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EPIDEMIOLOGICAL STUDIES OF WESTERN GALL RUST (*Endocronartium harknessii*) ON LODGEPOLE PINE (*Pinus contorta* var. *latifolia*)

BY

KAN -FA CHANG

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN

FOREST PATHOLOGY

DEPARTMENT OF PLANT SCIENCE

EDMONTON, ALBERTA

SPRING 1990

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ISBN 0-315-60172-8

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NAME OF AUTHOR: KAN -FA CHANG

TITLE OF THESIS: EPIDEMIOLOGICAL STUDIES OF WESTERN GALL RUST (*Endocronartium harknessii*) ON LODGEPOLE PINE (*Pinus contorta* var. *latifolia*)

DEGREE: DOCTOR OF PHILOSOPHY

YEAR THIS DEGREE GRANTED: 1990

Kan Fa Chang

KAN -FA CHANG

300 Reeves Way,
Edmonton, Alberta
Canada T6R 2B9

Date: April 26, 1990

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

THE UNDERSIGNED CERTIFY THEY HAVE READ, AND RECOMMEND TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH FOR ACCEPTANCE, A THESIS ENTITLED EPIDEMIOLOGICAL STUDIES OF WESTERN GALL RUST (*Endocronartium harknessii*) ON LODGEPOLE PINE (*Pinus contorta* var. *latifolia*) SUBMITTED BY KAN -FA CHANG IN PARTIAL FUFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN PLANT PATHOLOGY.

Supervisor, Dr. P. V. Blenis

Dr. Y. Hiratsuka

Dr. J. P. Tewari

Dr. V. J. Lieffers

Dr. E. J. van der Kamp
(External Examiner)

Date: April 5, 1990

I would like to dedicate this thesis to the memory of my father

Da -Sen Chang

Abstract

Spore release of *Endocronartium harknes* (J. P. Moore) Y. Hiratsuka was studied by placing two spore traps beside each of tw sporulating galls. On rainless days, most spores were trapped between 0800 and 1800 hr, when relative humidity (RH) was relatively low, and temperature, light intensity, and wind velocity were relatively high. On rainy days, most spores were released, on average, over a longer time interval than on dry days. Spore production stimulated by high RH was proved by moving galls alternatively between humid and dry conditions. Thus, it is proposed that the diurnal periodicity of spore release on dry days results from the production of spores during the night and subsequent passive release during the next day. Fresh and liquid-nitrogen-stored spores impacted on spider web and plastic threads were incubated in the darkness at the combination of 6, 15, and 24 ^{o}C and RH of 39% and 98% for 1, 2, 4, 8, and 16 days. Repeated-measures of ANOVA indicated that fresh and stored spores survived equally well. Survival was, however, significantly affected by temperature, RH, time, and all interactions except temperature x RH. Spore survival under full sunlight decreased linearly with time of exposure and averaged 33 percent after 12 hr exposure. Thus, spores have considerable potential for long distance dispersal.

Spore dispersal and disease gradients were studied in a cut block located near Hinton, Alta., at an elevation of about 1,280 m. spore concentration at 0.5 m above ground level decreased sharply between 2 and 5 m from an infected stand, and more gradually between 10 and 80 m from that stand. Spore concentrations and the slopes of spore dispersal gradients were significantly affected by wind values, although there was considerable unexplained variation. Seedling infection between 0 and 120 m from the stand edge averaged 4.2%, with an average of 7.7% infection occurring in the plots 2.5 m from the edge. Regression analysis failed to detect a disease gradient.

Acknowledgements

I wish to express my sincere gratitude to my adviser, Dr. P. V. Blenis, for his guidance during the course of this study. Special thanks are extended to Drs. Y. Hiratsuka, J. P. Tewari, V. J. Lieffers, and B. J. van der Kamp for their counseling and critical review of the thesis.

Financial supports from the Natural Sciences and Engineering Research Council of Canada and the Canadian Forest Service Human Resources Development Program are gratefully acknowledged.

I also thank Mr. W. Mattes of Weiwood of Canada Ltd. for his cooperation in using the experimental field in Hinton. The following assistance was appreciated: D. Alexander, E. Johnson, D. Holland, and G. Wonders for the field and laboratory studies; data analysis was adviced by T. Taerum, C. K. Humphrey, R. Weingardt, and H. Klein-Gebbinck; K. Conn and N. Leenders were consulted for graphs; T. Tribe prepared some photographs; fruit flies were provided by E. Wolyskyn; and spiders were identified by G. Hilchie.

I am indebted to S. Brezden for her assistance in various aspects of this thesis. Thanks are extended to my wife, Dr. Sheau-fang Hwang for her patience and encouragement during the course of this study.

TABLE OF CONTENTS

Chapter		Page
1.	INTRODUCTION	1
	References	19
2.	MECHANISM AND PATTERN OF SPORE RELEASE BY ENDOCRONARTIUM HARKNESSII	27
	References	51
3.	SURVIVAL OF *ENDOCRONARTIUM HARKNESSII* PERIDERMIOID TELIOSPORES IN A SIMULATED AIRBORNE STATE	52
	References	70
4.	DISPERSAL AND DISEASE GRADIENTS OF WESTERN GALL RUST	72
	References	87
5.	GENERAL Discussion and Conclusion	89
	References	100
	CURRICULUM VITAE	104

TABLE OF CONTENTS

Chapter		Page
1.	Introduction	1
	References	19
2.	Mechanism and Pattern of Spore Release by *Endocronartium harknessii*	27
	References	51
3.	Survival of *Endocronartium harknessii* Peridermioid Teliospores in a Simulated Airborne	52
	References	70
4.	Dispersal and Disease Gradients of Western Gall Rust	72
	References	87
5.	General Discussion and Conclusion	89
	References	100
	Curriculum Vitae	104

