JACK PINE [*PINUS BANKSIANA* LAMB.] POLLEN EFFECTS ON JACK PINE AND BLACK SPRUCE [*PICEA MARIANA* (P. MILL.) B.S.P.] SEED GERMINATION AND SEEDLING GROWTH

BY

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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

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ABSTRACT

Effects of jack pine pollen on jack pine and black spruce seed germination and jack pine seedling growth were investigated. Generally, *in vitro* speed of seed germination and radicle growth of both species were negatively affected by normal pollen levels (average annual jack pine pollen rain, i.e. 8.5 kg/ha.yr). Black spruce seeds under sterilized *in vitro* conditions germinated faster and produced longer hypocotyls when provided with jack pine pollen amounts equal to the annual rain.

On mineral soil under controlled conditions, seed germination of both species was unaffected by jack pine pollen additions at 10x the annual jack pine pollen rain. Additions at 50x and 100x the annual jack pine pollen rain significantly reduced radicle length and resulted in up to 65% of germlings with infested (e.g. pathogenic fungi) radicles. Pollen application, of levels equal to annual pollen rain, to potted jack pine seedlings under greenhouse conditions increased seedling biomass by up to 12% at the end of the first growing season. In mineral soil field sites of west-central Manitoba, biomass and growth of jack pine seedlings were positively affected by supplements of increasing pollen levels up to 100x annual pollen rain.

Possible nutritional and hormonal effects of pollen on seed germination and seedling growth are discussed and topics for future research are identified.

Keywords: jack pine, pollen, black spruce, seeds, germination, seedling growth, Manitoba

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Table of Contents

LIST OF TABLES

LIST OF FIGURES

Chapter I
General introduction

1.1. Study rationale

Although the biology of the ecologically distinct species, jack pine (*Pinus banksiana* Lamb.) and black spruce [*Picea mariana* (P. Mill.) B.S.P.], is generally known, little information on the effects of pine pollen on seed germination and seedling growth is available. This dissertation begins by considering some information known about jack pine and black spruce seeds and seed germination, seedling growth of the two species, pine pollen production, nutrients from pollen and pollen hormones. With vast regions of Western Canada subject to deforestation, it is essential to consider all factors that may affect and promote re-forestation of the disturbed sites. This pilot study is intended to look at the role of jack pine pollen in forest regeneration, and it is assumed it poses at least as many questions as it answers.

1.2 Jack pine and black spruce seeds and seed germination

In nature most jack pine seeds are developed within serotinous cones. Seed release occurs with exposure of cones to direct sunlight or with heat from a forest fire. Non serotinous jack pine trees are common in the southern limits of the range for this species, and seed release follows cone maturation. It is estimated that the quantity of jack pine seeds in serotinous cones can be more than 4 million seeds per hectare (Rudolph and Laidly 1990). Black spruce has semi-serotinous cones. Even though up to 1.2 million seeds per hectare are released each year, sufficient seeds remain in cones to assure successful regeneration of fire killed stands (Heinselman 1965).

Seed dormancy and germination are affected by three classes of phytohormones (*i.e.*, gibberellins, cytokinins, and inhibitors) (Khan 1975). Depending on the mix of internally borne phytohormones, seeds may be dormant or may even germinate in the presence of inhibitors.

Seed germination may be characterized as radicle emergence from the seed coat, or occurrence of the geotropic curvature of the radicle once it has emerged (Mayer and Poljakoff-Mayber 1989). Other characterizations are more conservative, and seeds are

deemed germinated only after the germling has utilized all the food reserves in the seed and is capable of independent life (Bradbeer 1988). In my study (Chapters 2 and 3 of this dissertation) a seed is considered to have germinated when its protruded radicle shows signs of geotropic curvature.

In laboratory studies, jack pine seeds may be germinated in a regime of 8 hr light in every 24 hr with 750 - 1250 lux light intensity, and at a temperature of 20-32 °C over 7 to 14 days (Edwards 1987). Temperature is generally held higher (~30 °C) during the light period and lower (~20 °C) during the dark period (Edwards 1987; Fraser 1970; Jeffers 1985; Sims 1968). Except for requirement of a longer period (21 days), similar conditions may be used to germinate black spruce seeds (Edwards 1987).

Jack pine seed germination is epigeal and, under optimum forest conditions, occurs within 15 to 60 days of exposure to air temperature above 18 °C and favorable soil moisture. Light exclusion markedly reduces jack pine seed germination (Rudolph and Laidly 1990). Normally, seed germination and seedling establishment under field conditions occur from May to June on burned and scarified areas. Depending on site and weather conditions, at least 450 seeds from broadcast cones are required to produce one successful yearling in a regenerating jack pine stand in southeastern Manitoba and other regions (Cayford 1958, 1959, 1961; Cayford and Dobbs 1967; Chrosciewicz 1983a, b; Chrosciewicz 1990; Dominy and Wood 1986; Sims 1970; Walker and Sims 1984;). On exposed mineral soils after intense burning in the Riding Mountains and Pine Falls areas of Manitoba, autumn seeding of jack pine results in survival of over six seedlings from 25 cleaned seeds planted two years previously (Jarvis 1966).

Black spruce seed germination and seedling establishment normally occur in wet sites. In open sites it is also possible for seeds to germinate and seedlings to establish in upland conditions during wet years. Dry season moisture is a critical factor for seedling survival (Heinselman 1965; Jarvis 1966). Successful seed germination and seedling establishment are generally achieved by late spring seeding in the White River and Cochrane Districts of Ontario (Brown 1973).

Hypocotyl plus radicle length for jack pine may be expected to be 7.1 cm by day 12 in contrast to 4.2 cm for black spruce over the same period (Thomas and Wein 1985). Such information serves to partially explain successful regeneration of jack pine, with its 'longer' radicle, in dry exposed sites, and the establishment of black spruce, with a 'shorter'

radicle, on continuously moist substrates. Generally, an inability to withstand periods of drought and desiccation confines black spruce regeneration to the relatively more moist sites.

1.3 Jack pine and black spruce seedling growth

In the end of the first growing season, jack pine seedling height is not affected by N, P, K fertilization. However, seedling diameter, as seen in Table 1.1, increases from 2.1 mm at 9.4 mg of N, 8.4 mg of P and 9.5 mg of K (nutrient quantities in a per seedling basis), to 2.4 mm, at doubled levels of N, P, and K (Calmé *et al.* 1993). Taking light as a nutrient, three different sources of artificial light on jack pine yearlings result in increases in height and shoot dry mass of approximately 20 to 25% under optimum conditions (Roberts and Zavitkovski 1981). Different substrate mixes (pure peat, peat mixed with sand, perlit or silt) affect the performance of jack pine yearlings by up to 56% in seedling diameter and up to 64% in seedling height (Govindaraju 1988).

Optimum soil temperature for eight-week-old jack pine seedlings, grown in controlled chambers, is 27 °C (Heninger and White 1974). Over eight weeks, seedlings grown at optimum temperature increase in height (from 28 to 37 mm), diameter (from 0.7 to 1.3 mm), shoot dry mass (from 35 to 148 mg), and root dry mass (from 13 to 87 mg). Soil provided to these plants included (in ppm): 8.2 NO_3^--N, 2.4 P, 65.3 K, 311 Ca, and 36.5 Mg. The soil used in the experiment, with a mean pH of 5.4, received amendments through a complete nutrient solution (plus minor elements) as required, and by the addition of 0.09 g of N, 0.013 g of P, and 0.025 g of K to each 40 cm x 4.7 cm glass tube containing two seedlings.

Jack pine seedlings grown in nursery beds begin to grow in height in May and complete their growth by the end of July. Increase in shoot mass occurs over the period of June to mid-October. By the end of the second growing season, shoot dry mass increases almost 15 times over the initial mass of yearling. Black spruce seedlings from the 45th parallel show a tendency to grow for a relatively longer period than those from the 49th parallel, although root dry mass at the end of the second growing season is very similar for the two provenances (Langlois *et al.* 1983).

Table 1.1. Ranges in some jack pine[1] and *Pinus jeffreyi* seedling characteristics under various growing conditions.

Growing conditions	Age	Parameters				Source
		Diameter per seedling	Height per seedling	Shoot dry mass per seedling	Root dry mass per seedling	
2:1 peat:vermiculit substrate,110 cm3/seedling	~ 5 months					Calmé et al. 1993
Normal fertilization 9,39 mg of N/seedling 8,37 mg of P/seedling 9.51 mg of K/seedling		2.1 mm	107 mm			
2x normal fertilization		2.4 mm	107 mm			
Potted seedlings, in several 1:1 substrate mixes	150 days					Govindaraju 1988
peat:sand pH= 6.00		1.2 mm	81.1 mm	829 mg of fresh weight		
peat:perlit pH= 4.52		1.9 mm	137.7 mm	2881 mg of fresh weight		
Tubed seedlings, in soil substrate ammended with chemical fertilizers	8 weeks	0,7 mm soil at 31 °C	28 mm soil at 15 and 30 °C			Heninger and White 1974
		1,3 mm soil at 19 °C	37 mm soil at 27 °C			
Nursery bed	Beginning 2nd year				191.3 mg	Langlois et al. 1983
Nursery bed	End 2nd year				1308.6 mg	
Potted seedlings, growing in greenhouse conditions under three artificially extended light periods	111 to 130 days		57 mm	140 mg		Roberts and Zavitkovski 1981
Potted Jeffrey pine seedlings, in sandy loam soil of pH= 5.4 to 6.00 and 1.6 to 6.0% organic matter	2-year old					Stark 1973
sandy soil only			113 mm	1060 mg	1090 mg	
sandy soil+litter			121 mm	1450 mg	1560 mg	
sandy soil+litter+10x pollen			149 mm	1640 mg	1820 mg	
sandy soil+litter+100x pollen			156 mm	1850 mg	2140 mg	
PVC pipes, filled with washed commercial sand	14 days			3.8 mg	1,4 mg	Wright et al. 1992
				4.9 mg	2,0 mg	
PVC pipes, filled with washed commercial sand	35 days			10.8 mg	7.2 mg	
				11.8 mg	8.4 mg	

1 All information in this table refers to jack pine seedlings, except for the case of Stark's work, in which Jeffrey pine seedlings were the subjects of the study.

1.4 Jack pine seedling growth under field conditions

Naturally established jack pine seedlings attain a height of 3 to 5 cm in the first year, and up to 15 cm in the second (Rudolph and Laidly 1990). At the end of the first growing season, on a typical sandy soil, root depth ranges from 13 to 25 cm. At the end of the second year, jack pine seedling roots are 28 to 33 cm long, dry mass ranges from 1 to 2 g, and seedling tops are 7 to 10 cm high (Rudolph 1965).

Jack pine growth under greenhouse conditions is reduced by the addition of lichen [*Cladina stellaris* (Opiz) Brodo, and *C. rangiferina* (L.) Nyl.] mulch. Seedling shoot dry mass, and nitrogen and phosphorus concentrations in seedling tissue are reduced when compared to seedlings grown on a control peat-moss mulch. Such reduction may result from interference in root growth, limitation in mycorrhizal association, impeded nitrogen and phosphorus uptake and lowered translocation (Fisher 1979). Despite these facts reported for seedlings grown under greenhouse conditions, no scarification or ground vegetation removal was considered necessary for successful jack pine regeneration on clear-cut sites with *Cladina* spp. and *Arctostaphylos uva-ursi* (L.) Spreng. ground vegetation in Saskatchewan (Jameson 1961).

1.5 Pine pollen production

In general, male strobili of pine produce an average of 0.15 liter of pollen from each liter of collected strobili. Over a 50-year period, a single *Pinus sylvestris* L. tree may produce 6 kg of pollen (Brooks 1971). However, annual production fluctuates and a pine stand varies from 40% to 146% of the potential average of stand pollen production (Stanley and Linskens 1974). Annual pine pollen production ranges from 0.6 kg/ha from *P. jeffreyi* Grev. & Balf (Stark 1972; Stark 1973) to 227 kg/ha from *P. elliottii* Engelm. (Maggs 1985). Jack pine begins to produce male strobili at five to ten years of age (Rudolph and Laidly 1990). In west-central Manitoba, annual jack pine pollen production is estimated to average 8.5 kg/ha in mature mixed boreal forest stands, and to range as high as 24.5 kg/ha in a 25-year-old regenerating pure jack pine stand (Lee 1997; Lee at al. 1996b). Such pollen production corresponds to a rain of 4.4 10^7 to 1.3 10^8 jack pine pollen grains/m^2 onto the forest floor each year (*e.g.*, Lee 1997) compared with 10^7 *Zea mays* L. pollen grains/m^2 .yr raining into monoculture of the species (Jiménez *et al.* 1983).

Pollen rain of pine species occurs over a short period of time each year depending on climatic conditions (Lee 1997; Lee *et al.* 1996a; Rudolph and Laidly 1990). For example, *Pinus elliottii* anthesis occurs within the month of August in Australia (Maggs 1985). Pollen rain of *P. radiata* D. Don takes place in July and August in South Africa (Versfeld and Donald 1991). *Pinus banksiana* undergoes anthesis over a two-week period in late May in southern Ontario (Ho 1991; Moore and Nozzolillo 1991) and in early to mid-June in west-central Manitoba (Lee 1997; Lee *et al.* 1996a).

Although there are examples of pine pollen being transported over great distances as a consequence of environmental factors including air turbulence and topography (Squillace and Long 1981), most of the pollen produced by individuals of *Pinus* spp. is normally deposited within 700 meters or less of the source tree (Buel 1947; Squillace and Long 1981; Stanley and Linskens 1974).

1.6 Pine pollen nutrients

The nutrient composition of pine pollen varies with the species, the clones, the individuals and the region of pollen origin (Doskey and Ugoagwu 1992; Jackson and Linskens 1982; Larionova *et al.* 1977). Several authors report nutrient composition of pine pollen, and values for some of the macronutrients are shown in Table 1.2 (total nutrients) and Table 1.3 (water-extractable nutrients). One third to four fifths of total P, K, S, and Mg in pine pollen are water extractable with pollen nitrogen low in water solubility. Pollen analyzed for nutrient content after water extraction is relatively high in nitrogen, in contrast to detected levels of P, K, S, and Mg (Lee 1997; Lee *et al.* 1996b).

Total annual litterfall in pine ecosystems averages from 2,700 kg/ha (Stark 1972; Stark 1973), to 6,480 kg/ha (Maggs 1985; Spain 1973). Pine pollen contributes from 0.6 to 227 kg of total annual litterfall. Pollen nutrient richness, short periods of pollen rain (2 to 3 weeks) and a concomitant period of decomposition (as short as 4 weeks) (Goldstein 1960) place the modest contribution of pollen to litterfall in a relatively important position in the nutrient budget of some forest ecosystems. Various workers have determined the annual macronutrient input to the forest floor from litterfall in pine dominated ecosystems (Table 1.4). Pollen represents an annual transfer of 3.83 kg/ha of N, 0.45 kg/ha of P, and 1.39 kg/ha of K to the forest floor, which are 16.8%, 30.6%, and 31.1% of N, P and K, respectively, of the total annual addition of nutrients through litterfall to the forest floor of a

Table 1.2. Total macronutrients* from pollen of some pine species.

Pine sp.	mg/g of pollen							Source
	C	N	P	K	S	Ca	Mg	
P. banksiana		20.1	2.9	8.4	0.7		0.8	Lee 1997; Lee et al. 1996,b
P. contorta Doug. ex Loud.			2.8					Lewis et al. 1985
P. contorta		19.6	1.7	9		0.2	0.9	Stark 1972; Stark 1973
P.contorta+ P.jeffreyi		12.6	1.7	5		0.1	2.8	Stark 1972; Stark1973
P. elliottii		16.8	2	6.1		0.2	0.8	Maggs 1985
P. mugo Turra.		22	3		1.8			Nielsen et al. 1955
P.radiata		21.5	3	8.8		0.3	1.1	Todd and Bretheric 1942
P. radiata		21.8	2.5	10.7		0.3	1.2	Versfeld and Donald 1991
P. resinosa Soland	497	19.7	2.7		2			Doskey and Ugoagwu 1992
P. sabiniana Dougl. ex Dougl.		18.2	3.6	8.7		0.4	0.9	Todd and Bretheric 1942
P. strobus L.	507	20.1	3		2.8			Doskey and Ugoagwu 1992

* Blank spaces in the body of the table mean that data were not available in the indicated literature.

Table 1.3. Water-extractable macronutrients* from pollen of some pine species.

Pine sp.	mg/g of pollen					Source
	nitrate-N	phosphate-P	K+	sulphate-S	Mg	
P. banksiana	0.02	1.8	7.4	0.5	0.5	Lee 1997; Lee et al. 1996,b
P. contorta		1				Lewis et al. 1985
P. monticola	0.05	1.2	5.1	1.3		Doskey and Ugoagwu-1992
Dougl. ex D. Don	0.22		5.1	3.8		Noll and Khalili 1990
P. ponderosa	0.06	1.6		0.7		Doskey and Ugoagwu-1992
P.& C. Lawson	0.26		3.3	2.1		Noll and Khalili 1990
P. resinosa	0.06	1.5	3.3	1		Doskey and Ugoagwu-1992
	0.21	2.1	9.9	0.6		Noll and Khalili 1988
	0.01	1.6	13.2	0.7		Noll and Khalili 1988
P. strobus	0.16	1.4	13.5	2.3		Noll and Khalili 1988

* Blank spaces in the body of the table mean that data were not available in the indicated literature.

Table 1.4. Annual macronutrient* input (kg/ha) from litterfall in various pine ecosystems.

Pine ecosystem	Component	kg/ha N	P	K	S	Ca	Mg	Source
P. banksiana	Litterfall & plant leaching	25.19	1.63	14.63		16.53	2.54	Foster 1974 (a)
P. banksiana	Litterfall	41	3.9	11		28	5	Perala and Alban 1982 (b)
P. banksiana	Pollen	0.45	0.07	0.19	0.02		0.02	Lee 1997; Lee et al. 1996,b (c)
P. elliottii	Litterfall	22.82	1.47	4.47		33.9	9.26	Maggs 1985 (d)
	Pollen	3.83	0.45	1.39		0.04	0.18	
P. contorta + *P. jeffreyi*	Pollen	0.04	0.01	0.02		0.001	0.01	Stark 1972, 1973 (e)
P. contorta	Pollen	0.06	0.01	0.03		0.001	0.003	Stark 1972, 1973 (e)
P. radiata	Litterfall	26.1	1.5	10.5		10.8	4.8	Versfeld and Donald 1991 (f)
	Pollen	0.62	0.07	0.3		0.01	0.03	
Pinus spp.	Pollen	<0.16	<0.23		<0.019			Doskey and Ugoagwu 1989 (g)

* Blank spaces in the body of the table mean that data were not available in the indicated literature.

a- from a 30-year-old pure, fire-regenerating *P. banksiana* stand in northern Ontario
b- from a 40-year-old *P. banksiana* plantation on sandy soils in Minnesota
c- from 25-year-old, pure post-scarification *P. banksiana* stands in west-central Manitoba
d- from a 15 -year-old pure plantation of *P. elliottii* in Queensland, Australia
e- from a *P. jeffreyii* temperate forest in Nevada
f- from a 40-year-old pure plantation of *P. radiata* in Cape Province, South Africa
g- from a grassy field in north Wisconsin surrounded by a *P. strobus*, *P. banksiana* and *P. resinosa* forest.

Pinus elliottii plantation (Maggs 1985). In jack pine stands, annual pollen rain cycles up to 0.45 kg/ha of N, 0.065 kg/ha of P, and 0.186 kg/ha of K (Lee 1997; Lee *et al.* 1996b). These values are 1.78%, 3.99%, and 1.27%, respectively, of the annual total quantity of N, P and K reaching the forest floor in litterfall in a 30-year-old jack pine stand in northern Ontario (Foster 1974; Foster and Gessel 1972; Foster and Morrison 1976).

Higher levels of N and P additions to the litter fermentation zone of temperate forests can be said to result from annual pollen rain. In a *Pinus jeffreyi* forest in Nevada, the annual pollen rain totals 0.6 to 3.0 kg/ha. These amounts are considered too low to be of direct importance to tree growth. However, pine pollen nutrients may be essential to decomposition processes in the litter fermentation zone of temperate forests (Stark 1972; Stark 1973). The mass loss of fresh needle litter and the number of hyphae in the litter are generally higher with pollen (up to 30 kg of pollen/ha) present. Also, *P. jeffreyi* pollen added to needle substrates at levels from 0x to 100x of the annual pollen rain positively affects the growth of potted *P. jeffreyi* seedlings (Stark 1972; Stark 1973). Seedling height ranges from 12.1 to 15.6 cm at pollen levels of 0x to 100x. Shoot dry mass ranges from 1.45 to 1.85 g/seedling, and root dry mass ranges from 1.56 to 2.14 g/seedling (Table 1.1).

In an oligotrophic lake in northern Wisconsin pine pollen contributes about 45% of total phosphorous (0.187 kg of P/ha.yr) and 20% of total potassium (0.294 kg of K/ha.yr) from external nutrient loading (Doskey and Ugoagwu 1989). Nitrate and sulfate additions from pollen to two lakes in northern Wisconsin are estimated to be 0.28 to 0.58 kg/ha NO_3^- and 0.45 to 1.18 kg/ha SO_4^{-2} (Noll and Khalili 1990). In contrast, precipitation measured at the Trout Lake Air Monitoring Station during the time of pollen rain, provides 0.91 kg of NO_3^-/ha and 1.03 kg of SO_4^{-2}/ha (Noll and Khalili 1990). Certainly, pollen adds to the total input of nitrate and sulfate to the environment.

1.7 Pollen hormones

1.7.1 Pollen hormones in general

Since the early 1900's, parthenocarpic development of fruits has been induced in certain curcubit, grape and orchid species with application of pollen or pollen extract to flower stigmas. Such induction occurs independently of the source of pollen extracts. In other words, parthenocarpic development does not require pollen extract from the plants bearing

fruit. Furthermore, parthenocarpy results in cases where extracts from living or dead pollen are applied. This suggests that the growth stimulus is chemical in nature (Gustafson 1937; Mitchell and Whitehead 1941).

Zea mays pollen extract induces stem cell elongation when applied to the cut surface of decapitated seedlings or to the epidermis of intact plants, particularly *Phaseolus* sp.. This effect resembles that from tryptophan application (Mitchell and Whitehead 1941). In contrast, *Zea mays* pollen application results in reduction in the length of hypocotyls and radicles of various species [*e.g.*, watermelon *Citrullus lanatus* (Thunb.) Matsumura & Nakai]. In such cases the meristematic tissue of the radicle is destroyed. Phenylacetic acid (PAA), a nonindolic auxin, is considered to be this phytotoxic substance from *Zea mays* pollen. This auxin acts to inhibit electron transport in cell respiration. Energy production is limited, explaining the >50% reduction in seedling mitotic activity. Some growth promoting substances are also present, but their action seems to be controlled by PAA (Anaya *et al.* 1992; Jiménez *et al.* 1983; Ortega *et al.* 1988).

Pollen and pollen water extract from some species are found to drastically reduce fruit formation and seed set when applied to the stigma of a number of species. This reduction results from the interference of pollen substances from one species with pollen germination and pollen tube growth of another species (Kanchan and Jayachandra 1980; Murphy and Aarssen 1995a, b). Compounds from pollen not only affect fruit formation and seed set but photosynthate production can be limited. Pollen substances from *Parthenium hysterophorus* L. are thought to cause reduction in the chlorophyll content of *Phaseolus vulgaris* L. plants (Kanchan and Jayachandra 1980).

1.7.2 Pine pollen hormones

A number of active substances, including auxins, gibberellins, cytokinin-like substances, and inhibitors, are known from pine pollen (*e.g.*, Ivonis 1969; Kamienska and Pharis 1971; Kamienska and Pharis 1975; Kamienska *et al.* 1976; Larionova *et al.* 1977; Michalski 1967; Stanley 1971). Sweet and Lewis (1971), who studied the dynamics of the hormone substances in pollen of *Pinus radiata* during the first 72 hr of pollen germination, show presence of five auxins, three inhibitors, four gibberellins and one compound with some properties characteristic of both gibberellins and cytokinins. As pollen germinates, *in vitro*, quantities of some of these substances vary. For example, after 72 hr of pollen germination on agar, sufficient auxins (*i.e.*, Aq 1, AEF 2 or NEF 4) are extracted from 12 mg of germinating pollen to cause an increase in *Avena* sp. coleoptile length from 0.24 to

0.41 mm. In contrast, application of the same auxins extracted prior to pollen germination results in only a 0.07 to 0.20 mm coleoptile increase. By comparison, 0.05 µg of pure IAA increases *Avena* sp. coleoptile length by 0.39 to 0.47 mm under the same bioassay conditions. The hormone Aq 1 is unique in its ability to diffuse from pollen grains into an agar substrate. This indicates a possible external role of this particular pollen hormone, possibly one of triggering ovule development (Sweet and Lewis 1969).

Sweet and Lewis (1971) also extracted 10^{-4} g of kinetin-like substance from 100 mg of *Pinus radiata* pollen. It is unclear whether this substance is kinetin, but its effect on *P. radiata* pollen tube growth is remarkably similar to that of pure kinetin. It is known that the gibberellin effect on pollen tube growth differs from that of pure kinetin and the kinetin-like substance extracted from pollen.

Gibberellic acid-(GA_1 and GA_2), IAA- (Indoleacetic acid) and IAN- (indoleacetonitrile) like substances are reported from *Pinus sylvestris* L.. The IAA- and IAN-like substances produce chromatographic R_fs and *Avena* sp. coleoptile section elongation similar to pure IAA and IAN, used as controls (Michalski 1967). Ivonis (1969) detected three different GAs in female cone extracts of *Pinus sylvestris*, while three other GA-like substances are identified from pollen extract. In bioassays with pea (*Pisum* sp.) shoots, all the above extracts cause significant increases in shoot length.

Larionova *et al.* (1977), investigating the auxin and inhibitor substances from pollen of 8 different individuals of *Pinus sibirica* Du Tour, report presence of ß-indolacetonitrile (ß-IAN). They also report extraction of one phenolic inhibitor from among the 8 pine individuals that sometimes acts as a weak promoter. The authors explain opposite actions resulting from differences in concentration of this inhibitor. They determine that pine individuals with low levels of pollen auxins also have low levels of pollen phenolic inhibitors. Individuals with high levels of pollen auxins have a corresponding high level of pollen inhibitors.

Some forms of gibberellic acid (GA) peak during the pollen germination process. In *Pinus attenuata* pollen, 0.0315 µg of GA-like substances (mainly GA_3) is present in each gram prior to germination, and 0.3358 µg of that substance is detected after 15 hr of germination. In contrast, no GA-like substance is found 72 hr after the beginning of germination (Kamienska and Pharis 1975). Other forms of GA are in greater concentrations prior to germination than at any time during or after germination. Again in

P. attenuata pollen, 0.0007 µg of GA_4 and 0.0002 µg of GA_7 are found in each gram 15 hr after germination. Prior to germination these concentrations are 0.0011 and 0.0013 µg/g, respectively (Kamienska *et al.* 1976). It is shown that some gibberellin-like substances (around 0.0025 µg/g of pollen) diffuse out of the germinating pollen into the surrounding medium, and remain stable throughout the germination period (Kamienska and Pharis 1971).

1.8 Working hypotheses and objectives

In light of limited knowledge of the effects of pine pollen on jack pine and black spruce seed germination and jack pine seedling growth, as well as the relatively large production of pine pollen in the boreal forest environment, as exhibited in the literature, various pilot studies were undertaken to determine possible roles of pollen in seed germination and seedling growth. Due to the probable hormonal load in jack pine pollen, and its nutrient composition, it was taken that various substances from the pollen may affect seed germination and plant growth. Specifically, it was thought that jack pine pollen may affect jack pine and black spruce seed germination, as well as the growth of jack pine yearlings.

To address the working hypothesis above, studies were conducted with the following objectives:

1. to determine if fresh whole jack pine pollen grains, sonicated pollen remains and pollen aqueous extract affect germination of jack pine and black spruce seeds under *in vitro* conditions;

2. to examine if the *in vitro* responses by jack pine and black spruce seeds to jack pine pollen were modified by germination on mineral soil;

3. to ascertain the effects of various loads of jack pine pollen on jack pine yearlings planted on lichen mat, mineral soil and needle litter substrates, under greenhouse conditions; and

4. to study the effects of various pollen levels on jack pine yearlings planted on lichen mat, exposed mineral soil and needle litter substrates in the field.

Chapter II

In vitro effects of jack pine pollen substances on jack pine [*Pinus banksiana* Lamb.] and black spruce [*Picea mariana* (P. Mill.) B.S.P.] seed germination

2.1 Introduction

Pollen has a reproductive function. However, pollen grains from one species may reduce seed and fruit set of other species (Kanchan and Jayachandra 1980; Murphy and Aarssen 1995a, b). Also, the chlorophyll content of pollen dusted leaves (*i.e.*, pollen from the weed *Parthenium hysterophorus* L.) of *Phaseolus vulgaris* L. plants is decreased (Kanchan and Jayachandra 1980). *Zea mays* L. pollen (10 to 200 mg/Petri dish) reduces the speed of seed germination and depresses by 25 to 90% the initial radicle and shoot growth of *Cassia jalapensis* (Britton) Lundell. (Jiménez *et al.* 1983) and *Citrullus lanatus* (Thunb.) Matsumura and Nakai, cv. Peacock improved (*i.e.*, watermelon) (Ortega *et al.* 1988). In contrast, treatment of young *Phaseolus* sp. seedlings with ether extract from *Zea mays* pollen promotes cell elongation resulting in longer stems, as if the seedlings had been treated with tryptophan (Mitchell and Whitehead 1941).

Pollen from many different plants has proven to stimulate pathogenic fungal spore germination and increase the number of leaf lesions by a factor of 3 to 20 (Borecka *et al.* 1969; Borecka and Millikan 1973; Chou and Preece 1968; Fokkema 1971; Warren 1972a, b; Warren 1976; Williams and Colotelo 1975). The stimulation of spore germination is probably non-nutritional (Warren 1976), and may be due to pollen hormones (Borecka *et al.* 1969; Borecka and Millikan 1973; Chou and Preece 1968). Furthermore, spore germination of *Claviceps purpurea* Fr. (Tul.), a fungal pathogen of rye (*Secale cereale* L.), is stimulated more by *S. cereale* pollen than by pollen from non-host species (Williams and Colotelo 1975).

Seed germination may be affected by many factors, such as light, temperature, and moisture. Changes in these factors trigger physiological processes, which are mediated, in many cases, by plant hormones. The application of exogenous hormones, such as gibberellins, is used to accelerate and improve germination of seeds of several species (*e.g.*, Bulard 1985; Crozier *et al.* 1970; Devlin *et al.* 1976). Ginkgo (*Ginkgo biloba* L.) seeds normally need a period of cold temperature to germinate. The stratification period is

not necessary if seeds are immersed in a 120 ppm gibberellin solution for 40 hr (West *et al.* 1970). At least thirteen active growth hormones and hormone-like substances (inc. auxins, gibberellins, phenolic inhibitors, and cytokinin-like substances) are known from pine pollen (*e.g.*, Ivonis 1969, Larionova *et al.* 1977, Michalski 1967; Sweet and Lewis 1971).

Pine pollen is also rich in nutrients (Tables 1.2-1.3), and may contribute up to one third of the nutrients cycled in some pine forest ecosystems (Maggs 1985). Jack pine pollen, specifically, is rich in nutrients (*e.g.*, 2% nitrogen, in a pollen dry mass basis), that are readily water-extractable (*e.g.*, phosphorus, potassium and sulphur) (Lee 1997). However, it remains to be determined if pollen has an effect on seed germination and seedling growth.

Normally, successful jack pine (*Pinus banksiana* Lamb.) seed germination and seedling establishment in southeastern Manitoba occur from May to June, coinciding partially with the time of jack pine pollen release. Depending on site and weather conditions, as many as 450 seeds may be required to produce a single year old seedling in a regenerating jack pine stand (Cayford 1958; Cayford 1959; Cayford 1961; Cayford and Dobbs 1967; Chrosciewicz 1983a, b; Chrosciewicz 1990; Dominy and Wood 1986; Sims 1970; Walker and Sims 1984). Successful black spruce seed germination and seedling establishment also coincide partially with the time of jack pine pollen release. Black spruce seeds normally germinate and establish themselves in wet sites, or in upland dryer sites during wet years, as dry season moisture is a critical factor for seedling survival (Heinselman 1965; Jarvis 1966).

Jack pine and black spruce are generally found in distinct habitats (upland dry sites x wet boggy sites, respectively), and differences in moisture level in each habitat are considered an important factor in determining the clear boundary between habitats (*e.g.*, Thomas and Wein 1985). However, jack pine pollen may reach sites favoring black spruce due to air movements or water run off, as low sites alternate regularly with upland, jack pine-dominated sites. Thus, it is possible that jack pine pollen interferes with the development of the two species. Together with moisture and other factors, pollen may even help to delimit the boundaries between the sites occupied by these two species.

In boreal forest sites, coniferous species account for over 90% of the pollen rain observed in May and June (Lee 1997; Lee *et al.* 1996a). The annual rain of airborne pollen ranges from 11.7 to 24.6 kg/ha and averages 8.5 kg/ha in mixed mature stands in west-central

Manitoba. Regionally, jack pine sheds its pollen in early to mid June and it accounts for approximately half, and in some local sites nearly 100%, of the annual pollen rain (Lee 1997; Lee *et al.* 1996,b). In general, pollen production by *Pinus* spp. may range from 0.6 to 227 kg/ha.yr, representing up to one third of the total of some nutrients cycled in a forest ecosystem (Doskey and Ugoagwu 1989; Doskey and Ugoagwu 1992; Jackson and Linskens 1982; Lee 1997; Lee *et al.* 1996b; Lewis *et al.* 1985; Maggs 1985; Nielsen *et al.* 1955; Stark 1972; Stark 1973; Versfeld and Donald 1991).

As pine pollen is potentially rich in nutrients and active growth substances, and is present in large quantities in the boreal forest, its role in seed germination, seedling establishment, and forest development requires detailed study. The objective of this study is to determine if whole fresh jack pine pollen, pollen aqueous extracts and sonicated pollen remains affect germination of jack pine and black spruce [*Picea mariana* (P. Mill.) B.S.P.] seeds in Petri dish systems.

2.2 Methods and materials

2.2.1 Jack pine pollen collection, handling, and storage

Jack pine pollen cones were collected in Sandilands Provincial Forest, 100 km south east of Winnipeg, in early May 1998, along Trails 17, 18, and 31. Young trees (10 to 15 years old), exposed to direct sunlight and heavily laden with male cones, were preferentially chosen for cone collection. Male cones were picked from branches a few days prior to anthesis (Stanley and Linskens 1974). Subsequent to picking, the cones were spread on a large cotton cloth in the shade of the forest floor for five to six hours. Then, the picked cones were transported in boxes from the field to University of Manitoba laboratories.

Collected cones were spread on aluminum foil in the laboratory and left to dry at room temperature (18 to 21 °C) for 4 to 6 days. After air drying, cones were sifted on a coarse #12 (1.7 mm aperture) sieve. Pollen derived from the cones was passed through a 140 mesh (105 µm aperture) sieve for cleaning and subsequently left to air dry for 3 days (Stanley and Linskens 1974). Water content of the cleaned pollen, gravimetrically determined after drying at 100 °C for 48 hr in a forced air oven, was 6%. The air dried and cleaned pollen was stored in tightly closed 500 ml glass bottles in a cold room at 3 to 5 °C, after Johnson (1943) and Bramlett and Matthews (1991).

2.2.2 Jack pine pollen processing

Initial experiments on seed germination utilized unsterilized pine pollen. Sonication and aqueous extraction procedures, developed for processing unsterilized pollen, were also applied in a second set of experiments with propylene oxide sterilized pollen.

Prior to preparing water extracts, 4.5 g of the stored pollen was sterilized for 12 hr in a desiccator with an atmosphere from 10 ml propylene oxide (see Appendix I). Sterilized pollen was de-gassed by repeated mixing of the material for half an hour in the open air and subsequent air exposure for 6 to 8 hr in the laboratory. Next, a slurry of sterilized and de-gassed pollen, prepared by adding the 4.5 g sterilized pollen to 165 ml sterilized distilled water, was sonicated. Pollen was sonicated for 15 min at 80 kc (after Jiménez *et al.* 1983; McIntyre and Norris 1964), using a Biosonik II sonicator with nominal output of 385 watts. The pollen slurry was protected from over heating in an ice bath. In the sonication process, pollen grains, initially floating on the water surface, sank to the bottom of the beaker. Next, the sonicated slurry was stirred for 15 min at room temperature. The slurry was then filtered through sterilized Whatman #1 filter paper into a sterile vacuum flask, and the aqueous extract restored to 165 ml with sterile distilled water. The sterile extract was immediately used in seed germination experiments.

Sonicated pollen remains were dried at 50 to 60 °C for 3 hr in a forced air oven. After drying, the remains were sterilized and de-gassed using the same techniques described for pollen used in the water extract preparation. A similar amount, *i.e.*, 4.5 g, of air dried and cleaned pollen (new pollen) was sterilized and de-gassed concomitantly with the sonicated pollen remains. Sterile pollen remains and unsonicated new pollen were applied in seed germination experiments immediately upon preparation.

2.2.3 Seed germination tests

2.2.3.a Seed storage, sources and sterilization

Subsequent to collection and maintenance at the Pineland Nurseries, seeds used in germination tests were stored at 3 to 5 °C for 3 to 6 months at the University of Manitoba (during the course of the experiments). The jack pine seeds were from the Northern Region, seed zone 4-6, seed lot 532, collection year 1994-1995, and the black spruce seeds were from the Northern Region, seed zone 11-7, seed lot 477, collection year 1994-1995.

In the first set of experiments ('block 0' for jack pine, and 'block 0' for black spruce) neither materials (filter paper, Petri dishes, pollen, distilled water, etc.) nor jack pine and black spruce seeds were sterilized. However, in the last four blocks (two blocks for jack pine, and two blocks for black spruce) all materials were sterilized by autoclaving or by propylene oxide in the case of pollen. Just before the experiments were established, the seeds were shaken in a 3% sodium hypochlorite solution for 30 seconds, and then rinsed three to five times in sterilized distilled water.

2.2.3.b Seed germination *in vitro*

Seed germination experiments were conducted in 9 cm diameter Petri dishes, with ten seeds scattered on moistened filter paper disks (Edwards 1987). One filter paper disk (Whatman #1) per Petri dish was provided in the first set of experiments (two 'blocks' 0), and two in the second set of experiments (two additional blocks for each species) (see Appendix I). The first set of experiments consisted of one round of data collection for each species (jack pine and black spruce), conducted under unsterile conditions. The second set of experiments, consisted of two rounds (two blocks) of data collection for each species, in which all materials were sterilized, including Petri dish set-ups, seeds, pollen and distilled water.

With the exception of controls, each Petri dish was supplied pollen or aqueous pollen extract, according to the treatment assigned to it. All dishes, irrespective of treatment, were provided 4.0 ml of water, either as aqueous extract of pollen (20 times normal seasonal pollen deposition on a unit area basis) (normal pollen deposition = 8.5 hg/ha.yr) (Lee 1997), 0.2 ml aqueous extract plus 3.8 ml distilled water (1 times pollen), or 2.0 aqueous extract plus 2.0 ml distilled water (10 times pollen). Control Petri dishes, as well as those ones that received additions of fresh pollen or sonicated pollen remains, received 4.0 ml distilled water. Pollen quantities (fresh pollen or sonication remnants) applied to each Petri dish were: 5 mg, 54 mg, and 108 mg, respectively for the 1x, 10x and 20x the normal seasonal pollen deposition amounts, on a unit area basis. After pollen treatment and addition of water, dishes were sealed with parafilm and placed in a growth chamber under a light regime of 8 hr of light and 16 hr of darkness. Light was provided by four 160 watt cool white fluorescent lights in a 78 cm by 185 cm growth chamber in the first set of experiments, and by six 215 watt cool white fluorescent lights in a 135 cm by 255 cm growth chamber in the second set of experiments. In each case the lights were positioned 1.4 m above the Petri dishes. In both sets of experiments, temperature was maintained at 28 °C during the light period, and at 20 °C during the dark period (Edwards 1987).

2.2.4 Data collection

Data collection occurred over 12 to 13 days. Total number of germinated seeds was recorded daily and at the end of the germination tests every germinated seed (*i.e.*, every seed with at least a protruded, bending radicle) was taken out of the Petri dish and measured for radicle and hypocotyl length. Also, the number of germlings with a damaged radicle was scored. (Some radicles were so severely damaged that virtually nothing remained.) The root collar, identified by the transition from a discolored tip to a pigmented region on the radicle-hypocotyl axis, was considered the starting point for determination of radicle and hypocotyl length and the end points were taken, respectively, as the tip of the radicle and the base of the cotyledon whorl. Length was measured with a plastic ruler to the nearest millimeter. The speed of germination was measured by the speed of germination index (S) (see Appendix I). According to Bradbeer 1988, this index is calculated by the formula:

$$S = \{\mathrm{Sum}(N_i/n_i)\} \times 100$$

where N_i stands for the proportion of seeds that germinated on day n_i, and n_i = 1,2, 3, .. N days after experimental initiation.

