brevipes* hyphae is generally better (high hyphal area) in cultures containing dextrose carbon than in plates without sugar (Fig. 2). Pollen additions increased the area covered by hyphae (Fig. 2A) and pollen-stimulated growth is greater when dextrose is available to *S. Brevipes* (Fig. 2B). Level 2 additions of pollen, representing mean pollen deposition (9 kg/ha) in Mistik Creek area, as well as level 3 (4 × mean deposition) and level 4 (16 × mean deposition) additions, notably increased hyphal area after 30 and 70 days of growth with dextrose availability.

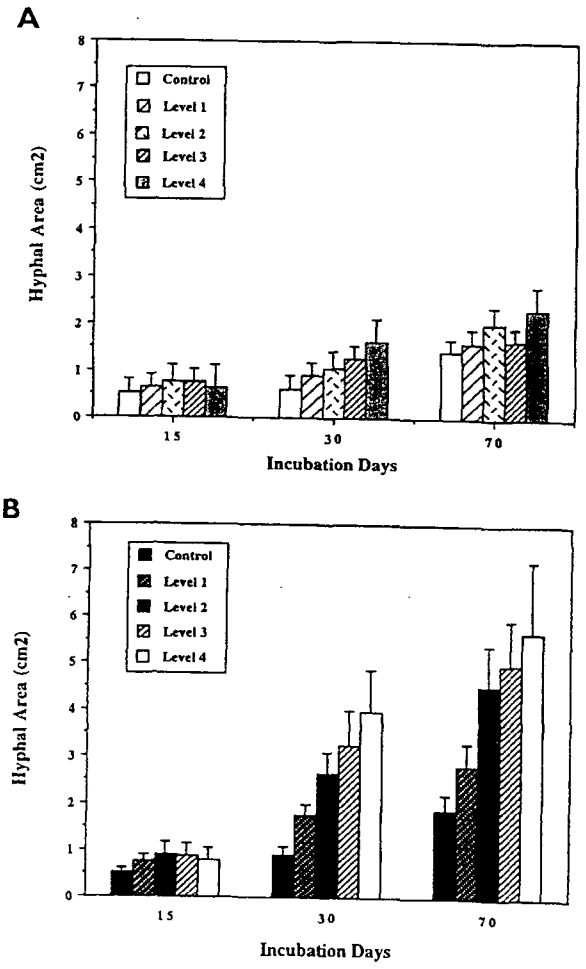

Fig. 2. Effects of jack pine (*Pinus banksiana*) pollen treatments on ectomycorrhizal fungal growth (*Suillus brevipes*). Control = no pollen added; level 1 = 1.56 mg of jack pine pollen / plate (55 cm^2); level 2 = 6.25 mg; level 3 = 25 mg; and level 4 = 100 mg. (Fig. A = no carbon substrate added to water agar medium; Fig. B = 10 g/l of dextrose was added as carbon substrate). Values are mean ± 1 SD.

DISCUSSION

The formation of ectomycorrhizas was seasonal in the Mistik Creek study area. Like other mycorrhizal studies the peak activity of ectomycorrhizal fungi is in the middle to end of each growing season in spruce and pine stands (Danielson and Pruden 1989, Wu *et al.* 1993). Generally lower ectomycorrhizal activities in wet habitat (Site 13) than in mesic habitat are explained by limited ectomycorrhizal activity in excess moisture habitats (Worley and Hackaylo 1959). The ectomycorrhizal fungi depend on available carbon for enhanced growth, as demonstrated for *Suillus brevipes* in this study, is shown for soils and litters by Harvey *et al.* (1978, 1979) and Read (1984). Plant-derived organic materials, such as litter, humus and decayed wood, support ectomycorrhizal activity. In addition to carbon, ectomycorrhizal fungi require elements from organic and inorganic components of litter and soil (Linderberg 1986, Cairney and Ashford 1991, Pankow *et al.* 1991). Because ecotomycorrhizal fungi are limited by low soil temperature (Bowen 1970, Marx *et al.* 1970, Theodorou and Bowen 1971) and available nutrients (Muttiah 1972), the results of this study show the peak activity of ectomycorrhizal fungi in summer in spruce and pine stands.

As early as the 1930's, Bransvheidt (1930) showed the nutritional and stimulatory value of pollen to fungal growth. Stark (1972, 1973) reports that pine pollen enhances decomposition by providing an early-summer macronutrient pulse to decomposers in the litter fermentation zone. The cytoplasm of pollen grains is rich in vitamins, carbohydrates, lipids, amino acids, and proteins (Stanley and Linskens 1974). Stark (1973) reports that after the spring pollen rain, the fermentation zone shows an increase in nitrogen and phosphorus.

Pollen studies show that significant amounts of important elements are delivered to the forest floor by pollen (Stark 1972, Doskey and Ugoagwu 1989, Lee *et al.* 1996b). Annual deposition of jack pine pollen averages 7.7 to 9.0 kg/ha in the Mistik Creek study area, corresponding to nutrient inputs of 0.34 to 0.49 kg N/ha, 0.04 to 0.07 kg P/ha, and 0.11 to 0.20 kg K/ha (Lee *et al.* 1996b). These values are greater than the 0.9~3.0 kg/ha range reported for Jeffrey pine (*Pinus jeffreyi Grev.* & Balf.) stands in Nevada (Stark 1972), but fall within the broad 3.5~80.0 kg/ha range estimated for white pine (*Pinus strobus* L.) and red pine (*Pinus resinosa* Ait.) stands in northern Wisconsin (Doskey and Ugoagwu 1989). Amounts

of nutrient in pollen added to the boreal forest floor during the summer (about 1/20 of annual nutrient input in a pine stand) is not likely to be significant to tree growth directly (Foster and Morrison 1976, Lee *et al.* 1996b). However, pollen treatment fungal growth experiments suggest that pollen nutrients are of a magnitude to be of value to ectomycorrhizal fungal growth. This study result shows that the peak ectomycorrhizal activity is followed by pollen deposition in the study area (Lee *et al.* 1996a). Therefore macronutrient deposition by pollen is concentrated both temporally and spatially combined with warm temperature, can be an important seasonal nutrient source for ectomycorrhizal growth in a boreal forest

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