LIST OF TABLES

Table		Page
3.1	Analysis of variance table showing the effects of spore freshness (fresh vs. stored), temperature (T), relative humidity (RH), and time (TI) on the survival of *Endocronartium harknessii* spores	68
3.2	A comparison of the outdoor survival of the peridermioid teliospores of *Endocronartium harknessii* with the urediniospores of three other rusts	69
4.1	Slopes (b), correlation coefficients (r^2), and the average number of peridermioid teliospores (spores/m^3 air) caught at various distances from the eastern edge of an infected lodgepole pine stand in June, 1985 and June, 1986	84
4.2	Summary of climatic data for the two-hour periods of spore sampling in June, 1985 and 1986	85
4.3	Number of pine seedlings infected by gall rust (and total number of seedlings) in 5 x 15 m plots, at eight distances along seven transects extending perpendicularly from the edge of an infected stand	86

LIST OF FIGURES

Figure — Page

1.1 Sporulating gall and witches' broom on a lodgepole pine seedling naturally infected by western gall rust in an open field. 3

1.2 A basal stem gall causing poor growth in a 20-year-old lodgepole pine. 3

1.3 The stunting effect of western gall rust on a 20-year-old lodgepole pine. 3

1.4 Twenty-year-old lodgepole pine killed by galls girdling the stem, only remaining lower branch is alive. 3

2.1 Design of spore traps used in the field. Top view of a 24-h impaction spore trap. 38

2.2 Arrangement of spore traps used in the field. Two impaction spore traps (st) were connected to 12-V battery (b). 38

2.3 Apparatus for simulating diurnal fluctuations of light, relative humidity (RH), and wind. The acrylic chamber was placed inside an incubator kept at 24.5 ± 0.5 °C and 2.16 ± 0.22 kPa VPD (30 ± 5% RH). 40

2.4 Apparatus for simulating diurnal fluctuations of light, relative humidity, and wind. Close-up view of part of the chamber. 40

2.5 Changes in spore concentration (x $10^4/m^3$) of *Endocronartium harknessii* in relation to climatic conditions in Hinton, Alberta during June, 1985. 42

2.6 Changes in spore concentration (x $10^4/m^3$) of *Endocronartium harknessii* in relation to climatic conditions in Hinton, Alberta during June, 1986. 44

2.7 Spore release by *Endocronartium harknessii* in response to changes of vapor pressure deficit, exposure to fluorescent light, and air speed. 46

2.8 Spore production under conditions of low and high VPD. 48

2.9 The process of peridial rupture in *Endocronartium harknessii*. Aecium with intact peridium after 24 h on a laboratory bench. 50

2.10 The process of peridial rupture in *Endocronartium harknessii*. After 9 h in a dew chamber, the aecium has expanded. 50

2.11 The process of peridial rupture in *Endocronartium harknessii*. After an additional 2 days on the laboratory bench, many of the spores had dried up and fallen away, thus making the openings in the peridium more conspicuous. 50

3.1 Apparatus for exposing spores to constant RH and temperature in a simulated airborne state. 61

3.2 Apparatus for exposing spores to sunlight.---------- 61

3.3 Survival of spores of *Endocronartium harknessii* incubated at 39% and 98% RHs and 6, 15, and 24 °C for 1, 2, 4, 8 and 16 days in darkness.---- 63

3.4 Survival of spores of *Endocronartium harknessii* exposed to full sunlight on clear days from 08:30 to 20:30.---------- 65

3.5 The relationship between temperature, RH, VPD, and sunlight, and the slopes of nine survival curves obtained by placing spores out of doors and regressing percent survival against time.---------- 67

4.1 Map of the experimental site, showing the location of the rotorods, infection plots and surrounding area.---------- 81

4.2 Detailed map of the experimental site, showing the location of the inoculum source, rotorod samplers, and datalogger.---------- 83

Chapter 1. Introduction

Western gall rust (WGR) caused by *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka was first described by H. W. Harkness on ponderosa pine (*Pinus ponderosa* Laws.) in 1876 (Nelson, 1971). In Canada, this disease is common on susceptible hard pines (Baranyay and Stevenson, 1964; Hiratsuka *et al.*, 1966; Gross, 1983) on which it causes globose galls (Figs. 1.1-1.4).

1.1. Occurrence and Distribution

In the past, western gall rust has often been confused with eastern gall rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai) because the two fungi produce morphologically identical galls. The two rusts, however, can be distinguished by certain characteristics such as germ-tube development and nuclear behaviour during spore germination (Anderson and French, 1965; Hiratsuka and Maruyama, 1968; Hiratsuka, 1969; Dietrich *et al.*, 1985). The germ tube length of *C. quercuum* is three times that of *E. harknessii* when spores are germinated on 2% water agar at 18.5 ^{0}C (Anderson and French, 1965). The distribution of both rusts overlap to some extent, and the presence of these two rusts has been reported on a single jack pine tree in northern Minnesota (Anderson, 1968). WGR is considered to be autoecious while eastern gall rust is heteroecious and infects oak (*Quercus* spp.) in the basidiospore stage. So far, WGR is restricted to the North America. The distribution of WGR extends throughout Canada and the northeastern United States and as far south as northern Mexico (Anderson, 1965; Hiratsuka and Maruyama, 1968; Peterson, 1967).