2.2.5 Experimental design

Both sets of experiments were carried out in a factorial design, with two factors: jack pine pollen substances levels (the equivalent of 1x, 10x and 20x the expected 8.5 kg/ha annual jack pine pollen deposition on mixed mature stands, on a unit area basis), and sources (fresh jack pine pollen, sonicated jack pine pollen remains, and aqueous jack pine pollen extract) (see Appendices II, III and IV for raw data, means and standard errors for both sets of experiments). The pollen substance levels were chosen to span a wide range of pollen rain in multiples of annual jack pine pollen rain of mixed mature forest from west-central Manitoba (*i.e.*, 8.5 kg/ha.yr = 1x) (Lee 1997), and after preliminary tests that showed pollen at 20x level, in *in vitro* conditions, as already toxic to seed germination. The use of sonication was meant to break external and internal components of pollen grains cells in order to make stronger and clear the effects of pollen substances on seed germination. Aqueous extract resulting from the sonication process may give an idea of the degree of pollen degradation resulting from the sonication process and of water solubility

of pollen substances during the 30 minutes in which pollen grains were suspended in water solution.

In the first set of experiments, each one of the nine combinations of the three levels for each factor had ten replicates (*i.e.*, ten Petri dishes) along with the ten control replicates. This totaled 100 Petri dishes for the first experiment on jack pine seed germination and 100 Petri dishes for the first experiment on black spruce seed germination. The first set of Petri dishes for each species is referred as 'block 0' in the analysis of variance (ANOVA) layout (Snedecor and Cochran, 1967; Underwood, 1997) shown in Table 2.1, although the design was actually a completely randomized design. In the second set of experiments, the same factor combinations were tested in two blocks of 100 Petri dishes for each species, so that the number of replications for each one of the nine combinations of the three levels of each factor was doubled to 20. The block (B) factor was introduced in the design as an artifact to isolate the effect of two successive batches of Petri dishes that were not accommodated at the same time in the growth chamber. Pollen substance levels (L) and sources (S) were considered fixed factors, while the block (B) factor was considered random (see ANOVA design in Table 2.1). The 100 Petri dishes for each species in each block (blocks '0', 1 or 2) were randomized at the same time in the same growth chamber, although the results for each species were analyzed separately.

Values from control Petri dishes were used to calculate a control mean for each variable in each block, and subsequently applied as divisors to germination results of each species and block variable. Although all these control divisors have an inherent error component that was not accounted for, the analyses, run through DataDesk® 4.1 (Velleman 1992), were performed on data expressed as a proportion of the control means. This results in differences between means being interpreted as more significant than they should be (Mead 1988).

Significance levels (p) of 10% , 5% or 1% for the F test are indicated by one, two or three asterisks, respectively. Significance levels for the Least Significant Difference (LSD) (Snedecor and Cochran 1967) test refer to the least pronounced difference of individual pairwise comparisons between means with $p<10\%$. Although relatively high, the significance level of 10% may be considered acceptable. According to Snedecor and Cochran 1967, the use of the LSD test may be advised if the F statistic of the analysis of variance is significant. Also, this test should not be applied to specific comparisons that attracted attention after a preliminary data examination. LSD test is applied in my study as

Table 2.1. Designs of the ANOVAs performed on jack pine and black spruce *in vitro* seed germination data.

SV	Maximum DF*		F divisor	
	'block' 0	blocks 1 plus 2	'block' 0	blocks 1 plus 2
Pollen substance level (L)	2	2	Error MS**	(B x L) MS
Pollen substance source (S)	2	2	Error MS	(B x S) MS
L x S	4	4	Error MS	(B x L x S) MS
Blocks (B)		1		Error MS
B x L		2		Error MS
B x S		2		Error MS
B x L x S		4		Error MS
Error	81	162		
Total	89	179		

*DF Degrees of Freedom
**MS= Mean Square

an additional tool for data analysis, along with the presentation of ANOVA results, mean values, and graphical display of the information considered relevant for the understanding of the effect of pollen substances on germling characteristics.

The standard error for each pollen substance level means in each pollen substance source was estimated by $\sqrt{}$(Error Mean Square/10), in the case of the unsterilized system experiments, and by $\sqrt{}$[(Block x Pollen substance level) Mean Square/20], in the case of the sterilized system experiments. The standard error of pollen substance level means in each pollen substance source, in either block 1 or block 2 of the sterilized system experiments, was estimated by $\sqrt{}$(Error Mean Square/10) (Underwood 1997). These estimates were used in the LSD test.

No ANOVA was performed on the variable number of germlings/replicate as many replicate values have shown no numerical variation. According to Mead (1988), the inclusion in the ANOVA of data that present no quantitative variation results in a pooled variance that is too low and not suitable for treatment comparisons. Mead advises a special analysis (which was not performed) on the data set that presents variation or simply the presentation of the data. When the interaction between block and the other factor(s) was non significant, means, standard errors and graphs were based on values obtained after pooling together the blocks. This improved the number of replicates of each mean, decreased the standard error, and avoided the need of plotting too many points in each graph. Although there could be differences between blocks, the trends of the effects of levels and/or sources of pollen substances were deemed statistically similar in each block, as indicated by the non significant interactions.

2.3 Results

Jack pine seed germination

Speed of germination of jack pine seeds in sterilized Petri dishes was significantly affected by pollen substance levels (1x, 10x and 20x) interacting with the pollen sources (fresh pollen, sonicated pollen remains and aqueous extract of fresh pollen) (Table 2.2). Addition of any level of any source of pollen substances reduced the speed of germination below the value of the control mean (Table 2.3). Specifically, the proportion means for levels 1x and 10x of fresh pollen were similar ($p>0.10$), and both were higher than the 20x mean.

Table 2.2. ANOVA[1] results of jack pine pollen substance effects on *in vitro* jack pine and black spruce seeds over a 12-day germination period.

Variable	Factors	Jack pine				Black spruce			
		unsterilized Petri dishes		sterilized Petri dishes		unsterilized Petri dishes		sterilized Petri dishes	
		DF	F	DF	F	DF	F	DF	F
Speed of germination	Pollen subst. level (L)		no data collection	2	21.1**		no data collection	2	29.1**
	Pollen subst. source (S)			2	11.7*			2	103.7***
	LxS			4	16.7***			4	6.91**
	Block (B)			1	26.2*			1	0.39
	BxL			2	2.23			2	2.38*
	BxS			2	1.9			2	0.91
	BxLxS			4	1.59			4	2.88**
	Error			161				161	
Hypocotyl length	Pollen subst. level (L)	2	37.5***	2	116.7***	2	44.95***	2	24.7**
	Pollen subst. source (S)	2	9.51***	2	5.42	2	5.43***	2	85.6**
	LxS	4	2.70**	4	19.0***	4	4.30***	4	2.74
	Block (B)			1	12.0***			1	4.45**
	BxL			2	0.45			2	2.91*
	BxS			2	4.41**			2	0.77
	BxLxS			4	1.08			4	4.73***
	Error	81		162		81		162	
Radicle length	Pollen subst. level (L)	2	85.9***	2	69.0**	2	125.1***	2	49.5**
	Pollen subst. source (S)	2	20.1***	2	4.39	2	24.6***	2	16.5*
	LxS	4	6.27***	4	7.89**	4	0.7	4	1.43
	Block (B)			1	19.6***			1	1.26
	BxL			2	1.58			2	1.47
	BxS			2	8.68***			2	1.42
	BxLxS			4	1.1			4	4.47***
	Error	81		162		81		162	

1 ANOVAs were performed on data expressed as proportion of control averages.
*, **, *** Significance levels of 10%, 5%, and 1%, respectively.

Table 2.3. LSD[1] test of jack pine pollen substance effects on *in vitro* jack pine seeds over a 12-day germination period under sterile conditions.

VARIABLE	CONTROL MEANS (SE) in the original scale	FRESH POLLEN MEANS			SONICATED POLLEN MEANS			AQUEOUS EXTRACT MEANS		
		1 x	10x	20x	1 x	10x	20x	1 x	10x	20x
Speed of germination	both blocks	0.9 A p<0.07	0.84 A	0.73 B	0.96 A p<0.01	0.81 B	0.28 C	0.91 A p>0.10	0.84 A	0.89 A
	block 1 17.02 (0.94)	0.99	0.91	0.83	0.95	0.79	0.41	0.95	0.93	0.98
	block 2 15.26 (0.90)	0.81	0.77	0.64	0.98	0.83	0.16	0.87	0.74	0.81
Number of germlings 2	block 1 9.00 (0.30)	1	0.98	0.92	0.97	0.86	0.5	0.96	0.91	0.98
	block 2 7.70 (0.40)	0.9	0.99	0.99	1.05	1.09	0.26	1.05	0.88	0.99
Hypocotyl length	both blocks	0.83 A p<0.02	0.82 A	0.53 B	0.96 A p<0.01	0.54 B	0.12 C	0.83 A p>0.10	0.76 A	0.8 A
	block 1 163.7 (9.0)	0.9	0.86	0.53	0.97	0.48	0.19	0.94	0.87	0.93
	block 2 122.1 (10.4)	0.76	0.77	0.53	0.95	0.6	0.05	0.72	0.65	0.67
Radicle length	both blocks	0.56 A p<0.06	0.26 B	0.11 C	0.66 A p<0.01	0.08 B	0.01 B	0.67	0.5	0.39
	block 1 144.0 (15.6)	0.59	0.32	0.13	0.7	0.05	0.02	0.85	0.56	0.55
	block 2 114.2 (11.7)	0.53	0.21	0.08	0.63	0.11	0.01	0.49	0.44	0.23

1 Least Significant Difference (LSD) test performed on means expressed as proportions of control averages.
2 No LSD test was performed on this variable.

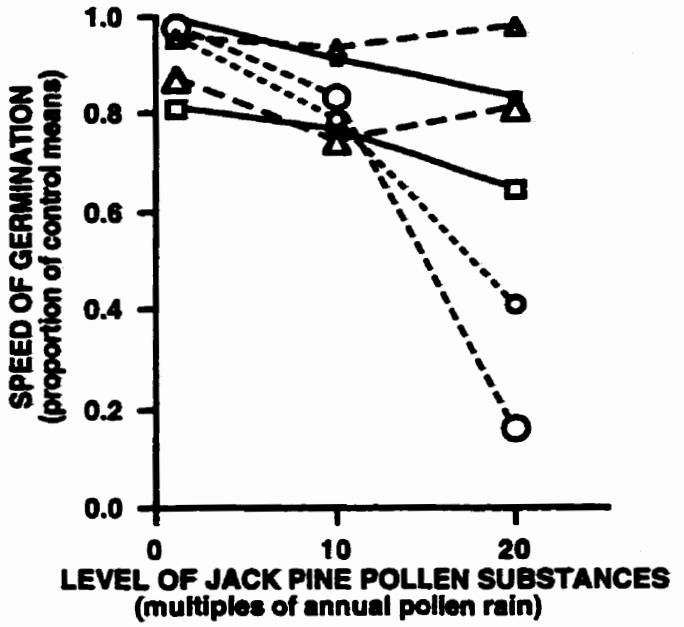
Sonicated pollen remains results showed significant differences in all three means ($p<0.01$), and the 20x mean value was markedly reduced to 28% of the control. Increases in aqueous pollen extract from 1x to 10x to 20x did not affect significantly the speed of germination of jack pine seeds (Figure 2.1.a).

The number of jack pine germlings per replicate (*i.e.*, each Petri dish) was affected interactively by blocks and pollen substance levels and sources, under sterilized conditions. In unsterilized Petri dishes, pollen substance levels and sources affected marginally the number of germlings (Table 2.4) (Figure 2.2a). With exception of 10x and 20x levels in sterilized sonicated pollen remains, all means of the number of jack pine germlings were less than 10% variant from the controls (Tables 2.4 and Table 2.5). The numbers of jack pine germlings in blocks 1 and 2 of the sterilized treatments basically did not differ from each other when considering each combination of pollen substances level and source, except for the quite reduced block means for sonicated pollen remains at level 20x, and, to a lesser extent, for the mean of aqueous extract at level 10x in block 2 (Table 2.3).

Jack pine hypocotyl length per replicate was interactively affected ($p<0.01$) by levels and sources of jack pine pollen substances (Table 2.2). There was also a significant effect ($p<0.05$) on hypocotyl length due to interaction between pollen sources and blocks. Sterile additions of pollen substances, at any level of fresh pollen and at levels 10x and 20x of sonicated pollen remains, reduced hypocotyl length significantly from the hypocotyl length of control germlings (Table 2.3). At levels 10x and 20x of jack pine pollen substances, jack pine hypocotyl growth was negatively affected, especially by fresh pollen and extracted pollen (Tables 2.3 and 2.4). Hypocotyl lengths in sterile Petri dishes were particularly shortened by treatments of fresh pollen at 20x (53% of control length) and sonicated pollen remains at 10x (54% of control length) and 20x (12% of control length) (Table 2.3). Under unsterilized treatment conditions, additions of pollen substances at 1x did not affect the hypocotyl length when compared with the control means (Table 2.4). Total hypocotyl lengths/replicate in unsterilized Petri dishes were notably shorter for fresh pollen additions at 20x (72% of control length) and for sonicated pollen remains treatment at 20x (63% of control length). Although no formal measurements were made, hypocotyls also reacted to high levels of jack pine pollen substances by perceptible coiling growth and propensity to breakage if straightened.

Radicle lengths of the germlings were significantly affected ($p<0.05$) by interaction of levels and sources of jack pine pollen substances (Table 2.2). Blocks and pollen sources

a) Jack Pine



b) Black spruce

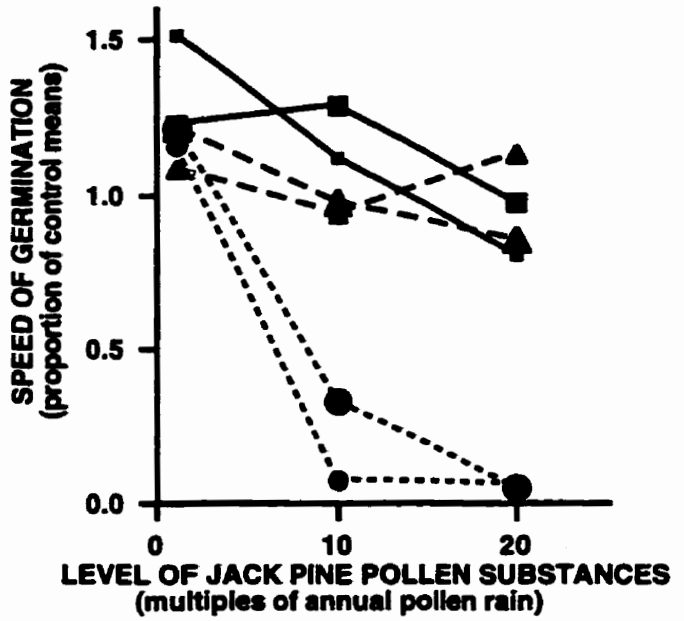


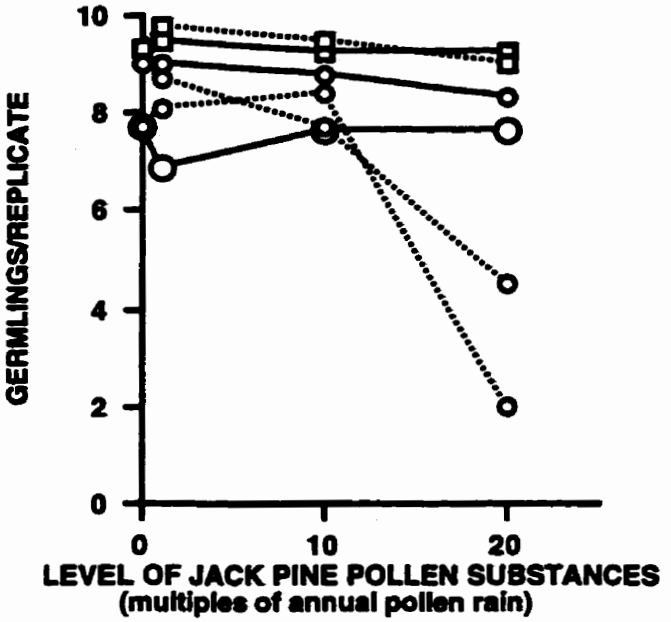
Figure 2.1. Effects of fresh jack pine pollen (squares), sonicated pollen remains (circles), and aqueous pollen extract (triangles) on the speed of germination of jack pine and black spruce seeds, in two blocks (different symbol sizes) under sterile conditions.

Table 2.4. LSD[1] test of jack pine pollen substance effects on *in vitro* jack pine seeds over a 12-day germination period under unsterile conditions.

VARIABLE	CONTROL MEANS (SE)	FRESH POLLEN MEANS			SONICATED POLLEN MEANS			AQUEOUS EXTRACT MEANS		
plot total basis	in the original scale	1 x	10x	20x	1 x	10x	20x	1 x	10x	20x
Number of germlings 2	9.30 (1.57)	1.02	0.99	0.99	1.05	1.02	0.97	1.05	1.03	1.03
Hypocotyl length	221.0 (42.5)	0.96 A p<0.01	0.89 A	0.72 B	1.03 A p<0.02	0.9 B	0.63 C	1.05 A p<0.10	0.98 A	0.89 B
Radicle length	180.7 (52.3)	0.64 A p<0.09	0.52 B	0.4 C	0.75 A p<0.01	0.38 B	0.17 C	1.01 A p<0.01	0.66 B	0.34 C

1 Least Significant Difference (LSD) test was performed on means expressed as proportions of control averages.
2 No LSD test was performed on means of this variable.

a) Jack pine



b) Black spruce

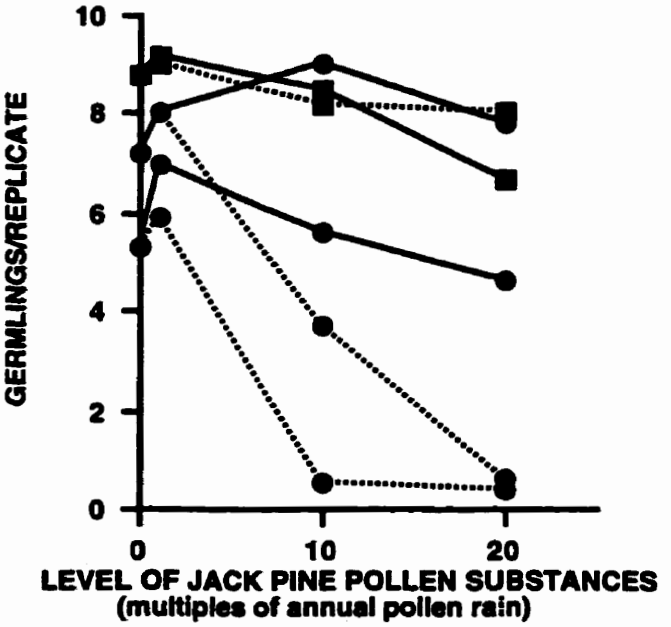


Figure 2.2. Effects of fresh jack pine pollen (full line) and sonicated pollen remains (dotted line) on the number of germlings/replicate, under unsterile (squares) and sterile (circles) block conditions.

Table 2.5. LSD[1] test of jack pine pollen substance effects on *in vitro* black spruce seeds over a 12-day germination period under sterile conditions.

VARIABLE	BLOCK	CONTROL MEANS (SE) in the original scale	FRESH POLLEN MEANS			SONICATED POLLEN MEANS			AQUEOUS EXTRACT MEANS		
			1 x	10x	20x	1 x	10x	20x	1 x	10x	20x
Speed of germination	Block 1	6.62 (0.95)	1.51 A p<0.02	1.12 B	0.8 C	1.15 A p<0.01	0.07 B	0.06 B	1.08 A p>0.10	0.93 A	1.13 A
	Block 2	11.93 (0.54)	1.23 A p<0.05	1.29 A	0.97 B	1.21 A p<0.03	0.33 B	0.05 C	1.21 A p<0.07	0.97 B	0.85 B
Number of germlings 2	Block 1	5.30 (0.58)	1.32	1.06	0.87	1.11	0.09	0.08	0.98	0.98	1.09
	Block 2	7.20 (0.25)	1.11	1.25	1.08	1.11	0.51	0.08	1.1	1.03	0.99
Hypocotyl length	Block 1	35.20 (6.46)	1.51 A p<0.09	0.88 B	0.61 C	0.97 A p<0.01	0.02 B	0.02 B	0.9 A p>0.10	0.83 A	0.96 A
	Block 2	71.10 (5.51)	1.31 A p<0.01	1.41 A	0.76 B	1.27 A p<0.01	0.13 B	0.02 B	1.27 A p<0.01	0.85 B	0.64 B
Radicle length	Block 1	12.50 (2.27)	1.26 A p<0.02	0.58 B	0.22 C	0.86 A p<0.01	0.02 B	0.02 B	0.69 A p>0.10	0.7 A	0.82 A
	Block 2	34.90 (3.01)	0.94 A p<0.01	0.4 B	0.24 B	0.92 A p<0.01	0.08 B	0.01 B	1.12 A p<0.01	0.6 B	0.36 B

1 Least Significant Difference (LSD) test was performed on means expressed as proportions of control means.
2 No LSD tes was performed on this variable.

showed significant interaction ($p<0.01$) in determining radicle length under sterilized conditions. Block effect was seen in the results of the aqueous extract treatment in sterilized Petri dishes (Table 2.3) where radicle lengths values were affected in different ways in block one and two (LSD test results ABB in block one and AAB in block two). Results from sterilized fresh pollen and sonicated pollen remains did not manifest similar block differences.

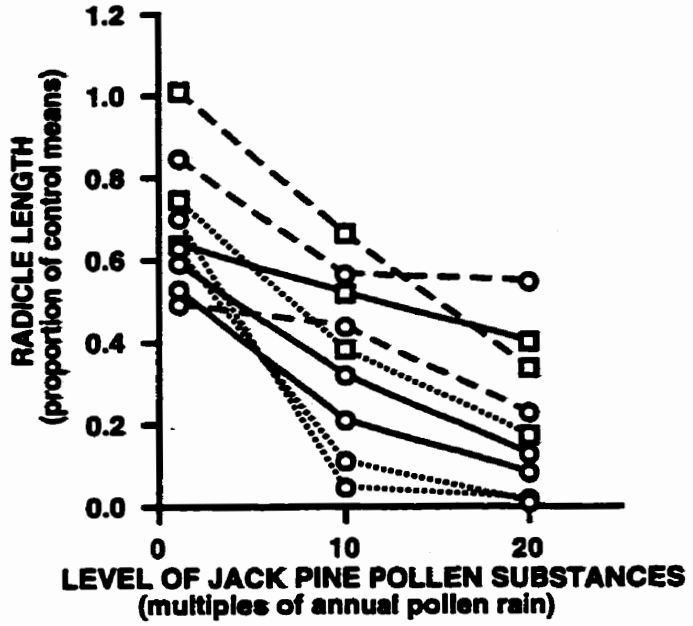
Pollen substance additions at all levels and from all sources under sterile and unsterile conditions significantly reduced radicle length if compared to the control means, except for aqueous extract at level 1x in unsterilized conditions (Table 2.4). In both types of experiments (*i.e.*, those with sterilized and unsterilized Petri dish set-ups), all pairwise comparisons of radicle length means were significantly different within each pollen source, except for comparisons in sterilized conditions, as follows: the 10x to 20x comparison for sonicated pollen remains, and the 10x to 20x and 1x to 10x comparisons for aqueous extract, in block one and block two, respectively (Tables 2.3) (Figure 2.3a).

In both sterilized and unsterilized Petri dish experiments, radicle lengths were generally shorter than the control values (around less than 50% at 10x, and <40% at 20x fresh pollen; <40% at 10x and <20% at 20x with sonicated pollen remains; and <70% at 10x and <40% at 20x with application of aqueous pollen extract). On average, increases in the pollen substance levels in aqueous extracts affected radicle length less than increased levels of fresh pollen and sonicated pollen remains (Figure 2.3.a). Practically no radicle was formed by seeds germinated at 20x application of fresh pollen and sonicated pollen remains.

Black spruce seed germination

Experimental blocks, and sources and quantity of pollen substances interacted to affect the speed of germination of black spruce (Table 2.2). At 1x sterile pollen substance additions, proportion means for black spruce germination characteristics in any one of the sources of pollen substance were higher than the control mean, and at 1x fresh pollen treatment up to 50% higher than the speed of germination of the controls (Table 2.5). Sterile fresh pollen additions at 1x and 10x (in blocks 1 and 2) stimulated more rapid germination of black spruce seeds, and aqueous pollen extract may have stimulated (level 1x in block 2) or decreased (level 20x block 2) the speed of germination, but in general aqueous extract seemed to have a non significant effect . In contrast, the speed of germination was

a) Jack Pine



b) Black spruce

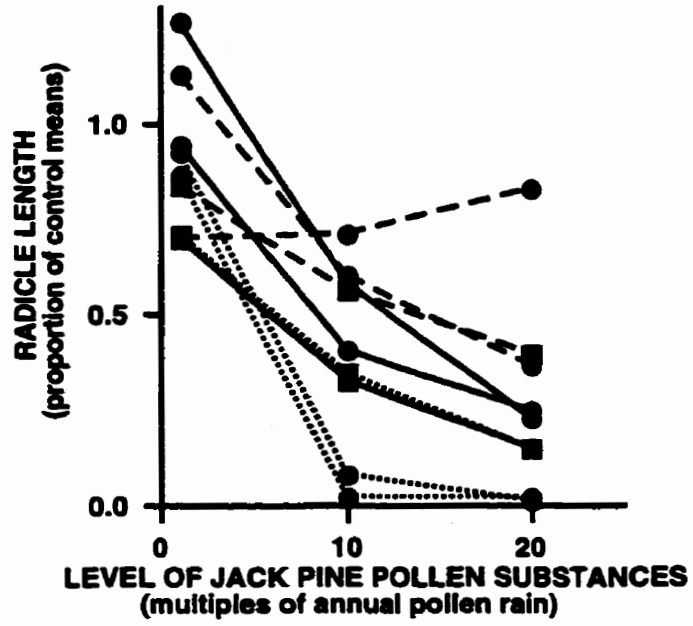


Figure 2.3. Effects of fresh jack pine pollen (full line), sonicated pollen remains (dotted line), and aqueous pollen extract (dashed line) on radicle length of jack pine and black spruce germlings, under unsterile (squares) and sterile (circles) block conditions.

significantly below the control (one third to one twentieth of the control) when treated with sonicated pollen remains at levels 10x and 20x (Figure 2.1b).

The number of black spruce germinated seeds was most affected by the pollen substances in unsterilized Petri dish set-ups (Table 2.5). In the sterilized Petri dish experiment, additions of sterile fresh pollen and aqueous extract of pollen at 1x, 10x, and 20x generally increased the number of black spruce germinated seeds above the control means, or resulted in equivalent numbers with the controls, when blocks 1 and 2 were considered together (Table 2.5). Exception was seen under sterilized fresh pollen treatment at 20x (block 1) where germinated seeds were approximately 90% of the control mean. With treatment by sterilized sonicated pollen remains, the numbers of germinated seeds were greater than the control mean at 1x and reduced at 10x and 20x pollen additions. In contrast, in unsterilized Petri dishes the proportion of germinated seeds was around the control mean level across all three pollen sources and levels with exception of fresh pollen and sonicated pollen remains at 20x (Table 2.6) (Figure 2.2.b).

In unsterilized Petri dishes, pollen substances sources and levels interacted to determine hypocotyl length of the developing germlings. In sterilized Petri dishes, blocks interacted with pollen substances source and levels (Table 2.2). One times addition of sterilized pollen substances generally increased hypocotyl length when both blocks were considered (Table 2.5). With few exceptions, hypocotyl length was around 85% or less of the control mean lengths for all three sterilized pollen substances at 10x and 20x additions. Hypocotyl lengths of developing germlings treated with unsterilized pollen substances at level 1x were around 90% to 95% of the control mean length, and hypocotyl/replicate length at levels 10x and 20x of any source of pollen substance was reduced to around 75% or less of the control mean (Table 2.6). Reductions in hypocotyl length below 70% occurred with treatments by sterilized sonicated pollen remains at 10x (<15%) and 20x (<5%), unsterilized sonicated pollen remains at 20x (55%) and 10x (~65%), unsterilized fresh pollen at 20x (35%), sterilized fresh pollen at 20x (~60%) (block 1), and aqueous extract of pollen at 20x (~65%) (block 2). Although no formal measurements were made, hypocotyls also reacted to high levels of jack pine pollen substances by a perceptible coiling growth and propensity to breakage if straightened.

In unsterilized Petri dishes, radicle length of developing germlings was significantly affected by pollen substance levels and sources, and in sterilized Petri dishes there was significant interaction between blocks and pollen substance levels and sources (Table 2.2).

Table 2.6. LSD[1] test of jack pine pollen substance effects on *in vitro* black spruce seeds over a 12-day germination period under unsterile conditions.

VARIABLE plot total basis	CONTROL MEANS (SE) in the original scale	FRESH POLLEN MEANS 1 x	10x	20x	SONICATED POLLEN MEANS 1 x	10x	20x	AQUEOUS EXTRACT MEANS 1 x	10x	20x
Number of germlings 2	8.80 (1.32)	1.05	0.97	0.76	1.02	0.93	0.91	0.98	0.94	0.99
Hypocotyl length	137.5 (24.8)	0.96 A p<0.01	0.72 B	0.35 C	0.91 A p<0.01	0.66 B	0.55 B	0.94 A p<0.03	0.77 B	0.71 B
Radicle length	77.5 (19.3)	0.69 A p<0.01	0.32 B	0.15 C	0.7 A p<0.01	0.34 B	0.15 C	0.83 A p<0.01	0.56 B	0.39 C

1 Least Significant Difference (LSD) test was performed on means expressed as proportions of control means.
2 No LSD test was performed on this variable.

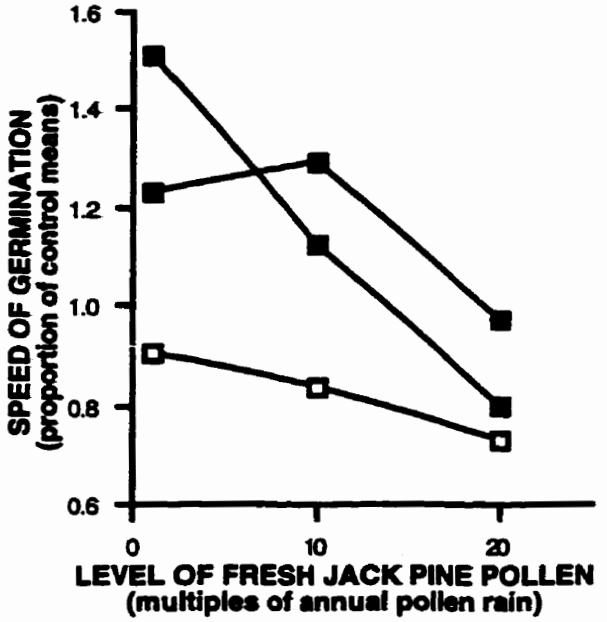
In general, the pairwise comparisons between means of levels 1x, 10x and 20x of each pollen substance source, sterilized or unsterilized, were significant ($p<0.01$) (Table 2.5 and 2.6).

Nearly all levels and sources of jack pine pollen substances tested in this study reduced radicle length of black spruce germlings to less than 95% of the control means (Tables 2.5 and 2.6). Sterilized fresh pollen treatment at 1x (block 1) with radicles ~1.25 longer than the control mean was the exception. Fresh pollen and sonicated pollen remains additions at 20x reduced radicle growth to less than 25% of the means of the control germlings under sterilized conditions and to ~15% in unsterilized conditions. Under sterilized conditions, the most pronounced reduction in radicle lengths (<10% of control lengths) was seen in 10x and 20x additions of sterilized sonicated pollen remains, while sterilized aqueous extract at any level resulted, in general, in less drastic radicle reduction (Table 2.5) (Figure 2.3b).

Comparison of jack pine and black spruce seed germination

As a percentage of control means, speed of germination and radicle length of jack pine and black spruce seeds were negatively affected by sterilized fresh pollen additions in increasing amounts, *i.e.*, 1x to 10x to 20x normal pollen rain (Figure 2.4). Although initially stimulated by propylene oxide sterilized fresh pollen addition at 1x, the decline in speed of germination and radicle length percentages from level 1x to 20x of fresh pollen observed in black spruce appeared greater than for jack pine. Further, in unsterilized fresh pollen levels, total radicle length/replicate of jack pine was reduced 2.5 times from the 0x to the 20x pollen levels (germinated seeds basis: reduction of 2.5 times). In contrast, total black spruce radicle length/replicate was reduced by 6.0 times from unsterilized fresh pollen level 0x to 20x (germinated seeds basis: reduction of 4.5 times, data no shown) (Figure 2.5.a, b). However, in sterilized fresh pollen treatment, total jack pine radicle length/replicate was reduced in length by 7.0 times (germinated seeds basis: reduction of 8 times) in block 1 results, and 13 times (germinated seeds basis: reduction of 15 times) in block 2, while total black spruce radicle length/replicate was 4 times shorter at the 20x than in the 0x fresh pollen level for both blocks 1 and 2 (germinated seeds basis: reductions from 3 to 5 times). With no pollen additions and in unsterilized Petri dishes, jack pine radicles (replicate total or germinated seed basis) were double the length of black spruce radicles grown under the same conditions (Figure 2.5a, b). In sterilized treatments with no

a) Speed of germination



b Radicle length

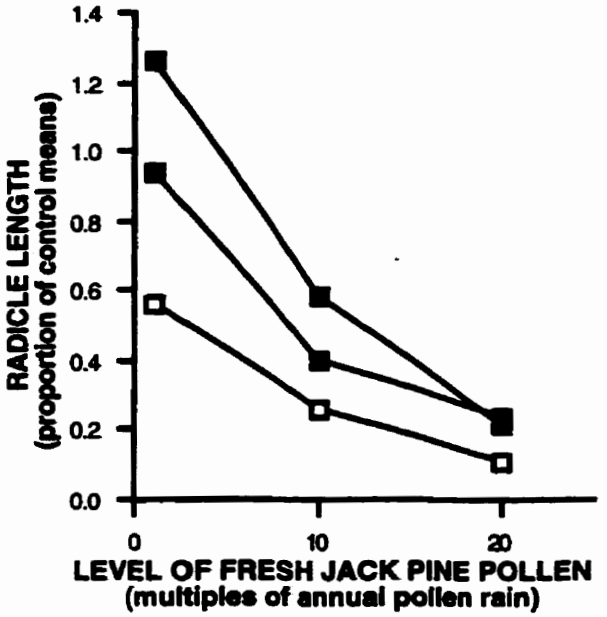
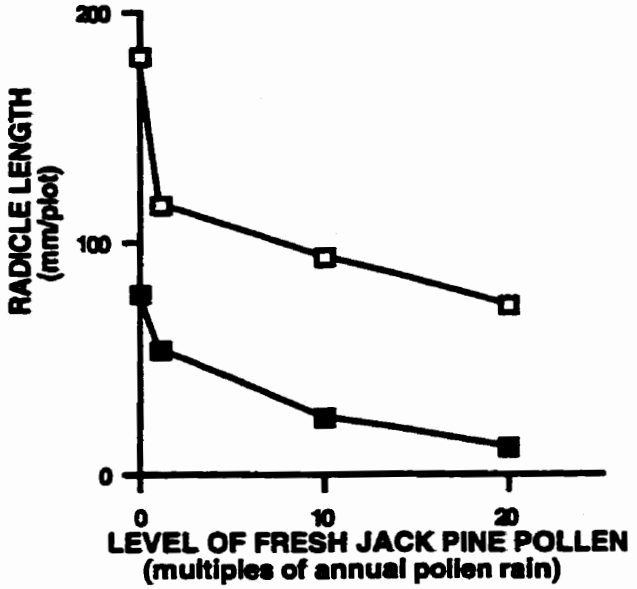


Figure 2.4. Effects of fresh jack pine pollen on the speed of germination and radicle length of jack pine (open square) (means after pooling the 2 blocks) and black spruce (solid square) germlings (means from each block), under sterile conditions.

a) Unsterile block conditions



b) Sterile block conditions

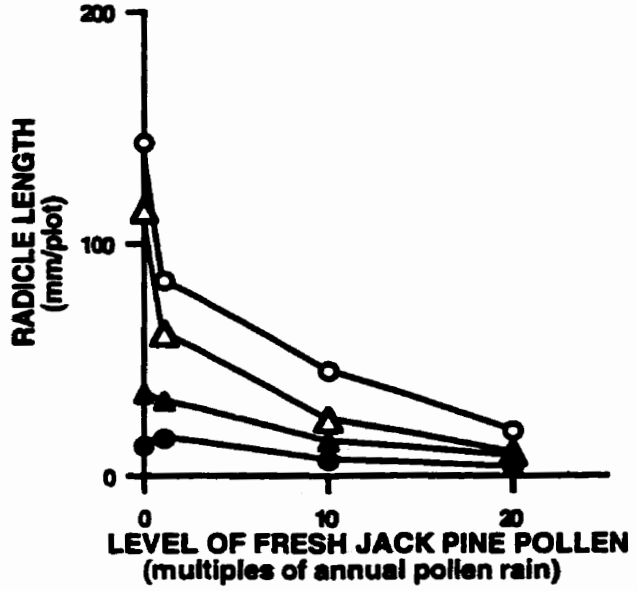


Figure 2.5. Effects of fresh jack pine pollen on radicle length of jack pine (open symbols) and black spruce (solid symbols) germlings, under unsterile and sterile block conditions.

pollen addition, total black spruce radicles/replicate were one tenth to one fourth the jack pine total. Based on germinated seeds, black spruce radicles/replicate were one eighth to one third, as long as jack pine radicles (Figure 2.5,b). Finally, with 10x and 20x unsterilized and sterilized fresh pollen additions, the radicle of each jack pine germling was at, or less than, 1.0 cm long (approximately one half or less of the control length) and black spruce radicles were at, or less than, 0.3 cm long (approximately one half or less of the control length) (Figure 2.5b).

2.4 Discussion

The results of my study show that jack pine pollen substances affect jack pine and black spruce *in vitro* seed germination. Pollen effects on seed germination are reported in a series of related papers by Anaya *et al.* (1992), Jiménez *et al.* (1983), and Ortega *et al.* (1988). These authors show that *Zea mays* pollen negatively affects seed germination of some species. The negative effects are due to the presence of phenylacetic acid, which disrupts the electron transport pathway in cellular respiration. The authors conclude that *Zea mays* pollen may play a role in the control of competing weeds. In contrast, several other authors (Borecka *et al.* 1969; Borecka and Millikan 1973; Chou and Preece 1968; Fokkema 1971; Warren 1972 a, b; Warren 1976; Williams and Colotelo 1975) observe a positive effect of pollen substances from several species on the germination of spores and growth of pathogenic fungi, due to nutritional and non-nutritional factors from pollen.

Light, temperature and moisture (Edwards 1987) trigger a chain of physiological processes in seeds, which is mediated by a dynamic balance of endogenous hormones (Khan 1975). Various workers demonstrate mimicry of endogenous seed hormone effects with exogenous applications of hormones to seeds (*e.g.*, Anderson and Widmer 1975; Ballington *et al.* 1976; Bulard 1985; Crozier *et al.* 1970; Devlin *et al.* 1976; Dweikat and Lyrene 1989; Scott and Leopold 1967; West *et al.* 1970).

Bioassays involving application of growth substances from pine pollen of various species to sensitive test plants, as well as chromatographic separations, show that pine pollen is rich in hormones, such as auxins (*e.g.*, Michalski 1967, from *Pinus sylvestris* L., and Sweet and Lewis 1971, from *Pinus radiata* D. Don), gibberellins (*e.g.*, Ivonis 1969, from *Pinus sylvestris*, and Kamienska and Pharis 1975, from *Pinus attenuata* Lemm., *P. coulteri* D. Don, and *P. ponderosa* Laws., var. ponderosa), kinetin (*e.g.*, Sweet and Lewis 1971,

from *Pinus radiata*), and phenolic compounds acting as inhibitors (*e.g.*, Larionova *et al.* 1977, from *Pinus sibirica* Du Tour).

Pine and spruce seeds are sensitive to hormones detected in pine pollen. Sandberg (1988) observed that seeds of *Pinus sylvestris* and *Picea abies* (L.) Karst. are negatively affected by indole-3-acetic acid and indole-3-butyric acid at concentrations from 1 to 1000 µM. Gibberellin improves speed of germination and/or percent germination in *Pinus taeda* L. (100 to 2888 µM) (Biswas *et al.* 1972) and in some seed lots of *Pinus sylvestris* (10 to 100 µM) (Sandberg 1988). Gibberellins may also be beneficial to *Picea abies* (10 to 100 µM) (Sandberg 1988) and *Picea smithiana* (Wall.) Boiss. (up to 2888 µM) (Singh 1989). Kinetin (at 46 µM) positively affects *Pinus taeda* seed germination (Biswas *et al.* 1972) and, at 1 to 1000 µM, it negatively affects *Pinus sylvestris* seed germination (Sandberg 1988). *Pinus monticola* Dougl. ex D. Don seed germination is improved by the simultaneous use of kinetin (at 4.6 µM) and gibberellin (at 114.3 µM) (Pitel and Wang 1985). *Picea abies* seed germination is also negatively affected by kinetin (1 to 1000 µM) (Sandberg 1988).

Based on the amounts (5 to 108 mg) of jack pine pollen added to 4 ml of water in the Petri dishes in my experiments, and assuming the hormone concentrations from jack pine pollen to be equivalent to hormone levels from other pine species, an auxin concentration range of 2.97×10^{-2} to 6.62×10^{-1} µM (Sweet and Lewis 1971), a joint gibberellin concentration range of 1.1×10^{-4} to 2.5×10^{-3} µM (Kamienska and Pharis 1975), and a kinetin-like substance concentration range of 6 to 125 µM (Sweet and Lewis 1971) might be expected. The predicted concentrations of auxin and kinetin from added jack pine pollen are close to the levels of hormones demonstrated to affect pine and spruce seed germination in the literature.