Many hard pines serve as hosts for WGR but there is considerable variability in susceptibility both within and among species of pine (Thomas *et al.*, 1984). In the Pinaceae, 15 species of hard pines are infected under field conditions, while 22 species have been infected in artificial inoculations (Nelson, 1971). In Canada, *P. banksiana* Lamb., *P. contorta* Dougl., *P. mugo* Turra., *P. muricata* D. Don, *P. nigra* Arnold, *P.*

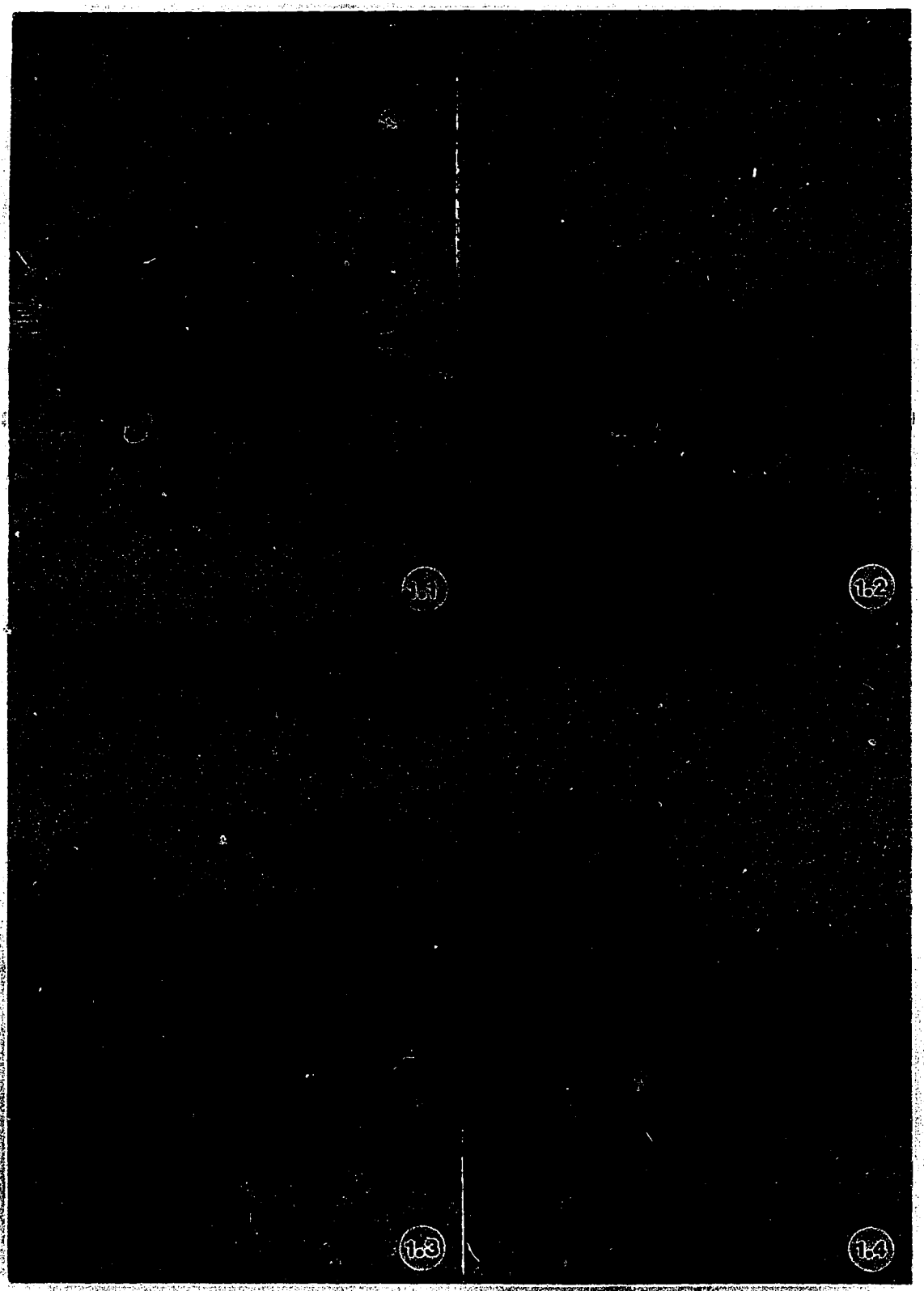
Fig. 1. Symptoms of western gall rust and the effect of the rust on the growth of lodgepole pine.

Fig 1.1. Sporulating gall and witches' broom on a lodgepole pine seedling naturally infected by western gall rust in an open field.

Fig. 1.2. A basal stem gall causing poor growth in a 20-year-old lodgepole pine.

Fig. 1.3. The stunting effect of western gall rust on a 20-year-old lodgepole pine. The infected sapling (left) is 2 m in height while the healthy one (right site) is 4 m tall.

Fig. 1.4. Twenty-year old lodgepole pine killed by galls girdling the stem. Only the lower branch below the main stem gall is alive.


1.1
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pinaster Ait., *P. ponderosa* Laws., *P. radiata* D. Don, and *P. sylvestris* L. have been reported to be susceptible to WGR (Ziller, 1974; Hiratsuka and Powell, 1976).

The mature peridermioid teliospores of WGR, measuring about 17 ~ 24 x 23 ~ 34 µm (Sutherland *et al.*, 1987), are usually orange-yellow, verrucose, and formed in chains. However, an albino type of *E. harknessii* has been reported to be widespread in the Rocky Mountain-Intermountain area (Mielke and Peterson, 1967; Christenson, 1969).

1.2. Damage

WGR infections are usually most numerous on vigorously growing trees because such trees have many young shoots (Bella, 1985; Gross, 1983). In an extremely severe case, more than 18,000 galls occurred on a single 4.6 m high tree (York, 1929). Although a high incidence of branch galls reduces tree growth (Baranyay and Stevenson, 1964), economic loss is mainly caused by degrading and culling of trees with stem galls (Hiratsuka and Powell, 1976; Howse *et al.*, 1981; Peterson, 1961; Walla and Stack, 1979). Pine seedlings with girdling stem galls are stunted and distorted and eventually die out as the stand matures (Figs. 1.1 and 1.4). In addition, trees with stem galls are susceptible to wind or snow load breakage. Van der Kamp (1981) has suggested that the impact of WGR is "best measured by the proportion of trees with stem infections rather than the total number of infected trees (branch or stem)". Zagory (1979) mentioned that the number of stem galls on 7-year-old *P. radiata* had a detrimental effect on tree height, although branch galls did not have this effect. In some cases, 60 ~ 75% infection of Scots pine trees resulted in the abandonment of plantations (Merrill and Kistler, 1976). Christmas trees in New Brunswick were abandoned because of WGR infections in the tops and branches (Forbes *et al.*, 1972). Sixty-three percent of the 6 to 12-year-old lodgepole pines were infected in a Christmas tree plantation in Alberta (Powell and

Hiratsuka, 1973). Due to deformation, those trees were of little commercial value. In North Central Ontario, as much as 5% mortality among jack pine seedlings was caused by WGR (Howse *et al.*, 1981). In a few cases, WGR spores can infect peduncles and cones (Byler and Platt, 1972), although the impact of this pathogen on pine seed production, dispersal, and viability remains unknown (Nelson, 1971).