The instances in my study of enhanced germination of black spruce at the equivalent to 1x and 10x the annual rain of fresh jack pine pollen (*e.g.*, speed of germination) (Figure 2.1.b), under sterile conditions, contrast with the generally negative effect auxin and kinetin have on spruce seed germination (Sandberg 1988). However, Sandberg (1988) also reports a positive effect of etephone (an ethylene precursor) on germination of some seed lots of *Picea abies*. Possibly, an ethylene-like product is present in my experiments in sufficient quantity to enhance black spruce speed of germination, number of germinated seeds, and hypocotyl growth at pollen levels equivalent to 1x and 10x the annual jack pine pollen rain.

Burg and Burg (1966) report a model in which ethylene formation by plant tissues is induced by exogenously applied auxin at concentrations of 1 to 10 μM. Ethylene reduces cell elongation by up to 50% and interferes in radial auxin diffusion through plant tissues (Burg and Burg 1966) . In *Vigna radiata* (L.) R. Wilczec, var. radiata (syn. *Phaseolus radiatus*) seedlings, ethylene promotes a hypocotyl coiling response due to its effect on auxin distribution through the hypocotyl axis (Sankhla and Shukla 1970). This auxin induced ethylene formation model may explain, in part, hypocotyl growth inhibition in jack pine, the observed coiling hypocotyl growth of the germlings, and also the improvement in the speed of germination of black spruce seeds. Furthermore, it is known that pollen of some other species is rich in an ethylene precursor (*e.g.*, Hill *et al.* 1987). Finally, organic soil from spruce forests may produce a substantial amount of ethylene (Lindberg *et al.* 1979; Sexstone and Mains 1990). Seed germination of several species is affected by ethylene and other volatile compounds in the soil (Holm 1972; Taylorson 1979). The positive response (*e.g.*, increased speed of germination) of black spruce observed *in vitro* in the sterilized conditions of my experiments may actually reflect the adaptation of this species to the presence of this gas in the natural substrate.

It is also possible that, after pollen sterilization, some propylene oxide residue was left in fresh pollen and sonicated pollen remains. Although propylene oxide may be harmful to seed (Ramakrishna *et al.* 1991), it is possible that this residue makes the seed especially sensitive to ethylene in the same way as ethylene oxide has been shown to increase tissue sensitivity to ethylene (Beyer 1985).

The negative pollen effect on radicle length is of special importance. In my study, a normal level of jack pine pollen (*i.e.*, 1x) reduced, *in vitro*, radicle length of jack pine and black spruce developing germlings by up to 30%. The normal variation in the pollen rain deposition shows an upper limit of 18.9 kg/ha.yr, in mature, mixed forest, and 28.7 kg/ha.yr in a pure regenerating jack pine stand (Lee 1997, Lee *et al.* 1996b). These figures are 2 to 3 times higher than the average annual pollen rain per unit area (*i.e.*, 8.5 kg/ha.yr) (Lee 1997) level used in 1x additions in my study. Such variation indicates the possibility of encountering microsites in the field with a significant potential for radicle reduction. In contrast to the post fire establishment of jack pine, the recruitment of black spruce may also occur over a long period of time and under the influence of already established jack pine trees. Black spruce germlings may be exposed to the negative effect of jack pine pollen on radicle growth. It is known that radicle length of black spruce is considered one of the

factors confining this species to moist sites (Thomas and Wein 1985). Further reductions in an already short radicle would restrict black spruce to moist environments and might even impact on successful establishment of seedlings in the natural environment. Furthermore, the effects of jack pine pollen on seed germination reported in my study are possible effects on seeds of other boreal plant species. This might be addressed in future studies.

There certainly are differences in pollen hormone composition among species (*e.g.*, Kamienska and Pharis 1975). It may be that jack pine pollen substances, as well as jack pine and black spruce responses to hormones, are different from those described for other species in the literature. Studies on jack pine pollen hormone composition are necessary to validate the suggested effects on seeds and germlings. Development of a model to fully explain the role of jack pine pollen on seed germination of jack pine and black spruce, as well as on seeds of other boreal forest plant species, is necessary. Mechanisms involved in such a model remain to be studied. Furthermore, the effect of jack pine pollen substances on seed germination should be tested on soil to determine if *in vitro* trends also occur on soils, which is the subject of the next chapter.

Chapter III

Effects of jack pine pollen on jack pine [*Pinus banksiana* Lamb.] and black spruce [*Picea mariana* (P. Mill.) B.S.P.] seed germination on mineral soil

3.1 Introduction

Seed germination is frequently tested with plant extracts. However, *in situ* effects of these extracts may differ from results observed *in vitro* (*i.e.*, in bioassays run on filter paper in Petri dishes) (Stowe 1979). Filtered water extract of *Empetrum hermaphroditum* Hagerup reduces radicle length, produces necroses of *Pinus sylvestris* L. and *Populus tremula* L. seedling radicles and may even suppress germination of seeds of these species, *in vitro* (Zackrisson and Nilsson 1992). In contrast, these authors also observed that humus soaked with aqueous *Empetrum* leaf extract shows no negative effect on aspen seed germination and seedling growth. Leachate from the washed humus also shows no negative effect on aspen seeds and seedlings. However, if microorganisms are inactivated (*i.e.*, sterilized humus), aspen seed germination is strongly and negatively affected by *Empetrum* leaf extract.

Similarly, *Zea mays* L. pollen (10 to 200 mg/Petri dish) reduces radicle length of *Cassia jalapensis* (Britton) Lundell. by 25 to 90%, *in vitro*. When this pollen is mixed with soil (0.7 g of pollen to 120 g of soil) radicles are reduced by 25% in unsterilized soil and by 50% in sterilized soil (Jiménez *et al.* 1983).

High levels of nutrients (*e.g.*, 2% of nitrogen, by weight) (*e.g.*, Doskey and Ugoagwu 1992; Stark 1973), and at least thirteen growth active substances, such as auxins and phenolic inhibitors (Ivonis 1969; Larionova *et al.* 1977; Michalski 1967; Sweet and Lewis 1971), are known from pine pollen. Pine species may release annually 0.6 to 227 kg of pollen/hectare (*e.g.*, Maggs 1985; Stark 1972; Stark 1973; Versfeld and Donald 1991). In central-west Manitoba, the annual rain of airborne pollen in May and June ranges from 11.7 to 24.6 kg/hectare, and over the range of forest microsites jack pine (*Pinus banksiana* Lamb.) alone accounts for more than half of that rain. In a mature, mixed stand of the region, annual pollen rain provides an average of 8.5 kg of jack pine pollen/ha, in early to mid June of each year (Lee 1997; Lee *et al.* 1996b).

As described in the previous chapter, jack pine pollen has been shown to affect jack pine and black spruce (*Picea mariana* (P. Mill.) B.S.P.) seed germination *in vitro* . However, it remains to be determined if the same is true when pollen is present on soil at the time of seed germination. Normally, successful jack pine and black spruce seed germination and seedling establishment in southern Manitoba and Ontario occur from May to June (*e.g.*, Brown 1973; Cayford 1961). For every successfully implanted one-year old jack pine seedling in regenerating stands in southeastern Manitoba, at least 450 seeds from broadcast cones are necessary (Cayford 1958; Cayford 1959; Cayford 1961; Cayford and Dobbs 1967; Chrosciewicz 1983a, b; Chrosciewicz 1990; Dominy and Wood 1986; Heinselman 1965; Sims 1970; Walker and Sims 1984). When jack pine seeds are cleaned, approximately every four seeds will produce a successful seedling (Jarvis 1966). Black spruce seed germination and seedling establishment occur normally in wet sites or in upland dryer sites during wet years. Successful germination and seedling establishment also coincide partially with the time of jack pine pollen release (Heinselman 1965; Jarvis 1966).

It is generally known that adequate light, temperature and moisture regimes trigger the physiological processes of seed germination (Edwards 1987; Rudolph and Laidly 1990). Although these processes are generally mediated by hormones endogenous to the seed, the application of exogenous hormones, such as gibberellins, is used to accelerate and improve germination of seeds of several species (*e.g.*, Bulard 1985; Devlin *et al.* 1976) (see Appendix I, item 5). Exogenous gibberellin is known to reduce or replace the cold stratification period necessary for *Picea smithiana* (Wall.) Boiss. (Singh 1989) or ginkgo (*Ginkgo biloba* L.) seed germination (West *et al.* 1970), respectively.

Jack pine and black spruce are generally found in distinct habitats (upland dry sites *vs.* wet boggy sites, respectively). In any case, jack pine pollen reaches black spruce prone sites due to air movements and water run off as low sites alternate regularly with upland, jack pine-dominated sites. Thus, it is possible that jack pine pollen interferes with black spruce establishment as it is shown to reduce black spruce radicle length (see Chapter II).

As pollen is rich in active growth substances, and is present in large quantities in the boreal forest, its role in seed germination, seedling implantation, the early stages of forest re-establishment and seasonal phases in tree growth requires detailed study. The objective of this study is to examine if the *in vitro* responses of jack pine and black spruce seeds to fresh jack pine pollen are modified by seed germination on mineral soil.

3.2 Methods and materials

3.2.1 Pollen, seeds and seed germination

Jack pine pollen collection, handling and storage, seed sources and storage are described in the Methods and materials section of Chapter II.

Plastic containers (7.1 cm diameter, and 4 cm deep) (*i.e.*, bottom of plastic soft drink bottles) were filled with 85 ml (~100 g, air dried basis), unsterilized, sieved mineral soil from the Payuk Lake region (54°39′ N, 101°28′ W) (see soil characteristics in section 3.3). Although jack pine and black spruce were known to occupy sites with distinct characteristics (jack pine in sandy soils with low organic matter content, and black spruce in organic, boggy soils), both jack pine and black spruce seed germination were tested on a sandy loam, after Edwards (1987).

No pollen or the equivalent of 1x (3 mg), 10x (34 mg), 50x (168 mg) or 100x (337 mg) the annual jack pine pollen rain (8.5 kg/ha.yr) (Lee 1997) was added, on a pollen mass/container area basis, to each replicate (*i.e.*, each container), and mixed into the surface of the soil. Prior to adding 32 ml of distilled water to each replicate, 10 seeds of jack pine or black spruce (nine seeds of black spruce/replicate in the last five blocks) were scattered on the soil surface. The replicates were placed in plastic trays (25 cm x 50 cm x 10 cm), and covered with a transparent plastic sheet to prevent soil desiccation.

The plastic trays were placed in a growth chamber (135 cm by 255 cm) under a 8 hr light : 16 hr darkness regime (six 215 watt white cool fluorescent light, positioned 1.4 m above the trays), for 12 to 13 days. The temperature was kept at 28 °C, during the light period, and at 20 °C, during the dark period (Edwards 1987). Five trays for each species were placed in the growth chamber from August 31 to September 12, 1998 (13 days). A further set of five trays for each species stayed in the growth chamber from October 24 to November 5, 1998 (12 days).

3.2.2 Data collection

Each germinated seed (*i.e.*, with a protruded and bending radicle) (Mayer and Poljakoff-Mayber 1989) was measured individually for root and shoot length, to the nearest millimeter, by means of a plastic ruler. Further data on the total number of germlings and number of germlings with radicle infestation were collected. Radicles were considered infested when demonstrating a brownish/black area, generally just below the root collar region.

The root collar, identified by transition from a discolored to a pigmented region on the radicle-hypocotyl axis, was the initial point for measurement of the radicle and shoot length. The end points for shoot and radicle length measurements were the tip of the radicle (or what was left) and the base of the cotyledon whorl, respectively.

3.2.3 Experimental design

Each one of the 10 plastic trays (blocks - B) contained two replicates of each one of the five pollen levels (PL) (0x, 1x, 10x, 50x and 100x the annual jack pine pollen rain of 8.5 kg/ha in mixed mature boreal forests in west-central Manitoba) (Lee 1997), totaling 20 replicates for each pollen level for jack pine and black spruce (raw data sets, means and standard errors are shown in Appendix V). [These pollen levels were chosen to cover critical pollen level values, such as control (0x), normal pollen rain (1x) (Lee 1997), a moderately high level (10x), shown to promote enhanced fungal activity in the substrate (Stark 1972; Stark 1973), and high levels of pollen (50x and 100x) to test for possible pollen toxicity.]

Pollen level (PL) factor was considered a fixed factor, while the block (B) factor was considered random. Data of each species were analyzed separately through DataDesk® 4.1 software (Velleman 1992), using the general ANOVA design (Snedecor and Cochran 1967; Underwood 1997) shown in Table 3.1. When the PL x B interaction was deemed to be non significant in the F test of the analysis of variance, the means for each variable were usually estimated after pooling together all the 20 replicates of the 10 blocks for each pollen level. This procedure increased the number of replicates of each mean, decreased the standard error, and satisfactorily summarized the statistically similar effects of pollen levels among blocks, as detected by the F test.

Table 3.1. Designs of the ANOVAs performed on data of seed germination of jack pine and black spruce on mineral soil substrate.

SV	Maximum DF*	F divisor
Pollen level (PL)	4	B x PL mean square
Block (B)	9	Error mean square
B x PL	36	Error mean square
Error	50	
Total	99	

*DF= Degrees of freedom

Log transformation (base 10) was used for diameter and length variables. Data transformation was performed independently of any test for homogeneity of variance or data distribution. Such transformation does not alter the reliability of the data and contributes toward improvement of homocedasticy (Mead 1988). Mead (1988) recommends the regular use of log transformation on continuous data to ensure compliance to the assumptions of the ANOVA model. The opposite transformation (*i.e.*, 10^{log}) was used on each transformed mean shown in tables in order to get means of the original measures. Prior to transformation, diameter and length variables were on a mm/replicate basis or on a mm/germinated seed basis, as indicated. The variables number of germlings and number of germlings with infested radicles are shown on a replicate total basis. No analysis of variance was performed on these two variables, nor was a multiple comparison test applied to them. According to Mead (1988), the inclusion in the ANOVA of data that presents no quantitative variation results in a pooled variance that is too low and inappropriate for treatment comparisons. Simple visual inspection of such data may be used for decisions, especially if the trends are clear. Thus, means for these variables are shown in graphs and in a table, along with the log transformed means of the variables diameter and length variables, on which ANOVAs and LSD test were applied.

Significance levels (p) of 10% , 5% or 1% for the F test are indicated by one, two or three asterisks, respectively. In the Least significant Difference (LSD) test results, significance levels refer to the least pronounced individual pairwise comparison between means with $p<0.10$ (see comments on significance level in the previous chapter). Some restrictions apply to LSD test (Snedecor and Cochran 1967) (see comments on this multiple comparison test in the previous chapter). However, this test was used for data interpretation, complementing the ANOVA procedure, the use of numerical information from tables, and the visual display of data in graphs.

The standard error for each pollen level mean, calculated after pooling the 20 replications from all 10 blocks, was estimated by $\sqrt{[}$(Block x Pollen Level) Mean Square/20] (Underwood 1997), and used in the LSD test.

For black spruce variables, additional ANOVAs were performed on the log of the total length of each replicate divided by the actual number of germlings/replicate. These ANOVA results are reproduced in Appendix VI, and are cited in the Results section.

3.3 Results

Soil analysis showed that the sandy soil used in my experiments had the following nutrient content: <1 ppm nitrate N, >60 ppm of phosphate P, 45 ppm K, 2 ppm sulphate-S, 303 ppm Ca, 17 ppm Na, 30 ppm Mg, 84 ppm Fe, 0.7 ppm Cu, 7.2 ppm Zn, 0.8 ppm B, 7.8 ppm Mn, and 2 ppm Cl. The soil pH was 5.6, the organic matter content was 1.6%, and the total cation exchange capacity was 8.05 meq/100g.

According to the ANOVA results in Table 3.2, the effect of pollen levels on germling variables of both species was statistically similar in all blocks (non significant PL x B interaction). Block effects were non significant for jack pine (Table 3.2). Block effects observed for black spruce (Table 3.2) were mainly a result of the different number of seeds used in the first five (10 seeds/replicate) and the number of seeds used in the last five (9 seeds/replicate) blocks (see Appendix VI). Pollen level (PL) affected significantly jack pine length variables and the number of seedlings with infested radicles. In black spruce seed germination, pollen level significantly affected the number of germlings with infested radicles and radicle lengths.

The number of jack pine germlings was not considered to be affected by jack pine pollen applied to the soil (Table 3.3). By contrast, the number of germlings with infested radicles, for both species, seemed to get significantly higher at pollen levels 50x and 100x, especially for jack pine. At these levels, one third to two thirds of the jack pine germlings, and 20 to 25% of black spruce germlings had infested radicles (Table 3.3, Figure 3.1a, b).

Hypocotyl length of jack pine germlings was negatively affected by jack pine pollen at 50x and 100x the annual pollen rain (Table 3.3). Based on means from 10 blocks, jack pine hypocotyl length may be significantly reduced by 11 to 13% (30 to 35 mm/replicate) (calculations done on data from Table 3.3 after re-transformation to the original scale) (Figure 3.2a). Considering just those germinated seeds, hypocotyl reduction was from 5 to 8% (1.3 to 2.2 mm/germling) (data shown in Figure 3.2b).

Compared to the 0x radicle mean from 10 blocks, jack pine radicles were reduced by 20 to 37% (57 to 82 mm/replicate) (Figure 3.3a) (calculations done after re-transformation of data on Table 3.3), or 4.6 to 7.7 mm/germling (data shown in Figure 3.4a) at pollen levels 50x and 100x. Radicle reduction for black spruce at 50x and 100x was approximately 9 to

Table 3.2. ANOVA[1] results of fresh jack pine pollen effects on jack pine and black spruce seed germination on mineral soil.

Variable	Factor	DF	Jack pine		Black spruce	
			MS	F	MS	F
Hypocotyl length	Pollen level (PL)	4	0.0139	3.02**	0.00006	0.01
	Block (B)	9	0.0034	0.81	0.01913	3.45***
	PL x B	36	0.0046	1.08	0.00555	1
	Error	50	0.0043		0.00555	
Radicle length	Pollen level (PL)	4	0.1792	27.2***	0.0229	2.55*
	Block (B)	9	0.0061	0.58	0.0107	1.43
	PL x B	36	0.0066	0.63	0.009	1.2
	Error	50	0.0105		0.0075	
Hypocotyl plus radicle length	Pollen level (PL)	4	0.0564	13.1***	0.0015	0.25
	Block (B)	9	0.0032	0.6	0.0137	2.52**
	PL x B	36	0.0043	0.8	0.0058	1.06
	Error	50	0.0054		0.0055	

1 ANOVAs were performed on log transformed data [log(mm/replicate)].
*, **, *** Significance levels of 10%, 5% and 1%, respectively.

Table 3.3. LSD[1] test of fresh jack pine pollen effects on jack pine and black spruce seed germination on mineral soil.

Variable	Species	Level of fresh pollen				
		0x	1x	10x	50x	100x
Number of germlings per replicate 2	Jack pine	9.65	9.25	9.75	9.05	9.15
	Black spruce	8	8.15	8.25	8.25	8.35
Number of germlings with infested radicles per replicate 2	Jack pine	0	0	0.4	3.4	6.6
	Black spruce	0	0.1	0.2	2	2.5
Hypocotyl length	Jack pine	2.434 A p<0.09	2.412 AB	2.425 A	2.382 BC	2.374 C
	Black spruce	2.17 A p>0.10	2.166 A	2.164 A	2.167 A	2.168 A
Radicle length	Jack pine	2.345 A p<0.01	2.343 A	2.35 A	2.215 B	2.144 C
	Black spruce	1.786 AB p<0.07	1.791 AB	1.81 A	1.746 BC	1.728 C
Hypocotyl plus radicle length	Jack pine	2.693 A p<0.01	2.68 A	2.691 A	2.609 B	2.578 B
	Black spruce	2.319 A	2.32 A	2.323 A	2.308 A	2.304 A

1 Least Significant Difference (LSD) test was performed on log transformed means [log(mm/replicate)].
2 No LSD test was performed on this variable. Means are expressed on a replicate total basis.

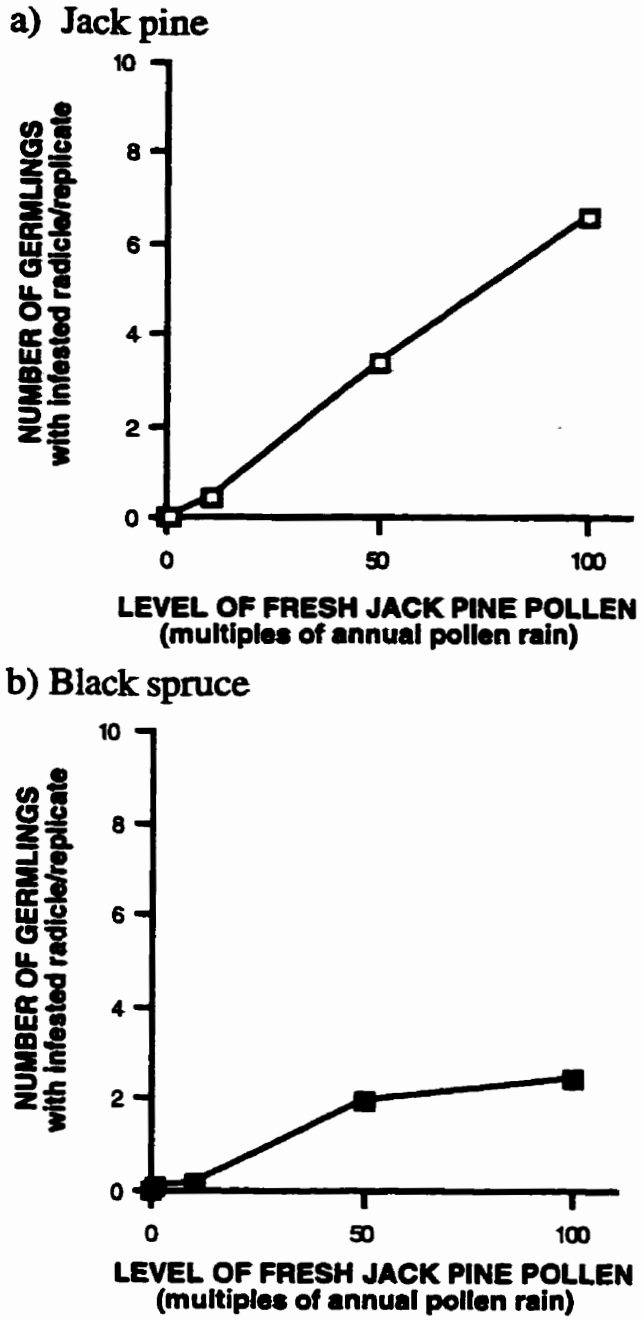
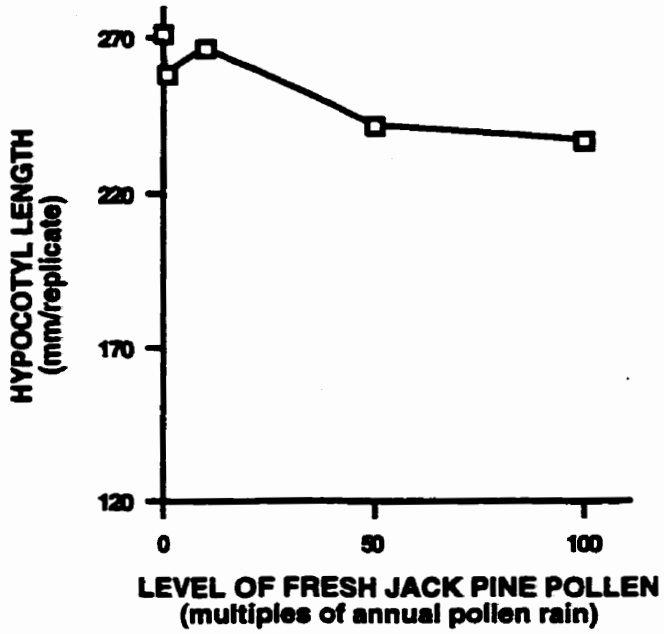


Figure 3.1. Effects of fresh jack pine pollen on the number of jack pine and black spruce germlings with infested radicles per replicate

a) Plot total basis



b) Germinated seed basis

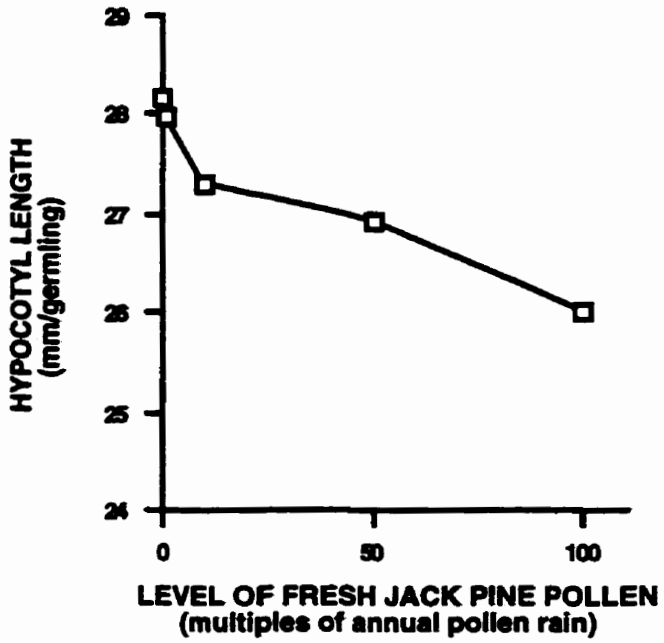
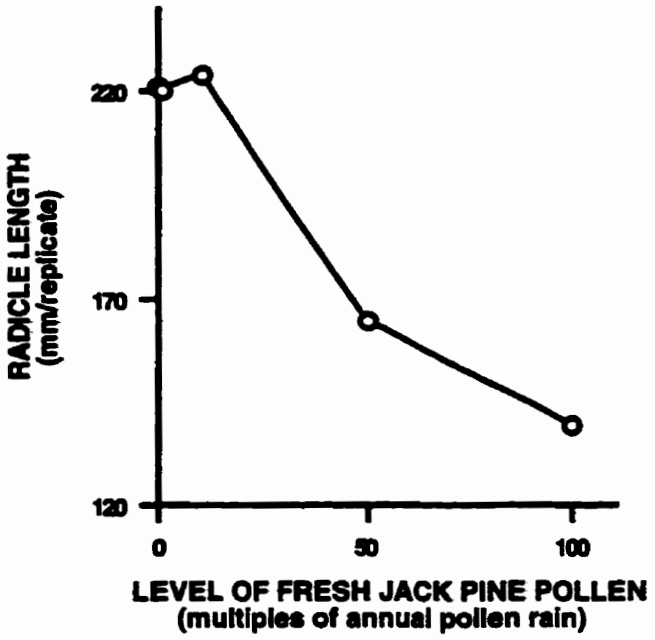


Figure 3.2. Effects of fresh jack pine pollen on hypocotyl lengths of jack pine germlings.

a) Jack pine



b) Black spruce

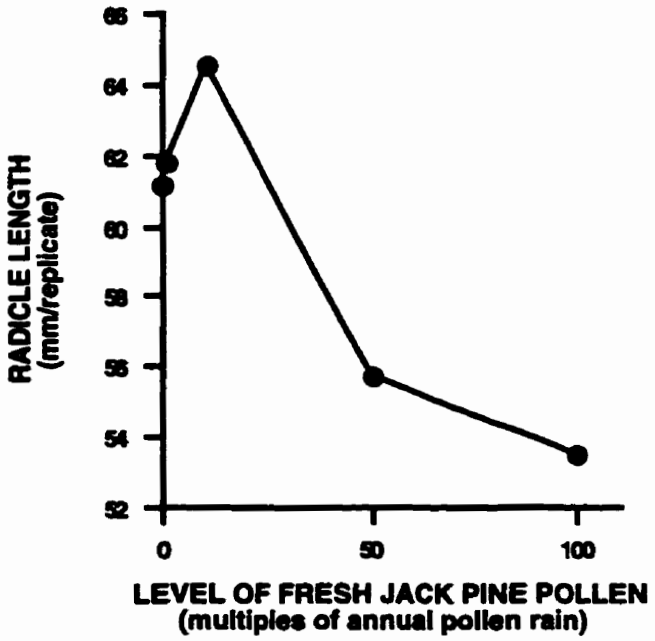


Figure 3.3. Effects of fresh jack pine pollen on radicle lengths of jack pine and black spruce germlings.

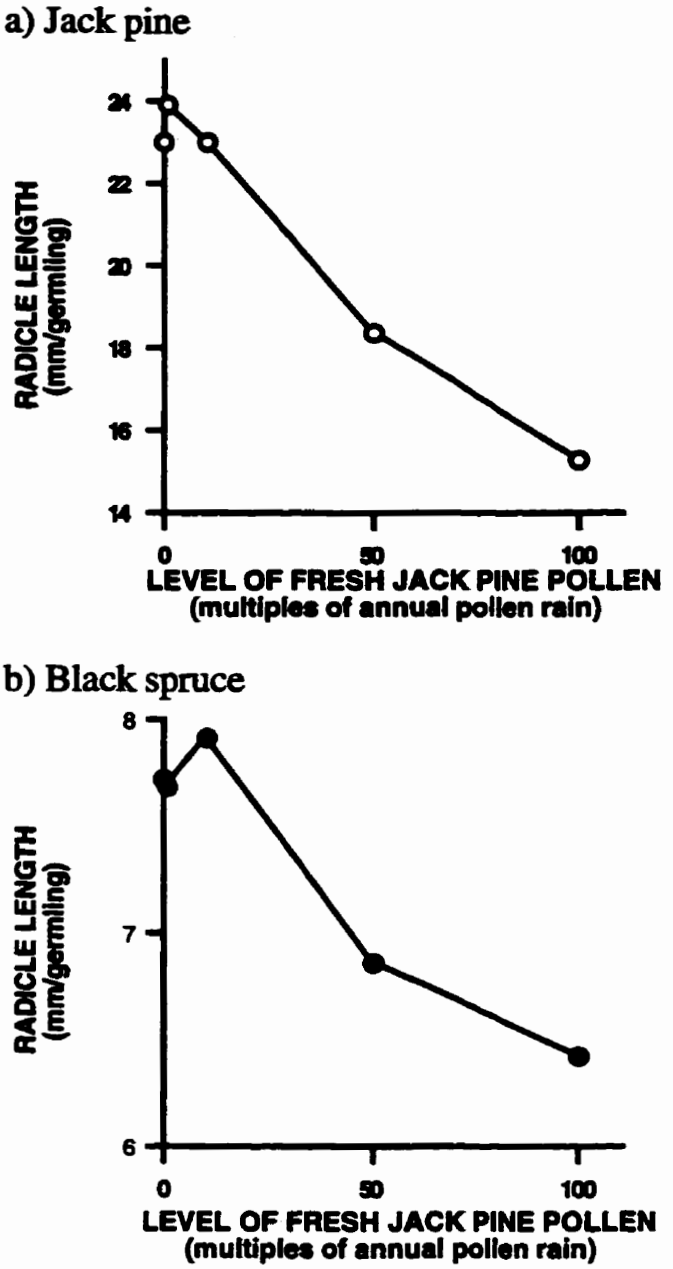


Figure 3.4. Individual jack pine and black spruce radicle lengths from seeds germinated in the presence of various levels of fresh jack pine pollen.

13% (5.4 to 7.6 mm/replicate) (Table 3.3) (Figure 3.3b), or 11 to 17% (0.8 to 1.3 mm/germling) (data shown on Figure 3.4b).

There was a non significant trend of increase in radicle length from pollen level 0x to 1x or 10x for both species. For jack pine, the non significant trend represented 2.5 mm of additional radicle length in each replicate, and 3.5 mm/replicate for black spruce (Table 3.3) (Figures 3.3a and 3.3b). Based on individual germlings the increase was 0.9 mm for jack pine, and 0.2 mm for black spruce (data shown on Figures 3.4a and 3.4b).

Significant reductions in total germling length (*i.e.*, hypocotyl plus radicle) were observed for jack pine germlings at pollen levels 50x and 100x. These reductions were, respectively, 18 and 23% (*i.e.*, 86.7 and 114.7 mm/replicate, respectively) (Table 3.3) (Figure 3.5a). Total black spruce germling length was not affected by increases in pollen levels, although there were non significant increases from pollen level 0x to 1x to 10x (Figure 3.5b).

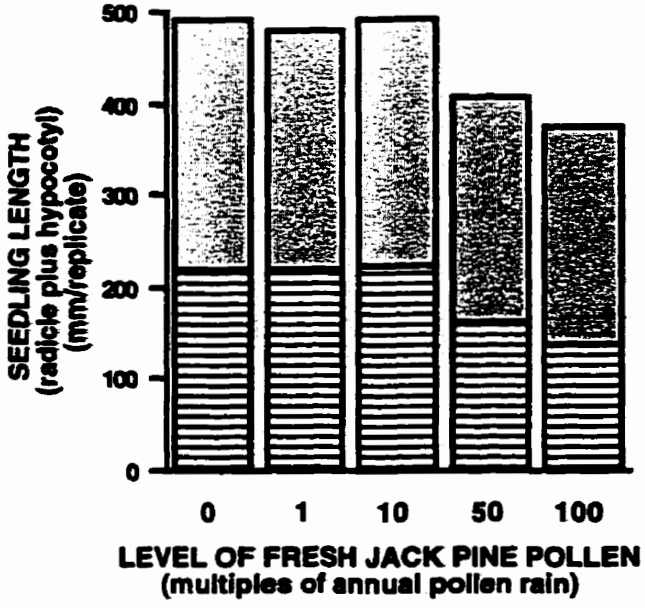
3.4 Discussion

Light, temperature and seedbed moisture are widely known to affect seed germination. Pollen may also play a role in seed germination of some species. Successful jack pine and black spruce seed germination and seedling establishment in scarified soil occur after broadcast seeding at the end of May through to mid June (Brown 1973; Cayford 1961). This coincides with pollen release by many species (*e.g.*, Lee *et al.* 1996a). Jack pine, in particular, contributes half or more of the total pollen rain in late May, in the Deep River, Ontario region (46°0' N, 77°3' W) (Moore and Nozzolillo 1991).

Jack pine pollen is known to negatively affect jack pine and black spruce seed germination, *in vitro* (see Chapter II). This is especially the case for speed of germination and radicle length at one or more times the annual jack pine pollen rain. However, black spruce seeds may benefit from low levels of jack pine pollen, *in vitro*. At 1x and 10x pollen additions under sterile *in vitro* conditions, germination is faster, there are increased number of germlings and longer hypocotyls are produced.

The *in vitro* positive effect of low levels (1x to 10x) of pollen on black spruce hypocotyl length (and other variables) is accompanied by a non significant positive trend on radicle

a)Jack pine total length



b)Black spruce total length

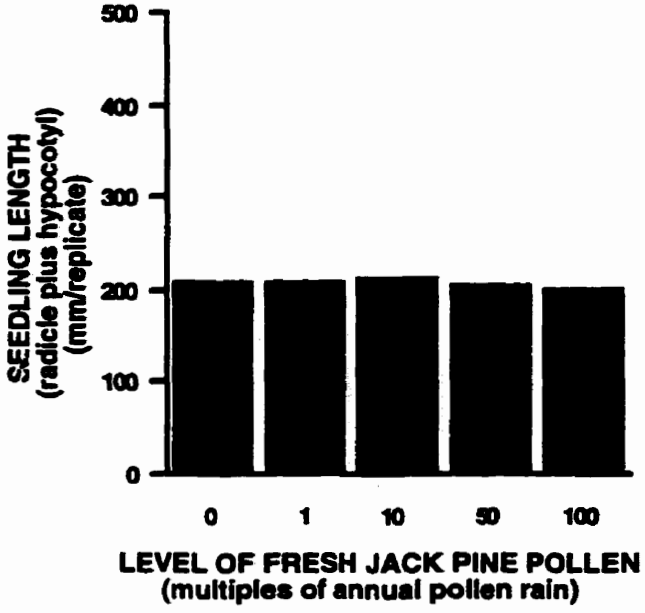


Figure 3.5. Effects of fresh jack pine pollen on hypocotyl (upper portion of the bars) plus radicle (lower portion of the bars) length of jack pine and black spruce germlings.

length of both species when pollen is applied to mineral soil at those same levels (*i.e.*, 1x to 10x) (Figures 3.3, 3.4). Thus, the negative effect of jack pine pollen substances on radicle length observed *in vitro* at all pollen levels differ from effects on radicle length with seed germination on mineral soil. This non significant increase in radicle length may be important for seedling establishment, especially for black spruce, as its characteristic short radicle is considered one of the factors confining this species to permanently moist sites (Thomas and Wein 1985).

On the other hand, the magnitude of jack pine and black spruce radicle reduction by jack pine pollen on soil at 100x normal jack pine pollen rain is comparable to radicle reduction in *Cassia jalapensis* brought about by almost six times more *Zea mays* pollen/soil quantity (Jiménez *et al.* 1983) than jack pine pollen used in my study. Also, radicle infestation of both jack pine and black spruce germlings, at pollen levels 50x and 100x, has the potential to be even more restrictive than the concomitantly occurring radicle length reduction. Also, during data collection most of the germlings with infested radicles could be identified before removal from the substrate by partial wilt of the cotyledons, suggesting impairment of water absorption by the infested radicle (unpublished data). Tissue infestation can prevent, respectively, the establishment of 35-65% and 20-25% of the jack pine and black spruce germlings that germinate on sites where jack pine pollen accumulates.

Enhancement of activity and infectivity of pathogenic fungi by pollen is reported by several authors (Borecka *et al.* 1969; Borecka and Millikan 1973; Chou and Preece 1968; Fokkema 1971; Warren 1976; Williams and Colotelo 1975). Sometimes, pollen can promote fungal infection in the very plant that produced the pollen (Williams and Colotelo 1975). This might explain radicle contamination of both species, as well as the higher level of radicle infestation of jack pine germlings. Perhaps, soil moisture and air humidity were elevated in the experimental set-up, and, therefore, detrimental to the dry site colonizer, *i.e.*, jack pine, as opposed to black spruce, which is known to be adapted to wetter sites (Thomas and Wein 1985).

Apart from the nutrient content of pollen, supposedly of a greater importance at a later stage of seedling development, pollen hormones could be important to seed germination and establishment of seedlings. Based on pollen hormonal concentrations of other pine species, jack pine pollen rain potentially delivers at least 35 mg of auxin/ha (Sweet and Lewis 1971), 268 µg of gibberellins/ha (Kamienska and Pharis 1975) and 8.5 g of kinetin/ha (Sweet and Lewis 1971) annually. In pure, young jack pine stands these

hormone levels might be two to three times higher. However, as no significant effect on seed germination and initial phases of seedling development is observed in up to 10x pollen addition in my study, the soil complex might somehow neutralize the effect of most of the growth active substances in pollen.

Microsite variability in jack pine pollen air-borne deposition observed in a mature mixed forest in west-central Manitoba (Lee 1997) ranges from 3.4 to 18.9 kg/ha.yr, and from 18 to 28.7 kg/ha.yr in a pure regenerating jack pine stand. These figures are within the levels of jack pine pollen (0x to 10x = 0 to 85 kg of pollen/ha) in which no significant negative effect could be observed on seed germination and germling growth. Although most of the forest floor environment receives less than 10x normal pollen rain, and therefore is not restrictive to seed germination and initial phases of seedling establishment, it remains to be determined if jack pine pollen is present in certain microsites at levels much higher than 10x. Also, it should be kept in mind that in a mature, mixed forest, jack pine contributes no less than 50% of the annual pollen rain, and spruce and hardwoods the other half or less (Lee 1997, Lee *et al.* 1996a, b). Studies involving the whole pollen rain mix might lead to development of a model showing cumulative or antagonistic effects of pollen from different tree species on seed germination.

Chapter IV

Effects of jack pine pollen on jack pine (*Pinus banksiana* Lamb.) seedling growth under laboratory conditions

4.1 Introduction

The boreal forest environment receives a thin layer of pollen grains every year (*e.g.*, Brooks 1971; Ho 1991; Moore and Nozzolillo 1991). Among the species with anemophilous pollen, pine species, and jack pine (*Pinus banksiana* Lamb.) in particular in west-central Manitoba, are expected to contribute 50-90% of this pollen rain, estimated to be around 8.5 to 24.5 kg/ha.yr. The annual pine pollen rain from a pure regenerating jack pine stand in west-central Manitoba represents up to 1.3×10^{12} pollen grains/ha (Lee 1997; Lee *et al.* 1996b).

Certainly, pine pollen is conspicuous in the forest environment, and it is efficient in carrying the male gamete into close proximity with its female counterpart. An average of 2-3 pollen grains may be expected to arrive in each ovule of *Pinus sylvestris* L. (Sarvas 1962), *Pinus elliottii* Engelm. (Bramlett 1981), and *Pinus radiata* D. Don. (Lill and Sweet 1977) seed cones. Fertilization of the egg cells in these cones results in seed development in 90% of the viable ovules (Bramlett 1981). It is estimated that serotinous cones of mature jack pine stands contain and store more than four million seeds/ha (Rudolph 1965; Rudolph and Laidly 1990). Such seed set numbers suggest need for less than 20 million pollen grains to produce seeds, leaving most of the pollen grains as an excess available to other components of the boreal forest ecosystem.