1.3. Fungal Life Cycle and Disease Cycle

The autoecious nature of WGR on hard pines has been demonstrated by artificial inoculation tests (Meinecke, 1920, 1928, 1929; Ziller, 1970). Several workers also confirmed that infections previously thought to be *Peridermium cerebroides* and Woodgate gall rust were actually *E. harknessii* (Hedgcock and Hunt, 1920; York,1929,1938; Hutchinson, 1935; True, 1938; Peterson, 1959; Anderson and French, 1965). Successful infection of *Castilleja* spp. by WGR spores has been reported by several workers (Rhoads *et al.*, 1918; Meinecke, 1929; Weir, 1928; Anderson and French, 1965). However, inoculum used in these experiments may have been contaminated with spores of other rust species, since attempts to repeat these experiments were unsuccessful (Lorbeer, 1955; Wagener, 1958; Peterson, 1959; Zalasky and Riley, 1963). To date, there are no reports of an alternate host for this rust in western Canada (Ziller, 1974).

Detailed cytological studies of WGR by Hiratsuka *et al.* (1966) have shown that the young spores of WGR are usually binucleate but become uninucleate as the spores mature, thus suggesting nuclear fusion. Nuclear division occurs in the spores upon germination and meiosis occurs in the germ tubes which divide into 4 uninucleate cells. Basidiospores are absent and the germ tubes of *E. harknessii* act as basidia. Based on its nuclear behaviour during spore germination, germ tube morphology, the absence of an alternate host, and the existence of only two spore types (i. e. spermatium and peridermioid teliospore), Hiratsuka (1969) created a new genus, *Endocronartium*, to

accommondate endo-species having morphological similarities to the imperfect genus *Peridermium* Lk. and aecioid telia of the *Cronartium* type (Hiratsuka, 1973, 1974, and 1981). Similar observation of nuclear characteristics have been reported in *E. pini* (Pers.) Lev. (Hiratsuka, 1968). The heteroecious rusts have binucleate aeciospores and retain their dikaryotic condition through the periods of germ tube formation, nuclear migration, and appressorium production (Allen, 1989). More recent studies of the nuclear behavior in spores of *E. harknessii* (Epstein and Buurlage, 1988) and *E. pini* (Gibbs *et al.*, 1988) suggest that the pathogen should be returned to the genus *Peridermium*. Further studies are required to clarify the nuclear condition throughout the life cycle of these fungi.

Spores of *E. harknessii* germinate over a temperature range of 10 ~ 30 ^{0}C with optimum germination occurring between 15 and 20 ^{0}C. The initiation of germination is much slower at 5 ~ 10 ^{0}C than at 15 ~ 20 ^{0}C (Powell and Morf, 1966; Nelson, 1971). After germination, germ tubes of the spore germlings grow either perpendicular or parallel to the epidermal ridges of hypocotyls (Hopkin *et al.*, 1988). Polymorphic appressoria form and the infection peg penetrates at the junctions of epidermal cells of hypocotyls (Hopkin *et al.*, 1988) or through the cuticle of the candles (True, 1938), or young ovulate cones (Byler and Platt, 1972; Merrill and Kistler, 1976). The earliest reported symptoms consisted of red spots or stripes which appeared on the epicotyls of lodgepole pine 3 ~ 7 days after infection (Allen, 1989; Hopkin *et al.*, 1988). Successful infection through artificial wounds or bark slits also have been reported (Boyce, 1957; Anderson and French, 1965). The haploid and monokaryotic mycelium remains localized and once it reaches the cambium it stimulates repeated division of cambial cells, causing the production of excess xylem and ray parenchyma (Peterson, 1960; Hiratsuka and Powell, 1976), thereby resulting in gall formation 2 or 3 years after infection. Dikaryotic cells in the cortex divide to produce aecia which annually sporulate on the gall surface in late spring until the middle of July. A spermogonial stage of questionable function has been

reported on *P. contorta, P. ponderosa, and P. sylvestris* (Weir and Hubert, 1917; Gill, 1932; True, 1938; Boyce, 1957; Nelson, 1971). The fungus usually does not kill infected bark, but secondary organisms including hyperparasitic fungi (Tsuneda *et al.*, 1980; Tsuneda and Hiratsuka, 1981; Byler *et al.*, 1972), rodents (van der Kamp, 1981), and insects (Powell, 1982) eventually invade or feed on the bark and kill the gall. A persistent mycelium which remains alive in the gall tissues enables the fungus to survive during the winter period.

1.4. Control Measures

Various control efforts, including silvicultural, chemical, and biological methods and resistant seedling stocks have been considered as possible ways to minimize the incidence of WGR.

Effective control of WGR in nurseries can be achieved by removal of the inoculum source (Peterson, 1960). Carlson (1969) recommended that infected trees within 274 m of a nursery should be removed to prevent further spread of the pathogen. Trees with stem galls that completely girdle the stem should be rogued during thinning operations. Not only do such trees contribute inoculum and eventually die, but they also occupy space and hinder the growth of surrounding healthy trees. However, thinning practices may affect the incidence of WGR. Bella (1985) reported that in west-central Alberta, 30 and 18% WGR infection rate occurred in thinned and unthinned stands of lodgepole pine, respectively. The higher percentage of tree infection occurring in the thinned stand than the unthinned stand may have been caused by removing the short and healthy trees and leaving behind the tall and infected trees when the stands were thinned. Therefore, care must be taken to remove all trees with main stem galls during thinning operations.