Pine pollen is rich in nutrients, especially nitrogen (approximately 2% of N, by weight) (*e.g.*, Lee 1997; Stark 1973; Versfeld and Donald 1991), as well as gibberellins and auxins (*e.g.*, Kamienska and Pharis 1971; Kamienska and Pharis 1975; Sweet and Lewis 1971). Upon deposition, pollen acts on the surrounding environment to enhance microbial activity in the needle fermentation zone (Stark 1973) and on the phylloplane of host plants (*e.g.*, Borecka and Millikan 1973; Fokkema 1971). Stark (1973) observes that *Pinus jeffreyi* Grev. & Balf. pollen enhances fungal activity, resulting in increased nutrient cycling rates in a Nevada temperate pine forest. Aside from the effects of pollen on microbes, it has other roles. For example, *Zea mays* L. pollen is indicated as a means of weed control by Jiménez *et al.* (1983).

Among a number of possible pollen interactions with other organisms (see Appendix I), the direct or indirect action of pollen on the early stages of the growth of forest seedlings is of particular interest in my studies. It is possible that pollen derived processes are occurring in jack pine stands in west-central Manitoba, a region of significant pollen rain, affecting litter accumulation and weed competition. Three common substrate surfaces, excluding bare rock and bog peat, are present in the region (*i.e.*, lichen mat, exposed mineral soil, and needle litter) and pollen is present on, and certainly interactive with them. Greenhouse experiments were undertaken with the objective to determine the effects of jack pine pollen deposited on lichen mat, exposed mineral soil and needle litter substrates on jack pine seedlings during their first growing season.

4.2 Methods and materials

4.2.1 Pollen, mineral soil, lichen mat, and needle litter

Jack pine pollen collection, handling and storage are described in the Methods and materials section of Chapter II.

Mineral soil for each experiment was removed from a single site in the Payuk Lake region (54°39' N, 101°28' W) and transported to the University of Manitoba. The soil was air dried and sifted to remove stones and coarse organic materials before use in laboratory (greenhouse) experiments.

Lichen mat and needle litter monoliths (after Herr and Duchesne 1995), 25 cm wide x 50 cm long x 10 cm deep, were removed intact from the forest floor, placed into plastic trays and transported from the Payuk Lake region to laboratories at the University of Manitoba .

4.2.2 Seed origin and seedling production

Jack pine seeds used for seedling production in 1997 were from the northern region, seed zone 4-6, seed lot 532, collection year 94-95. For the 1998 seedlings, seed identification was 98033403 Pj Repap. In both years, seeds were provided by Manitoba Forestry.

The seedlings used in the experiments were grown at the Pineland Forest Nursery in Hadashville, MB in plastic trays of 200 slots (15/16" square at the top, by 1 1/2" deep each slot) (10 cm^3/slot). The nursery substrate was peat moss, and the seeds were sown on April 15, 1997 and 1998, respectively. Seedlings were kept under greenhouse conditions until transplantation to pots in late June.

4.2.3 Experiment set up and maintenance

Between May 18 and May 25, 1997, 120 pots (25 cm diameter, 19 cm deep) were prepared for seedling transplant. First, each one of the pots were filled with 4.5 liters of sieved soil. A 3 cm thick by 24 cm diameter layer of lichen mat was placed on the surface of 40 of these pots. These pots were considered the first block of the lichen substrate experiment. To another 40 pots, a covering of mineral soil of around 2 cm was added. These constituted the first block of the mineral soil substrate experiment. A 4 cm thick by 24 cm diameter layer of needle litter mat was placed on the remaining 40 pots, and formed the first block of the needle litter substrate experiment. These prepared pots remained undisturbed for over a month in the greenhouse, and were kept moist by watering every other day.

Nine seedlings were transplanted to each pot on June 28, 1997, into holes made previously in the substrate, in a 7 cm x 7 cm grid. Jack pine pollen was added to the pots on July 3, 1997, by mixing pollen at different levels with 100 ml of reverse osmosis water and spreading the slurry on the top layer of the substrate in each pot. Reverse osmosis water only was added to the control pots that received no pollen. Pollen levels were established to represent 0x (0 g pollen/pot), 1x (0.042 g pollen/pot), 10x (0.417 g pollen/pot), 50x (2.086 g pollen/pot), and 100x (4.172 g pollen/pot) the average annual jack pine pollen rain in the Payuk Lake region (8.5 kg/ha.yr) (Lee 1997, Lee *et al.* 1996b). Every pot received an average of 130 ml of reverse osmosis water every 3 days, during 1997.

The experiments were repeated (*i.e.*, the second block of each substrate experiment) in June 1998. In the 1998 experimental run the seedlings were watered with an average of 215 ml of reverse osmosis water every four to five days.

The pots were randomized within each substrate type at the beginning of the experiments, and re-randomized every two to three weeks.

4.2.4 Light and temperature conditions in the greenhouse

No artificial light was applied to the seedlings, and direct sunlight was attenuated by a bamboo screen rolled down over the greenhouse glass roof during most of the time the seedlings were kept in the greenhouse. Mean maximum and minimum temperatures recorded for each month in the greenhouse are shown is Table 4.1.

4.2.5 Seedling harvesting, handling and measurement

Seedlings were harvested on October 20, 1997 (at 157 days age, and 83 days after transplanting), and on October 7, 1998 (at 144 days age, and 76 days after transplanting). On harvesting, soil was washed away from the seedling roots with tap water. Excess water was toweled from the seedlings, and they were stored in paper envelopes at room temperature until measurements were made.

The root collar, characterized as a slight S-shaped protuberance at the junction between radicle and hypocotyl, usually at the point of germination at ground level (Anonymous 1983), was used as the reference point for measurement of seedling diameter and height, and the point where the seedling, just after diameter measurement, was cut into two separate pieces (root and shoot) for biomass determination. Seedling diameter was measured at 50 times magnification. Seedling height (*i.e.*, the length from the root collar to the base of the apical bud) was measured by means of a plastic ruler, and rounded to the nearest millimeter. Shoot length and root collar diameter measurements were completed within a week of harvesting.

After 24 hr in a forced air circulation oven, at 80 °C, seedling root and shoot dry mass were determined gravimetrically using an electronic balance accurate to one thousandth of a gram. Biomass measurements were completed by the middle of November of each year.

4.2.6 Experimental design and data analysis

Greenhouse experiments were conducted in a block design (years as blocks and labeled as B) for each substrate type, with eight replicates/block for each one of the five pollen levels (labeled PL) (0x, 1x, 10x, 50x, and 100x) (raw data sets, means and standard errors are shown in Appendices VII, VIII and IX). These pollen levels cover critical pollen level values, such as control (0x), normal pollen rain (1x), pollen level in which pollen is

Table 4.1. Mean maximum and minimum temperatures recorded for each month in the greenhouse during the period of the experiment.

	1997		1998	
	minimum	maximum	minimum	maximum
June	NA*	NA	11.9 °C	30.0 °C
July	NA	NA	13.4 °C	33.3 °C
August	NA	NA	13.4 °C	33.6 °C
September	8.4 °C	25.0 °C	9.8 °C	30.9 °C
October	10.3 °C**	23.0 °C**	13.1 °C***	26.0 °C***

*NA = Not Available **From Oct. 1 to Oct. 19, 1997 ***From Oct. 1 to Oct. 6, 1998

expected to be optimum to fungal activity in the substrate (10x) (Stark 1972; Stark 1973), and high levels of pollen (50x and 100x). These high pollen levels may not be common in the forest environment, but gives an indication of seedling response in unexpectedly high pollen levels.

DataDesk® 4.1 software (Velleman 1992) was used to perform analyses of variance (ANOVAs) (Snedecor and Cochran 1967; Underwood 1998) on data of each substrate experiment, as shown in Table 4.2. PL was considered a fixed factor, and B a random factor. Mean square of the interaction between PL x B was pooled with the error mean square when the interaction showed a significance level >0.25 (Underwood 1997). Results of the pooled analyses are seen in Appendix X.

Log transformation (base 10) was applied to the seedling diameter, height, shoot dry mass, root dry mass, and shoot plus root dry mass variables (Mead 1988) (see comments on log transformation in the previous chapter). The opposite transformation (*i.e.*, $10^{\log}$) was used on each transformed mean to derive original means. Prior to log transformation, diameter, height and mass were expressed in mm/measured seedling or mg/measured seedling.

Significance levels (p) of 10%, 5%, and 1% in the F test are indicated by one, two or three asterisks, respectively. Significance level in the Least Significant Difference (LSD) test (Snedecor and Cochran 1967) refers to the least pronounced individual pairwise comparison between means with $p<0.10$ (see comments on significance levels in Chapter II). According to these authors, some restrictions apply to LSD test (see comments on this multiple comparison test in the previous chapters). However, this test was used as an additional tool for data interpretation, complementing the ANOVA procedure, the use of numerical information from tables, and the visual display of data in graphs.

The standard error of each pollen level mean, calculated after polling the two blocks together, was estimated by $\sqrt{}$[(pollen Level x Block)Mean Square/16], or by $\sqrt{}$(Pooled Error Mean Square/16) when the Pollen level x Block and Error Mean Squares were pooled together. The standard error of each pollen level mean in either block 1 or block 2 was estimated by $\sqrt{}$(Error Mean Square/8) (Underwood 1997). These estimates were used in the LSD test.

Table 4.2. Designs of the ANOVAs of fresh jack pine pollen effects on jack pine seedlings after the first growing season under greenhouse conditions.

Source of Variation	Degrees of Freedom (DF)	F divisor	F divisor after mean square pooling
Pollen level (PL)	4	PL x B MS*	Pooled Error MS
Block (B)	1	Error MS	Pooled Error MS
PL x B	4	Error MS	
Error	70, or 74 after MS pooling		
Total	79		

*MS= Mean Square

No formal analyses were performed on the number of surviving seedlings/pot, as numerical variation could be observed in only twelve plots, out of the total of 240 pots over the blocks and substrates. Inclusion in the ANOVA of data that present no quantitative variation results in a pooled variance that is too low and not suitable for treatment comparisons (Mead 1988). However, mean values for this variable are available in the Results section together with the transformed means of the variables on which analysis of variance and multiple test comparisons were performed.

4.3 Results

Analysis performed by NorWest Labs indicated the following nutrient contents in the soil used in the experiments: < 1 ppm nitrate N, >60 ppm of phosphate P, 45 ppm K, 2 ppm sulphate S, 303 ppm Ca, 17 ppm Na, 30 ppm Mg, 84 ppm Fe, 0.7 ppm Cu, 7.2 ppm Zn, 0.8 ppm B, 7.8 ppm Mn, and 2 ppm Cl. Soil pH was 5.6, the organic matter content 1.6%, and the total cation exchange capacity 8.05 meq/100g.

Generally, pollen levels significantly affected seedlings growing on lichen and mineral soil substrates (Table 4.3). Over the three substrates, the mean number of surviving seedlings/pot ranged from 8.38 to 9.0, out of nine seedlings transplanted to each pot at the beginning of the growing season (Tables 4.4 to 4.6). Although pollen levels and blocks interacted over all variables in mineral soil substrate, and over height in the lichen substrate, I considered that the general trends were quite similar for all variables in both blocks (Tables 4.4 and 4.5) (*e.g.*, Figure 4.1a, b, c). For this reason, the graphs in Figures 4.2-4.4 were plotted with the 2-block pooled means for each pollen level.

Two-block means for diameter, shoot dry mass and shoot plus root dry mass of seedling grown in lichen substrate with normal (1x) pollen additions were, in general, higher than the means at levels 0x and 10x (Table 4.4) (Figures 4.2 to 4.4). After re-transformation to the original scale, diameter means were (mm/seedling) 1.20 at 0x pollen additions, 1.28 at 1x pollen additions, and 1.25 at 10x pollen additions. Shoot dry mass means were, respectively, 265.5, 306.9 and 289.1 mg/seedling at 0x, 1x and 10x pollen additions, and shoot plus root dry mass means (mg/seedling) were 391.7 at 0x, 438.5 at 1x, and 414.0 at 10x. This represented a 6% increase in diameter, 16% in shoot dry mass, and 12% in

Table 4.3. ANOVA[1] results of fresh jack pine pollen effects on jack pine seedlings after the first growing season under greenhouse conditions.

Variable	Factors	DF	Lichen substrate		Mineral soil substrate		Needle litter substrate	
			MS	F	MS	F	MS	F
Diameter								
	Pollen level (PL)	4	0.00697	11.4**	0.00714	2.87	0.00191	2.56
	Block (B)	1	0.02109	21.6***	0.00198	2.90*	0.06865	48.6***
	PLxB	4	0.00061	0.63	0.00248	3.64***	0.00075	0.53
	Error	70	0.00098		0.00068		0.00141	
Height								
	Pollen level (PL)	4	0.01071	1.15	0.00224	0.46	0.00646	2.24
	Block (B)	1	0.09383	50.8***	0.05367	33.1***	0.39063	138.0***
	PLxB	4	0.00928	5.03***	0.00484	2.99**	0.00288	1.02
	Error	70	0.00185		0.00162		0.00283	
Shoot dry mass								
	Pollen level (PL)	4	0.03727	6.70**	0.0423	6.50**	0.00782	2.04
	Block (B)	1	0.80541	149.3***	0.5273	180.6***	0.12546	12.0***
	PLxB	4	0.00556	1.03	0.00651	2.23*	0.00383	0.36
	Error	70	0.0054		0.00292		0.0105	
Root dry mass								
	Pollen level (PL)	4	0.02051	2.63	0.03882	2.6	0.0064	5.59*
	Block (B)	1	0.00843	1.03	0.02095	7.62***	0.87161	58.6***
	PLxB	4	0.0078	0.95	0.01491	5.42***	0.00115	0.08
	Error	70	0.00819		0.00275		0.01486	
Shoot plus Root dry mass								
	Pollen level (PL)	4	0.03065	5.22*	0.04	5.73*	0.00601	2.11
	Block (B)	1	0.43051	86.8***	0.211	115.6***	0.00118	0.12
	PLxB	4	0.00587	1.18	0.00697	3.82***	0.00284	0.3
	Error	70	0.00496		0.00183		0.00959	

1 ANOVA performed after log transformation of data expressed on a seedling basis [log(mm/seedling) or log(mg/seedling)].
*, **, *** Significance levels of 10%, 5% and 1%, respectively.

Table 4.4. LSD[1] test of fresh jack pine pollen effects on jack pine seedlings after the first growing season on lichen substrate.

Variable	Block	Level of pollen				
		0x	1x	10x	50x	100x
Number of surviving seedlings per replicate [2]	Block 1	9	8.75	9	9	9
	Block 2	9	8.88	9	9	9
Diameter	Block 1	0.058	0.0902	0.0804	0.1058	0.1174
	Block 2	0.1025	0.1207	0.1155	0.1182	0.1573
	Both blocks	0.0803 C p<0.05	0.1054 B	0.098 BC	0.112 B	0.1373 A
Height	Block 1	1.778 C p<0.06	1.801 BC	1.819 B	1.864 A	1.899 A
	Block 2	1.765 A p>0.10	1.77 A	1.754 A	1.753 A	1.776 A
Shoot dry mass	Block 1	2.497	2.608	2.557	2.618	2.65
	Block 2	2.351	2.366	2.365	2.39	2.456
	Both blocks	2.424 C p<0.08	2.487 B	2.461 BC	2.504 AB	2.553 A
Root dry mass [3]	Block 1	2.068	2.142	2.117	2.138	2.178
	Block 2	2.113	2.079	2.058	2.117	2.172
	Both blocks	2.091	2.111	2.087	2.127	2.175
Shoot plus Root dry mass	Block 1	2.637	2.737	2.692	2.744	2.778
	Block 2	2.55	2.548	2.543	2.576	2.638
	Both blocks	2.593 C p<0.08	2.642 BC	2.617 BC	2.66 AB	2.708 A

1 Least Significant Difference (LSD) test applied on log transformed means [log(mm/seedling) or log (mg/seedling)].
2 No LSD test was performed on this variable.
3 No LSD tes was performed on this variable, although F is significant after pooling PL x B and Error MS (see App. X).

Table 4.5. LSD[1] test of fresh jack pine pollen effects on jack pine seedlings after the first growing season on mineral soil substrate.

Variable	Block	Level of pollen				
		0x	1x	10x	50x	100x
Number of surviving seedlings per replicate [2]	Block 1	8.875	8.375	8.875	8.875	8.875
	Block 2	8.875	9	9	9	9
Diameter	Block 1	0.0191 C p<0.06	0.0643 A	0.0343 B	0.0486 AB	0.0445 AB
	Block 2	0.0224 C p<0.06	0.0519 B	0.0219 C	0.0768 A	0.0877 A
Height	Block 1	1.706 C p<0.08	1.745 B	1.744 B	1.744 B	1.781 A
	Block 2	1.694 AB p<0.07	1.713 A	1.689 AB	1.689 AB	1.676 B
Shoot dry mass	Block 1	2.267 C p<0.06	2.351 AB	2.32 B	2.33 B	2.389 A
	Block 2	2.112 C p<0.05	2.128 C	2.14 C	2.195 B	2.269 A
Root dry mass	Block 1	2.191 BC p<0.08	2.174 C	2.226 B	2.178 C	2.273 A
	Block 2	2.106 C p<0.086	2.147 C	2.127 C	2.227 B	2.273 A
Shoot plus Root dry mass	Block 1	2.532 C p<0.06	2.574 B	2.577 B	2.566 BC	2.636 A
	Block 2	2.411 C p<0.01	2.439 C	2.435 C	2.513 B	2.572 A

1 Least Significant Difference (LSD) test applied on log transformed data [log(mm/seedling) or log(mg/seedling)].
2 No LSD test was applied on this variable.

Table 4.6. LSD[1] test of fresh jack pine pollen effects on jack pine seedlings after the first growing season on needle litter substrate.

Variable	Block	Level of pollen				
		0x	1x	10x	50x	100x
Number of surviving seedlings per replicate [2]	Block 1	9	9	8.875	9	9
	Block 2	9	9	9	9	9
Diameter [3]	Block 1	0.113	0.0907	0.0948	0.0919	0.1065
	Block 2	0.1534	0.145	0.152	0.1548	0.1846
	Both blocks	0.1332	0.1179	0.1234	0.1233	0.1455
Height [3]	Block 1	1.957	1.878	1.907	1.889	1.924
	Block 2	1.772	1.758	1.764	1.768	1.795
	Both blocks	1.865	1.818	1.836	1.828	1.859
Shoot dry mass [3]	Block 1	2.575	2.54	2.555	2.561	2.563
	Block 2	2.492	2.453	2.453	2.462	2.537
	Both blocks	2.534	2.497	2.504	2.512	2.55
Root dry mass [3]	Block 1	1.938	1.939	1.942	1.93	1.967
	Block 2	2.142	2.164	2.129	2.131	2.194
	Both blocks	2.04	2.051	2.036	2.03	2.081
Shoot plus Root dry mass [3]	Block 1	2.67	2.64	2.651	2.654	2.663
	Block 2	2.653	2.634	2.623	2.63	2.7
	Both blocks	2.661	2.637	2.637	2.642	2.681

1 No Least Significant Difference (LSD) test was applied on means from the needle litter substrate experiment.

2 Means are expressed in a number of surviving seedlings/pot basis.

3 Means are expressed as log (mm/seedling) or log (mg/seedling).

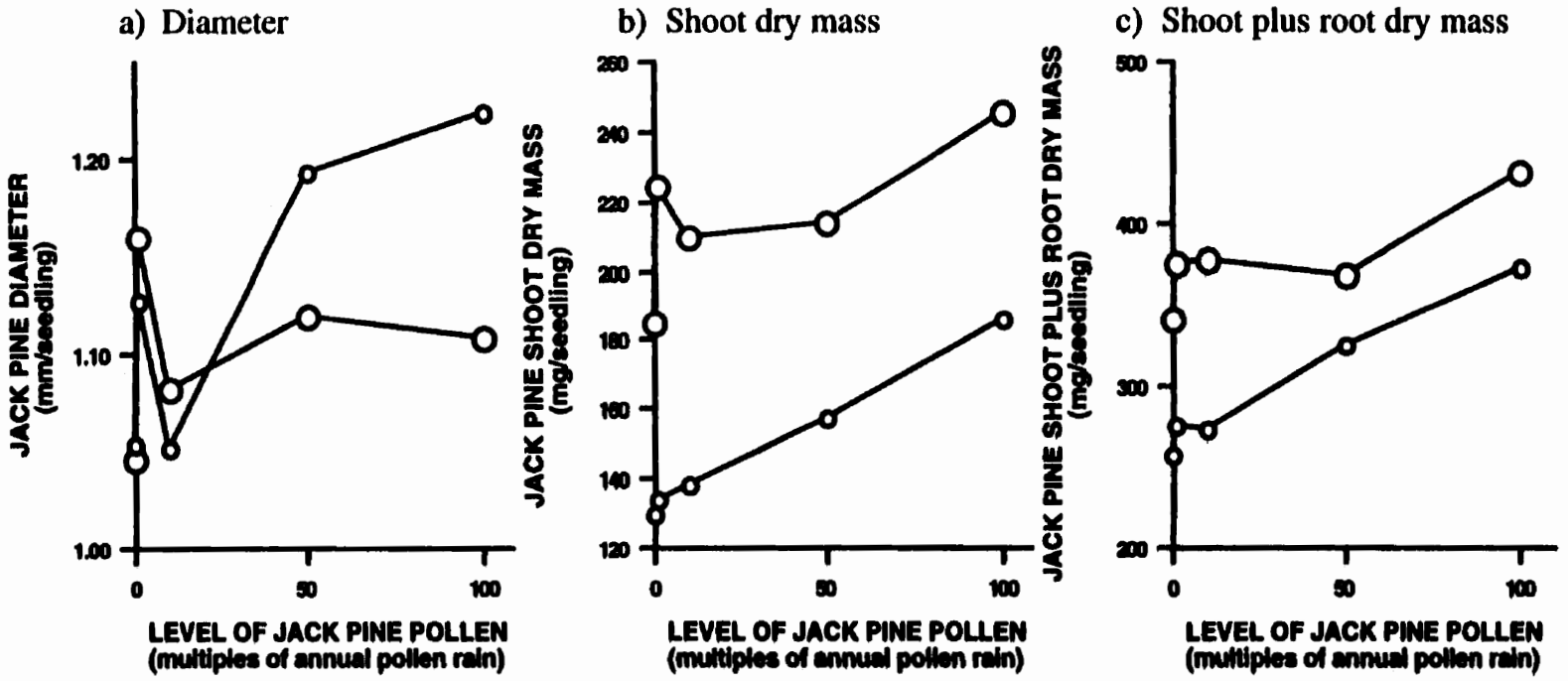


Figure 4.1. Effects of fresh jack pine pollen on diameter, shoot dry mass and shoot plus root dry mass of one-season old jack pine seedlings, in two blocks (circles of different sizes) from the mineral soil substrate experiment.

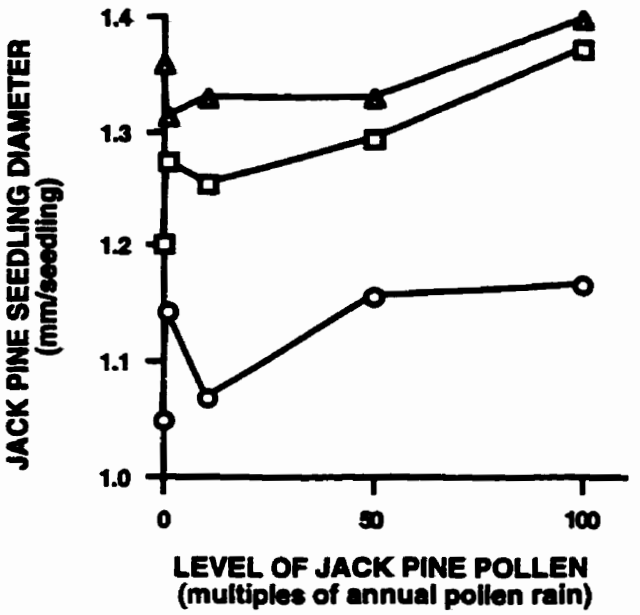


Figure 4.2. Effects of fresh jack pine pollen on diameter of one-season old jack pine seedlings grown on lichen (squares), mineral soil (circles) or needle litter (triangles) substrates.

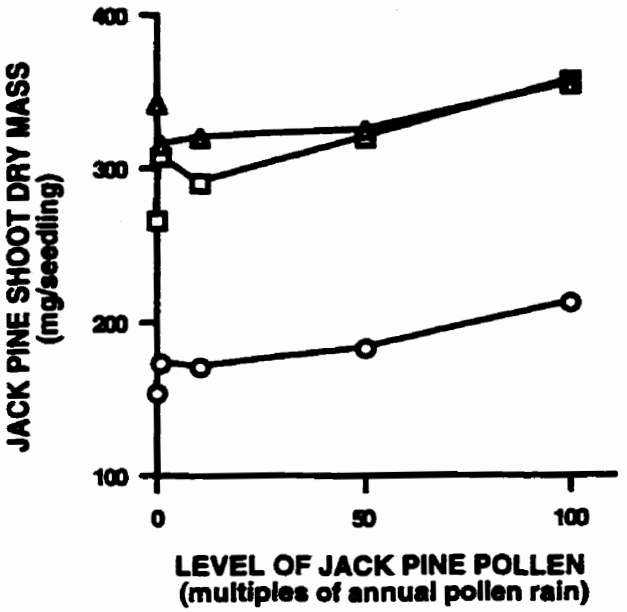


Figure 4.3. Effects of fresh jack pine pollen on shoot dry mass of one-season old jack pine seedlings grown on lichen (squares), mineral soil (circles) or needle litter (triangles) substrates.

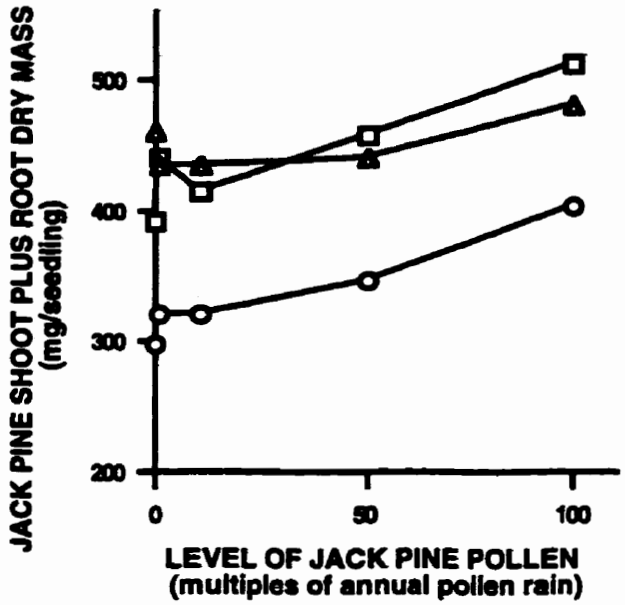


Figure 4.4. Effects of fresh jack pine pollen on shoot plus root dry mass of one-season old jack pine seedlings grown on lichen (squares), mineral soil (circles) or needle litter (triangles) substrates.

shoot plus root dry mass from pollen levels 0x to 1x, and decreases of 2% in diameter, 6% in shoot dry mass, and 6% in shoot plus root dry mass from pollen levels 1x to 10x.

Two-block means for diameter, shoot dry mass and shoot plus root dry mass mean of seedlings grown in lichen substrate (Table 4.4) were higher at 50x and 100x pollen additions than the values observed at 1x normal pollen. After re-transformation to the original scale, diameter means (mm/seedling) were 1.28 at 1x pollen additions, 1.29 at 50x pollen additions, and 1.37 at 100x pollen additions (Figure 4.2). Shoot dry mass means at 1x, 50x and 100x pollen additions were, respectively, 306.9, 319.2 and 357.3 mg/seedling (Figure 4.3), and shoot plus root dry mass means were, respectively, 438.5, 457.1 and 510.5 mg/seedling (Figure 4.4). This represented increases of 7% in diameter, 16% in shoot dry mass and 16% in shoot plus root dry mass from pollen levels 1x to 100x.

Similarly for seedlings grown in mineral soil substrate (Table 4.5), two-block means for diameter (mm/seedling) were 1.05 at 0x pollen additions, 1.14 at 1x pollen additions, and 1.07 at 10x pollen additions (Figure 4.2). For shoot dry mass, means at 0x, 1x and 10x pollen levels were, respectively, 154.5, 173.4 and 169.8 mg/seedling (Figure 4.3), and for shoot plus root dry mass the means were 296.5, 320.6 and 320.6 mg/seedling, respectively (Figure 4.4). This represented increases of, respectively, 9% in diameter, 12% in shoot dry mass and 8% in shoot plus root dry mass, from pollen levels 0x to 1x, and decreases of 7, 2 and 0% respectively, in diameter, shoot dry mass and shoot plus root dry mass, from pollen levels 1x to 10x.

In mineral soil substrate (Table 4.5), two-block means (mm/seedling) for diameter were 1.14 at 1x pollen additions, 1.16 at 50x pollen additions, and 1.16 at 100x pollen additions (Figure 4.2). For shoot dry mass, means at the same pollen levels were, respectively, 173.4, 183.2 and 213.3 mg/seedling (Figure 4.3), and for shoot plus root dry mass the means were, respectively, 320.6, 345.9 and 401.8 mg/seedling (Figure 4.4). This represented increases of 2% in diameter, 23% in shoot dry mass and 25% in shoot plus root dry mass, from pollen levels 1x to 100x.

ANOVA results (Table 4.3) generally showed a non significant effect of pollen levels on seedling growing on needle litter substrate. However, the values of mean diameter, shoot dry mass and shoot plus root dry mass means (Table 4.6) showed a general trend of lower means at pollen levels 1x, 10x and 50x, resulting in an asymmetric U-shaped seedling

response to jack pine pollen. The lowest point for each of the three variables corresponded to means at 1x pollen addition (Figures 4.2-4.4)

4.4 Discussion

The general response of jack pine seedlings to increasing jack pine pollen levels is positive when pollen is added to lichen or mineral soil substrates. Jack pine seedlings respond to various levels of chemical fertilization (Calmé *et al.* 1993), type and chemical characteristics of growth media (Govindaraju 1988), soil temperature (Heninger and White 1974), and to many other factors (Fisher 1979; Langlois *et al.* 1983; Wright *et al.* 1992) (see Table 1.4 in Chapter I). For example, the diameter of jack pine seedlings treated with 9 to 18 mg/seedling of N, P and K, growing in a 2:1 peat-vermiculite substrate, is increased by ~16% by the end of the first growing season, while height is not significantly affected by N, P, K fertilization (Calmé *et al.* 1993). Also, after one-growing-season, jack pine seedlings of four families on four substrates differ in diameter by a maximum of 57% (from a mean diameter of 1.19 mm/seedling in a peat and sand substrate at pH= 6.00 to 1.87 mm/seedling in a peat and perlit substrate at pH= 4.52).

Pine pollen is known to be rich in nutrients (*e.g.*, Lewis *et al.* 1985; Maggs 1985). A gram of jack pine pollen contains 20.1 mg of N, 2.9 mg of P, 8.4 mg of K, and other nutrients as well (Lee 1997; Lee *et al.* 1996b). From the normal pollen rain level (1x) to 100x addition of pollen the amounts of these nutrients range from ~0.09 to 9.30 mg/seedling, for nitrogen; from 0.014 to 1.35 mg/seedling, for phosphorus; and from 0.039 to 3.92 mg/seedling, for potassium. Pollen nitrogen addition at level 100x is on the same order as the basic nitrogen addition used by Calmé *et al.* (1993) in their experiments. Increases in jack pine diameter due to pollen additions (100x) to the lichen or mineral soil substrates, as high as 16% are in the same order of magnitude as the increases in jack pine diameter produced by the levels of N, P, K additions reported by Calmé *et al.* (1993). Diameter differences among the three substrates (up to 34%) from my experiments are also comparable to the 57% difference in jack pine diameter seen over four different substrates by Govindaraju (1988). Increases in seedling total biomass from pollen additions to the lichen or mineral soil substrates, as high as 12% with 1x jack pine pollen level addition (Table 4.4) or as high as 45% with 100x pollen addition (Table 4.5), suggest that pollen may act as an effective a fertilizing agent as N, P, K additions. As nitrogen is one of the most limiting nutrients in soil, nitrogen addition from pollen may contribute to the general

positive response of jack pine seedlings to increasing jack pine pollen levels applied in my study.

Alternatively, and perhaps additionally to the nutritional component of pollen, jack pine seedling growth pattern in lichen and mineral soil resembles a hormonal-like response to substances from pollen at 1x addition. In those substrates, there is a relatively sharp increase in seedling diameter, shoot dry mass and shoot plus root dry mass from no pollen addition (0x) to normal pollen addition (1x), followed by a decrease from pollen level 0x to 10x. Pine pollen is known to be rich in hormones (Ivonis 1969; Kamienska and Pharis 1971; Kamienska and Pharis 1975; Larionova *et al.* 1977; Michalski 1967; Sweet and Lewis 1971). Based on the hormonal composition of pollen of other pine species, 42 mg of jack pine pollen (*i.e.*, the amount of pollen added to the substrate in each pot in my experiments to simulate the normal 1x-annual jack pine pollen rain) might provide 1.3×10^{-3} µg of gibberellic acid-like substances (Kamienska and Pharis 1975), and perhaps up to 1.75×10^{-1} µg of one of the five auxins detected in pine pollen by Sweet and Lewis (1971).

Assuming the pollen effect on seedlings exposed to 1x pollen additions to be a result of hormonal action, it is interesting that seedling growth responses are not negative at higher levels (50x and 100x) in lichen and mineral substrates. Nutritional factors might predominate at higher pollen levels, as nutrient added to the substrates in pollen are sufficiently high to promote jack pine seedling growth (Calmé *et al.* 1993).

Two lichen species, *Cladina stellaris* (Opiz) Brodo and *C. rangiferina* (L.) Nyl., are known to reduce jack pine seedling growth over a period of 17 to 52 weeks (Fisher 1979). Lichen mulch negatively affects jack pine seedling growth in a sand substrate of pH 5.1 and organic carbon content of 1.8%, when compared with growth in a peat mulch washed into the sand medium. Lichen mulch reduces shoot dry mass in 17-week old seedlings to 0.28 mg, as compared to 0.53 mg/seedling in the peat moss mulch control. Reductions in the biomass of the root system can also be observed. *Cladina* ground covers reduce the growth of jack pine seedlings, probably due to impairment of root system development, and reduction in N and P uptake by the seedlings (Fisher 1979).

In my study, lichen substrate did not seem to limit the growth of jack pine seedlings. Seedling means in diameter, shoot dry mass and shot plus root dry mass on the lichen substrate were higher than the equivalent means on mineral substrate. These differences in total seedling dry mass may have been due to the use of lichen mulch removed from the

field (Fisher 1979), versus the use of 'intact' monoliths of soil with a supposedly active lichen mat, in my study.

The pattern of jack pine seedling growth response on needle litter with increasing additions of jack pine pollen is a decrease from pollen level 0x to 1x, a gradual recovery from pollen levels 1x to 50x and the return to previous 0x mean levels at pollen addition 100x. A possible explanation for this seedling growth pattern might involve the immobilization of litter nutrients and the deactivation of pollen substances by the wide range of microbial functional groups present in needle litter (Millar 1974). Also, the high water holding capacity of needle litter that favors microbial activity (Dickinson 1974) and results in generally rapid infestation and breakdown of pollen with immobilization of nutrients, and the duration (one growing season) of my experiment are other factors that may contribute to the decrease in seedling response at pollen level 1x.

Indeed, pollen of several species is known to stimulate fungal growth (Borecka *et al.* 1969; Borecka and Millikan 1973; Chou and Preece 1968; Fokkema 1971; Stark 1972; Stark 1973; Warren 1972a, b; Warren 1976; Williams and Colotelo 1975). Pollen effect on fungi may be nutritional, as suggested by Fokkema (1971) and Stark (1973), or it may also be hormonal as suggested by Borecka *et al.* (1969), Borecka and Millikan (1973) and Warren (1976).

Also, *Pinus jeffreyi* pollen enhances microorganism activity in the litter fermentation zone (Stark 1972; Stark 1973). Microorganisms intercept substances from pollen and temporarily immobilize pollen and litter nutrients during litter decomposition. A build up of available nutrients in the substrate is expected from both enhanced litter decomposition and the decrease in microorganism populations to 'normal' levels at the end of the favorable period of decomposition. However, these extra nutrients are most likely to be available for seedling growth in the following growing season. In my study, seedlings grown in needle litter pots that receive no pollen addition perform initially better than the seedlings grown in needle litter pots receiving pollen. Were my seedlings grown for another year with pollen additions, they may well be expected to grow taller and heavier than the seedlings in pots receiving no pollen additions. This added growth, in part, would result from the pool of nutrients remaining in the soil after the preceding decomposition season.

Thus, the pattern of one-season old jack pine seedling response to jack pine pollen additions on the needle litter substrate, observed in my study, shows the same general

positive response of two-year old *Pinus jeffreyi* seedlings to *P. jeffreyi* pollen additions to needle litter substrate of 0x, 10x and 100x the 3 kg/ha.yr pollen rain for this pine species, reported by Stark (1973). Furthermore, the pattern of seedling growth from my study complements Stark's work. The detected trend of decrease in seedling growth at 1x pollen level, seen in my work, suggests that the microorganism population from needle litter is somehow affected by the normal pollen rain. Differences in the duration of the experiments and the suggested cumulative effect of pollen on litter decomposition and nutrient cycling make it possible to conclude that pollen may have a positive long-term effect on seedling growth in needle litter, as well as an immediate effect on seedling growth on lichen and mineral soil substrates.

Chapter V

Effects of jack pine pollen on jack pine (*Pinus banksiana* Lamb.) seedling growth under field conditions

5.1 Introduction

Pines annually produce millions of pollen grains per hectare. There are an estimated 1.3 x 10^{12} pollen grains/ha produced in young regenerating jack pine (*Pinus banksiana* Lamb.) stands in west-central Manitoba (Lee 1997). It is thought that the production of such large numbers of pollen grains insures seed production by pine species. Indeed, an average of 1.9 to 2.5 pine pollen grains are encountered in each ovule of *Pinus sylvestris* L. (Sarvas 1962), *P. elliottii* Engelm. (Bramlett 1981), or *P. radiata* D. Don. (Lill and Sweet 1977) seed cones. Such numbers of pollen grains in the ovules result in seed development in 90% of the viable ovules (Bramlett 1981).

More than four million seeds/ha (Rudolph 1965; Rudolph and Laidly 1990) may be stored in serotinous cones in a mature jack pine stand. Assuming as 90% pollination efficiency in jack pine from approximately two pollen grains per ovule chamber, less than 30 million pollen grains are required to produce that number of seeds. After the reproductive requirements, there remain more than 1.2 x 10^{12} pollen grains per hectare. Thus, practically all the produced pollen grains with their nutrient contents (*e.g.*, Doskey and Ugoagwu 1992; Lee 1997; Stark 1973) and active growth substances (*e.g.*, Ivonis 1969; Kamienska and Pharis 1975; Sweet and Lewis 1971) are made available to other forest ecosystem processes.

Generally, pollen affects seed production, leaf chlorophyll, growth of germinating seedlings, and stem growth. Free pollen from one species may reduce seed set of other species (Kanchan and Jayachandra 1980; Murphy and Aarssen 1995a, b). Pollen from a weed species, *Parthenium hysterophorus* L., is known to decrease the chlorophyll content of leaves of *Phaseolus vulgaris* L. plants (Kanchan and Jayachandra 1980). *Zea mays* L. pollen, in quantities of 10 to 200 mg per Petri dish, diminishes the growth of radicles and hypocotyls of *Cassia jalapensis* (Britton) Lundell. and *Citrullus lanatus* (Thunb.) Matsumura and Nakai (Jiménez *et al.* 1983; Ortega et al. 1988). Cell elongation and, hence, longer stems are reported (Mitchell and Whitehead 1941) for young *Phaseolus* sp. seedlings treated with *Zea mays* pollen extract.

Seedling growth of various species is also affected by the presence of pollen on the substrate. *Zea mays* pollen mixed into soil (0.7 g in 120 g of soil) reduces *Cassia jalapensis* radicle growth by up to 54% after 20 days of germination in sterilized soil (Jiménez *et al.* 1983). Radicle growth reduction in unsterilized soil with the same level of pollen addition is 28%. Potted *Pinus jeffreyi* Grev. & Balf. seedlings grown for two years in needle litter on mineral soil are taller and heavier with application of *P. jeffreyi* pollen equivalent to 30 to 300 kg/ha (Stark 1972; Stark 1973). Stark suggests that *P. jeffreyi* pollen rain acts indirectly, through enhanced microorganism activity and litter decomposition, to promote seedling and tree growth.

Jack pine pollen seems to be richer in such nutrients as nitrogen than *Pinus jeffreyi* pollen (Lee 1997; Stark 1972; Stark 1973). The annual jack pine pollen rain, in west-central Manitoba, is three to nine times greater than the pollen rain from *P. jeffreyi*, in Nevada. Possibly, the annual jack pine pollen rain has an equal or greater effect on the growth of seedlings of the species and the seedlings of other species than detected for *P. jeffreyi* . Thus, in my study, three experiments were conducted under field conditions, to determine the effect of several levels of pollen on jack pine seedling growth during the first growing season on lichen, mineral soil and needle litter substrates.

5.2 Methods and materials

5.2.1 Site descriptions and pollen

Two experimental sites (A and B) were chosen as representatives of the three general substrates surfaces present in the region (*i.e.*, lichen, mineral soil, and needle litter substrates). Site A was located south of the Twin Lakes access road (Figure 5.1, site A), outside of an AC transmission line right-of-way that cross the study area. The second site (B) was on the north side of the Payuk Lake access road (Figure 1, site B).