Rust pathogens can be introduced into a new area or country through the importation of contaminated or diseased plant materials (Savile, 1973). For example, *Puccinia*

arthami Cda. was introduced into America through the contaminated seeds of safflower. The introduction of *Chrysomyxa ledi* var. *rhododendri* on *Rhododendron* sp. into coastal Oregon or Washington may have resulted from the importation of plants with infected, symptomless leaves. Since WGR has not been reported outside North America (Martinsson, 1980), a strict three-year quarantine for pine propagating material is required to prevent the spread of this disease to other continents.

Application of fungicides during the sporulating period can protect susceptible nursery stock from infection (Carlson, 1969). However, application of 200 ppm cycloheximide (Actidione) and phytoactin to the lower 1.1 m of lodgepole and ponderosa pine trees (up to 4.6 m in height) only temporarily reduced fungal sporulation, due to poor translocation of these chemicals to the fruiting galls (Hinds and Peterson, 1966). Merrill and Kistler (1976) sprayed the fungicides benomyl (50% W.P., 3.6 g/L water), oxycarboxin (75% W.P., 12.0 g/L water), and Dowco 261 (3.6 g/L water) on 1 to 1.5 m tall Scots pines (*P. sylvestris*) and found that none of them prevented sporulation. Nevertheless, a single spray of Maneb (80% W.P., 3.6 g/L water) when the needles were breaking through the fasicle sheath reduced WGR infection.

Biological control of WGR, although not yet practical, has some potential. More than 11 fungi have been isolated from living or dead galls (Byler *et al.*, 1972). In studies with *P. radiata*, *P. muricata*, and *P. contorta*, Byler and Cobb (1969) found that *Penicillium* sp. and *Cladosporium* sp. killed the pathogen and prevented spore release whereas *Gibberella lateritium* (Nees.) Snyder and Hansen, *Diplodia pinea* (Desm.) Kicks, and *Nectria* sp. invaded the phloem and xylem tissue of galls. *Nectria fuckeliana* Booth was the most virulent parasite and was frequently isolated from damaged galls (Byler, 1970). In some localities in western Canada, 80% of the rust galls on jack and lodgepole pines were infected by *Scytalidium uredinicola* Kuhlman, *et al.* (Tsuneda *et al.*, 1980). Hyphae of *S. uredinicola* invaded the wood tissue and destroyed the rust hyphae to a depth of 300 µm below the sori (Tsuneda *et al.*, 1980). Maltol (3-hydroxy-2-methyl-4H-

pyranone), a metabolite of *S. uredinicola*, caused a 50% decrease in spore germination in vitro when applied at a concentration of 50 µg/ml (Cunningham and Pickard, 1985). These mycoparasites have some potential for controlling WGR, but none of them have been tested under field conditions.

The best long-term control for WGR may be to plant seedlings with disease resistance. Hoff (1986) studied the susceptibility of ponderosa pine to WGR and found that resistance among provenances of pine trees varied from 5 ~ 45%, with the average level being 21%. Allen (1989) postulated three sites of resistance in the interaction between *E. harknessii* and *P. contorta*, namely: the occurrence of a hypersensitive reaction in epidermis, the formation of necrophylactic periderm and the rhytidome in the cortex, and the inactivation of cambial initials in cambium. Hiratsuka and Maruyama (1983) and Hopkin (1986) also reported that the resistant reaction to WGR in *P. thunbergiana* Franco. and *P. densiflora* Sieb. and Zucc. involved the formation of periderm tissue in the infected cortex area.

1.5. Epidemiology

The incidence of infection varies from year to year and single "wave years" of heavy infection may be followed by many years of light infection (Peterson, 1973). For example, a survey of ponderosa pines in five North Dakota shelterbelts showed that 60% infection had occurred in a single year during a cool and very wet summer (Meyer *et al.*, 1982).

1.5.1. Spore Production

Spore production is one of the most important stages in the disease cycle. Generally, obligate (biotrophic) parasites -such as rusts, powdery mildews and downy mildews - sporulate best on living or greenish tissue while facultative (necrotrophic) parasites sporulate best on necrotic lesions and on dead leaves (Rotem *et al.*, 1978). Spore

production is very sensitive to environmental conditions, especially temperature and relative humidity (RH).

Higher temperatures may favor spore production in some pathogens. For example, more *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. and Henn. urediniospores were produced at temperatures of 10 to 30 ^{0}C cycle than at temperatures of 2 to 18 ^{0}C (Milus and Line, 1980). Similarly, more *P. graminis* Pers. f. sp. *tritici* Eriks. & Henn. urediniospores were produced at 29 ^{0}C than at 13 ^{0}C (Prabhu and Wallin, 1971) . On the other hand, high temperatures negatively affect spore production by some other fungi. For example, uredinia of *C. quercuum* (Berk.) Miyabe ex Shirai developed at temperatures of 16, 20, and 24 ^{0}C but rarely formed at 28 ^{0}C (Nighswander and Patton, 1965) and urediniospore production by the fusiform rust (*C. quercuum* f. sp. *fusiforme* Burdsall and Snow) on northern red oak (*Quercus rubra* L.) was six times greater at 16 ^{0}C than at 18 ^{0}C (Kuhlman, 1987).

Spore production is also influenced by ambient RH and soil water content. Yarwood (1961) found that short periods of free moisture or high RH increased sporulation of *Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth. on bean leaves. Spore production by *Septoria nodorum* (Berk.) Berk. occurred following exposure to 12 hr of water-satured air alternated with 12 hr at 85 ~ 90% RH, whereas sporulation ceased at a constant 85 ~ 90% RH (Shearer and Zadoks, 1972). Urediniospore production of *P. recondita* was greater at a higher level (-200 J/kg) than at a lower level (-800 J/kg) of soil water potential (Cowan and Zadoks, 1973) .