Site A was characterized by scattered jack pine individuals, seedlings and adults, and clumps of *Arctostaphylos uva-ursi* (L.) Spreng. in a dense *Cladina* spp. dominated herb layer. The nutrient content in the top 15 cm of the mineral portion in the lichen dominated site was determined to be: <1 ppm nitrate N, >60 ppm of phosphate P, 48 ppm K, 7 ppm sulphate S, 210 ppm Ca, 12 ppm Na, 24 ppm Mg, 80 ppm Fe, 0.6 ppm Cu, 5.7 ppm Zn,

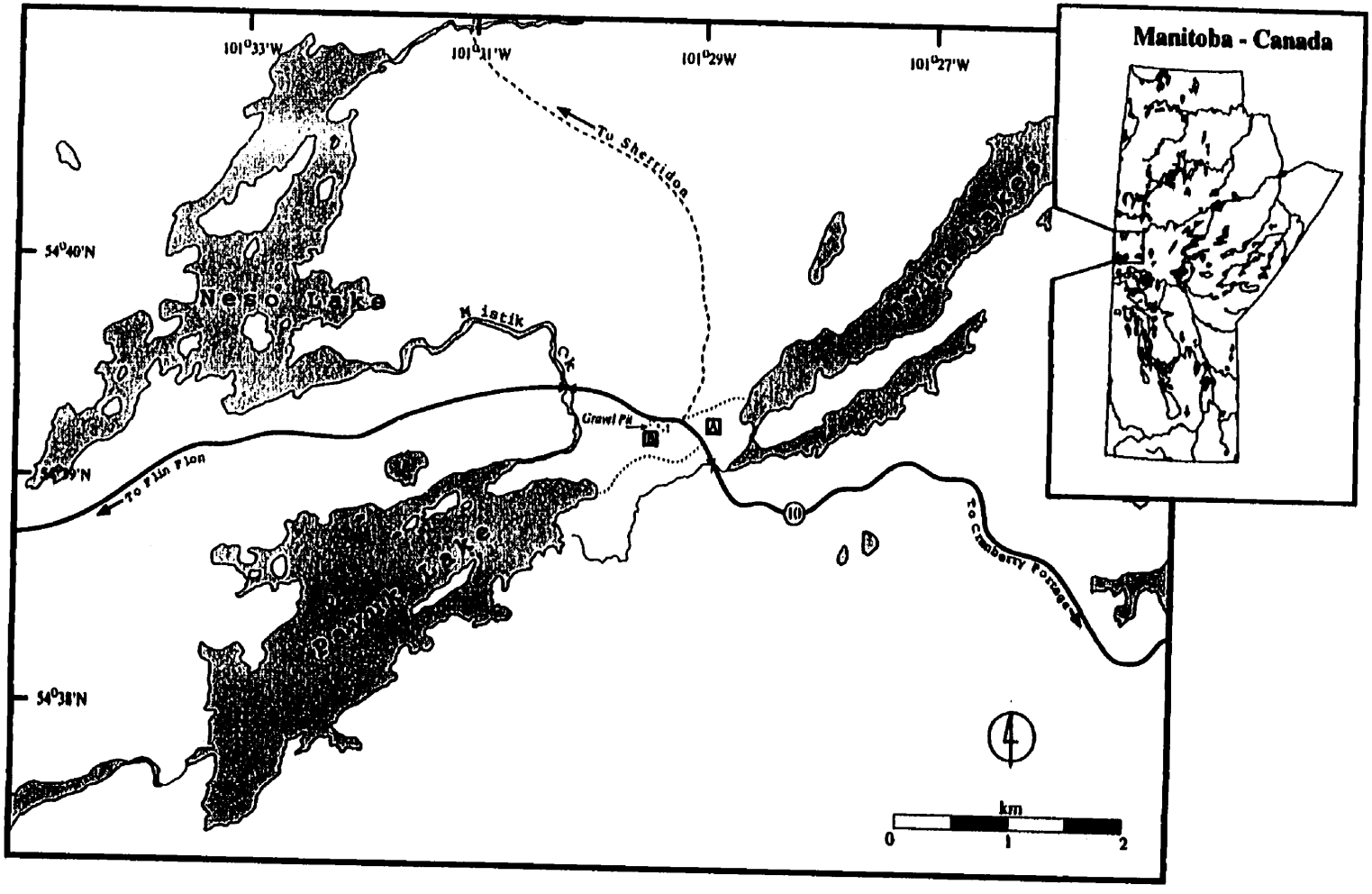


Figure 5.1. Lichen, mineral soil and needle litter substrate study sites A and B.

0.5 ppm B, 4.6 ppm Mn, and 2 ppm Cl. The soil pH was 5.6, the organic matter content was 1.6% and the total exchange capacity was 6.82 meq/100g.

Experimental plots in site B were located at a position 50 m on the south-west side of an abandoned gravel pit. The experimental site and the area around it had been clear cut five years previously. Major woody species in the regenerating clear cut jack pine ridge site included jack pine, *Picea glauca* (Moench.) Voss., *Populus balsamifera* L. Mill. and *P. tremuloides* Michx. *Arctostaphylos uva-ursi* and *Vaccinium vitis-idaea* L. were common as low shrubs. No appreciable living lichen or moss layer was seen in the site, however dead material of these organisms on the surface of the mineral soil was common. Nutrients in the top 15 cm of the mineral portion of the soil included: <1 ppm nitrate N, >60 ppm of phosphate P, 45 ppm K, 2 ppm sulphate S, 303 ppm Ca, 17 ppm Na, 30 ppm Mg, 84 ppm Fe, 0.7 ppm Cu, 7.2 ppm Zn, 0.8 ppm B, 7.8 ppm Mn, and 2 ppm Cl. The soil pH was 5.6, the organic matter content was 1.6%, and the total cation exchange capacity was 8.05 meq/100g.

Jack pine pollen collection, handling and storage are described in the Methods and materials section of Chapter II.

5.2.2 Preparation of experimental plots

Experimental plots were established in an undisturbed lichen mat (site A). No cleaning or perturbation occurred prior to the two seedling plantings in 1997 and 1998. The plots of the lichen substrate experiment were placed where the soil was at least 15 cm deep, by probing the soil depth with a steel nail, prior to the set up of the experiment.

Prior to transplanting seedlings into the gravel pit plots (site B), contiguous areas were cleaned of debris, roots, shrubs and regenerating trees after the period of forest tree anthesis. Boulders were removed when detected during systematic spading of the mineral soil to a 25 cm depth. Subsequent to cleaning and spading, and prior to seedling transplanting, freshly cut monoliths (Herr and Duchesne 1995) of needle litter from the floor of a nearby mature jack pine stand were placed side by side on half of the cleaned area. These plots were considered to be representative of needle litter substrate. The other half of the cleaned area was left bare and taken to represent mineral soil plots. Site cleaning, spading and plot monolith provision were undertaken in each of two years during the course, 1997 and 1998, of my experiments.

5.2.3 Seedling origins and field transplanting

For details on seedling sources and transplanting the reader may wish to consult sections 4.2.2 and 4.2.3, in the previous chapter.

The 1997 plots measured 49 cm by 49 cm, in which 49, 2-month-old seedlings were transplanted to them (June 19 to 22, 1997), on a 7 by 7 seedling grid, spaced 7 cm between and within rows. At the time of transplanting soil moisture from rainfall was deemed sufficient for the seedlings and no watering occurred. The 1998 plots measured 28 cm by 49 cm, in which 28, 2-month-old seedlings were transplanted to them (June 12 to June 14, 1998), on a 7 by 7 seedling grid, spaced 7 cm between and within rows. Due to dry conditions, the 1998 plantings were watered with untreated well water.

The first two blocks (from 1997) of the mineral soil substrate and needle litter substrate field experiments were protected by a garden wire fence. No fence protection was provided to the lichen mat substrate experiment, nor to the last two blocks (1998) in the mineral and needle litter substrate experiments.

5.2.4 Pollen applications

Pollen quantities added as a slurry in 250 ml of well water, to the 49 cm by 49 cm field plots (1997) were 0 mg for plots with no pollen addition, and 2.041 g, 10.204 g, and 20.409 g for plots receiving the equivalent of 10, 50 and 100 times, respectively, the annual jack pine pollen rain. For the 28 cm by 49 cm plots (1998), pollen additions, as a slurry in 500 ml well water, were 0 g, 1.166 g, 5.831 g and 11.662 g for plots supplied with the equivalent of 0, 10, 50 and 100 times, respectively, the annual jack pine pollen rain. Two-week old pollen, with around 6% moisture content, was provided to the seedlings of both blocks in each year, two to five days after seedling transplanting.

5.2.5 Seedling harvesting, storage, and measurement characteristics

Seedlings were harvested in early October, 1997 and late September, 1998. In the 1997 harvest, surviving seedlings from two of the 5 inner rows (always the same inner rows) of each plot were harvested, while in 1998 the surviving seedlings from the two inner rows were collected. Each harvested seedling was washed with water and dried on paper

toweling. Cleaned seedlings were stored at room temperature prior to measurements of seedling diameter, shoot length, shoot biomass, and root biomass. Seedling diameter at the root collar was measured using a dissecting microscope at 50X magnification and an eyepiece micrometer. Seedling height was determined by unaided eye using a plastic ruler with millimeter divisions. Height was taken to run from the root collar to the base of the apical bud. After drying for 24 hr at 80 °C, root and shoot dry masses were determined gravimetrically using an electronic balance (Mettler PE 360 Delta Range) accurate to one thousandth of a gram.

Many seedlings were browsed (*e.g.*, by snow shoe hare), particularly in the unfenced plots of 1998. Browsing occurred in the mineral soil and needle litter substrate experiments and not in the lichen mat experiment. Seedling height and biomass are reduced after grazing and it seemed reasonable to correct each plot value by a multiplication factor f>1. By factoring in the browsed and non-browsed seedlings in each plot, general estimates of the f factor for height and biomass correction, derived from the formula f= mean of the non-browsed seedlings divided by the mean of the browsed seedlings, were possible from the 1998 results on mineral soil and needle litter. (The procedure to estimate the f factor can be exemplified using data from Appendix XII. In the left half of that appendix there are 4 columns headed 'Number of browsed shoots/plot', 'Number of non-browsed shoots/plot', Height of browsed shoots - mm/plot', and 'Height of non-browsed shoots - mm/plot'. The four columns totals are, respectively, $T_1 = 95$, $T_2 = 657$, $T_3 = 2907$, and $T_4 = 30703$. Then, the correction factor for height, considered as the correction value for biomass variables as well, in the mineral soil substrate is estimated by the formula $(T_4 / T_2) / (T_3/T_1) = 1.527$.) This value for mineral soil was approximated to 1.5. Similarly for height and biomass corrections in needle litter, the f value was approximated to 1.3. The approximated f values for diameter are f= 0.95 and f= 0.92, respectively for the mineral soil and needle litter substrate experiments (see footnote in Appendix XII for examples of application of the f correction to plot values).

5.2.6 Experimental design and data analysis

The field experiment on each substrate type was set in a block (B) design (two blocks for each of the two years), with 10 randomized replicates/block for each one of the four pollen level (PL) additions (0x, 10x, 50x, and 100x) (raw data sets, means and standard errors are shown in Appendixes XI, XII, and XIII). The natural pollen rain may be considered and added to the pollen artificially added to plots, resulting in pollen levels of 1x, 11x, 51

and 101x the annual jack pine pollen rain. However, pollen levels are referred throughout this chapter as the actual pollen addition to each plot.

In order to improve the power of the F test for the PL factor, the mean square of the interaction between PL and B was pooled with the error mean square when the interaction showed a significance level $p>0.25$ (Underwood 1997). The data from each substrate (corrected by the f factor in the case of the mineral soil and needle litter substrate experiments) were analyzed separately, according to the ANOVA model (Snedecor and Cochran 1967; Underwood 1997) in Table 5.1, where PL was considered a fixed factor, and B a random factor (the reader may wish to see the uncorrected ANOVAs for the data from the mineral soil and needle litter experiments in Appendix XIV).

No analysis of variance and multiple comparison test were performed on the variable number of surviving seedlings, as the inclusion in the ANOVA of data that present no quantitative variation results in a pooled variance that is too low and not suitable for treatment comparisons (Mead 1988). Log (base 10) transformation was used on diameter, height, shoot dry mass, root dry mass, and shoot plus root dry mass (Mead 1988) before analyses of variance (see comments on the use of log transformation in Chapter III). Prior to log transformation, diameter and height variables were expressed as mm/surviving seedling, and biomass variables were seen as mg/surviving seedling.

Significance levels (p) of 10%, 5% and 1% in the F test are indicated by one, two or three asterisks, respectively. Significance level in the Least Significant Difference (LSD) test (Snedecor and Cochran 1967) refers to the least pronounced individual pairwise comparison between means with $p\leq 0.10$ (see comments on significance level in Chapter II). Some restrictions apply to the LSD test (see comments on this multiple comparison test in the previous chapters). However, this test was used as an additional tool for data interpretation, complementing the ANOVA procedure, the use of numerical information from tables, and the visual display of data in graphs.

The standard error of each pollen level mean calculated over the four blocks considered together was estimated by $\sqrt{[}$(Pollen Level x Block) Mean Square/40], from the regular ANOVA, or by $\sqrt{}$(Pooled Error Mean Square/40), when the Pollen Level x Block and the Error Mean Squares are pooled. If it would be necessary to estimate the standard error of each pollen level mean in each one of the four blocks, it would be estimated by $\sqrt{}$(Error mean Square/10).

Table 5.1. Designs of the ANOVAs of the effects of jack pine pollen levels on the growth of jack pine seedlings, under field conditions.

Source of Variation	Maximum Degrees of Freedom (DF)	F divisor	F after mean square pooling
Pollen level (PL)	3	PL x B MS*	Pooled Error MS
Block (B)	3	Error MS	Pooled Error MS
PL x B	9	Error MS	
Error	144		
Total	159		

*MS= Mean Square

5.3 Results

The mean number of surviving jack pine seedlings/plot was higher on mineral soil than on lichen and needle litter substrates, at any pollen level (Table 5.1). Approximately 90% of the seedlings transplanted to the field in mid June remained alive at the end of the growing season on mineral soil, 80% on lichen substrate, and 70% on needle litter substrate.

ANOVA (Table 5.2) shows that there was no significant PL x B interaction for all variables, except for diameter and root dry mass ($p<0.10$) on lichen substrate. In contrast, there was a significant (generally $p<0.01$) block effect for all variables, except for shoot dry mass on needle litter. On lichen substrate pollen level effects, taken independently of blocks, were not significant for any of the measured seedling growth characteristics. On mineral soil substrate, pollen level effect was significant for seedling height ($p<0.05$), shoot dry mass ($p<0.01$), and shoot plus root dry mass ($p<0.05$). On needle litter substrate, pollen level effect was significant ($p<0.05$) for height and shoot dry mass. However, after pooling PL x B and error mean squares in the cases when PL x B interaction significance level was $p>0.25$ (*i.e.*, all the ANOVAs from mineral soil and needle litter substrates) (Table 5.3), pollen levels significantly affected height, shoot dry mass, root dry mass, and shoot plus root dry mass on mineral soil, while on needle litter substrate pollen level was not significant.

The significant PL x B interactions on lichen substrate were the result of the negative response of jack pine seedling diameter and root dry mass to increasing pollen levels in blocks 1 and 2, which contrasts with the positive response in blocks three and four. After re-transformation to the original scale, diameter and root dry mass means at pollen level 100x were, in general, significantly lower than means at pollen level 0x, in blocks 1 and 2. For example, seedling diameter at pollen level 100x was up to 0.05 mm lower (Figure 5.2a) and root dry mass was up to 14.3 mg lighter (Figure 5.2b) than in pollen level 0x. In contrast, in blocks 3 and 4, diameter and root dry mass means at pollen level 100x were higher than means at pollen level 0x. At pollen level 100x diameter was up to 0.05 mm thicker (Figure 5.2a) and root dry mass was up to 8 mg heavier (Figure 5.2b) than at pollen level 0x. Similar trends were also observed for the other measured seedling characteristics, although to a lesser extent.

Table 5.2. Number of surviving* jack pine seedlings/plot after one growing season on three different substrates.

Substrate	Pollen level				Substrate average
	0x	10x	50x	100x	
Lichen	7.6	7.8	8.2	7.7	7.8
Mineral soil	9	9.3	9.1	9.6	9.2
Needle litter	6.9	7	6.8	6.8	6.9

* surviving seedlings, out of the 10 collected seedlings/plot at the end of the first growing season.

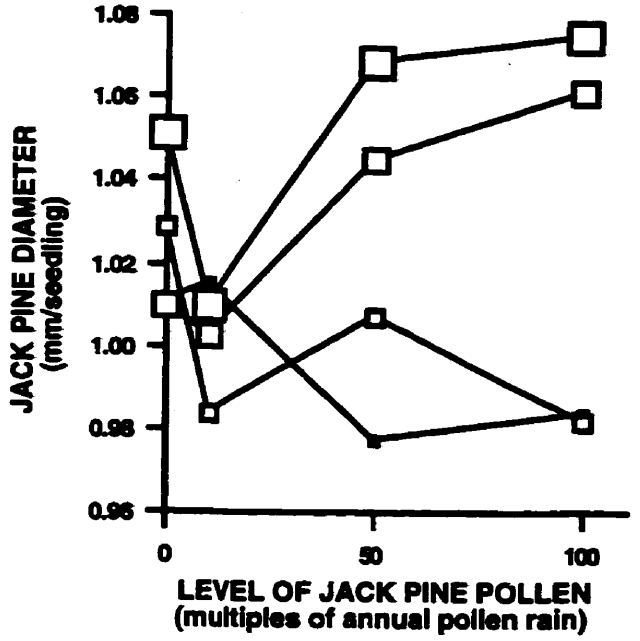
Table 5.3. ANOVA[1] results of fresh jack pine pollen effects on jack pine seedlings after the first growing season under field conditions.

Variable	Factors	DF (2)	Lichen substrate		Mineral soil substrate		Needle litter substrate	
			MS	F	MS	F	MS	F
Diameter	Pollen level (PL)	3	0.000751	0.58	0.003282	1.82	0.002319	1.72
	Block (B)	3	0.004559	6.77***	0.338594	188.4***	0.092443	51.8***
	PL x B	9	0.001304	1.94*	0.001805	1	0.001352	0.76
	Error	144	0.000673		0.001798		0.001784	
Height	Pollen level (PL)	3	0.00173	0.88	0.007602	5.14**	0.00329	3.71*
	Block (B)	3	0.156195	105.9***	0.229737	115.7***	0.125543	38.4***
	PL x B	9	0.001965	1.33	0.001478	0.74	0.000889	0.27
	Error	144	0.001475		0.001986		0.003272	
Shoot dry mass	Pollen level (PL)	3	0.008493	2	0.035538	9.31***	0.023429	3.08*
	Block (B)	3	0.042072	12.8***	0.427993	23.5***	0.051918	1.84
	PL x B	9	0.004246	1.3	0.003815	0.21	0.007616	0.27
	Error	144	0.003278		0.018232		0.028286	
Root dry mass	Pollen level (PL)	3	0.015149	0.23	0.070944	2.63	0.002489	0.17
	Block (B)	3	0.443915	110.6***	2.90543	93.7***	0.108459	8.61***
	PL x B	9	0.006681	1.66*	0.026988	0.87	0.015028	1.19
	Error	144	0.004015		0.031002		0.012595	
Shoot plus root dry mass	Pollen level (PL)	3	0.003858	0.84	0.034144	3.98**	0.010086	0.96
	Block (B)	3	0.168231	56.8***	1.09072	85.8***	0.060595	4.88***
	PL x B	9	0.004613	1.56	0.008585	0.68	0.010532	0.85
	Error	144	0.002962		0.012717		0.012411	

1 Data were corrected by the number of browsed seedlings/plot, log transformed [log(mm/seedling) or log(mg/seedling)], and analyzed.

2 There were three 'missing' plots in the lichen substrate experiment, and the actual Error DF for the lichen substrate experiment is141.

a) Jack pine diameter



b) Jack pine root dry mass

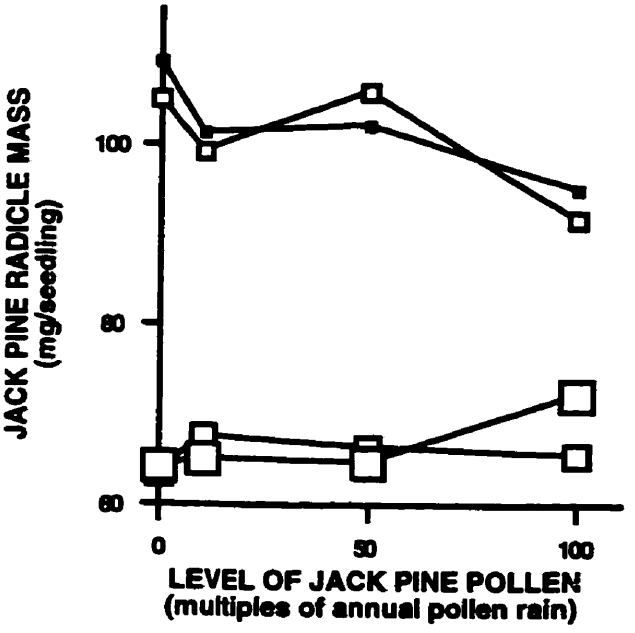


Figure 5.2. Effects of jack pine pollen on jack pine seedling diameter and root dry mass after one growing season in different blocks (different symbol sizes) of the lichen substrate experiment.

On mineral soil substrate, height, shoot dry mass, root dry mass, and shoot plus root dry mass means at pollen level 0x were significantly lower than means at pollen levels 50x, 100x, and, specifically for root dry mass, it was also lower than the mean at pollen level 10x (Table 5.4 and Table 5.5). Mean height increased from 38.7 to 39.7 to 41.1 to 41.5 mm/seedling, respectively for pollen levels 0x, 10x, 50x and 100x (Figure 5.3a). Shoot dry mass averaged 175.8, 182.0, 197.7 and 204.2 mg/seedling, respectively at pollen levels 0x, 10x, 50x and 100x. Root dry mass 0x mean was 118.3 mg/seedling. This mean was significantly ($p<0.06$) lower than the mg/seedling means 141.6 at 10x pollen level, 146.2 at 50x, and 142.6 at 100x (Figure 5.3c). Shoot plus root dry mass increased from 309.0 mg/seedling at 0x to 331.9 at 10x to 354.3 at 50x to 357.3 at 100x (Figure 5.3d). For this seedling characteristic these responses represented a non significant increase in seedling biomass by approximately 7% from pollen levels 0x to 10x, and significant increases by approximately 15% from the 0x pollen level compared with shoot plus root dry mass at the 50x or 100x pollen levels.

Although non significant, as indicated by the ANOVA (Table 5.3), on needle litter there was a trend of positive pollen effect on seedling variables, in general.

5.4 Discussion

In my study, response of jack pine seedlings to pollen additions may be directly related to nutrients derived from pollen, as the soils of the experimental sites are deficient in nitrogen. Considering the jack pine pollen nutrient composition (Lee 1997) (Table 1.1, Chapter I), pollen additions at levels 50 or 100x the annual jack pine pollen rain could provide approximately 4 to 8 mg nitrogen/seedling. Nitrogen available in jack pine pollen rain is at about the same level as nitrogen levels shown to produce a positive growth response in jack pine seedling diameter previously (Calmé *et al.* 1993).

As seen in my study, pollen effects on seedling characteristics on lichen substrate differ from blocks 1 and 2 (1997) to blocks 3 and 4 (1998). Pollen effects are, in general, negative in 1997 and positive in 1998. Probably, many factors are acting simultaneously in the lichen substrate. For example, shrub and tree roots were left undisturbed at the time of seedling transplant. Perhaps, these undisturbed roots differentially take up nutrient from pollen and introduce a source of variation in the growth characteristics data.

Table 5.4. Pooled ANOVA[1] results of fresh jack pine pollen effects on jack pine seedlings after the first growing season under field conditions.

Variable	Factors	DF	Mineral soil substrate		Needle litter substrate	
			MS	F	MS	F
Diameter	Pollen level (PL)	3	0.003282	1.83	0.002319	1.32
	Block (B)	3	0.338594	188.3***	0.092443	52.6***
	PL x B	pooled	pooled		pooled	
	Error	153	0.001798		0.001759	
Height	Pollen level (PL)	3	0.007602	3.89**	0.003296	1.05
	Block (B)	3	0.229737	117.4***	0.125543	40.1***
	PL x B	pooled	pooled		pooled	
	Error	153	0.001956		0.003132	
Shoot dry mass	Pollen level (PL)	3	0.035538	2.04*	0.023429	0.87
	Block (B)	3	0.427993	24.6***	0.051918	1.92
	PL x B	pooled	pooled		pooled	
	Error	153	0.017384		0.02707	
Root dry mass	Pollen level (PL)	3	0.070944	2.31*	0.002489	0.2
	Block (B)	3	2.90543	94.4***	0.10845	8.51***
	PL x B	pooled	pooled		pooled	
	Error	153	0.030766		0.012738	
Shoot plus root dry mass	Pollen level (PL)	3	0.034144	2.74**	0.010086	0.82
	Block (B)	3	1.09072	87.4***	0.060595	4.93***
	PL x B	pooled	pooled		pooled	
	Error	153	0.012474		0.0123	

1 Date were corrected by the number of browsed seedlings/plot, and log transformed [log(mm/seedling) or log(mg/seedling)] prior to analyses.

Table 5.5. LSD[1] test, corrected for the number of browsed seedlings/plot, of fresh jack pine pollen effects on jack pine seedlings after the first growing season on mineral soil substrate.

Variable	Block	Pollen level			
		0x	10x	50x	100x
Number of surviving seedlings/plot [2]	4-block means	9	9.3	9.1	9.6
Diameter [3]	4-block means	0.1179	0.1232	0.1393	0.1264
Height	4-block means	1.588 C p<0.06	1.599 BC	1.614 AB	1.618 A
Shoot dry mass	4-block means	2.246 C p<0.10	2.26 BC	2.296 AB	2.31 A
Root dry mass	4-block means	2.073 B p<0.06	2.151 A	2.165 A	2.154 A
Shoot plus root dry mass	4-block means	2.49 B p<0.02	2.521 AB	2.55 A	2.553 A

1 Least Significant Difference (LSD) test was performed on means expressed on a log tranformed scale [log (mm/seedling[or log (mg/seedling)].

2 No LSD tes was performed, and the means are expressed in a number of surviving seedlings/plot.

3 No LSD was performed, as no significance was detected by the analysis of variance.

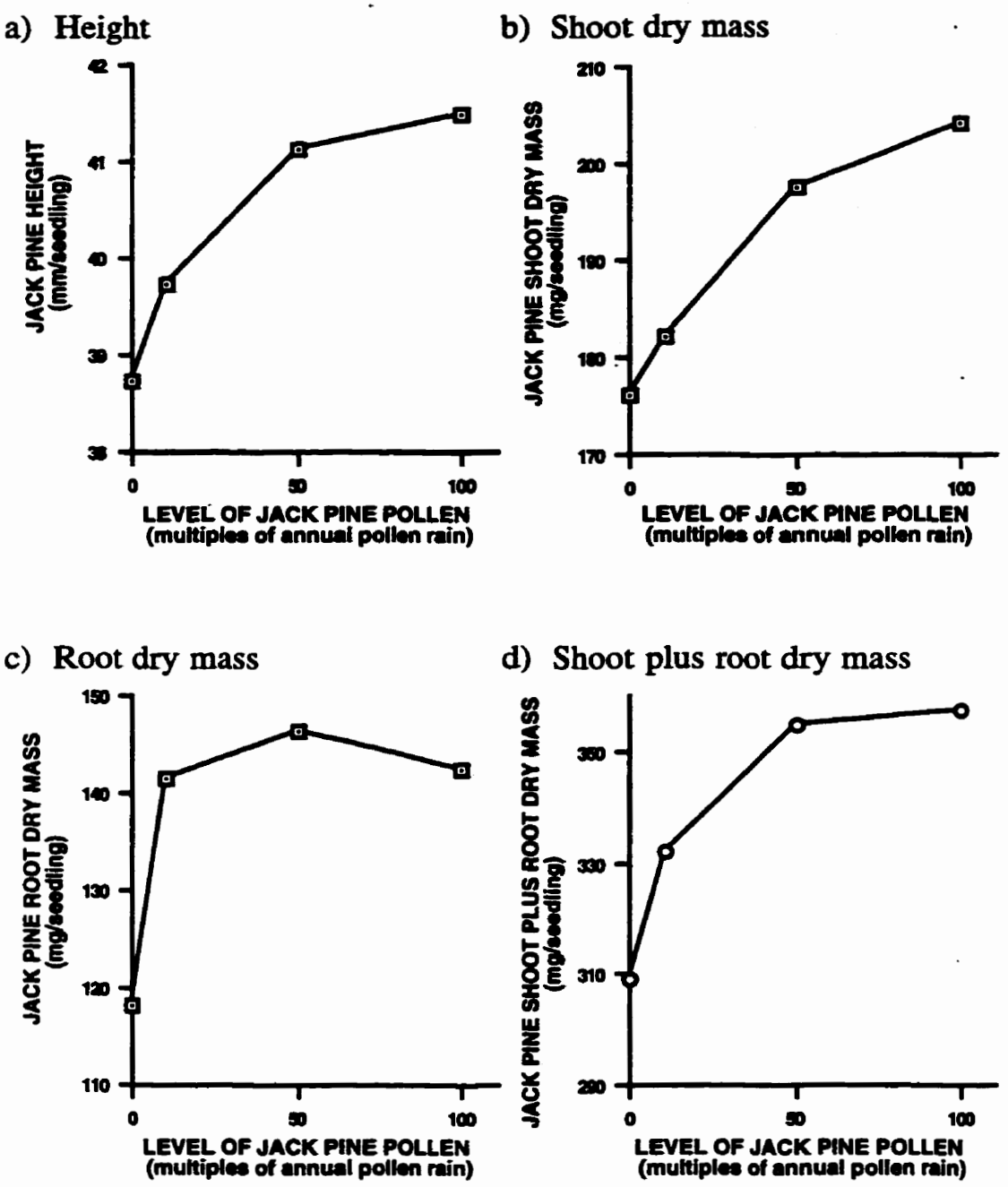


Figure 5.3. Effects of jack pine pollen on jack pine seedling height, and seedling dry masses of shoot, root, and shoot plus root, after one growing season, on mineral soil substrate.

Before planting seedlings on mineral soil the substrate was perturbed, and the natural heterogeneity in the soil environment was reduced. Observed increases of seedling height, shoot dry mass, root dry mass and shoot plus root dry mass means from pollen levels 0x to 10x on mineral soil (and on needle litter to a lesser extent) are higher than increases in means from pollen levels 10x to 50x and 100x. These trends conform with generally accepted models of plant response to fertilizer applications (Colwell 1994; Cook 1982).

Positive seedling growth response to pollen was also reported by Stark (1973). Two-year old potted *Pinus jeffreyi* seedlings get taller and heavier with the addition of increasing *P. jeffreyi* pollen levels equivalent to 10x or 100x the annual Jeffrey pine pollen rain of 0.3 kg/ha (30 or 300 kg/ha.yr). At levels up to 10x, pollen enhances the activity of microorganisms in the needle litter fermentation zone, and seedling growth through increase in the rates of litter decomposition and nutrient cycling. Thus, increases observed in seedling parameters at levels higher than pollen levels equivalent to 10x the annual pollen rain in my study and in Stark's (1973) work are, perhaps, partly due to pollen nutrients from pollen made available to, and directly utilized by, seedlings.

The inclusion of a true control 0x fresh pollen level in this kind of field experiment should be included in future work in order to check the effect of the lack of fresh pollen on seedling growth. Greenhouse experiments (Chapter IV of this dissertation) and field results suggest that annual jack pine pollen rain has the potential to significantly benefit the growth of jack pine seedlings in the field. Certainly this is the case on mineral soils.

Chapter VI

Discussion

6.1 Summary statements and conclusions

Annual pine pollen rain has the potential to affect forest development through various stages of tree seed germination through to tree maturity. *In vitro* results in my study suggest, in general, that pollen prolongs the time required for seed germination and reduces radicle length of jack pine and black spruce seedlings by at least 20% with pollen applications equal to the annual jack pine pollen rain. However, when seeds germinate on mineral soil, the negative effect of jack pine pollen is lessened and no significant negative effect is observed on germinating seed characteristics with pollen applications up to 10x the annual jack pine pollen rain. Perhaps, at 0x to 10x additions, compounds and nutrients from pollen on soil are modified and temporarily immobilized by soil organisms, resulting in diminished negative pollen effect on germinating seeds.

Positive *in vitro* response to pollen is shown by black spruce speed of germination and germling hypocotyl length after treatment with pollen sterilized in propylene oxide. This result may be interpreted in at least two ways: 1) there were fewer microorganisms to directly interfere with seed germination or deactivate seed germination promoting substances available from pollen; and 2) the presence of propylene oxide residue in sterilized pollen (fresh pollen or pollen remains) somehow promotes speed of germination and hypocotyl growth. The chemical composition of propylene oxide is similar to propylene, which is known to be an ethylene analog and a weak promoter of seed germination. This fact and other evidence from the literature, including the knowledge of an ethylene precursor in pollen of various species and the formation of ethylene in organic soils of spruce forests, indicates the necessity of further study of the role of pollen in controlling the distribution of jack pine and black spruce in the natural environment.

Slight positive response of jack pine and black spruce seeds germinating on soil exposed to jack pine pollen levels up to 10x the annual pollen rain mirrors positive *in vitro* responses to pollen additions observed for some black spruce germling characteristics. Positive germination response on soil may be of ecological importance (*e.g.*, slight increase in radicle length), especially if concomitant with other significant positive effects in advancing stages of seedling growth.

Natural microsite variation in jack pine pollen deposition ranges from 3.4 to 28.8 kg/ha.yr (Lee 1997). Levels at which jack pine pollen is detrimental to jack pine and black spruce seed germination and germling growth on soil in my studies are higher than 10x the average annual jack pine pollen rain (the equivalent of 85 kg/ha.yr). Thus, with natural microsite variation in jack pine pollen deposition below the 10x pollen level it is possible to conclude that in most sites of my study region pollen has no detrimental effect on the seed and germling characteristics which were studied. However, further studies in pollen relocation by wind and water movements, as well as pollen deposition on open areas, are necessary in order to determine if pollen concentrations at the microsite level may exceed the 10x pollen level and, therefore, affect seed germination and the early stages of seedling establishment in the boreal forest. For example, points in open sites with exposed mineral soil, where no pollen is intercepted by branches or other plant parts, may be subject to fresh pollen concentrations of 50x or more annual pollen rain. The 15-30% radicle reduction due to levels of jack pine pollen exceeding 10x annual pollen rain is also associated with radicle infestation by microorganisms in up to 65% of the seedlings. Radicle infestation probably limits water uptake by developing seedlings, resulting in reduced survival and, therefore, seedling implantation.

It is already known that nutrient additions, genetics and light are important factors for jack pine seedling development. However, the inclusion of pollen among factors of such importance is something not often encountered in the literature. For example, the jack pine pollen component equivalent to annual pollen rain in mixed mature boreal forests may significantly increase total seedling dry mass by 10-12% in lichen (Tables 4.2) and in mineral soil (Table 4.3), under greenhouse conditions. Considering additions of 100x the annual jack pine pollen rain to lichen or mineral soil substrates, seedling biomass is increased by 27-45% compared to biomass means at 0x. These increases are comparable to the effects of heavy nitrogen fertilization on adult jack pine trees (36% wood volume increase) (Weetman *et al.* 1995), or to light source effects observed on jack pine seedling height (up to 25%) and shoot dry mass (19 to 85%) (Roberts and Zavitkovski 1981).

Further studies on jack pine pollen, as well as total pollen rain (from pine, spruce and other trees) should be carried out in order to determine further effects of pollen rain on seedling growth and forest development. Certainly, the potential role of pollen to stimulate or inhibit germling, seedling and juvenile tree growth requires further study.

6.2 Laboratory and field results compared

Except for the two blocks in the lichen experiment, the field results show the same general positive trends observed in greenhouse conditions. Considering that pollen added to the plots in the field experiments is supplemented with the natural pollen rain, then the actual pollen level in each field plot is 1x, 11x, 51x and 101x. Seedling response in the field from pollen levels 1x to 11x to 51 to 101x in needle litter substrate resembles response to equivalent pollen levels observed for seedlings on needle litter in the greenhouse (see Appendix XIV).

On mineral soils in the field, no decrease in seedling measurements is observed from level 1x to 11x, as generally occurred on mineral soil in the greenhouse from pollen 1x to 10x. This difference may be a result of field site treatments prior to the planting of seedlings. In the spading of the mineral soil and the removal of organic debris the pollen naturally deposited on the ground during pollen rain was buried in a lower soil layer than that occupied by the root system of the transplanted seedlings. If true, pollen levels in the field plots might be considered as 0x, 10x, 50x, and 100x. With this supposition, seedling responses in mineral soil substrate from pollen levels 0x to 10x, to 50x, to 100x may be considered very similar under both field and greenhouse conditions (see Appendix XIV).

6.3 Recommendations for future study

The potential effect of jack pine pollen to act in forest development, such as the 12% increase observed in jack pine seedling dry mass and the enhancement of black spruce seed germination at normal seasonal pollen levels, deserves further analysis and study. Also, some of the general areas listed below might be considered worthy of further research in the future:

1) pattern of initial and final pollen deposition on the forest floor and on the surface of exposed sites;

2) hormone content and nature in jack pine pollen;

3) effect of pollen on soil organisms;

4) effect of propylene oxide, propylene and ethylene on jack pine and black spruce seed germination;

5) effect of the pollen mix of pine, spruce and other trees on seed germination and seedling growth; and

6) long term studies on the cumulative effect of the pollen rain on seedling growth and forest development.

Specifically, it might be interesting to consider:

1) effects of jack pine pollen on *Picea glauca* (Moench) Voss (white spruce) seed germination, germling growth and seedling development; and

2) effects of the pollen of various boreal plant species on jack pine seed germination, germling growth and seedling development.

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APPENDIX I

Additional information from the literature

ADDITIONAL INFORMATION FROM THE LITERATURE

1 Pollen and its products in the environment

1.1 Pollen interactions with fungi and other organisms

Increased levels of N and P in the litter fermentation zone of temperate forests are associated with the annual pollen rain. In a *Pinus jeffreyi* Grev. & Balf. forest in Nevada, the annual pollen rain totaled 0.6 to 3.0 kg/ha. These amounts were considered too low to be of direct importance to tree growth. However, pine pollen nutrients (see Tables 1.1 to 1.3) may be essential to the litter fermentation zone decomposition that occurs during the summer in temperate forests (Stark 1972; Stark 1973). The rate of mass loss of fresh needle litter is greater, and the number of hyphae in the litter are generally higher, when pollen (equivalent to 30 kg/ha) is present.

In wet substrates, chytrids are known to perforate pollen walls and feed on the inner content of pollen grains (Goldstein 1960; Stark 1972; Stark 1973). Pine pollen grains are an especially favored substrata for these zoosporic fungi (Goldstein 1960). Perforation and inner content consumption of pollen grains occur within 30 to 60 days (Goldstein 1960; Stark 1973). Pine pollen is known to support a number of lignicolous fungi (Hutchison and Barron 1997). Pollen grains are in the diet of some soil microorganisms, such as certain species of Colembola (Harding and Stuttart 1974) and earthworms (Walch *et al.* 1970). Some rock dwelling oribatids were found to feed almost exclusively on *Alnus* pollen in the spring (Harding and Stuttard 1974).

Pollen from several species stimulates fungal growth and infectivity by a factor of up to 20 (Chou and Preece 1968; Warren 1972a, b; Williams and Colotelo 1975). For example, pollen from several species influences germination of conidia of a fungus pathogen of *Secale cereale* L. It was shown that pollen from host and non-host species stimulates germination, but the greatest effect was always caused by pollen from the host species. Nutrients (Fokkema 1971), and hormones, such as abscisic acid present in strawberry pollen, or other substance present in gladiolus and bent-grass pollen (Borecka *et al.* 1969; Borecka and Millikan 1973; Warren 1976) may stimulate fungal growth and infectivity.

1.2 Pollen degradation and decomposition

Fungi are the main agent of pollen degradation. Several fungal species digest the pollen wall, and perforate tiny holes through it. The fungi gets nourishment from the inner content of the pollen grain and leave behind an empty and chemically resistant shell, composed basically by sporopollenin. In *Taxus baccata* L. pollen grains, after the assimilation by fungi of the substances easily absorbed, activity diminishes and fungal hyphae are subsequently destroyed by bacterial attack (Rohr and Kilbertus 1977). The same process occurs with pine pollen grains (Goldstein 1960; Stark 1972; Stark 1973).

Pollen wall decomposition begins with its oxidation. Oxidation may occur when pollen is exposure to the air (autoxidation), to some biochemical oxidative material produced by microorganisms, and especially to fire (Havinga 1964, 1967).

Ultrasound may have a destructive mechanical effect on pollen grains. Frequencies of 250 or 500 kc is sometimes necessary to damage some types of pollen grain (Havinga 1967). Untreated *Pinus radiata* D. Don pollen is resistant to ultrasound frequency of 40 kc at an average 60 watt output. At an initial rate of 10% damaged grains, it took almost 30 minutes to reach the 50% mark of split or broken grains, and 50 minutes to reach the 80% mark. Jiménez *et al.* (1983) found that a 10-minute sonication in water or methylene chloride, in an unspecified frequency, extracted enough allelopathic substances from *Zea mays* pollen to negatively affect root and shoot growth of *Cassia jalapensis* and of other species.