There may be a relationship between levels of survivability and levels of productivity of fungal spores (Rotem *et al.*, 1978) . For example, the prolific sporulation of rusts may compensate for low rates of spore survival, while in less fertile fungi (i. e. *Alternaria porri* (ELL.) Neerg. f. sp. *solani* (E. & M.)), the high survival rate of spores may compensate for low productivity. Christensen (1942) estimated that wheat,

moderately infected by *P. graminis*, would produce a minimum of 25 million urediniospores /m^2 area.

1.5.2. Spore Release

Spore release by many fungi shows a diurnal periodicity (Pady *et al.*, 1968; Peterson, 1973; Powell, 1972). For example, the number of peridermioid teliospores released by *E. harknessii* increased sharply at 0700 and reached its maximum at about 1100 (Peterson, 1973). Similarly, most aeciospores of *Cronartium comandrae* Pk. were released between 0800 and 1900 (Powell, 1972). Release of aeciospores of *Gymnosporangium juniperi-virginianae* Schw. peaked between 6 and 8 AM, when RH was declining (Pady *et al.*, 1968).

Aeciospores of different rusts may vary in their release mechanisms. For example, the aecia of *G. juniperi-virginianae* are of the roestelioid type. The peridia are hygroscopic and curl inward over the opening of the aecium during the wet conditions and curl outward and expose the aeciospores when RH is decreasing (Pady *et al.*, 1969). In the aecidioid rusts with non-hygroscopic peridia *Uromyces psoraleae* Peck and *Puccinia andropogonis* Schw. aeciospores are forcibly discharged from the aecium when water is condensing on the aecium (Kramer *et al.*, 1968). This mechanism explains the occurrence of aeciospore release peaks between 2000 hr and midnight. The caeomoid aecia of *Phragmidium speciosum* (Fr.) Cooke has a release mechanism that is similar to those of aecidioid aecia (Kramer *et al.*, 1968).

Based on the daily succession of spores in the air, many fungi can be categorized into different groups (Meredith, 1973). The spores of the forenoon group are usually released between 7 AM and noon, when RH is decreasing early in the morning. Members of this group are *Phytophthora infestans* De Bary, *Nigrospora* spp. and *Deightoniella torulosa* (Syd.) Ellis. The afternoon group releases most spores when the ambient temperature and wind speed or turbulence are greatest. Fungi in this group include *Cladosporium* spp., *Alternaria* spp., *Ustilago* spp., and *Erysiphe* spp. The nocturnal group, discharging

spores between midnight and 6 AM, includes all types of basidiospores such as those produced by *C. comandrae* and *C. ribicola* (Van Arsdel, 1967; Krebill, 1968). Gregory (1973) even created a fourth group of fungi with a double-peak of daytime release pattern, including *Helminthosporium* spp., some *Cercospora* spp., and *Aspergillus* spp.

Within the daily cycle, spore release of rusts is strongly influenced by rain and wind speed and direction (Meredith, 1973). Stepanov (1935), cited by Meredith (1973), found that the minimum wind speed required for spore release varied among different fungi. For example, urediniospore release by *P. graminis* was proportional to wind speed, with a minimum wind speed of about 4.5 km/hr required to initiate spore release (Smith, 1966). For release of urediniospores of *Hemileia vastatrix* Berk. and Br., a strong wind, up to 19 km/hr, was necessary (Nutman *et al.*, 1960). Increasing wind speed probably increases spore release by reducing the thickness of the boundary layer of air at the leaf surface, thereby exposing more spores to moving air (Gregory and Lacey, 1963).

Rainfall may also promote spore release. For instance, Ormrod *et al.* (1984) noticed that release of *Gymnosporangium fuscum* DC. aeciospores was dramatically increased when rain followed one or more days of dry weather. Ascospores of *Venturia inaequalis* (Cooke) Wint. can be released from dead leaves on the ground with as low as 0.2 mm of rainfall (Hirst and Stedman, 1962).

1.5.3. Spore Dispersal

Spores are the most important units of dispersal (Sussman, 1968). Theoretically, vegetative cells can become airborne as do spores, but their survivability is much reduced because of radiation and desiccation (Sussman and Halvorson, 1966). The effectiveness of spore dispersal plays an important role in determining the development of plant diseases. Although much information has been reported on spore dispersal of cereal rusts (Eversmeyer and Kramer, 1980; Roelfs, 1972; Roelfs and

Martell, 1984; Smith, 1966) and other diseases (Cammack, 1958; Fried *et al.*, 1979; Gregory, 1968; McCartney and Bainbridge, 1984), information on tree rusts is scarce.

Forces influencing spore dispersal include gravity, wind eddies, and electrostatic forces (Gregory, 1973, 1982; Leach, 1980; Benninghoff, 1984). In stationary air, spores fall gradually under the influence of gravity. Although still air rarely occurs under field conditions, the rate at which spores fall in still air affects spore dispersal. McCubbin (1918) and Ukkelberg (1933) studied the terminal velocity of urediniospores and aeciospores of rusts in vertical cylinders and found that spores reached the bottom of the cylinder in consecutive time intervals and showed a negatively skewed distribution. Although spore size and terminal velocity had little effect on the spore deposition gradient of *Lycopodium* sp. and *Podaxis* sp. over short distances from their source, the rate of deposition of *Lycopodium* sp. and *Podaxis* sp. from suspension was approximately proportional to the volumes of the individual spores (Sreeramulu and Ramalingam, 1961).

Long-distance spore dispersal is very important in terms of the spread of diseases since those spores can create new disease centres. Well known examples are the annual northward migration of *P. graminis* urediniospores from Mexico to the U. S. A. and Canada (Stakman and Harrar, 1957) and the spread of *H. vastatrix* from north to south in Brazil, in the same direction as prevailing wind currents (Schieber, 1972).

Spore dispersal gradients may or may not result in disease gradients, depending on the suitability and uniformity of environmental conditions, the virulence of pathogens, and the susceptibility of hosts (Gregory, 1968, 1973).