1.3 Pine pollen collection, processing and storage

Standard collection of pine pollen involves removal of mature male strobili from the trees a few days before anthesis. Male strobili are subsequently spread on trays, paper or cellophane sheets for air drying. After dehiscence, pollen is separated by shaking the catkins on a sieve, and the collected pollen is then transferred to storage containers (Stanley and Linskens 1974). Cryopreservation at -20 °C may increase *in vitro* germinability and pollen tube growth, and permits long term storage of living pollen grains (Braggio *et al.* 1990). *Pinus taeda* L. pollen may be stored in a living condition in a refrigerator at 3 °C for up to one year if the moisture content is kept below 10%. Optionally, this low moisture (<10%) loblolly pine pollen may be successfully stored for 3 years in tightly closed, half

filled, 100 ml glass bottles, maintained at -20 °C. Dried living pollen can also be stored in vacuum-sealed ampoules at 3 °C for at least 10 years (Bramlett and Matthews 1991).

Jack pine pollen can be stored in living conditions for up to one year in cotton-stoppered vials kept in darkness at room temperature at a relative humidity between 10 and 35%. Alternatively, jack pine is at 92% viability after one year of storage at 2 °C and a relative humidity between 5% and 75% (Johnson 1943).

2. Seed germination in laboratory and controlled environment

Under laboratory conditions, the usual substrates used for seed germination are sand, soil or paper (Edwards 1987). Usually seeds are germinated in Petri dishes (5 or 9 cm diameter), which are normally sealed with parafilm to prevent desiccation during the seed germination process. Depending on seed size and study objectives, 10 to 250 seeds are placed in each Petri dish (Anaya *et al.* 1992; Bradbeer 1988; El-Khatib 1998; Jiménez *et al.* 1983: Ortega *et al.* 1988; Wardle *et al.* 1991; Wardle *et al.* 1992: Wardle *et al.* 1993; Zackrisson and Nilsson 1992).

3. Seed germination characteristics

Normally, percent germination, speed of germination, radicle growth, and hypocotyl growth are recorded along 3 weeks of seed germination test (Anaya *et al.* 1992; Bradbeer 1988; El-Khatib 1998; Jiménez *et al.* 1983: Ortega *et al.* 1988; Wardle *et al.* 1991; Wardle *et al.* 1992: Wardle *et al.* 1993; Zackrisson and Nilsson 1992).

Speed of germination is assessed by the speed of germination index (S) (Bradbeer 1988), calculated by:

$$S = \{\mathrm{Sum}(N_i/n_i)\} \times 100$$

where N_i stands for the proportion of seeds that germinate on day n_i, and $n_i = 1,2\ 3, \ldots N$ days following the set up of the experiment. Values for S range from zero, when no seed germinates during the germination test, to 100, when all the seeds germinate on day one of the germination test. Intermediate S values may correspond to several sets of N_i and n_i.

Other measures of speed of germination are germination value (GV) index (Czabator 1962), and germination energy (GE) (Sandberg 1988). The index S is the measure of speed of germination used in my study.

4. The effect of plant materials on seed germination characteristics

In general, speed of germination and radicle growth are considered to be more affected by plant materials or extracts than is percent germination and hypocotyl growth (Anaya *et al.* 1992; El-Khatib 1998; Ortega *et al.* 1988; Wardle *et al.* 1991; Wardle *et al.* 1992: Wardle *et al.* 1993; Zackrisson and Nilsson 1992). Faster seed germination is considered to be a desirable seed characteristic for many agricultural species (Bradbeer 1988). It has been shown that increased speed of germination is advantageous for growth and survival of seedlings of *Acer sp.* under broad-leaved forests in Japan (Seiwa 1998), and white pine (Thomas and Wein 1985). Also, plant substances from a species used in bioassays may be detrimental to its seed germination or seedling development (Stowe 1979; Wardle *et al.* 1991). However, the detrimental effect to the species of origin may be less than the detrimental effect to other species, resulting in a 'positive net result' (Wardle *et al.* 1991).

Stowe (1979) shows the effects of plant extracts from seven different species on seed germination among the species of extract origin. Autotoxicity is, in many cases, as severe as allotoxicity, and all the species studied can be shown to have active allelopathic substances in bioassays. Types of allelopathy detected through the most common types of bioassays are not demonstrably effective under field conditions, as the species composition and distribution in the field were not correlated to the patterns of detected toxicity.

5 The effect of water potential and hormones on jack pine and black spruce seed germination

5.1 Osmotic potential

Osmotic effects, primarily dehydration, are known to induce stress responses in plants (Slayter 1967). Significant reduction in percent germination, speed of germination and radicle length in seeds and seedlings of grass seeds are due to increasing osmotic potential

(0 to 0.0439 MPa) (Wardle *et al.* 1992). They concluded that in bioassays designed to test plant extracts, the eventually detected effects should not be considered due only to allelopathic compounds, but also due to the osmotic potentials of plant extract.

Under controlled conditions (20 °C, 16 hr light : 8 hr dark, in Petri dishes), germination of jack pine and black spruce seeds is not affected by osmotic pressures up to 1.2 MPa, but at 1.6 MPa the number of germinated seeds drops by 40% of the control at 0 MPa . The estimated mean time to reach 50% of total potential germination increases from 10 to 14 days, for jack pine, and from 10 to 21 days for black spruce, when osmotic pressure is increased from 0 to 1.2 MPa. At 2.0 MPa practically no seed germination occurs (Thomas and Wein 1985).

5.2. Ethylene

Ethylene is a plant hormone produced in the tissues near the lateral buds. This hormone is synthesized as a reaction to auxin (Burg and Burg 1966; Raven and Johnson 1992). Ethylene participates in many process of plant growth (Abeles *et al.* 1992), and it stimulates the germination of seeds (*e.g.*, Abeles 1986; Abeles and Lonski 1969; Deuber 1931; Raven and Jonhson 1992; Taylorson 1979).

Exogenous supply of ethylene increases germination of non dormant, soaked lettuce seeds from less than 20% to close to 60% germination (Abeles and Lonski 1969) .

Indoleacetic acid (IAA) induces ethylene formation in many plant species (Burg and Burg 1966). The reduction in growth in length of plant tissues under high levels of IAA results from high levels of auxin-induced ethylene formation by the tissue. Ethylene changes the IAA mediated length cell growth to radial cell growth, which is seen to cause swelling of *Pisun sativum* L. stem section (Burg and Burg 1966). Also, ethylene inhibits lateral transport of auxin in *P. sativum* stem tissue, suggesting the possibility of a feedback mechanism in which hormones interacts to control rates of synthesis.

In germinating seedlings of *Vigna radiata* (L.) R. Wilczec, var. radiata, (syn. *Phaseolus radiatus*), ethylene lowers levels of diffusible auxins and reduces cell elongation (Sankhla and Shukla 1970). Ethylene drastically reduces cell elongation and fresh masses of root, hypocotyl and epicotyl. It does not affect dry weight. Seedlings also have a pronounced

hypocotyl coiling response. It is thought that the ethylene precursor, ethrel, causes asymmetric distribution of auxin, resulting in localized surplus of auxin and consequent hypocotyl coiling.

Ethylene and gibberellin cause opposite effects in plant tissues. Gibberellin applied simultaneously with ethrel acts independently and limits ethrel action in growth extension, but not in hypocotyl coiling (Sankhla and Shukla 1970). Ethylene suppresses GA effects in lettuce hypocotyl growth, invertase formation in sugar beet, and the initiation of alfa-amylase activity in barley endosperm (Scott and Leopold 1967). Ethylene is also effective in alleviating the strong negative effect of high gibberellin concentrations on *Picea abies* (L.) Karst. seed germination (Sandberg 1988). In general, ethylene has a high hormonal activity, and propylene, an ethylene analog, has about 1% of the activity of ethylene (Abeles 1986; Abeles *et al.* 1992; Burg and Burg 1965; Taylorson 1979).

Pollen of a number of species contains high levels of the ethylene precursor 1-aminocyclopropane-1-carboxilic acid (ACC) (*e.g.*, Stead 1985; Whitehead *et al.* 1983). In *Nicotiana tabacum* L., cv. White Burley, a mean concentration of 2675 nmol of ACC/g of pollen is encountered (Hill *et al.* 1987). Up to 60% of the ACC readily diffuses from the pollen grains. This pollen ACC acts in post-pollination changes to *N. tabacum* flowers. For example, ethylene from pollen induces *de novo* synthesis of ethylene by gynoecial tissue.

Not only does ethylene act in post-pollination plant activity and interact with other hormones to affect above ground plant growth, but it also affects below-ground growth and activity. Sexstone and Mains (1990) demonstrate high levels of ethylene production from wet coniferous forest soils. It is known that ethylene causes adverse effects on plant root growth (Lindberg et al. 1979).

5.3. Cytokinin (Kinetin)

Germination of unstratified *Pinus taeda* L. seeds is not improved by high levels (up to 465 μM) kinetin (Biswas *et al.* 1972). However, at 46.5 μM kinetin concentration, percent germination of 21-day stratified seeds rose from 46%, at 0 M kinetin solution, to 91%. Speed of germination of stratified *P. taeda* seeds is also improved by kinetin.

5.4. Gibberellins

Gibberellins are synthesized in stem and root apices. These hormones promote cell elongation in tissues of mature trees and shrubs. Gibberellins also mediate mobilization of food reserves during seed germination (Raven and Johnson 1992)

Exogenous gibberellins have proven to affect seed dormancy and germination of several species. After seed immersion in gibberellin solutions at concentrations from 0.1 to 500 ppm, during 2 to 48 hr, Anderson and Widmer (1975), Ballington *et al.* (976), Biswas *et al.* (1972), Bulard (1985), Dweikat and Lyrene (1989), Raven and Johnson (1992), and West *et al.* (1970) report various effects on seed germination. High doses of gibberellin result in production of unviable expelled embryos (Anderson and Widmer 1975), and a decrease in seed germination (*e.g.*, Biswas *et al.* 1972; Sandberg 1988; Singh 1989). The growth of the embryonic root of apple is also negatively affected by high levels of gibberellin (Bulard 1985).

Effects of exogenous gibberellin applied to seeds are counteracted by other hormones, such as abscisic acid (*e.g.*, Bulard 1985) or ethylene (Sandberg 1988). This suggests that the germination process results from a dynamic balance of hormones rather than the action of one specific hormone alone Khan 1975).

Seed stratification affects the levels of endogenous hormones. *Ginkgo biloba* L. embryos of stratified seeds contain 100 times more extractable GA than embryos of unstratified seeds. Germination numbers of unstratified *G. biloba* seeds immersed for 40 hr in a 120 ppm GA_3 solution (0.346 μM) are the same as for stratified seeds germinated under the same conditions (West *et al.* 1970). The authors suggest that the role of gibberellin on *Ginkgo biloba* seed germination may be similar to its role on seed germination of many grass species, although *Ginkgo biloba* seeds has no aleurone layer or true endosperm.

Pinus taeda seeds require stratification for proper germination. Unstratified seeds are little affected by the application of gibberellin (GA_3), and percent germination ranges from 20% (control) to a maximum of 30%, after soaking for 1 hr in a 288.6 μM GA_3 solution. However, after 1 hr soaking in a 288.6 μM GA_3 solution, percent germination of 21-day stratified seeds increases from 46% to 96%. Speed of germination of stratified seeds is also positively affected by exogenous gibberellin (Biswas *et al.* 1972).

Unstratified and stratified (4 °C, 21 to 63 days) *Pinus monticola* Dougl. ex D. Don seeds, germinated at 18-20 °C/24 hr light and soaked in a solution of (1 mg kinetin + 500 mg GA_3)/liter for 24 hr, followed by soaking in 35% (w/v) hydrogen peroxide for 1.5 hr, performed differently. Germination of unstratified seeds increased from 6.5-14.5%, at 18-20 °C, to 53% in both temperatures, after the hormone treatment. The similarly treated 42-day stratified seeds increase in germination from 38-20%, at 18-20 °C, to 74-83% germination, at 18-20 °C, after the hormone treatment (Pitel and Wang (1985).

Effects of IAA, IBA, GA_1, GA_3, $GA_{4/7}$, GA_9, kinetin, and ethrel (according to Abeles *et al.* 1992, 2 chloroethylphosphonic acid) on *Pinus sylvestris* L. seed germination are recorded (Sandberg 1988). Increases of 5 to 10% in the number of seeds germinating and germination energy (GE) in pine seeds, after 1 hr soaking, occurs in some sensitive seed lots as a response to low concentrations (100 μM) of GA_9. In general, gibberellins increase GE by up to 5%, but percent germination is reduced (Sandberg 1988).

No positive effect on percent germination of *Picea abies* seeds is detected after one hr soaking in auxin, GA, kinetin or etephone solutions under vacuum. However, some *Picea* seed lots respond positively to GA_9, $GA_{4/7}$ and/or etephone with increases of 5 to 10% in GE. Germination energy is particularly increased by GA_9 (Sandberg 1988). By interaction of GA_9 or $GA_{4/7}$ with etephone GE further increases by 5% Sandberg 1988). *Picea smithiana* (Wall.) Boiss. seeds soaked in 120 to 2888 μM GA_3 solutions for 24 to 72 hr are increased in percent germination by 20% (Singh 1989), which contrasted with no increase in *Picea abies* percent germination in similar conditions (Sandberg 1988).

6. Surface sterilization with propylene oxide

Experimental materials, including filter paper and pollen, is sterilized by overnight exposure to 1 ml of propylene oxide/liter in a closed vessel. Prior to use, the sterilized material should be well aerated to allow volatilization of propylene oxide (Johnston and Booth 1983).

Seeds and microorganisms exposed for 24 hr to high doses (400 mg/liter of container) of propylene oxide are killed. Even at lower (200 mg/liter of container) propylene oxide doses, seed (barley) germination may be negatively affected, aside from the fact that the

seed microflora is not killed and the seed surfaces are not sterilized (Ramakrishna *et al.* 1991).

APPENDIX II

Levels and sources of unsterilized jack pine pollen substances,
and per replicate number of germlings,
hypocotyl length and radicle length

Raw data from the experiment of jack pine and black spruce in vitro seed germination in unsterilized Petri dishes

JACK PINE DATA SET					BLACK SPRUCE DATA SET				
Pollen level*	Source of pollen subst.	Number of jack pine germlings/plot	Jack pine hypocotyl length mm/plot	jack pine radicle length mm/plot	Pollen level*	Source of pollen subst.	Number of black spruce germlings/plot	Black spruce hypocotyl length mm/plot	Black spruce radicle length mm/plot
1	1= FP**	10	192	87	1	1	10	160	62
1	1	9	203	106	1	1	8	127	43
1	1	10	247	148	1	1	10	118	47
1	1	10	208	133	1	1	9	121	48
1	1	8	174	92	1	1	8	144	75
1	1	10	218	105	1	1	10	140	54
1	1	10	234	144	1	1	9	118	55
1	1	10	208	78	1	1	9	142	53
1	1	9	201	100	1	1	9	134	55
1	1	9	226	161	1	1	10	121	45
10	1	9	193	89	10	1	10	123	31
10	1	9	173	63	10	1	9	121	17
10	1	10	242	130	10	1	8	97	33
10	1	10	228	147	10	1	9	80	17
10	1	10	197	87	10	1	8	107	25
10	1	9	204	80	10	1	8	59	22
10	1	9	197	83	10	1	8	93	30
10	1	8	175	69	10	1	8	95	33
10	1	9	163	81	10	1	10	119	29
10	1	9	192	102	10	1	7	95	8
20	1	9	164	117	20	1	6	25	8
20	1	10	178	82	20	1	9	65	20
20	1	8	177	71	20	1	7	77	7
20	1	9	153	74	20	1	7	37	12
20	1	9	141	55	20	1	8	96	17
20	1	10	162	42	20	1	7	56	19
20	1	9	130	68	20	1	9	58	16
20	1	10	173	52	20	1	4	42	10
20	1	9	154	79	20	1	8	24	9
20	1	9	167	83	20	1	2	2	2

* In multiples of annual jack pine pollen rain.

**FP= fresh pollen; SR= sonicated remains; AE= aqueous extract; NP= no pollen.

CONTINUED: JACK PINE DATA SET

Pollen level*	Source of pollen subst.	Number of jack pine germlings/plot	Jack pine hypocotyl length mm/plot	Jack pine radicle length mm/plot
1	2= SR**	10	232	154
1	2	10	210	106
1	2	10	237	148
1	2	10	215	113
1	2	10	236	112
1	2	10	240	193
1	2	10	221	109
1	2	9	231	142
1	2	9	223	155
1	2	10	238	116
10	2	10	211	42
10	2	10	190	81
10	2	9	212	82
10	2	10	204	55
10	2	9	192	55
10	2	9	180	101
10	2	10	183	65
10	2	10	231	73
10	2	9	216	83
10	2	9	171	42
20	2	7	99	11
20	2	10	203	91
20	2	9	201	31
20	2	8	115	38
20	2	9	68	30
20	2	10	146	12
20	2	10	119	15
20	2	9	164	18
20	2	9	69	6
20	2	9	213	57

CONTINUED: BLACK SPRUCE DATA SET

Pollen level*	Source of pollen subst.	Number of black spruce germlings/plot	Black spruce hypocotyl length mm/plot	Black spruce radicle length mm/plot
1	2	7	103	41
1	2	8	113	42
1	2	10	140	63
1	2	10	124	55
1	2	8	114	46
1	2	9	119	52
1	2	10	142	93
1	2	9	137	65
1	2	10	132	47
1	2	9	121	40
10	2	7	88	12
10	2	10	113	38
10	2	9	107	26
10	2	8	109	26
10	2	9	74	28
10	2	9	94	31
10	2	8	76	22
10	2	7	67	25
10	2	9	123	39
10	2	6	53	18
20	2	9	99	27
20	2	9	137	16
20	2	9	82	15
20	2	5	51	8
20	2	9	82	4
20	2	8	53	17
20	2	9	108	8
20	2	8	24	8
20	2	7	73	3
20	2	7	47	10

* In multiples of annual jack pine pollen rain.

**FP= fresh pollen; SR= sonicated remains; AE= aqueous extract; NP= no pollen.

CONTINUED: JACK PINE DATA SET

Pollen level*	Source of pollen subst.	Number of jack pine germlings/plot	Jack pine hypocotyl length mm/plot	jack pine radicle length mm/plot
1	3= AE**	9	216	222
1	3	10	243	193
1	3	10	234	144
1	3	10	237	122
1	3	10	220	178
1	3	10	235	189
1	3	10	243	222
1	3	10	235	197
1	3	9	223	165
1	3	10	244	186
10	3	10	240	111
10	3	10	231	168
10	3	10	214	90
10	3	10	243	131
10	3	10	229	115
10	3	10	210	115
10	3	8	181	129
10	3	9	206	88
10	3	9	191	85
10	3	10	219	161
20	3	10	241	78
20	3	8	113	27
20	3	10	219	67
20	3	9	189	45
20	3	9	187	46
20	3	10	178	39
20	3	10	187	46
20	3	10	217	79
20	3	10	210	68
20	3	10	220	114

CONTINUED: BLACK SPRUCE DATA SET

Pollen level*	Source of pollen subst.	Number of black spruce germlings/plot	Black spruce hypocotyl length mm/plot	Black spruce radicle length mm/plot
1	3	9	129	50
1	3	9	142	77
1	3	6	78	43
1	3	8	117	65
1	3	8	115	65
1	3	10	159	66
1	3	10	126	58
1	3	10	151	81
1	3	7	114	52
1	3	9	163	87
10	3	7	94	60
10	3	10	109	41
10	3	8	120	39
10	3	7	95	41
10	3	10	128	50
10	3	9	136	43
10	3	8	75	32
10	3	7	72	36
10	3	8	98	38
10	3	9	134	54
20	3	9	109	32
20	3	8	97	38
20	3	9	94	33
20	3	9	115	30
20	3	8	76	26
20	3	8	91	25
20	3	10	107	26
20	3	8	113	25
20	3	9	81	26
20	3	9	99	38

* In multiples of annual jack pine pollen rain.

**FP= fresh pollen; SR= sonicated remains; AE= aqueous extract; NP= no pollen.

CONTINUED: JACK PINE DATA SET

Pollen level*	Source of pollen subst.	Number of jack pine germlings/plot	Jack pine hypocotyl length mm/plot	Jack pine radicle length mm/plot
0	0= NP**	10	231	119
0	0	5	115	82
0	0	10	234	237
0	0	10	258	179
0	0	9	199	173
0	0	9	211	234
0	0	10	267	241
0	0	10	218	170
0	0	10	234	162
0	0	10	243	210

CONTINUED: BLACK SPRUCE DATA SET

Pollen level*	Source of pollen subst.	Number of black spruce germlings/plot	Black spruce hypocotyl length mm/plot	Black spruce radicle length mm/plot
0	0	8	119	65
0	0	9	141	67
0	0	10	159	107
0	0	10	177	115
0	0	9	141	79
0	0	8	127	61
0	0	8	108	66
0	0	6	97	62
0	0	10	157	85
0	0	10	149	68

* In multiples of annual jack pine pollen rain.

**FP= fresh pollen; SR= sonicated remains; AE= aqueous extract; NP= no pollen.

Mean and standard error (SE) of several characteristics of seed germination in Petri dishes, under unsterilized conditions.

JACK PINE

VARIABLES	CONTROL MEANS (SE)	FRESH POLLEN MEANS (SE)			SONICATED POLLEN MEANS (SE)			AQUEOUS EXTRACT MEANS (SE)		
		1 x	10x	20x	1 x	10x	20x	1 x	10x	20x
Number of germlings per replicate	9.30 (1.57)	9.50 (0.22)	9.20 (0.20)	9.20 (0.20)	9.80 0.13)	9.50 (0.17)	9.00 (0.30)	9.80 (0.13)	9.60 (0.22)	9.60 (0.22)
Hypocotyl length mm/replicate	221.0 (42.5)	211.1 (6.7)	196.4 (7.7)	159.9 (4.9)	228.3 (3.3)	199.0 (5.9)	139.7 (17.2)	233.0 (3.2)	216.4 (6.4)	196.1 (11.2)
Radicle length mm/replicate	180.7 (52.3)	115.4 (9.1)	93.1 (8.4)	72.3 (6.6)	134.8 (9.0)	67.9 (6.2)	30.9 (8.3)	181.8 (10.0)	119.3 (9.1)	60.9 (8.1)

BLACK SPRUCE

VARIABLES	CONTROL MEANS (SE)	FRESH POLLEN MEANS (SE)			SONICATED POLLEN MEANS (SE)			AQUEOUS EXTRACT MEANS (SE)		
		1 x	10x	20x	1 x	10x	20x	1 x	10x	20x
Number of germlings per replicate	8.80 (1.32)	9.20 (0.25)	8.50 (0.31)	6.70 (0.70)	9.00 (0.33)	8.20 (0.39)	8.00 (0.42)	8.60 (0.43)	8.30 (0.37)	8.70 (0.21)
Hypocotyl length mm/replicate	137.5 (24.8)	132.5 (4.4)	98.9 (6.3)	48.2 (8.8)	124.5 (4.1)	90.4 (7.2)	75.6 (10.6)	129.4 (8.1)	106.1 (7.3)	98.2 (4.2)
Radicle length mm/replicate	77.5 (19.3)	53.7 (3.0)	24.5 (2.6)	12.0 (1.9)	54.4 (5.1)	26.5 (2.6)	11.6 (2.3)	64.4 (4.5)	43.4 (2.7)	29.9 (1.6)

APPENDIX III

Levels and sources of sterilized jack pine pollen substances, and per replicate speed of jack pine seed germination, number of germlings, hypocotyl length and radicle length

Data for JACK PINE *in vitro* seed germination under sterilized conditions.

Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicle	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicle	Hypocotyl length mm/plot	Radicle length mm/plot
1	1= FP**	1	17.5	9	0	160	66	1	1	2	14.67	9	0	100	78
1	1	1	17.19	10	0	154	128	1	1	2	11.78	7	0	80	62
1	1	1	19.33	9	0	177	116	1	1	2	10.65	6	1	59	47
1	1	1	18.86	10	2	165	107	1	1	2	10.41	6	1	77	44
1	1	1	10.5	6	0	80	37	1	1	2	12.5	7	0	103	48
1	1	1	17.29	9	0	145	109	1	1	2	13.75	7	1	102	30
1	1	1	16.47	10	1	132	67	1	1	2	8.67	5	1	66	61
1	1	1	19.5	9	0	169	102	1	1	2	13.6	7	0	125	82
1	1	1	15.85	8	0	130	61	1	1	2	14.85	8	0	128	84
1	1	1	16.94	10	1	160	51	1	1	2	12.75	7	0	94	69
10	1	1	17.45	9	3	157	65	10	1	2	11.38	7	6	93	12
10	1	1	16.67	8	1	163	46	10	1	2	12.1	6	1	98	60
10	1	1	15.35	9	2	175	49	10	1	2	11.54	8	4	121	19
10	1	1	14.6	9	2	115	24	10	1	2	16.23	9	3	125	32
10	1	1	17.76	10	7	125	16	10	1	2	9.96	7	3	93	35
10	1	1	16.55	10	3	146	36	10	1	2	14.25	9	6	103	21
10	1	1	10.36	7	0	111	39	10	1	2	9.93	8	2	68	14
10	1	1	18.75	9	1	193	65	10	1	2	9.27	7	1	53	14
10	1	1	14.89	9	2	98	42	10	1	2	13.76	9	4	104	22
10	1	1	11.86	8	0	131	74	10	1	2	8.46	6	5	80	10
20	1	1	12.4	8	5	107	14	20	1	2	14.08	10	10	83	5
20	1	1	12.63	9	3	94	33	20	1	2	7.26	7	5	34	9
20	1	1	M*	7	7	70	4	20	1	2	5.76	6	4	18	5
20	1	1	13.65	9	3	106	20	20	1	2	6.33	5	2	58	17
20	1	1	14.48	9	4	98	16	20	1	2	6.31	6	5	34	6
20	1	1	14.11	8	1	87	48	20	1	2	12.62	9	9	73	7
20	1	1	15.6	9	9	83	5	20	1	2	12.12	8	8	77	2
20	1	1	17.74	9	4	116	17	20	1	2	12.61	8	3	118	20
20	1	1	17.1	9	9	30	5	20	1	2	9.77	8	8	66	3
20	1	1	9.76	6	3	72	31	20	1	2	11.11	9	8	86	15

* In multiples of annual jack pine pollen rain.

**FP= fresh pollen; SR= sonicated pollen remains; AE= aqueous extract; NP= no pollen; M= missing plot.

Continued: Data for JACK PINE *in vitro* seed germination under sterilized conditions.

Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicle	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicle	Hypocotyl length mm/plot	Radicle length mm/plot
1	2= SR**	1	17.87	10	2	156	73	1	2	2	15.72	9	3	116	45
1	2	1	17.77	10	0	172	117	1	2	2	12.71	8	4	76	24
1	2	1	14.44	8	0	133	87	1	2	2	14	8	0	85	63
1	2	1	15.6	8	0	160	102	1	2	2	12.84	8	1	92	59
1	2	1	19.86	10	0	200	136	1	2	2	15	9	0	135	109
1	2	1	18.18	10	0	180	100	1	2	2	14.6	7	0	118	77
1	2	1	14.43	8	0	153	99	1	2	2	14.93	7	0	118	76
1	2	1	14.58	8	0	139	87	1	2	2	16.5	7	0	148	108
1	2	1	12.76	7	0	130	83	1	2	2	16.6	9	0	144	71
1	2	1	16.67	8	0	172	127	1	2	2	15.96	9	0	123	82
10	2	1	13.22	8	8	61	7	10	2	2	12.63	9	8	110	6
10	2	1	16.1	9	9	77	1	10	2	2	10.9	8	8	70	8
10	2	1	10.35	6	5	97	19	10	2	2	14.6	10	0	29	4
10	2	1	11.96	7	7	19	0	10	2	2	7.74	6	6	38	0
10	2	1	10.95	7	7	51	3	10	2	2	14.89	10	4	121	18
10	2	1	15.67	9	7	123	16	10	2	2	13.62	8	2	109	29
10	2	1	7.95	5	5	25	0	10	2	2	10.48	7	7	22	0
10	2	1	19.43	10	10	81	7	10	2	2	16.94	10	3	124	44
10	2	1	16.26	8	8	135	14	10	2	2	14.11	9	9	80	13
10	2	1	12.79	8	8	113	0	10	2	2	11.44	7	7	32	0
20	2	1	5.97	4	4	14	5	20	2	2	0	0	0	0	0
20	2	1	6.52	4	4	22	3	20	2	2	0	0	0	0	0
20	2	1	5.13	4	4	21	1	20	2	2	5.56	5	5	13	0
20	2	1	2.78	2	2	6	2	20	2	2	3.36	3	3	14	4
20	2	1	8.01	5	5	53	0	20	2	2	3.75	3	3	5	0
20	2	1	8.52	5	5	54	3	20	2	2	5.04	4	4	19	2
20	2	1	11.63	7	6	99	7	20	2	2	1.11	1	1	3	0
20	2	1	9.02	6	6	15	2	20	2	2	1.43	1	1	3	0
20	2	1	6.65	5	5	18	0	20	2	2	4.21	3	3	10	0
20	2	1	4.78	3	3	14	3	20	2	2	0	0	0	0	0

* In multiples of annual jack pine pollen rain.

**FP= fresh pollen; SR= sonicated pollen remains; AE= aqueous extract; NP= no pollen; M= missing plot.

Continued: Data for JACK PINE *in vitro* seed germination under sterilized conditions.

Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicle	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicle	Hypocotyl length mm/plot	Radicle length mm/plot
1	3= AE**	1	20.42	10	0	206	159	1	3	2	15.43	9	0	113	57
1	3	1	13.61	7	0	115	99	1	3	2	13.36	9	0	86	63
1	3	1	11.76	6	0	100	127	1	3	2	11.41	7	0	51	33
1	3	1	14.74	9	0	145	92	1	3	2	15.11	9	0	112	95
1	3	1	18.01	9	0	182	149	1	3	2	12.61	9	0	78	35
1	3	1	18.17	9	0	182	109	1	3	2	17.93	10	0	116	55
1	3	1	17.63	10	0	149	115	1	3	2	15.66	8	1	136	117
1	3	1	20.1	10	0	192	163	1	3	2	13.56	8	0	94	50
1	3	1	13.8	9	0	108	73	1	3	2	10.45	7	0	61	28
1	3	1	14.17	7	0	158	145	1	3	2	7.12	5	1	38	22
10	3	1	19.63	10	0	171	80	10	3	2	11.5	6	0	88	28
10	3	1	15.69	9	0	143	71	10	3	2	6.88	5	4	29	7
10	3	1	11.63	7	0	104	49	10	3	2	13.67	8	0	113	59
10	3	1	16.87	9	0	156	60	10	3	2	13.12	8	0	100	59
10	3	1	13.7	7	0	115	70	10	3	2	10.36	7	3	51	21
10	3	1	19.22	9	0	161	120	10	3	2	13.1	9	3	105	37
10	3	1	18.35	9	1	154	112	10	3	2	13.33	7	0	113	138
10	3	1	10.43	5	0	117	72	10	3	2	4.27	4	2	9	4
10	3	1	15.26	8	0	127	84	10	3	2	10.75	6	3	76	31
10	3	1	17.58	9	0	184	87	10	3	2	16.08	8	0	106	123
20	3	1	16.58	8	0	144	105	20	3	2	12.74	10	6	53	9
20	3	1	14.77	8	0	119	58	20	3	2	11.5	6	0	104	52
20	3	1	14.5	8	1	129	51	20	3	2	14.18	8	2	112	36
20	3	1	15.29	10	2	141	56	20	3	2	12.21	8	7	66	7
20	3	1	18.2	9	1	165	113	20	3	2	16.99	9	1	116	26
20	3	1	17.26	9	0	176	127	20	3	2	14.91	9	3	107	31
20	3	1	18	9	0	161	92	20	3	2	11.93	7	1	87	34
20	3	1	16.51	9	0	159	48	20	3	2	9.12	6	4	48	9
20	3	1	18.36	9	0	174	86	20	3	2	7.4	6	4	31	6
20	3	1	16.62	9	0	156	62	20	3	2	13.21	7	3	94	50

* In multiples of annual jack pine pollen rain.

**FP= fresh pollen; SR= sonicated pollen remains; AE= aqueous extract; NP= no pollen; M= missing plot.

Continued: Data for JACK PINE *in vitro* seed germination under sterilized conditions.

Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicle	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicle	Hypocotyl length mm/plot	Radicle length mm/plot
0	0= NP**	1	16.67	9	0	151	108	0	0	2	10.36	6	0	68	50
0	0	1	21.42	10	0	186	220	0	0	2	13.5	6	0	137	115
0	0	1	17.29	9	0	154	138	0	0	2	14.18	7	0	103	117
0	0	1	17.18	9	0	186	209	0	0	2	18.5	8	0	140	120
0	0	1	16.45	9	0	202	159	0	0	2	17.33	8	1	131	158
0	0	1	M*	8	0	166	155	0	0	2	12.03	7	0	83	54
0	0	1	19.5	10	0	190	177	0	0	2	16.08	8	0	129	118
0	0	1	11.3	7	0	119	93	0	0	2	16.66	8	0	135	137
0	0	1	18.1	10	0	162	110	0	0	2	14.66	9	0	110	113
0	0	1	15.24	9	0	121	71	0	0	2	19.33	10	0	185	160

* In multiples of annual jack pine pollen rain.

**FP= fresh pollen; SR= sonicated pollen remains; AE= aqueous extract; NP= no pollen; M= missing plot.

Mean and standard error (SE) of several characteristics of jack pine seed germination in Petri dishes, under sterilized conditions.

VARIABLES		CONTROL MEANS (SE)	FRESH POLLEN MEANS (SE)			SONICATED POLLEN MEANS (SE)			AQUEOUS EXTRACT MEANS (SE)		
			1 x	10x	20x	1 x	10x	20x	1 x	10x	20x
Speed of germination	block 1	17.02 (0.94)	16.94 (0.81)	15.42 (0.84)	14.16 (0.82)	16.22 (0.70)	13.47 (1.08)	6.9 (0.79)	16.24 (0.95)	15.84 (0.99)	16.61 (0.44)
	block 2	15.26 (0.90)	12.36 (0.63)	11.69 (0.78)	9.8 (0.99)	14.89 (0.44)	12.74 (0.84)	2.45 (0.69)	13.26 (0.97)	11.31 (1.10)	12.42 (0.87)
Number of germlings per replicate	block 1	9.00 (0.30)	9 (0.39)	8.8 (0.29)	8.3 (0.33)	8.7 (0.37)	7.7 (0.47)	4.5 (0.45)	8.6 (0.45)	8.2 (0.47)	8.8 (0.20)
	block 2	7.70 (0.40)	6.9 (0.35)	7.6 (0.37)	7.6 (0.50)	8.1 (0.28)	8.4 (0.45)	2 (0.58)	8.1 (0.46)	6.8 (0.49	7.6 (0.45)
Hypocotyl length mm/replicate	block 1	163.7 (9.0)	147.2 (8.9)	141.4 (9.67)	86.3 (7.9)	159.5 (7.0)	78.2 (12.6)	31.6 (9.1)	153.7 (11.7)	143.2 (8.4)	152.4 (5.9)
	block 2	122.1 (10.4)	93.4 (7.3)	93.8 (7.0)	64.7 (9.4)	115.5 (7.7)	73.5 (12.9)	6.7 (2.2)	88.5 (10.0)	79 (11.8)	81.8 (9.5)
Radicle length mm/replicate	block 1	144.0 (15.6)	84.4 (9.9)	45.6 (5.8)	19.3 (4.5)	101.1 (6.3)	6.7 (2.3)	2.6 (0.7)	123.1 (9.6)	80.5 (6.9)	79.8 (9.0)
	block 2	114.2 (11.7)	60.5 (5.7)	23.9 (4.8)	8.9 (2.0)	71.4 (8.22)	12.2 (4.6)	0.6 (0.4)	55.5 (9.6)	50.7 (14.6)	26 (5.6)

APPENDIX IV

Levels and sources of sterilized jack pine pollen substances, and per replicate speed of black spruce seed germination, number of germlings, hypocotyl length and radicle length

Data for BLACK SPRUCE *in vitro* seed germination under sterilized conditions.

Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings w/ damaged radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings w/ damaged radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot
1	1= FP**	2	13.19	9	4	74	17	1	1	3	12.93	7	1	87	41
1	1	2	12.21	7	1	70	22	1	1	3	19.83	10	1	128	69
1	1	2	10.86	9	2	65	24	1	1	3	19.94	10	2	121	32
1	1	2	8.11	6	3	31	9	1	1	3	16.99	9	2	86	31
1	1	2	7.25	5	2	43	22	1	1	3	9.93	6	0	52	20
1	1	2	12.17	7	5	61	23	1	1	3	8.86	5	0	57	20
1	1	2	8.48	7	4	44	7	1	1	3	16	9	2	108	35
1	1	2	10.37	8	3	39	9	1	1	3	11.33	6	2	76	18
1	1	2	8.17	4	0	61	19	1	1	3	15.68	9	4	120	20
1	1	2	9.37	8	8	42	6	1	1	3	15.6	9	1	96	41
10	1	2	5.35	4	4	19	3	10	1	3	17.19	10	4	134	27
10	1	2	8.29	7	4	24	7	10	1	3	16.77	10	M*	132	14
10	1	2	10.14	7	7	39	13	10	1	3	16.67	9	M*	34	6
10	1	2	2	1	1	4	1	10	1	3	18.1	10	M*	162	13
10	1	2	6.43	5	5	34	7	10	1	3	15.33	10	M*	91	19
10	1	2	9.11	7	6	33	2	10	1	3	14.67	9	M*	98	10
10	1	2	10.66	8	3	54	12	10	1	3	13.44	8	M*	94	11
10	1	2	7.54	6	2	32	12	10	1	3	13.43	8	M*	103	14
10	1	2	7.44	5	1	42	11	10	1	3	14.78	9	M*	74	14
10	1	2	6.96	6	6	27	4	10	1	3	13.33	7	M*	79	13
20	1	2	6.72	6	6	31	10	20	1	3	12.02	8	M*	74	15
20	1	2	7.16	6	6	37	6	20	1	3	14.62	9	M*	98	16
20	1	2	5.96	6	6	21	7	20	1	3	*	8	M*	28	9
20	1	2	2.92	2	2	22	1	20	1	3	14.95	10	M*	100	8
20	1	2	4.35	3	3	8	0	20	1	3	13.27	9	M*	32	3
20	1	2	3.48	3	0	6	3	20	1	3	12.93	8	M*	81	11
20	1	2	4.99	5	5	23	0	20	1	3	8.69	6	M*	22	4
20	1	2	10.6	9	9	47	0	20	1	3	12.94	8	M*	82	7
20	1	2	3.13	3	3	10	1	20	1	3	7.77	5	M*	9	1
20	1	2	3.68	3	3	11	0	20	1	3	6.71	7	M*	16	8

*In multiples of annual jack pine pollen rain

**FP= fresh pollen; SR= sonicated remains; AE= aqueous extract; NP= no pollen.

Continued: Data for BLACK SPRUCE *in vitro* seed germination under sterilized conditions.

Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot
1	2=SR**	2	5.71	5	3	23	7	1	2	3	14.76	8	0	93	32
1	2	2	7.82	7	7	36	9	1	2	3	16.43	9	0	123	42
1	2	2	11.8	7	5	44	15	1	2	3	12.13	7	1	65	36
1	2	2	8.31	7	7	36	9	1	2	3	12.43	7	0	83	21
1	2	2	8.43	6	2	49	15	1	2	3	9.68	6	1	55	18
1	2	2	6.36	5	4	26	9	1	2	3	15.93	8	0	99	43
1	2	2	10.62	8	0	56	22	1	2	3	14.53	8	6	77	11
1	2	2	11.2	8	2	52	14	1	2	3	15.25	9	1	110	37
1	2	2	5.25	5	5	16	7	1	2	3	15.91	9	1	103	46
1	2	2	0.91	1	1	4	0	1	2	3	16.62	9	0	96	35
10	2	2	0.83	1	1	2	0	10	2	3	0.83	1	M*	0	1
10	2	2	0	0	0	0	0	10	2	3	3.83	4	M*	0	4
10	2	2	0	0	0	0	0	10	2	3	2.65	3	3	4	2
10	2	2	1.77	2	2	4	0	10	2	3	0.83	1	M*	0	1
10	2	2	0.91	1	0	0	1	10	2	3	4.62	4	4	6	1
10	2	2	0	0	0	0	0	10	2	3	2.36	2	M*	1	2
10	2	2	0	0	0	0	0	10	2	3	6.03	6	M*	19	4
10	2	2	0	0	0	0	0	10	2	3	5.31	5	5	14	6
10	2	2	0.91	1	0	0	1	10	2	3	5.73	5	5	16	3
10	2	2	0	0	0	0	0	10	2	3	6.98	6	M*	29	4
20	2	2	0	0	0	0	0	20	2	3	0	0	0	0	0
20	2	2	0	0	0	0	0	20	2	3	0	0	0	0	0
20	2	2	0	0	0	0	0	20	2	3	2.74	3	M*	5	2
20	2	2	0	0	0	0	0	20	2	3	0	0	0	0	0
20	2	2	2.91	3	3	6	1	20	2	3	0	0	0	0	0
20	2	2	0	0	0	0	0	20	2	3	0	0	0	0	0
20	2	2	0	0	0	0	0	20	2	3	1.74	2	2	4	0
20	2	2	0	0	0	0	0	20	2	3	0.91	1	M*	2	1
20	2	2	0	0	0	0	0	20	2	3	0	0	0	0	0
20	2	2	0.91	1	1	2	1	20	2	3	0	0	0	0	0

*In multiples of annual jack pine pollen rain

**FP= fresh pollen; SR= sonicated remains; AE= aqueous extract; NP= no pollen.

Continued: Data for BLACK SPRUCE *in vitro* seed germination under sterilized conditions.

Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot
1	3= AE**	2	4.48	4	1	20	6	1	3	3	15.5	8	1	110	46
1	3	2	2.02	2	2	10	0	1	3	3	13.69	9	2	72	29
1	3	2	8.35	6	4	46	20	1	3	3	13	7	1	81	34
1	3	2	9.35	8	2	49	13	1	3	3	15.61	8	2	83	37
1	3	2	11.67	4	2	27	4	1	3	3	14.94	8	0	91	40
1	3	2	11.21	7	4	44	13	1	3	3	16.1	9	0	112	44
1	3	2	7.79	6	2	45	19	1	3	3	7.83	4	1	44	21
1	3	2	4.24	4	3	24	4	1	3	3	16.6	9	0	111	43
1	3	2	6.12	5	5	26	4	1	3	3	18.87	10	1	121	53
1	3	2	6.35	6	5	27	3	1	3	3	11.51	7	1	75	42
10	3	2	7.61	7	5	31	6	10	3	3	11.94	8	1	66	18
10	3	2	7.79	7	3	34	14	10	3	3	10.21	6	0	60	26
10	3	2	5.36	5	3	24	3	10	3	3	11.56	7	0	56	23
10	3	2	1.82	2	1	7	1	10	3	3	10.33	6	0	61	21
10	3	2	9.39	8	4	50	23	10	3	3	13.62	8	1	65	23
10	3	2	2.86	2	1	13	4	10	3	3	12.02	9	1	48	20
10	3	2	5.45	4	3	22	6	10	3	3	14.35	9	1	84	26
10	3	2	9.38	7	2	47	15	10	3	3	12.1	8	0	72	28
10	3	2	5.93	4	1	37	9	10	3	3	10.12	6	1	42	15
10	3	2	6.11	6	4	28	7	10	3	3	9.69	7	1	50	11
20	3	2	5.65	4	2	31	6	20	3	3	12.49	9	2	57	23
20	3	2	9.62	7	6	49	11	20	3	3	9.4	7	5	31	9
20	3	2	3.5	2	2	20	8	20	3	3	9.58	6	2	36	10
20	3	2	9.65	8	4	36	14	20	3	3	11.12	8	4	45	14
20	3	2	7.71	6	6	29	4	20	3	3	12.93	9	1	78	20
20	3	2	6.92	5	2	38	16	20	3	3	8.59	6	3	29	8
20	3	2	6.76	6	1	30	14	20	3	3	10.19	7	1	53	18
20	3	2	7.43	6	1	34	12	20	3	3	10.93	8	4	53	13
20	3	2	7.39	6	4	28	6	20	3	3	8.65	6	5	31	7
20	3	2	10.31	8	4	43	12	20	3	3	6.94	5	4	40	5

*In multiples of annual jack pine pollen rain

**FP= fresh pollen; SR= sonicated remains; AE= aqueous extract; NP= no pollen.

Continued: Data for BLACK SPRUCE *in vitro* seed germination under sterilized conditions.

Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen level*	Source of pollen subst.	Block	Speed of germin.	Number of germlings/ plot	Number of germlings with damaged radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot
0	0= NP**	2	2.85	3	0	12	4	0	0	3	9.83	6	0	58	30
0	0	2	9.58	7	0	48	19	0	0	3	15.33	8	0	101	48
0	0	2	8.11	6	0	43	15	0	0	3	11.27	8	1	61	22
0	0	2	9.96	8	0	68	26	0	0	3	11.67	7	1	69	28
0	0	2	5.77	5	0	27	14	0	0	3	13.5	8	0	69	44
0	0	2	5.04	5	0	22	9	0	0	3	12.52	7	0	94	50
0	0	2	3.43	3	0	17	6	0	0	3	10.63	7	1	51	31
0	0	2	9.27	6	1	45	16	0	0	3	12.33	7	1	89	35
0	0	2	9.56	7	0	60	13	0	0	3	12.55	8	3	55	35
0	0	2	2.65	3	2	10	3	0	0	3	9.62	6	0	64	26

*In multiples of annual jack pine pollen rain

**FP= fresh pollen; SR= sonicated remains; AE= aqueous extract; NP= no pollen.

Mean and standard error (SE) of several characteristics of black spruce seed germination in Petri dishes, under sterilized conditions.

VARIABLES		CONTROL MEANS (SE)	FRESH POLLEN MEANS (SE)			SONICATED POLLEN MEANS (SE)			AQUEOUS EXTRACT MEANS (SE)		
			1 x	10x	20x	1 x	10x	20x	1 x	10x	20x
Speed of germination	block 1	6.62 (0.95)	10.02 (0.79)	7.39 (0.79)	5.30 (0.76)	7.64 (1.03)	0.44 (0.20)	0.38 (0.30)	7.16 (0.98)	6.17 (0.79)	7.49 (0.64)
	block 2	11.93 (0.54)	14.71 (1.22)	15.37 (0.55)	11.54 (0.96)	14.37 (0.71)	3.92 (0.69)	0.54 (0.31)	14.36 (0.97)	11.59 (0.49)	10.08 (0.58)
Number of germlings per replicate	block 1	5.30 (0.58)	7.0 (0.52)	5.6 (0.64)	4.6 (0.69)	5.9 (0.66)	0.5 (0.22)	0.4 (0.31)	5.2 (0.55)	5.2 (0.68)	5.6 (0.57)
	block 2	7.20 (0.25)	8.0 (0.58)	9.0 (0.33)	7.8 (0.47)	8.0 (0.33)	3.7 (0.60)	0.6 (0.34)	7.9 (0.53)	7.4 (0.37)	7.1 (0.43)
Hypocotyl length mm/replicate	block 1	35.20 (6.46)	53.00 (4.70)	30.80 (4.30)	21.60 (4.28)	34.20 (5.32)	0.60 (0.43)	0.80 (0.61)	31.80 (4.18)	29.30 (4.31)	33.80 (2.60)
	block 2	71.10 (5.51)	93.10 (8.38)	100.1 (11.37)	54.20 (11.36)	90.40 (6.54)	8.90 (3.19)	1.10 (0.60)	90.00 (7.51)	60.40 (3.89)	45.30 (4.85)
Radicle length mm/replicate	block 1	12.50 (2.27)	15.60 (2.29)	7.20 (1.44)	2.80 (1.14)	10.70 (1.90)	0.20 (0.13)	0.20 (0.13)	8.60 (2.24)	8.80 (2.12)	10.30 (1.28)
	block 2	34.90 (3.01)	32.70 (4.90)	14.10 (1.78)	8.20 (1.54)	32.10 (3.69)	2.80 (0.54)	0.30 (0.21)	38.90 (2.88)	21.10 (1.68)	12.70 (1.90)

APPENDIX V

Levels of jack pine pollen applied to the soil, and per replicate numbers of seeds, germlings, and germlings with infested radicles, hypocotyl length and radicle length

JACK PINE DATA							BLACK SPRUCE DATA						
Pollen levels*	Blocks	Number of seeds/plot	Number of germlings/ plot	Number of germlings with infested radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen levels*	Blocks	Number of seeds/plot	Number of germlings/ plot	Number of germlings with infested radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot
0	1	10	10	0	273	204	0	1	10	10	0	197	85
0	1	10	9	0	238	230	0	1	10	6	0	114	50
1	1	10	8	0	225	203	1	1	10	9	0	174	74
1	1	10	9	0	267	232	1	1	10	9	0	158	73
10	1	10	10	0	262	250	10	1	10	8	0	133	64
10	1	10	10	4	282	254	10	1	10	10	1	154	79
50	1	10	7	3	193	169	50	1	10	10	2	168	70
50	1	10	10	3	293	186	50	1	10	6	2	117	41
100	1	10	8	6	215	151	100	1	10	8	2	121	50
100	1	10	8	6	217	116	100	1	10	8	3	141	51
0	2	10	9	0	240	185	0	2	10	8	0	141	70
0	2	10	9	0	252	205	0	2	10	9	0	177	76
1	2	10	10	0	275	204	1	2	10	10	0	204	81
1	2	10	9	0	268	226	1	2	10	9	0	175	71
10	2	10	9	0	258	210	10	2	10	8	0	140	55
10	2	10	10	0	263	251	10	2	10	6	0	104	39
50	2	10	10	4	264	174	50	2	10	8	0	164	65
50	2	10	10	4	291	218	50	2	10	6	0	107	52
100	2	10	10	7	250	116	100	2	10	9	1	156	70
100	2	10	9	7	257	138	100	2	10	8	2	147	56
0	3	10	10	0	311	198	0	3	10	7	0	129	52
0	3	10	10	0	282	206	0	3	10	9	0	165	62
1	3	10	9	0	252	201	1	3	10	9	0	173	59
1	3	10	9	0	251	243	1	3	10	9	0	174	64
10	3	10	10	0	291	247	10	3	10	9	0	169	77
10	3	10	9	0	266	227	10	3	10	9	0	160	68
50	3	10	9	5	254	173	50	3	10	10	2	180	54
50	3	10	10	6	258	164	50	3	10	9	3	168	60
100	3	10	9	6	243	121	100	3	10	9	3	151	64
100	3	10	10	8	260	181	100	3	10	9	5	159	51
0	4	10	10	0	286	230	0	4	10	8	0	163	54
0	4	10	9	0.	253	212	0	4	10	9	0	189	61
1	4	10	10	0	280	226	1	4	10	9	0	172	75
1	4	10	9	0	256	231	1	4	10	9	0	183	75

*Pollen levels in multiples of the annual jack pine pollen rain.

Continued: Data for jack pine and black spruce seed germination on soil.

JACK PINE DATA							BLACK SPRUCE DATA						
Pollen levels*	Blocks	Number of seeds/plot	Number of germlings/ plot	Number of germlings with infested radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot	Pollen levels*	Blocks	Number of seeds/plot	Number of germlings/ plot	Number of germlings with infested radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot
10	4	10	9	2	254	168	10	4	10	8	0	126	49
10	4	10	10	0	277	247	10	4	10	9	0	170	73
50	4	10	9	2	211	171	50	4	10	10	3	191	67
50	4	10	10	5	272	188	50	4	10	8	2	157	48
100	4	10	10	9	279	128	100	4	10	10	6	183	61
100	4	10	10	4	269	166	100	4	10	10	4	175	45
0	5	10	10	0	283	211	0	5	10	9	0	173	60
0	5	10	10	0	264	257	0	5	10	9	0	161	58
1	5	10	9	0	237	178	1	5	10	8	0	131	67
1	5	10	9	0	244	202	1	5	10	9	0	167	69
10	5	10	10	1	266	196	10	5	10	10	0	194	101
10	5	10	9	0	242	210	10	5	10	10	0	197	72
50	5	10	9	3	226	180	50	5	10	9	3	173	60
50	5	10	10	0	256	211	50	5	10	10	1	176	66
100	5	10	8	6	215	115	100	5	10	8	0	124	61
100	5	10	9	7	233	169	100	5	10	9	0	166	59
0	6	10	10	0	291	218	0	6	9	9	0	173	63
0	6	10	10	0	296	259	0	6	9	5	0	75	35
1	6	10	9	0	239	262	1	6	9	7	0	120	65
1	6	10	9	0	239	169	1	6	9	6	0	101	46
10	6	10	10	0	271	242	10	6	9	9	0	155	84
10	6	10	10	1	273	211	10	6	9	8	0	153	75
50	6	10	8	6	231	104	50	6	9	8	1	149	57
50	6	10	10	7	271	139	50	6	9	6	4	105	33
100	6	10	10	6	287	226	100	6	9	8	2	156	47
100	6	10	10	7	252	148	100	6	9	8	3	152	57
0	7	10	10	0	287	260	0	7	9	7	0	120	56
0	7	10	10	0	283	254	0	7	9	7	0	120	65
1	7	10	9	0	257	244	1	7	9	8	0	143	69
1	7	10	10	0	278	244	1	7	9	7	1	133	59

*Pollen levels in multiples of the annual jack pine pollen rain.

CONTINUED JACK PINE DATA

Pollen levels*	Blocks	Number of seeds/plot	Number of germlings/ plot	Number of germlings with infested radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot
10	7	10	10	0	279	248
10	7	10	9	0	255	199
50	7	10	9	4	259	165
50	7	10	9	2	248	200
100	7	10	10	7	282	205
100	7	10	9	9	254	154
0	8	10	10	0	275	231
0	8	10	10	0	282	221
1	8	10	9	0	256	213
1	8	10	10	0	270	254
10	8	10	10	0	274	214
10	8	10	10	0	275	239
50	8	10	10	2	258	151
50	8	10	10	4	284	244
100	8	10	10	4	260	226
100	8	10	6	4	82	60
0	9	10	10	0	289	253
0	9	10	9	0	250	232
1	9	10	10	0	291	252
1	9	10	10	0	288	220
10	9	10	10	0	280	253
10	9	10	10	0	256	237
50	9	10	6	0	140	95
50	9	10	9	6	235	134
100	9	10	10	6	287	202
100	9	10	9	9	224	67
0	10	10	9	0	248	201
0	10	10	9	0	264	182
1	10	10	9	0	265	224
1	10	10	9	0	238	205
10	10	10	10	0	238	178
10	10	10	10	0	264	229
50	10	10	9	0	247	178
50	10	10	7	2	194	122
100	10	10	9	8	240	92
100	10	10	9	5	247	166

CONTINUED BLACK SPRUCE DATA

Pollen levels*	Blocks	Number of seeds/plot	Number of germlings/ plot	Number of germlings with infested radicles/plot	Hypocotyl length mm/plot	Radicle length mm/plot
10	7	9	8	0	137	61
10	7	9	5	0	95	43
50	7	9	9	0	119	63
50	7	9	8	0	140	70
100	7	9	7	0	119	52
100	7	9	7	3	128	39
0	8	9	7	0	135	55
0	8	9	8	0	139	70
1	8	9	6	0	105	37
1	8	9	9	1	136	49
10	8	9	8	1	143	62
10	8	9	8	0	152	60
50	8	9	7	1	130	57
50	8	9	8	4	137	38
100	8	9	9	6	168	56
100	8	9	8	1	148	56
0	9	9	8	0	153	55
0	9	9	9	0	181	81
1	9	9	9	0	173	69
1	9	9	6	0	105	34
10	9	9	8	0	150	65
10	9	9	8	0	141	53
50	9	9	9	3	171	61
50	9	9	9	2	178	65
100	9	9	7	1	119	42
100	9	9	8	3	143	42
0	10	9	7	0	127	59
0	10	9	9	0	178	76
1	10	9	6	0	110	56
1	10	9	9	0	158	75
10	10	9	8	0	146	72
10	10	9	8	1	138	69
50	10	9	8	2	141	69
50	10	9	7	4	113	43
100	10	9	8	2	163	55
100	10	9	9	2	151	67

*Pollen levels in multiples of the annual jack pine pollen rain.

Mean and standard error (SE) of several characteristics of jack pine and black spruce seed germination on soil.

Variable	Species	Level of fresh pollen				
		0x	1x	10x	50x	100x
Number of germlings per replicate	Jack pine	9.65(0.11)	9.25(0.12)	9.75(0.10)	9.05(0.27)	9.15(0.23)
	Black spruce*	0.92 (0.02)	0.92(0.01)	0.93(0.01)	0.93(0.02)	0.94(0.01)
Number of germlings with infested radicles per replicate	Jack pine	0 (0)	0 (0)	0.40(0.22)	3.40(0.47)	6.55(0.35)
	Black spruce*	0 (0)	0.01(0.01)	0.0.2(0.01)	0.21(0.03)	0.26(0.04)
Hypocotyl length mm/replicate	Jack pine	272.4(4.5)	258.8(4.1)	266.3(3.0)	2.44.3(8.4)	242.7(9.9)
	Black spruce	150.5(6.9)	149.8(6.8)	147.9(5.5)	149.2(6.1)	148.5(4.2)
Radicle length mm/replicate	Jack pine	222.5(5.5)	221.7(5.6)	225.5(5.8)	168.3(8.3)	147.4(10.6)
	Black spruce	62.2(2.6)	63.4(2.9)	66.1(3.2)	57.0(2.5)	54.1(1.9)
Hypocotyl plus radicle length mm/replicate	Jack pine	494.8(8.1)	480.5(8.3)	491.8(8.1)	412.6(15.1)	390.0(18.7)
	Black spruce	212.7(9.0)	213.1(9.3)	213.9(8.4)	206.2(7.8)	202.6(5.1)

* Means expressed as proportions of the number of seeds in each replicate.

APPENDIX VI

ANOVA results of jack pine pollen effects on black spruce seed germination on mineral soil, corrected by the number of seeds/replicate

ANOVA results of jack pine pollen effects on black spruce seed germination on mineral soil, corrected by the number of seeds/replicate.

Variable	Factor	DF	MS	F
Hypocotyl length	Pollen level (PL)	4	0.00006	0.01
	Block (B)	9	0.00885	1.59
	PL x B	36	0.00556	1
	Error	50	0.00555	
Radicle length	Pollen level (PL)	4	0.0229	2.55*
	Block (B)	9	0.0055	0.73
	PL x B	36	0.009	1.2
	Error	50	0.0075	
Hypocotyl plus radicle length	Pollen level (PL)	4	0.00146	0.25
	Block (B)	9	0.005	0.92
	PL x B	36	0.00577	1.06
	Error	50	0.00545	

APPENDIX VII

Levels of jack pine pollen application to lichen substrate, and per pot jack pine seedling diameter, height, shoot dry mass and root dry mass under greenhouse conditions

Raw data of the effects of pollen levels on jack pine seedling characteristics under greenhouse conditions.

Pollen levels*	Blocks	Number of surviving seedling/ pot	Number of measured seedling/ pot	Diameter** total/ pot	Height mm/ pot	Shoot dry weight mg/ pot	Root dry weight mg/ pot	Pollen levels*	Blocks	Number of surviving seedling/ pot	Number of measured seedling/ pot	Diameter* total/ pot	Height mm/ pot	Shoot dry weight mg/ pot	Root dry weight mg/ pot
0	1	9	9	240	447	1888	1114	0	2	9	9	282	532	1813	842
0	1	9	8	242	469	2365	938	0	2	9	9	312	523	1999	1316
0	1	9	9	301	638	3626	1210	0	2	9	9	283	509	2038	1379
0	1	9	9	273	460	2388	1012	0	2	9	9	304	501	2056	1123
0	1	9	9	292	610	3421	1185	0	2	9	9	298	529	1876	1068
0	1	9	8	241	458	2329	707	0	2	9	9	316	523	1878	1119
0	1	9	9	276	513	2797	1080	0	2	9	9	317	522	2440	1289
0	1	9	8	256	569	3345	908	0	2	9	9	339	551	2112	1314
1	1	9	9	285	528	3248	1151	1	2	8	8	258	444	1795	1044
1	1	7	6	193	339	2502	837	1	2	9	9	311	515	2268	1231
1	1	9	9	320	690	4331	1327	1	2	9	9	339	522	2691	1438
1	1	9	8	263	480	3321	1170	1	2	9	9	333	511	1856	1058
1	1	9	9	271	529	2676	900	1	2	9	9	353	551	2238	1108
1	1	9	8	278	423	3780	1352	1	2	9	9	320	565	1948	1111
1	1	9	9	313	696	3684	1678	1	2	9	9	288	537	1731	646
1	1	9	8	262	558	3414	899	1	2	9	9	323	539	2110	1060
10	1	9	9	272	525	2547	1167	10	2	9	9	296	501	2092	1206
10	1	9	8	250	465	2794	1099	10	2	9	9	321	524	2292	1217
10	1	9	9	302	641	3581	1118	10	2	9	9	320	498	2138	833
10	1	9	8	259	542	3065	1190	10	2	9	9	318	514	1908	508
10	1	9	9	283	596	2970	1202	10	2	9	9	320	484	2243	1335
10	1	9	8	266	535	3272	1107	10	2	9	9	332	535	2215	1198
10	1	9	9	278	592	2666	790	10	2	9	9	340	566	2270	1229
10	1	9	8	286	594	3788	1282	10	2	9	9	279	472	1625	1031
50	1	9	9	283	513	2429	832.2	50	2	9	9	347	546	2601	1134
50	1	9	8	282	587	3744	893	50	2	9	9	349	505	2706	1318
50	1	9	9	296	660	3148	1460	50	2	9	9	345	572	2473	1408
50	1	9	8	292	681	3671	1155	50	2	9	9	302	475	2166	1333
50	1	9	9	301	744	3821	1212	50	2	9	9	281	506	2296	1333
50	1	9	8	272	485	3457	1346	50	2	9	9	330	498	2218	1133
50	1	9	9	318	701	4286	1435	50	2	9	9	282	484	1458	816
50	1	9	8	283	636	3968	1171	50	2	9	9	310	496	2017	1065
100	1	9	9	354	778	4583	1760	100	2	9	9	350	518	2626	1388
100	1	9	8	293	781	4152	1368	100	2	9	9	378	551	2440	1287
100	1	9	9	278	613	3433	1099	100	2	9	9	357	524	2747	1528
100	1	9	8	261	548	2654	1146	100	2	9	9	342	525	2231	1097
100	1	9	9	316	802	4191	1052	100	2	9	9	393	601	3305	1758
100	1	9	8	307	614	4369	1524	100	2	9	9	350	556	3066	1529
100	1	9	9	310	678	3647	1603	100	2	9	9	344	506	2465	1324
100	1	9	8	278	614	3708	919	100	2	9	9	275	522	1963	969

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter im mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

Mean and standard error (SE) of several characteristics of jack pine seedlings growing on lichen substrate, under greenhouse conditions.

Variable	Block	Pollen level 0x	1x	10x	50x	100x
Number of surviving seedlings per pot	Block 1	9 (0)	8.75 (0.25)	9 (0)	9 (0)	9 (0)
	Block 2	9 (0)	8.88 (0.13)	9(0)	9 (0)	9 (0)
Diameter mm/seedling	Block 1	1.145 (0.027)	1.232 (0.024)	1.205 (0.024)	1.277 (0.022)	1.314 (0.037)
	Block 2	1.268 (0.028)	1.323 (0.033)	1.307 (0.029)	1.317 (0.042)	1.443 (0.051)
	2-block mean	1.207 (0.025)	1.278 (0.023)	1.256 (0.022)	1.297 (0.023)	1.379 (0.035)
Height mm/seedling	Block 1	60.4 (3.0)	63.8 (3.3)	66.1 (2.0)	73.7 (3.6)	79.8 (3.7)
	Block 2	58.2 (0.6)	58.9 (0.9)	56.9 (1.2)	56.7 (1.3)	59.8 (1.2)
	2-block mean	59.3 (1.5)	61.4 (1.8)	61.5 (1.6)	65.2 (2.9)	69.8 (3.2)
Shoot dry mass mg/seedling	Block 1	321.7 (25.6)	410.0 (20.9)	365.2 (22.4)	421.9 (26.8)	452.7 (26.2)
	Block 2	225.2 (7.7)	234.2 (11.7)	233.1 (9.0)	249.1 (15.4)	289.5 (17.1)
	2-block mean	273.4 (18.0)	322.1 (25.5)	299.2 (20.7)	335.5 (26.9)	371.1 (25.9)
Root dry mass mg/seedling	Block 1	117.7 (5.0)	141.1 (10.0)	132.5 (7.5)	139.9 (9.3)	154.0 (11.9)
	Block 2	131.2 (7.0)	122.6 (8.7)	118.8 (10.8)	132.5 (7.7)	151.1 (9.9)
	2-block mean	124.5 (4.5)	131.9 (6.8)	125.7 (6.6)	136.2 (5.9)	152.6 (7.5)
Shoot plus root dry mass mg/seedling	Block 1	439.4 (27.6)	551.1 (28.0)	497.7 (28.1)	561.9 (32.1)	606.8 (33.6)
	Block 2	356.4 (13.2)	356.8 (19.7)	351.9 (17.2)	381.6 (21.6)	440.6 (26.8)
	2-block mean	397.9 (18.3)	454.0 (30.1)	424.8 (24.6)	471.7 (29.9)	523.7 (29.9)

APPENDIX VIII

Levels of jack pine pollen application to mineral soil substrate, and per pot jack pine seedling diameter, height, shoot dry mass and root dry mass under greenhouse conditions

Pollen levels*	Blocks	Number of surviving seedl./pot	Number of measured seedl./pot	Diameter** total/pot	Height mm/pot	Shoot dry weight mg/pot	Root dry weight mg/pot	Pollen levels*	Blocks	Number of surviving seedl./pot	Number of measured seedl./pot	Diameter** total/pot	Height mm/pot	Shoot dry weight mg/pot	Root dry weight mg/pot
0	1	9	9	259	503	1740	1343	0	2	9	9	255	376	916	1237
0	1	9	8	237	497	1692	1560	0	2	9	9	259	486	1180	1123
0	1	8	8	219	334	1176	1028	0	2	8	8	221	393	1033	1071
0	1	9	8	224	391	1487	1403	0	2	9	9	255	458	1193	1032
0	1	9	8	228	419	1614	1101	0	2	9	9	268	469	1222	1126
0	1	9	8	220	395	1422	1069	0	2	9	9	256	483	1397	1155
0	1	9	9	248	419	1561	1453	0	2	9	9	244	422	1082	987
0	1	9	8	217	419	1566	1392	0	2	9	9	250	434	1224	1375
1	1	8	8	245	421	1738	1220	1	2	9	9	269	460	1090	1232
1	1	8	7	220	438	1769	1284	1	2	9	9	262	411	1229	1278
1	1	7	7	220	353	1497	1157	1	2	9	9	291	455	1189	1268
1	1	9	8	259	455	2014	1204	1	2	9	9	255	471	1182	1188
1	1	8	8	250	419	1786	878	1	2	9	9	245	448	1139	1258
1	1	9	8	255	462	1721	1362	1	2	9	9	295	482	1257	1346
1	1	9	9	270	505	1942	1389	1	2	9	9	288	513	1299	1208
1	1	9	8	241	455	1664	990	1	2	9	9	277	489	1306	1333
10	1	9	9	262	429	1709	1382	10	2	9	9	248	451	1442	1342
10	1	9	8	240	498	1947	1442	10	2	9	9	244	408	1236	1246
10	1	8	8	227	449	1706	1577	10	2	9	9	269	470	1252	1066
10	1	9	8	237	442	1867	1446	10	2	9	9	269	443	1275	1442
10	1	9	9	269	505	1901	1566	10	2	9	9	283	426	1168	1185
10	1	9	9	254	512	1987	1298	10	2	9	9	258	427	1238	1220
10	1	9	9	254	505	1681	1652	10	2	9	9	229	420	1153	1181
10	1	9	8	232	434	1439	1137	10	2	9	9	237	474	1188	1024
50	1	9	9	290	590	2508	1694	50	2	9	9	266	448	1410	1617
50	1	9	8	258	505	2177	830	50	2	9	9	320	513	1641	1473
50	1	8	8	266	550	2307	1386	50	2	9	9	264	360	1247	1471
50	1	9	8	263	462	2078	1434	50	2	9	9	294	458	1351	1706
50	1	9	9	258	463	1720	1432	50	2	9	9	297	477	1691	1534
50	1	9	8	224	421	1440	1146	50	2	9	9	314	437	1430	1486
50	1	9	9	238	374	1297	1266	50	2	9	9	297	430	1267	1493
50	1	9	8	219	384	1247	1097	50	2	9	9	261	410	1311	1390
100	1	9	9	271	545	2227	1955	100	2	9	9	277	413	1687	1656
100	1	9	8	238	455	1983	1614	100	2	9	9	306	415	1749	1495
100	1	9	9	280	529	2106	1708	100	2	9	9	281	416	1569	1690
100	1	9	8	228	437	1917	1352	100	2	9	9	312	433	1633	1789
100	1	9	9	263	594	2325	1687	100	2	9	9	308	441	1665	1597
100	1	8	7	193	412	1627	1203	100	2	9	9	279	421	1546	1797
100	1	9	9	257	549	1988	1486	100	2	9	9	331	462	1868	1698
100	1	9	8	268	540	2256	1620	100	2	9	9	276	415	1678	1799

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter im mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

Mean and standard error (SE) of several characteristics of jack pine seedlings growing on mineral soil substrate, under greenhouse conditions.

Variable	Block	Pollen level 0x	1x	10x	50x	100x
Number of surviving seedlings per pot	Block 1	8.88 (0.13)	8.38 (0.26)	8.88 (0.13)	8.88 (0.13)	8.88 (0.13)
	Block 2	8.88 (0.13)	9 (0)	9 (0)	9 (0)	9 (0)
Diameter mm/seedling	Block 1	1.045 (0.013)	1.160 (0.011)	1.082 (0.010)	1.123 (0.037)	1.110 (0.024)
	Block 2	1.053 (0.011)	1.129 (0.026)	1.054 (0.027)	1.197 (0.034)	1.226 (0.030)
	2-block mean	1.049 (0.008)	1.145 (0.014)	1.068 (0.014)	1.160 (0.026)	1.168 (0.024)
Height mm/seedling	Block 1	51.2 (2.2)	55.7 (1.4)	55.6 (1.4)	56.1 (3.3)	60.5 (1.5)
	Block 2	49.6 (1.4)	51.8 (1.2)	48.9 (0.9)	49.1 (1.8)	47.4 (0.7)
	2-block mean	50.4 (1.3)	53.7 (1.0)	52.2 (1.2)	52.6 (2.0)	54.0 (1.9)
Shoot dry mass mg/seedling	Block 1	185.8 (7.1)	224.7 (6.2)	209.8 (8.1)	221.3 (21.0)	245.3 (6.6)
	Block 2	130.2 (5.4)	134.6 (3.0)	138.2 (3.6)	157.6 (6.5)	186.0 (4.0)
	2-block mean	158.0 (8.4)	179.7 (12.1)	174.0 (10.2)	189.4 (13.4)	215.7 (8.5)
Root dry mass mg/seedling	Block 1	156.8 (8.3)	151.2 (8.5)	169.4 (7.2)	153.1 (9.7)	188.1 (6.5)
	Block 2	128.3 (4.8)	140.4 (2.2)	134.8 (5.3)	169.0 (3.9)	187.8 (4.2)
	2-block mean	142.6 (5.9)	145.8 (4.5)	152.1 (6.2)	161.1 (5.5)	187.9 (3.8)
Shoot plus root dry mass mg/seedling	Block 1	342.6 (13.8)	375.9 (12.2)	379.3 (12.7)	374.3 (26.1)	433.4 (11.8)
	Block 2	258.6 (7.2)	275.0 (4.4)	273.0 (7.9)	326.6 (7.7)	373.8 (4.5)
	2-block mean	300.6 (13.2)	325.5 (14.4)	326.1 (15.5)	350.5 (14.5)	403.6 (9.8)

APPENDIX IX

Levels of jack pine pollen application to needle litter substrate, and per pot jack pine seedling diameter, height, shoot dry mass and root dry mass under greenhouse conditions

Pollen levels*	Blocks	Number of surviving seedl./pot	Number of measured seedl./pot	Diameter** total/pot	Height mm/pot	Shoot dry weight mg/pot	Root dry weight mg/pot	Pollen levels*	Blocks	Number of surviving seedl./pot	Number of measured seedl./pot	Diameter** total/pot	Height mm/pot	Shoot dry weight mg/pot	Root dry weight mg/pot
0	1	9	9	276	773	2359	879	0	2	9	9	372	570	3036	1602
0	1	9	8	252	677	2362	634	0	2	9	9	322	511	2850	1190
0	1	9	9	335	921	4135	1023	0	2	9	9	332	513	2161	1029
0	1	9	8	260	616	2557	270	0	2	9	9	315	457	2354	1119
0	1	9	9	351	988	4089	1344	0	2	9	9	373	541	3385	1414
0	1	9	8	239	538	1881	479	0	2	9	9	368	599	3353	1409
0	1	9	9	357	959	5184	829	0	2	9	9	341	537	2461	1045
0	1	9	8	320	809	4581	1038	0	2	9	9	334	545	3043	1286
1	1	9	8	260	594	2545	330	1	2	9	9	306	406	1946	1089
1	1	9	8	283	584	3175	875	1	2	9	9	335	571	2884	1415
1	1	9	9	285	591	2851	642	1	2	9	9	353	540	3016	1762
1	1	9	8	264	689	2445	935	1	2	9	9	316	506	2102	998
1	1	9	9	305	821	3602	967	1	2	9	9	352	533	2893	1208
1	1	9	8	265	644	2937	638	1	2	9	9	376	566	2554	1371
1	1	9	9	282	616	2691	846	1	2	9	9	332	544	2900	1538
1	1	9	8	271	553	3118	854	1	2	9	9	334	478	2395	1282
10	1	9	9	252	550	1974	612	10	2	9	9	335	546	2325	1205
10	1	9	8	285	732	3683	772	10	2	9	9	348	513	2779	1228
10	1	9	9	304	812	3738	572	10	2	9	9	345	534	2566	1212
10	1	9	8	319	845	4353	1049	10	2	9	9	334	460	2211	1121
10	1	9	9	278	642	2463	681	10	2	9	9	336	533	2572	1270
10	1	9	8	282	684	3222	805	10	2	9	9	380	532	3397	1272
10	1	9	9	304	612	3259	772	10	2	9	9	328	547	2108	1151
10	1	8	7	219	567	2116	673	10	2	9	9	339	526	2704	1248
50	1	9	9	271	541	2401	745	50	2	9	9	351	608	2996	1255
50	1	9	8	225	516	1832	278	50	2	9	9	333	451	2161	1222
50	1	9	9	275	724	2615	722	50	2	9	9	327	538	2048	1139
50	1	9	8	281	781	3527	741	50	2	9	9	372	577	3209	975
50	1	9	9	369	730	5023	1334	50	2	9	9	377	551	3257	1679
50	1	9	8	262	622	3081	744	50	2	9	9	347	507	2593	1270
50	1	9	9	333	739	4904	1001	50	2	9	9	327	493	2270	1033
50	1	9	9	292	738	3048	761	50	2	9	9	330	509	2637	1270
100	1	9	9	329	828	4077	911	100	2	9	9	364	552	2886	1381
100	1	9	8	241	528	1869	611	100	2	9	9	390	575	3636	1559
100	1	9	9	256	737	2042	562	100	2	9	9	396	593	3608	1402
100	1	9	8	275	653	3296	683	100	2	9	9	356	597	2925	1291
100	1	9	9	370	841	4377	1604	100	2	9	9	354	532	2855	1502
100	1	9	8	254	617	2519	518	100	2	9	9	355	567	3047	1239
100	1	9	9	342	811	4463	1106	100	2	9	9	360	488	2856	1378
100	1	9	8	287	750	3400	749	100	2	9	9	384	591	3112	1532

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter im mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

Mean and standard error (SE) of several characteristics of jack pine seedlings growing on needle litter substrate, under greenhouse conditions.

Variable	Block	Pollen level 0x	1x	10x	50x	100x
Number of surviving seedlings per pot	Block 1	9 (0)	9 (0)	8.88 (0.13)	9 (0)	9 (0)
	Block 2	9 (0)	9 (0)	9 (0)	9 (0)	9 (0)
Diameter mm/seedling	Block 1	1.306 (0.057)	1.232 (0.017)	1.250 (0.047)	1.244 (0.055)	1.286 (0.056)
	Block 2	1.427 (0.034)	1.399 (0.032)	1.420 (0.024)	1.430 (0.029)	1.531 (0.025)
	2-block mean	1.366 (0.036)	1.316 (0.028)	1.335 (0.034)	1.337 (0.039)	1.409 (0.043)
Height mm/seedling	Block 1	91.8 (5.4)	76.0 (3.2)	81.8 (5.1)	78.2 (4.1)	84.5 (3.4)
	Block 2	59.3 (1.7)	57.6 (2.1)	58.2 (1.1)	58.8 (2.0)	62.4 (1.5)
	2-block mean	75.6 (5.0)	66.8 (3.0)	70.0 (4.0)	68.5 (3.3)	73.5 (3.4)
Shoot dry mass mg/seedling	Block 1	396.8 (48.3)	349.2 (15.4)	372.5 (37.4)	381.8 (43.9)	381.0 (38.3)
	Block 2	314.5 (18.1)	287.4 (15.9)	287.0 (15.9)	294.0 (18.5)	346.2 (12.8)
	2-block mean	355.7 (27.1)	318.3 (13.3)	329.7 (22.5)	337.9 (25.6)	363.6 (20.0)
Root dry mass mg/seedling	Block 1	94.4 (13.2)	90.8 (9.0)	89.7 (7.7)	90.9 (11.2)	98.1 (13.4)
	Block 2	140.2 (8.0)	148.1 (9.7)	134.8 (2.1)	136.7 (8.4)	156.7 (4.5)
	2-block mean	117.3 (9.5)	119.5 (9.8)	112.2 (7.0)	113.8 (9.0)	127.4 (10.2)
Shoot plus root dry mass mg/seedling	Block 1	491.2 (57.3)	440.0 (20.7)	462.2 (43.1)	472.7 (54.1)	479.1 (49.3)
	Block 2	454.7 (25.0)	435.5 (24.4)	421.8 (17.6)	430.8 (23.0)	502.9 (15.1)
	2-block mean	473.0 (30.6)	437.7 (15.4)	442.0 (23.1)	451.7 (28.9)	491.0 (25.1)

APPENDIX X

ANOVA results of jack pine pollen effects on jack pine seedlings grown on lichen and needle litter substrates under greenhouse conditions, after pooling PL x B and Error Mean Squares

ANOVA[1] results of jack pine pollen effects on jack pine seedlings under greenhouse conditions, after pooling PL x B and Error Mean Squares.

Variables	Factors	DF	Lichen substrate MS	Lichen substrate F	Needle litter substrate MS	Needle litter substrate F
Diameter						
	Pollen level (PL)	4	0.00697	7.3***		1.39
	Block (B)	1	0.02109	22.0***		49.9***
	PL x B	pooled	pooled		pooled	
	Error	74	0.00096			
Height						
	Pollen level (PL)		No MS			2.28*
	Block (B)		pooling			137.9***
	PL x B		was		pooled	
	Error		performed			
Shoot dry weight						
	Pollen level (PL)	4	0.03727	6.89***		0.77
	Block (B)	1	0.80541	149.0***		12.4***
	PL x B	pooled	pooled		pooled	
	Error	74	0.0054			
Root dry weight						
	Pollen level (PL)	4	0.02051	2.51**		0.45
	Block (B)	1	0.00843	1.03		61.7***
	PL x B	pooled	pooled		pooled	
	Error	74	0.00817			
Shoot plus root dry weight						
	Pollen level (PL)	4	0.03065	6.12***		0.65
	Block (B)	1	0.43051	86.0***		0.13
	PL x B	pooled	pooled		pooled	
	Error	74	0.00501			

1 ANOVA performed on log transformed data [log(mm/seedling) or log(mg/seedling)].

APPENDIX XI

Levels of jack pine pollen application to lichen substrate, and per plot jack pine seedling diameter, height, shoot dry mass and root dry mass under field conditions

Raw data from the field experiment on lichen substrate.

Pollen levels*	Blocks	Number of surviving seddling/plot	Diameter** total/plot	Height mm/plot	Shoot dry weight mg/plot	Root dry weight mg/plot	Pollen levels*	Blocks	Number of surviving seddling/plot	Diameter** total/plot	Height mm/plot	Shoot dry weight mg/plot	Root dry weight mg/plot
0	2	10	265	345	1086	1018	0	3	9	236	348	715	538
0	2	2	53	75	242	222	0	3	9	248	358	749	552
0	2	4	120	168	506	481	0	4	6	174	257	604	433
0	2	3	86	98	322	272	0	4	9	270	383	966	690
0	1	4	119	132	470	435	0	3	9	244	308	678	538
0	1	3	72	85	266	298	0	4	7	193	289	649	395
0	1	4	119	128	410	479	0	4	10	271	416	886	644
0	2	4	109	117	442	478	0	3	9	263	375	849	643
0	2	4	112	130	409	412	0	3	9	220	323	601	480
0	2	8	212	267	859	807	0	3	10	262	366	740	508
0	1	10	251	305	980	990	0	3	9	246	356	887	754
0	2	10	269	316	998	1037	0	4	10	267	402	852	562
0	1	10	271	342	1183	1253	0	4	10	301	417	914	568
0	1	10	289	332	1206	1342	0	3	9	232	350	717	564
0	1	9	239	290	998	1060	0	3	8	230	342	767	549
0	2	10	291	349	1112	940	0	4	9	230	339	719	487
0	1	3	84	96	327	330	0	3	10	264	485	1116	693
0	2	3	81	110	320	329	0	4	10	276	406	814	613
0	1	10	283	342	1210	1150	0	4	10	283	418	962	765
0	1	10	257	317	942	994	0	4	10	306	456	1019	730
0	1	10	259	301	928	829	10	4	10	259	391	804	734
10	1	9	239	284	828	762	10	3	9	241	382	798	638
10	1	8	221	264	954	873	10	3	10	273	435	938	626
10	2	2	57	76	215	215	10	4	8	207	329	617	495
10	1	3	84	95	264	276	10	3	10	289	427	947	701
10	2	0	0	0	0	0	10	3	10	240	341	579	671
10	2	0	0	0	0	0	10	3	10	299	434	989	910
10	2	10	270	283	971	929	10	4	7	208	347	812	566
10	2	10	272	336	1104	1064	10	4	10	276	412	904	613
10	1	10	256	306	830	941	10	4	7	193	293	614	459
10	2	9	247	288	921	935	10	3	10	261	401	755	546
10	2	10	245	282	869	1035	10	4	10	291	395	823	538
10	2	10	241	319	880	863	10	4	10	261	475	954	650
10	1	5	139	145	444	346	10	3	10	270	389	867	591
10	1	10	280	348	1135	1093	10	4	10	261	402	838	626
10	2	9	237	283	872	876	10	4	9	258	375	841	622
10	1	6	172	246	816	653	10	3	10	266	411	868	770
10	1	10	271	341	1108	1202	10	3	10	284	467	1006	727
10	1	10	253	278	960	1056	10	3	10	247	402	798	577
10	1	10	281	373	1317	1332	10	4	9	223	345	761	548

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter im mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

Continued: Raw data from the field experiment on lichen substrate.