The terminal velocity of falling spores can be calculated by Stokes's law ($V = (2/9)(x-y/\mu) . g . r^2$, where V = terminal velocity, x = density of the sphere, y = density of the medium, g = acceleration due to gravity, r = radius of the sphere, μ = viscosity of the medium. However, Stokes's law only applies to non-turbulent conditions and to spherical particles with a smooth surface.

Differing spore dispersal and disease gradients can be compared by comparing the values of the regression coefficient, b, obtained by plotting the logarithm of spore number against the logarithm of distance (Gregory, 1968; 1982). This is an inverse power law model, which can be expressed by the equation: $y = a \cdot x^b$, or $\ln (y) = \ln (a) + b \cdot \ln (x)$. The second commonly used empirical model is the log-linear transformation or the exponential model (Kiyosawa and Shiyomi, 1972); $y = c \cdot \exp(-dx)$ or $\ln (y) = \ln (c) - dx$. In both equations, y is the spore concentration, x is the distance, a and c are constants related to the source strength and b and d are the slopes of the regression line (dispersal gradient). Fitt and McCartney (1986) have discussed the advantages and disadvantages of both models. Fitt *et al.* (1987) found that dispersal gradients for spores less than 10 µm in diameter fit the power law model better than the exponential model. Schmidt *et al.* (1982) found the same phenomenon during their study of spore deposition gradients of *C. quercuum* f. sp. *fusiforme* over open ground. Both models are descriptive, not [illegible], and should not be extrapolated outside the range of the testing field (Gregory, 1968; 1982).

Information on spore dispersal is important for the evaluation of different methods of disease management such as chemical control, isolation, and sanitation (Gregory, 1982). Little infection by *E. harknessii* was found beyond 270 m from an inoculum source (Carlson, 1969). Peterson (1973) found that few spores of WGR were collected further than 90 m from an infected stand. Buchanan and Kimmey (1938) studied the spore dispersal gradients of *C. ribicola* J. C. Fischer ex Rabenh from a point source of *P. monticola* Dougl. and found that beyond 20 m from the source, spore numbers were quite low. In many cases, spore dispersal and disease gradients were also influenced by meteorological factors (Eversmeyer and Kramer, 1980). Cammack (1958) reported that local spread of *Puccinia polysora* Underw. was greater in the direction of the prevailing wind and decreased with distance from the sporulating pustules. Studies of urediniospore dispersal of stem and leaf rust of wheat showed that 3 and 6% of the

initial amounts, respectively, were trapped at 100 m downwind from the source (Roelfs, 1972).

Many researchers, during their studies of spore dispersal, have calculated the infection rate, the ratio between the number of uredia or telia per square centimeter of leaf surface and the number of spores caught at each distance from the inoculum source (Schmidt *et al.*, 1982; Smeltzer and French, 1981). Infection of sweetfern (*Comptonia peregrina* (L.) Coult.) by aeciospores of *C. comptoniae* Arth. was observed at a maximum of 30 m from the source (Smeltzer and French, 1981) and was explained by a reduction in spore viability as the distance from the source increased. However, the infection ratios for *C. fusiforme* decreased very little over a distance of 91.4 m (Schmidt *et al.*, 1982).

1.5.4. Effect of Environmental Factors on Spore Survival

In epidemiological studies, spore survival is an important factor in determining infection potential. The survival phase of monocyclic rust spores like *E. harknessii* can be defined as the period between spore production and infection. Spore viability is influenced by many interacting variables including the inherent properties of spores and environmental conditions (Anderson *et al.*, 1948; Sussman, 1968). Roane *et al.* (1960) found that differences in survivability existed among physiological races of *P. graminis* f. sp. *tritici*. Among them, races 38 and 56 had very good survivability; races 14, 15B, and 23 were intermediate; and races 23A, 48, 69, and 186A survived poorly. Under natural conditions, temperature, RH, and solar radiation are the main factors influencing spore viability.

1.5.4.1 Temperature Effect

Both in nature and in storage, spore longevity tends to decrease with increasing temperature. Aeciospore viability of *C. comandrae* declined rapidly when temperature

rose above the maximum temperature (above 25 ^{0}C) for germ tube formation (Powell, 1974). Temperatures of about 0 ^{0}C have no harmful effect on most fungi (Melhus and Durrel, 1919). Spores of *E. harknessii* retain their viability better than those of *C. coleosporioides* Arth. and *C. comandrae* when stored at low temperatures (Powell, 1971). Few spores of *E. harknessii* retained their viability for over a year when stored at 4 ~ 5 ^{0}C (Lightle, 1955). Sixty three percent of aeciospores of *C. quercuum* f. sp. *banksianae* germinated after 4 months storage at 4 ^{0}C whereas 17% germinated after 4 weeks at 20 ^{0}C. At 36 ^{0}C, spores of this species survived only for a few hours (Nighswander and Patton, 1965). The effects of high temperature on spore viability may be accompanied by desiccation injury since these two factors may not always be clearly distinguishable (Sussman, 1968).

1.5.4.2 Relative Humidity Effect

Humidity also influences spore viability, and high RH is the most detrimental (Anderson *et al.*, 1948; Snell, 1922; Sussman, 1968). For example, at 50% RH and 15 ^{0}C, all of the urediniospores of *P. coronata* f. sp. *avenae* Cda. were dead after six months to one year, but at 25% RH and 15 ^{0}C, a small percentage of spores remained viable for more than one year (Rosen and Weetman, 1939). Klingstrom (1963) found that spores of *P. pini* (syn. *E. pini*) germinated poorly during wet conditions. Powell (1971) found that wet aeciospores of *C. comandrae* rapidly lost their viability. Urediniospores of *P. glumarum* Eriks. and Henn. (syn. *P. striiformis* West.) survived best at 0 ~ 5 ^{0}C and 40% RH. (Manners, 1951) Relative humidity in the range of 20 ~ 40% was also optimal for survival of urediniospores of *P. coronata* (Rosen and Weetman, 1939). Conversely, storage under wet conditions is required to retain viability of some spores, including conidia of *Erysiphe polygoni* DC. (Yarwood *et al.*, 1954), and basidiospores of *Tilletia caries* (DC.) Tul. (Buller, 1933).