Pollen levels*	Blocks	Number of surviving seedling/plot	Diameter** total/plot	Height mm/plot	Shoot dry weight mg/plot	Root dry weight mg/plot	Pollen levels*	Blocks	Number of surviving seedling/plot	Diameter** total/plot	Height mm/plot	Shoot dry weight mg/plot	Root dry weight mg/plot
50	1	9	263	347	1237	1040	50	4	7	203	320	757	466
50	1	9	226	292	920	813	50	4	10	288	391	993	659
50	1	9	244	325	1156	912	50	3	6	155	236	515	386
50	1	9	244	320	980	1012	50	4	8	237	351	783	453
50	1	6	156	189	530	624	50	4	10	304	435	1077	634
50	2	2	53	62	212	180	50	3	10	275	397	862	631
50	2	1	30	48	181	138	50	4	10	261	409	931	592
50	2	8	212	243	795	831	50	3	10	290	429	1000	850
50	1	10	256	302	1027	1103	50	3	9	242	353	713	454
50	1	10	243	312	973	1086	50	3	10	269	439	935	577
50	2	10	252	296	813	829	50	4	10	289	421	970	569
50	2	10	281	324	1082	1041	50	3	9	266	421	1019	673
50	2	10	244	296	907	875	50	3	10	277	442	977	648
50	2	6	162	221	699	715	50	4	10	274	434	929	678
50	2	7	193	250	807	708	50	4	10	293	430	1025	833
50	2	9	250	302	1000	1040	50	3	10	261	426	885	530
50	2	10	277	343	1227	1280	50	4	10	300	455	1055	648
50	1	6	154	190	546	538	50	3	10	310	452	1087	768
50	1	2	55	66	187	166	50	3	8	245	378	859	648
50	1	10	251	292	918	1123	50	4	9	248	379	810	582
100	2	10	280	354	1151	920	100	4	9	276	386	853	614
100	1	9	224	269	900	844	100	4	8	242	358	885	755
100	2	2	50	58	196	206	100	3	8	239	334	766	425
100	1	5	145	184	469	461	100	3	9	250	369	830	698
100	1	7	187	186	667	654	100	4	10	331	440	1103	695
100	2	0	0	0	0	0	100	3	9	267	378	812	558
100	1	10	259	316	933	773	100	3	9	260	358	676	470
100	1	10	260	306	949	838	100	4	10	249	396	822	582
100	1	10	270	383	1300	1120	100	3	10	288	396	932	691
100	2	7	156	209	514	464	100	3	10	284	419	951	692
100	2	4	106	119	321	357	100	3	9	251	389	829	552
100	2	6	172	172	574	668	100	4	10	291	439	969	622
100	2	9	245	298	935	895	100	3	9	242	375	800	791
100	2	10	277	363	1101	947	100	3	9	266	348	721	457
100	2	5	124	155	450	401	100	4	8	222	340	733	526
100	1	10	279	325	1072	1022	100	3	10	272	449	1040	841
100	2	10	277	314	1180	976	100	4	10	284	476	1016	692
100	1	3	72	93	277	302	100	4	9	269	422	958	832
100	1	6	169	229	736	697	100	4	10	285	469	1064	838
100	1	4	99	115	372	334	100	4	10	267	448	968	677

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter im mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

Mean and standard error (SE) of several characteristics of jack pine seedlings growing on lichen substrate, under field conditions.

Variable	Block	Pollen level 0x	10x	50x	100x
Number of surviving seedlings per pot	Block 1	7.55 (0.98)	8.10 (0.81)	8.00 (0.82)	7.40 (0.87)
	Block 2	5.80 (1.04)	6.67 (1.52)	7.30 (1.06)	6.30 (1.13)
	Block 3	9.10 (0.18)	9.90 (0.10)	9.20 (0.42)	9.20 (0.20)
	Block 4	9.10 (0.46)	9.00 (0.39)	9.40 (0.34)	9.40 (0.27)
Diameter mm/seedling	Block 1	1.013(0.022)	1.016 (0.013)	0.979(0.017)	0.985(0.019)
	Block 2	1.030(0.015)	0.985(0.023)	1.009(0.018)	0.985(0.024)
	Block 3	1.002(0.017)	1.005(0.021)	1.047(0.022)	1.062(0.012)
	Block 4	1.053(0.019)	1.011(0.019)	1.070(0.016)	1.078(0.027)
	4-block means	1.024(0.009)	1.006(0.009)	1.026(0.010)	1.028(0.012)
Height mm/seedling	Block 1	31.9 (0.5)	33.1 (1.2)	32.9 (0.9)	32.4 (1.3)
	Block 2	34.5 (1.1)	31.9 (1.3)	34.1 (1.7)	31.6 (.9)
	Block 3	39.7 (1.3)	41.3 (1.1)	43.1 (0.9)	41.4 (0.6)
	Block 4	41.6 (0.6)	42.0 (1.2)	42.9 (0.6)	44.4 (0.8)
	4-block means	36.8 (0.8)	37.5 (0.9)	38.3 (0.9)	37.6 (1.0)
Shoot dry mass mg/seedling	Block 1	106.7 (3.6)	105.9 (6.0)	104.2 (5.2)	102.2 (4.3)
	Block 2	110.1 (2.6)	98.4 (3.4)	113.2 (8.5)	98.3 (4.8)
	Block 3	85.9 (4.3)	86.3 (4.0)	96.0 (3.6)	90.7 (2.6)
	Block 4	92.5 (2.9)	89.1 (3.5)	99.4 (2.0))	99.8 (2.9)
	4-block means	99.0 (2.3)	94.7 (2.6)	103.2 (2.8)	97.7 (1.9)
Root dry mass mg/seedling	Block 1	110.1 (4.4)	102.6 (5.8)	102.8 (3.6)	95.5 (3.9)
	Block 2	105.4 (3.1)	99.7 (3.0)	107.0 (5.7)	92.6 (4.2)
	Block 3	64.1 (3.0)	68.3 (3.4)	67.1 (3.7)	66.7 (4.2)
	Block 4	64.8 (2.9)	65.4 (2.4)	64.9 (2.7)	73.1 (4.0)
	4-block means	86.7 (3.8)	82.7 (3.5)	85.4 (3.7)	81.7 (2.8)
Shoot plus root dry mass mg/seedling	Block 1	216.8 (7.5)	208.5 (11.1)	206.9 (7.4)	197.8 (7.9)
	Block 2	215.6 (4.9)	198.1 (5.8)	220.2 (13.7)	190.9 (8.0)
	Block 3	150.0 (7.0)	154.6 (6.4)	163.1 (6.9)	157.4 (6.0)
	Block 4	157.3 (5.5)	154.6 (5.4)	164.3 (3.5)	172.9 (6.4)
	4-block means	185.7 (5.8)	177.4 (5.6)	188.6 (5.8)	179.5 (4.3)

APPENDIX XII

Levels of jack pine pollen application to mineral soil substrate, and per plot jack pine seedling diameter, height, shoot dry mass and root dry mass under field conditions

Raw data from the field experiment on mineral soil substrate.

Pollen levels*	Blocks	Number of surviving seedling/ plot	Number of browsed shoots/ plot	Number of non-browsed shhots/ plot	Diameter** total/plot	Height mm/plot	Shoot dry weight mm/plot	Root dry weight mg/plot	Pollen levels*	Blocks	Number of surviving seedling/ plot	Number of browsed shoots/ plot	Number of non-browsed shhots/ plot	Diameter** total/plot	Diam.** of browsed shoots total/plot	Diam.** of non-browsed shoots total/plot	Height mm/plot	Height of browsed shoots mm/plot	Height of non-browsed shoots mm/plot	Shoot dry weight mm/plot	Root dry weight mg/plot
0	2	8	0	8	211	279	905	209	0	4	9	0	9	410***		410	444		444	2889	2142
0	1	8	0	8	256	282	1049	300	0	4	9	0	9	405		405	402		402	2002	2159
0	2	10	0	10	330	377	1830	1934	0	3	9	0	9	435		435	458		458	3554	2260
0	1	9	0	9	280	361	1755	2314	0	4	8	0	8	370		370	356		356	2034	1702
0	2	9	0	9	245	277	976	360	0	4	10	0	10	443		443	455		455	2709	1898
0	1	10	0	10	338	393	1959	2086	0	3	10	0	10	352		352	424		424	1764	1876
0	2	10	0	10	273	319	1292	469	0	4	8	0	8	422		422	434		434	3190	2156
0	2	7	0	7	210	226	897	271	0	4	9	0	9	393		393	415		415	2372	1888
0	2	3	0	3	91	114	444	115	0	4	7	0	7	325		325	360		360	2059	1952
0	1	7	0	7	222	255	1163	391	0	4	10	2	8	423	93	330	286***	37	249	1046	2139
0	1	10	0	10	295	349	1540	1298	0	3	10	0	10	334		334	413		413	1725	1431
0	1	10	0	10	284	363	1736	2159	0	3	10	0	10	426		426	469		469	2190	1987
0	2	7	0	7	202	203	942	259	0	4	10	10	0	444	444		232	232		291	1911
0	1	10	0	10	310	340	1514	439	0	3	10	0	10	355		355	437		437	1679	1458
0	1	10	0	10	280	337	1256	698	0	3	8	0	8	365		365	343		343	1660	1601
0	2	9	0	9	234	254	942	193,8	0	4	10	0	10	497		497	491		491	2996	1856
0	1	10	0	10	264	349	1621	1400	0	3	10	0	10	366		366	459		459	2058	1576
0	2	9	0	9	262	254	1334	625	0	3	9	0	9	329		329	381		381	1803	1204
0	1	10	0	10	298	324	1629	1338	0	3	10	0	10	392		392	399		399	2078	1586
0	2	9	0	9	240	262	955	443	0	3	10	0	10	436		436	452		452	2931	2110
10	2	9	0	9	256	321	1224	394	10	3	9	0	9	353		353	437		437	1952	1285
10	2	9	0	9	295	363	1255	787	10	4	9	6	3	463	302	161	351	198	153	2013	2328
10	2	9	0	9	275	357	1513	910	10	4	10	7	3	372	270	102	295	183	112	616	1569
10	1	9	0	9	285	312	1371	679	10	4	9	0	9	402		402	385		385	1903	1668
10	1	9	0	9	291	306	1417	1240	10	3	10	0	10	397		397	446		446	1864	1970
10	1	9	0	9	282	335	1798	1564	10	4	9	0	9	401		401	411		411	2253	1486
10	2	10	0	10	287	370	1502	1005	10	3	10	0	10	444		444	506		506	3199	1826
10	2	9	0	9	262	275	1107	881	10	3	10	0	10	425		425	488		488	2428	2098
10	1	9	0	9	248	322	1118	1203	10	4	10	0	10	445		445	503		503	2383	2050
10	2	9	0	9	250	260	1071	577	10	4	10	3	7	379	106	273	391	98	293	1333	1550
10	1	9	0	9	265	264	1299	1316	10	3	10	0	10	397		397	486		486	2443	2034
10	1	10	0	10	348	367	2124	2261	10	3	10	0	10	395		395	481		481	2532	1944
10	1	10	0	10	329	373	2108	2103	10	3	10	0	10	492		492	543		543	4149	2373
10	1	10	0	10	278	338	1497	1752	10	4	10	5	5	547	283	264	477	197	280	2761	2675
10	2	10	0	10	280	301	1430	339	10	4	10	0	10	443		443	465		465	2592	2186
10	1	8	0	8	251	281	1369	795	10	4	10	4	6	447	181	266	375	98	277	1507	2311
10	1	9	0	9	268	294	1302	868	10	3	10	5	5	431	218	213	333	137	196	1466	1907
10	2	4	0	4	112	112	466	115	10	3	8	2	6	319	83	236	299	49	250	1181	1371
10	2	10	0	10	272	325	1271	534	10	4	9	1	8	350	40	310	343	29	314	1474	1772
10	2	10	0	10	281	306	1615	799	10	3	9	0	9	397		397	412		412	1989	2124

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter in mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

*** (410 x 0.037254901/9) x [1 - 0.05 x (0/9)] = 1.697 is the f-corrected diameter value in mm/seedling, and (286/10) x [1 + 0.5 x (2/10)] = 31.46 is the f-corrected height value in mm/seedling.

Continued: Raw data from the field experiment on mineral soil substrate.

Pollen levels*	Blocks	Number of surviving seedling/ plot	Number of browsed shoots/ plot	Number of non-browsed shhots/ plot	Diameter** total/plot	Height mm/plot	Shoot dry weight mm/plot	Root dry weight mg/plot	Pollen levels*	Blocks	Number of surviving seedling/ plot	Number of browsed shoots/ plot	Number of non-browsed shhots/ plot	Diameter** total/plot	Diam.** of browsed shoots total/plot	Diam.** of non-browsed shoots total/plot	Height mm/plot	Height of browsed shoots mm/plot	Height of non-browsed shoots mm/plot	Shoot dry weight mm/plot	Root dry weight mg/plot
50	1	10	0	10	355	372	2363	1614	50	3	10	2	8	364	74	290	374	53	321	1455	1420
50	2	9	0	9	299	355	1419	785	50	3	10	0	10	482		482	491		491	2787	1900
50	2	10	0	10	344	390	1907	2326	50	4	10	2	8	454	103	351	471	79	392	2172	2181
50	1	10	0	10	313	390	1573	1036	50	3	10	0	10	330		330	428		428	1691	1826
50	1	9	0	9	281	325	1608	1578	50	3	9	0	9	447		447	452		452	2772	2038
50	2	10	0	10	338	357	2005	1398	50	3	8	0	8	410		410	397		397	2985	1905
50	2	10	0	10	315	361	1554	1357	50	4	10	0	10	450		450	514		514	2853	2532
50	1	10	0	10	298	353	1447	1667	50	4	10	0	10	436		436	464		464	2150	2110
50	2	1	0	1	24	30	99	21	50	3	10	0	10	442		442	470		470	2374	2077
50	1	8	0	8	235	270	1066	421	50	4	10	1	9	585	52	533	559	41	518	4424	2517
50	2	8	0	8	236	219	814	209	50	4	8	0	8	380		380	416		416	2040	1758
50	1	10	0	10	318	363	1929	1762	50	4	7	7	0	386	386		221	221		880	1907
50	2	10	0	10	288	350	1839	1067	50	4	9	0	9	452		452	499		499	3230	2391
50	1	10	0	10	282	348	1543	891	50	3	8	0	8	371		371	363		363	2149	1696
50	1	10	0	10	282	347	1381	1297	50	4	9	9	0	400	400		270	270		570	1865
50	1	10	0	10	278	354	1510	1498	50	3	9	0	9	390		390	452		452	2266	1795
50	2	9	0	9	254	260	880	334	50	3	9	0	9	355		355	390		390	1935	1454
50	2	10	0	10	302	340	1951	440	50	3	9	1	8	408	52	356	407	35	372	2050	1835
50	2	10	0	10	254	323	1084	577	50	4	10	0	10	456		456	479		479	2986	2378
50	1	10	1	9	353	399	3365	1943	50	4	9	1	8	415	47	368	424	29	395	2518	1931
100	2	10	0	10	291	365	1600	727	100	3	10	0	10	339		339	435		435	1387	1100
100	1	10	0	10	299	349	1490	721	100	3	9	0	9	427		427	420		420	3170	1986
100	1	9	0	9	329	423	2445	2557	100	4	10	1	9	451	44	407	474	38	436	2897	2215
100	1	10	0	10	300	366	1672	1286	100	4	9	2	7	469	99	370	460	93	367	3561	2183
100	1	10	0	10	276	362	1708	1518	100	4	10	0	10	411		411	435		435	2317	1833
100	1	9	0	9	320	352	2258	2075	100	3	10	0	10	491		491	507		507	3276	1983
100	1	10	0	10	323	371	1653	2120	100	3	10	0	10	450		450	472		472	2976	1931
100	2	8	0	8	247	301	1350	664	100	4	10	0	10	411		411	484		484	2345	2178
100	1	10	0	10	319	377	1748	1688	100	4	9	7	2	418	331	87	275	191	84	918	2218
100	1	10	0	10	289	355	1489	1943	100	4	10	7	3	468	330	138	367	235	132	1529	2342
100	1	10	0	10	286	366	1752	1870	100	3	10	0	10	363		363	410		410	1807	1548
100	2	9	0	9	286	324	1366	346	100	3	10	0	10	367		367	437		437	2223	1887
100	1	10	0	10	345	397	2661	2194	100	3	8	0	8	361		361	432		432	2423	1790
100	2	10	0	10	294	333	1267	319	100	4	9	0	9	380		380	440		440	2628	1685
100	2	9	0	9	260	287	1290	346	100	4	8	3	5	353	148	205	351	120	231	1638	1400
100	2	10	0	10	278	332	1511	367	100	3	9	5	4	411	228	183	397	188	209	1789	1727
100	2	10	0	10	334	350	1674	540	100	3	10	0	10	331		331	377		377	1650	1297
100	2	10	0	10	296	343	1728	615	100	4	10	0	10	421		421	511		511	2516	2114
100	2	10	0	10	300	348	1775	889	100	4	9	2	7	339	87	252	309	56	253	1176	1610
100	2	10	0	10	308	359	1984	952	100	3	10	0	10	391		391	539		539	2754	2165

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter im mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

*** (410 x 0.037254901/9) x [1 + 0.05 x (0/9)] = 1.697 is the f-corrected diameter value in mm/seedling, and (286/10) x [1 + 0.5 x (2/10)] = 31.46 is the f-corrected height value in mm/seedling.

Mean and standard error (SE) of several characteristics of jack pine seedlings growing on mineral soil substrate, under field conditions.

Variable	Block	Pollen level 0x	10x	50x	100x
Number of surviving seedlings per pot	Block 1	9.4 (0.3)	9.2 (0.2)	9.7 (0.2)	9.8 (0.1)
	Block 2	8.1 (0.7)	8.9 (0.6)	8.7 (0.9)	9.6 (0.2)
	Block 3	9.6 (0.2)	9.6 (0.2)	9.2 (0.2)	9.6 (0.2)
	Block 4	9.0 (0.3)	9.6 (0.2)	9.2 (0.3)	9.4 (0.2)
Diameter mm/seedling	Block 1	1.124(0.026)	1.151(0.027)	1.149(0.033)	1.177(0.037)
	Block 2	1.061(0.026)	1.075(0.019)	1.114(0.041)	1.124(0.19)
	Block 3	1.478(0.060)	1.569(0.038)	1.629(0.069)	1.533(0.070)
	Block 4	1.715(0.036)	1.649(0.066)	1.794(0.059)	1.635(0.047)
	4-block means	1.344(0.047)	1.361(0.045)	1.422(0.054)	1.367(0.042)
Height mm/seedling	Block 1	36.0 (0.9)	34.7 (0.8)	36.3 (0.6)	38.0 (1.1)
	Block 2	32.2 (1.1)	33.3 (1.4)	33.8 (1.3)	34.9 (0.5)
	Block 3	44.1 (1.0)	46.0 (2.0)	46.0 (1.3)	46.3 (1.7)
	Block 4	43.7 (3.1)	41.6 (2.0)	46.5 (2.8)	43.6 (2.3)
	4-block means	39.0 (1.2)	38.9 (1.1)	40.6 (1.2)	40.7(1.0)
Shoot dry mass mg/seedling	Block 1	161.8 (7.3)	166.7 (9.7)	182.3 (19.7)	194.0 (15.4)
	Block 2	130.3 (7.8)	138.4 (5.6)	149.0 (13.7)	161.6 (6.2)
	Block 3	224.5 (22.0)	239.4 (25.4)	247.5 (20.8)	246.1 (23.5)
	Block 4	245.8 (33.8)	196.8 (21.4)	254.1 (34.3)	228.5 (27.5)
	4-block means	190.6 (12.5)	185.3 (10.3)	208.2 (13.3)	207.6 (10.9)
Root dry mass mg/seedling	Block 1	129.2 (2.2)	147.4 (15.6)	139.9 (14.3)	1184.9 (18.6)
	Block 2	56.07 (15.8)	69.1 (8.9)	88.8 (21.1)	60.3 (7.6)
	Block 3	178.7 (11.8)	196.5 (8.9)	196.4 (9.1)	182.7 (12.3)
	Block 4	222.9 (10.4)	204.0 (12.7)	235.1 (7.6)	209.8 (8.6)
	4-block means	146.7 (12.7)	154.3 (10.3)	165.0 (11.2)	159.4 (11.1)
Shoot plus root dry mass mg/seedling	Block 1	291.0 (31.1)	314.1 (23.4)	322.2 (30.6)	378.9 (31.8)
	Block 2	186.4 (22.6)	207.5 (12.6)	237.8 (32.0)	221.9 (13.3)
	Block 3	403.3 (32.4)	435.9 (30.6)	443.9 (29.0)	428.9 (34.7)
	Block 4	468.8 (39.9)	400.8 (30.3)	489.3 (37.7)	438.4 (29.6)
	4-block means	337.4 (23.2)	339.6 (18.6)	373.3 (22.3)	367.0 (19.5)

APPENDIX XIII

Levels of jack pine pollen application to needle litter substrate, and per plot jack pine seedling diameter, height, shoot dry mass and root dry mass under field conditions

Raw data of the field experiment on needle litter substrate.

Pollen levels*	Blocks	Number of surviving seedling/ plot	Number of browsed shoots/ plot	Number of non-browsed shoots/ plot	Diameter* total/plot	Height mm/plot	Shoot dry weight mg/plot	Root dry weight mg/plot	Pollen levels*	Blocks	Number of surviving seedling/ plot	Number of browsed shoots/ plot	Number of non-browsed shoots/ plot	Diameter** total/plot	Diam.** of browsed shoots total/plot	Diam.** of non-browsed shoots mm/plot	Height mm/plot	Height of browsed shoots mm/plot	Height of non-browsed shoots mm/plot	Shoot dry weight mg/plot	Root dry weight mg/plot
0	2	7	1	6	214	230	1225	857	0	3	8	8	0	359	359		227	227		724	1260
0	2	5	0	5	150	179	870	471	0	3	9	8	1	333	299	34	284	249	35	871	1169
0	1	10	0	10	330	346	1991	1582	0	4	9	8	1	366	330	36	289	254	35	1354	1525
0	2	10	0	10	321	324	1890	1517	0	3	9	4	5	339	161	178	318	122	196	1335	1302
0	1	9	0	9	301	307	1775	1554	0	4	8	5	3	313	204	109	275	156	119	970	1298
0	2	6	0	6	194	213	1190	1068	0	4	10	7	3	333	245	88	311	186	125	800	1421
0	2	4	0	4	124	125	495	722	0	4	9	8	1	299	271	28	291	256	35	692	1034
0	2	7	0	7	240	266	1545	1224	0	4	10	5	5	480	246	234	455	196	259	3226	2110
0	2	6	0	6	234	258	1627	1319	0	3	7	7	0	283	283		211	211		275	761
0	1	4	0	4	139	158	981	904	0	4	5	1	4	177	45	132	191	39	152	845	631
0	1	5	0	5	178	195	1166	1186	0	4	5	3	2	214	132	82	183	105	78	934	828
0	1	7	0	7	205	232	1129	1148	0	4	8	0	8	290		290	326		326	1623	1111
0	1	8	0	8	257	284	1617	1632	0	3	6	0	6	274		274	283		283	1487	1117
0	1	8	0	8	280	323	1925	1569	0	3	9	1	8	355	36	319	439	34	405	1837	1682
0	2	2	2	0	73	49	215	433	0	4	9	0	9	394		394	454		454	2853	1662
0	2	6	0	6	168	187	730	890	0	4	7	4	3	263	163	100	235	125	110	802	867
0	2	2	0	2	61	63	294	312	0	3	8	5	3	288	193	95	263	135	128	741	1158
0	1	7	0	7	214	226	1349	1188	0	3	7	0	7	231		231	280		280	992	985
0	1	8	1	7	232	273	1237	1097	0	3	6	0	6	223		223	242		242	996	763
0	1	5	0	5	170	172	901	815	0	3	9	0	9	391		391	429		429	2784	1677
10	2	8	0	8	244	255	1282	808	10	3	9	5	4	365	214	151	363	164	199	1830	1304
10	2	4	0	4	117	115	499	230	10	3	8	8	0	355	355		328	328		1458	1346
10	2	1	0	1	43	33	194	168	10	4	8	7	1	314	274	40	230	185	45	738	931
10	1	10	0	10	364	403	2642	2199	10	3	7	2	5	277	79	198	274	30	244	1359	1266
10	1	8	0	8	290	271	1656	1364	10	3	4	4	0	187	187		122	122		276	823
10	2	4	0	4	106	103	346	427	10	4	8	7	1	334	296	38	251	206	45	812	1279
10	1	7	0	7	254	271	1743	1559	10	4	8	5	3	344	213	131	323	164	159	2066	1314
10	1	10	0	10	349	391	2493	2044	10	4	8	3	5	346	142	204	344	106	238	1858	1389
10	1	6	0	6	215	239	1380	1129	10	3	10	10	0	488	488		413	413		2405	2348
10	2	4	0	4	124	128	644	661	10	3	9	2	7	318	71	247	315	45	270	1557	1062
10	2	10	0	10	350	401	2636	1884	10	4	9	9	0	320	320		241	241		546	1297
10	2	9	0	9	291	291	1693	1611	10	3	8	3	5	337	125	212	304	89	215	1696	1386
10	1	8	0	8	286	313	1900	1604	10	4	7	5	2	300	222	78	277	186	91	1135	1151
10	1	4	0	4	129	129	769	800	10	3	8	5	3	300	195	105	285	166	119	858	1138
10	1	7	0	7	211	245	1243	1248	10	3	10	2	8	410	79	331	460	77	383	2854	1976
10	2	6	0	6	220	261	1659	1442	10	3	10	7	3	337	245	92	299	188	111	647	1234
10	1	4	0	4	116	109	565	579	10	4	10	0	10	378		378	364		364	1990	1175
10	2	7	0	7	194	200	1078	1058	10	4	4	0	4	152		152	152		152	917	572
10	1	7	0	7	229	221	1443	1380	10	4	7	3	4	356	157	199	348	109	239	2548	1470
10	2	4	1	3	129	142	813	799	10	4	9	4	5	373	167	206	354	147	207	1765	1392

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter in mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

Continued: Raw data of the field experiment on needle litter substrate.

Pollen levels*	Blocks	Number of surviving seedling/ plot	Number of browsed shoots/ plot	Number of non-browsed shoots/ plot	Diameter* total/plot	Height mm/plot	Shoot dry weight mg/plot	Root dry weight mg/plot	Pollen levels*	Blocks	Number of surviving seedling/ plot	Number of browsed shoots/ plot	Number of non-browsed shoots/ plot	Diameter** total/plot	Diam.** of browsed shoots total/plot	Diam.** of non-browsed shoots mm/plot	Height mm/plot	Height of browsed shoots mm/plot	Height of non-browsed shoots mm/plot	Shoot dry weight mg/plot	Root dry weight mg/plot
50	2	6	0	6	172	181	848	473	50	3	8	7	1	370	325	45	302	262	40	1814	1882
50	2	4	0	4	120	141	606	180	50	4	8	6	2	332	240	92	275	198	77	1348	1140
50	2	3	0	3	97	92	414	339	50	4	10	6	4	374	233	141	300	167	133	1055	1204
50	2	6	0	6	195	199	1114	967	50	3	8	7	1	333	293	40	258	213	45	1097	1325
50	2	1	0	1	29	37	188	126	50	4	7	5	2	290	227	63	316	234	82	1612	1385
50	1	9	0	9	282	290	1633	1393	50	4	7	5	2	289	224	65	256	181	75	926	1244
50	1	10	0	10	300	317	1355	1235	50	3	6	3	3	242	125	117	211	104	107	1040	1106
50	1	10	0	10	330	356	2188	1655	50	3	9	8	1	377	339	38	327	271	56	1020	1768
50	2	9	0	9	275	289	1327	1375	50	3	9	1	8	404	51	353	527	63	464	3585	2153
50	1	7	0	7	249	260	1616	1329	50	4	10	5	5	408	210	198	384	156	228	1824	1932
50	2	8	0	8	253	295	1347	1449	50	4	8	5	3	361	253	108	261	167	94	1195	1247
50	2	3	0	3	94	97	450	419	50	3	9	2	7	375	93	282	418	88	330	2483	1585
50	1	5	0	5	149	156	778	692	50	4	8	5	3	317	198	119	279	147	132	1275	1499
50	1	8	0	8	261	299	1608	1584	50	3	6	5	1	215	183	32	137	99	38	259	787
50	1	3	0	3	122	129	917	840	50	4	7	0	7	312		312	334		334	2340	1545
50	2	6	0	6	207	217	1293	1249	50	4	7	0	7	321		321	323		323	1907	1160
50	2	3	0	3	105	105	670	601	50	4	8	4	4	355	180	175	296	131	165	1335	1180
50	1	9	0	9	291	311	2104	1786	50	3	4	1	3	178	50	128	189	35	154	747	737
50	1	9	0	9	332	374	2827	2236	50	3	8	2	6	337	80	257	361	51	310	2008	1240
50	1	6	0	6	170	204	1312	1014	50	3	9	0	9	359		359	400		400	2434	1781
100	2	6	0	6	174	206	761	524	100	3	6	5	1	299	250	49	235	192	43	1282	1214
100	2	4	1	3	123	136	520	356	100	4	8	6	2	289	216	73	189	117	72	671	1029
100	2	5	0	5	164	185	1078	817	100	3	7	7	0	345	345		235	235		1035	1348
100	1	7	0	7	193	244	944	531	100	3	7	7	0	270	270		160	160		236	1039
100	1	9	0	9	315	338	2125	1620	100	4	10	1	9	363	30	333	454	28	426	2596	1562
100	1	7	0	7	219	230	1243	1109	100	4	9	7	2	323	252	71	260	186	74	805	1436
100	2	9	0	9	310	312	1945	1778	100	3	10	10	0	432	432		300	300		636	1448
100	2	10	0	10	337	344	2257	1912	100	3	6	6	0	245	245		219	219		761	1003
100	1	9	0	9	291	334	1686	1673	100	3	7	0	7	304		304	350		350	2214	1425
100	1	7	0	7	229	247	1477	1385	100	4	7	3	4	298	139	159	287	105	182	1380	1036
100	2	4	0	4	125	133	569	660	100	4	6	4	2	227	157	70	205	129	76	805	703
100	1	8	0	8	257	278	1504	1590	100	3	8	4	4	328	184	144	364	195	169	1490	1310
100	1	9	0	9	301	325	1808	1762	100	3	9	1	8	388	40	348	462	27	435	2244	1724
100	1	3	0	3	106	132	881	735	100	4	9	4	5	399	184	215	381	143	238	2120	1390
100	2	6	0	6	199	220	1104	1108	100	3	10	2	8	445	95	350	458	69	389	2725	2030
100	2	7	0	7	247	257	1566	1359	100	3	10	1	9	455	58	397	573	81	492	3866	1986
100	1	4	0	4	130	163	998	908	100	4	9	2	7	357	94	263	368	63	305	1643	1373
100	2	1	0	1	31	32	155	136	100	4	10	2	8	476	95	381	520	99	421	3293	2147
100	2	2	0	2	67	70	314	308	100	4	7	7	0	287	287		226	226		653	1251
100	1	5	0	5	184	200	1420	1054	100	4	6	4	2	275	196	79	242	147	95	1123	927

* In multiples of annual jack pine pollen rain of 8.5 kg/ha.

**For diameter in mm/pot, multiply each value by the conversion factor = 0.037254901 of the readings from the dissecting microscope.

Mean and standard error (SE) of several characteristics of jack pine seedlings growing on needle litter substrate, under field conditions.

Variable	Block	Pollen level 0x	10x	50x	100x
Number of surviving seedlings per pot	Block 1	7.1 (0.6)	7.1 (0.7)	7.6 (0.7)	6.8 (0.7)
	Block 2	5.5 (0.8)	5.7 (0.9)	4.9 (0.8)	5.4 (0.9)
	Block 3	7.8 (0.4)	8.3 (0.6)	7.6 (0.5)	8.0 (0.5)
	Block 4	8.0 (0.6)	7.8 (0.5)	8.0 (0.4)	8.1 (0.5)
Diameter mm/seedling	Block 1	1.217 (0.028)	1.265 (0.032)	1.231 (0.044)	1.226 (0.030)
	Block 2	1.208 (0.039)	1.208 (0.057)	1.175 (0.025)	1.210 (0.023)
	Block 3	1.470 (0.049)	1.526 (0.056)	1.561 (0.035)	1.636 (0.042)
	Block 4	1.453 (0.057)	1.541 (0.050)	1.572 (0.032)	1.517 (0.050)
	4-block means	1.337 (0.029)	1.385 (0.034)	1.385 (0.034)	1.397 (0.035)
Height mm/seedling	Block 1	35.7 (0.9)	35.7 (1.4)	35.8 (1.3)	37.3 (1.1)
	Block 2	33.6 (1.6)	33.1(1.7)	33.9 (0.8)	34.8 (0.5)
	Block 3	38.2 (2.4)	37.7 (1.6)	40.6 (3.1)	41.2 (3.4)
	Block 4	37.5 (2.4)	37.3 (2.2)	38.3 (1.9)	38.1 (2.6)
	4-block means	36.3 (0.9)	36.0 (0.9)	33.2 (1.0)	37.9 (1.1)
Shoot dry mass mg/seedling	Block 1	200.6 (9.9)	215.4 (12.0)	219.5 (18.1)	216.3 (15.7)
	Block 2	172.8 (15.9)	181.2 (18.4)	170.9 (9.8)	177.6 (12.7)
	Block 3	153.7 (25.8)	173.2 (22.9)	207.6 (31.6)	199.7 (35.1)
	Block 4	174.1 (27.6)	189.5 (28.5)	190.1 (21.9)	179.2 (25.6)
	4-block means	175.1 (10.5)	189.8 (10.6)	197.1 (10.9)	193.2 (11.8)
Root dry mass mg/seedling	Block 1	182.8 (10.1)	192.6 (7.4)	186.6 (15.2)	187.5 (14.6)
	Block 2	164.2 (12.3)	155.7 (17.0)	140.6 (16.4)	156.2 (12.9)
	Block 3	151.3 (8.7)	168.9 (11.8)	186.5 (10.5)	181.6 (7.3)
	Block 4	153.9 (9.6)	154.8 (8.6)	170.9 (9.5)	156.4 (8.4)
	4-block means	163.1 (5.3)	168.0 (6.2)	171.2 (7.0)	170.4 (5.8)
Shoot plus root dry mass mg/seedling	Block 1	383.4 (19.2)	408.1 (18.8)	406.1 (32.9)	403.9 (28.9)
	Block 2	337.0 (21.6)	336.9 (33.8)	311.5 (24.4)	333.9 (24.5)
	Block 3	304.9 (33.3)	342.1 (29.8)	394.1 (39.4)	381.2 (41.4)
	Block 4	328.0 (35.6)	344.3 (34.9)	361.0 (29.3)	335.7 (31.0)
	4-block means	338.4 (14.4)	357.8 (15.1)	368.2 (16.4)	363.6 (16.2)

APPENDIX XIV

Uncorrected ANOVA results of the effect of pollen levels on jack pine seedling diameter, height, shoot dry mass and root dry mass under field conditions.

Uncorrected ANOVA results of the effect of pollen levels on jack pine seedling growth under field conditions.

Variable	Factor	DF	Mineral soil		Needle litter	
			MS	F	MS	F
Diameter log(mm/seedling)	Pollen level (PL)	3	0.0035	2.01	0.0025	1.6
	Block (B)	3	0.3525	194.6***	0.1352	79.3***
	PL x B	9	0.001726	0.95	0.0016	0.91
	Error	144	0.001811		0.0017	
Height log(mm/seedling)	Pollen level (PL)	3	0.0052	4.07*	0.0033	8.03**
	Block (B)	3	0.1574	44.9***	0.0253	4.85***
	PL x B	9	0.0013	0.37	0.0004	0.08
	Error	144	0.0035		0.0053	
Shoot dry mass log(mg/seedling)	Pollen level (PL)	3	0.0308	6.06**	0.0232	4.1**
	Block (B)	3	0.3398	14.5***	0.1143	3.29**
	PL x B	9	0.0051	0.22	0.0057	0.16
	Error	144	0.0234		0.0348	
Root dry mass log(mg/seedling)	Pollen level (PL)	3	0.0572	1.82	0.0019	0.15
	Block (B)	3	2.6313	88.2***	0.0731	5.98***
	PL x B	9	0.0315	1.05	0.0121	0.99
	Error	144	0.0298		0.0122	
Shoot plus root dry mass log(mg/seedling)	Pollen level (PL)	3	0.0277	2.42	0.0096	1.2
	Block (B)	3	0.9207	65.3***	0.0636	4.17***
	PL x B	9	0.0113	0.8	0.008	0.52
	Error	144	0.0141		0.0152	

*, **, *** Significance levels of 10%, 5%, and 1%, respectively.

APPENDIX XV

Response of jack pine seedling shoot dry mass and shoot plus root dry mass to pollen additions on needle litter and mineral soil substrate under greenhouse and field conditions

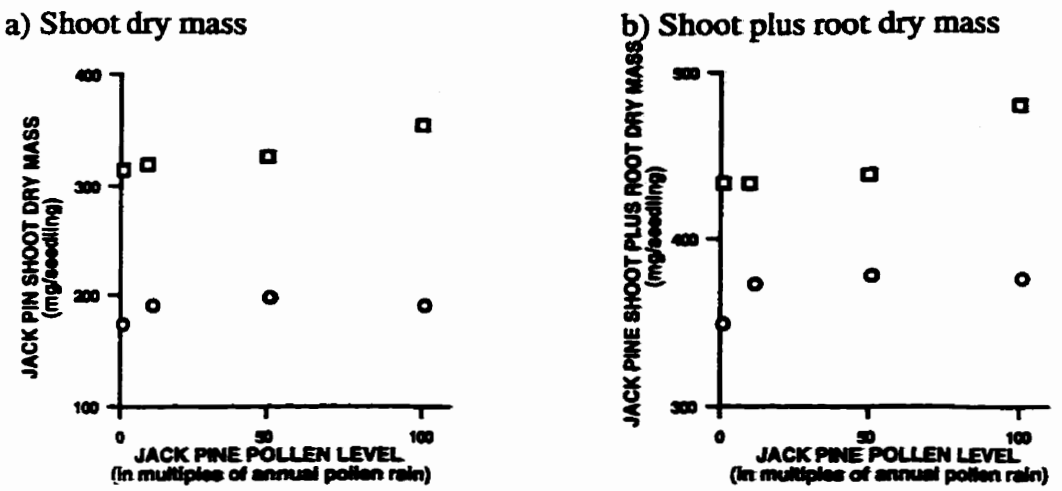

Figure XIV.1. Effects of jack pine pollen on dry masses of shoot and shoot plus root of one-growing-season old jack pine seedlings, under equivalent pollen levels in needle litter substrate in greenhouse (squares) and field (circles) conditions.

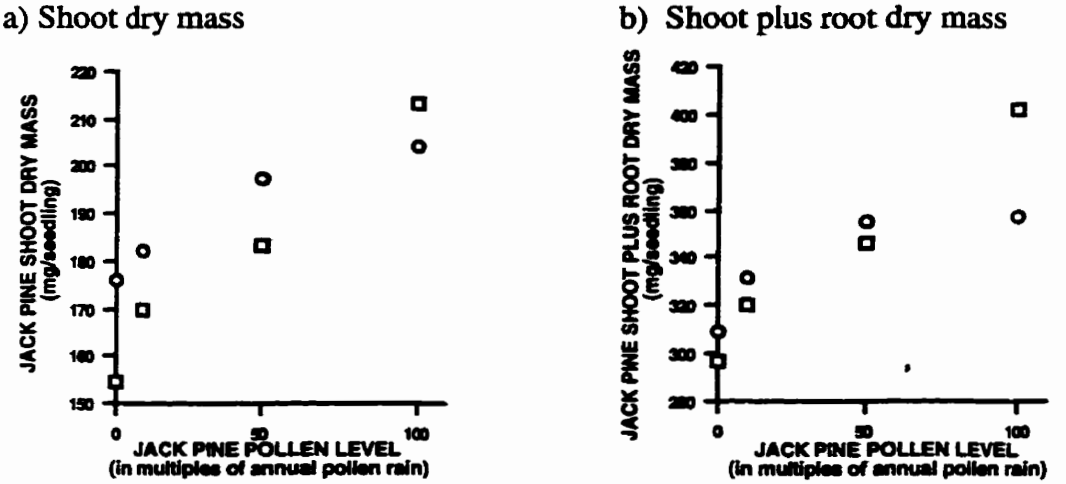

Figure XIV.2. Effects of jack pine pollen on dry masses of shoot and shoot plus root of one-growing-season old jack pine seedlings, under equivalent pollen levels in mineral soil substrate in greenhouse (squares) and field (circles) conditions.