Desiccation extends the lifespan of some spores, such as the sporidia of *Ustilago*

zeae (Beckm.) Unger., which survived five months under dry conditions (Piemeisel, 1917). Rapid desiccation at low temperatures is favorable for the survival of most fungi. In contrast, a fluctuating environment is most deleterious to spore survival. Most mature spores germinate very poorly or not at all when alternately moistened and dried or frozen and thawed (Piemeisel, 1917; Snell, 1922). Extremes in dryness and moisture during the formation of *C. fusiformis* aeciospores, reduced their viability (Siggers, 1947). However, it is generally agreed that dehydration is an effective way to extend spore longevity. Webb *et al.*, (1964) and Webb (1965) have suggested that a physical change occurs in the macromolecules, such as DNA, when structural water is removal from spores and that trehalose and mannitol may protect the spore against drought injury.

1.5.4.3. Effect of Sunlight

Light has a strong influence on growth, development, reproduction, and survival of fungi. Most sunlight at the surface of the earth has a wavelength greater than 290 nm (Sussman, 1968). However, a small amount of light with a wavelength of less than 290 nm may still reach the earth's surface although in very low amounts. The maximum effect of ultraviolet light on fungi is associated with wavelengths approximately 260 nm which corresponds to nucleic acid absorption. Spore viability is affected by solar radiation as reported by many researchers (Pomper and Atwood, 1955; Maddison and Manners, 1972). For example, none of the sporidia and telia of *C. ribicola* survived on ribes leaves when exposed to bright sunlight for 10 min at 25 ^{o}C and 30.5% RH (York, 1918; Taylor, 1919). Maddison and Manners (1972) found that germinability of *P. graminis* urediniospores was reduced to 10% after 20 hr exposure to sunlight whilst 6 - 10 hr exposure caused a similar result in *P. striiformis*. They believe that sunlight in the wavelength range close to 300 nm and up to 400 nm is responsible for inactivation.

There is evidence that visible light may also induce mutations in some microorganisms (Webb and Malina, 1967; Leff and Krinsky, 1967).

1.6. Study Objectives

Based on the previously mentioned life cycle of *E. harknessii*, it can be assumed that host susceptibility, spore longevity and environmental conditions during the critical period of spore production and release and infection will determine whether a certain year's infection will be heavy or light.

With this in mind, the objectives of this research were

(*i*) to describe the hourly fluctuations in spore release of *E. harknessii* under controlled and field conditions in Alberta; to determine the mechanism of spore release and to explain the observed pattern of spore release in terms of the effect of climatic factors on the mechanisms of spore production and release,

(*ii*) to determine the effects of temperature and RH on spore survival of *E. harknessii*, and the longevity of *E. harknessii* spores out of doors during daytime hours,

(*iii*) to describe spore dispersal gradients and infection gradients in a cut block adjacent to a heavily infected sapling stand.

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Chapter 2. Mechanism and Pattern of Spore Release by *Endocronartium harknesssii* [1]

2.1 Introduction

Western gall rust, caused by the fungus *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka, is a widespread disease of many hard pine species in North America (Hiratsuka *et al.*, 1987). In Western Canada, the disease is considered to be the most important stem rust of hard pines (Ziller, 1974). The fungus produces globose swellings on branches or on the main stem. Branch galls may not have a significant impact on tree growth. For example, there was no significant difference in height or diameter between infected and uninfected trees in two stands, aged 50 and 55 years old, which had an average of 57 and 46 galls per infected tree, respectively (Gross, 1983). Main-stem galls, however, may result in tree death and could cause significant losses in volume in poorly stocked stands (Bella, 1985b). Infection frequencies of 30 and 18% occurred in thinned and unthinned stands, respectively, in Alberta (Bella, 1985a), thus suggesting that this disease may become more important with increased application of intensive management practices, such as thinning.

Despite the importance of western gall rust, there have been very few epidemiological studies of this disease. In particular, little is known of the influence of environmental factors on spore production and release by this or related rusts. Aeciospore release by *Cronartium comandrae* Pk. occurred mainly during the daylight hours and was related to meteorological conditions (Powell, 1972). Spore release was increased by heavy rains but not by light rains. Few spores were released if the

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[1] A version of this chapter has been published in Can. J. Bot. 67 : 104-111., 1989

temperature was below 7 ^{o}C or if the relative humidity (RH) was high. Wind was important for initiation of spore release, although the numbers of spores caught were not well correlated with wind speed. Release of spores by *E. harknessii* in Nebraska also occurred primarily during the daytime (Peterson, 1973). The number of captured spores increased rapidly at approximately 0700, when wind speed and temperature were increasing and RH was decreasing. On rainy days, more spores were captured in the afternoon than on dry days.

Without knowledge of the mechanism of spore release, it is difficult to explain this pattern of spore release or predict whether such a pattern would be observed in Alberta. The objectives of this study were to (*i*) describe the hourly fluctuations in spore release by *E. harknessii* under field conditions in Alberta, and under controlled environmental conditions, (*ii*) determine the mechanism of spore release, and (*iii*) explain the observed pattern of spore release in terms of the effect of environmental factors on the mechanisms of spore production and release.

2.2 Materials and methods

2.2.1 Field Studies

These studies were conducted in a dense, heavily infected stand of lodgepole pine, *Pinus contorta* Doug. var. *latifolia* Engelm., approximately 20 years of age, located northeast of Hinton, Alta., at an elevation of about 1280 m.

Spores were collected using four impaction spore traps (Fig. 2.1) similar to the one designed by Powell and Morf (1967), with a suction rate of approximately 0.36 $m^3 \cdot h^{-1}$. Spores were sampled from two galls by placing two spore traps on opposite sides of both, with the orifice of the traps located 1 to 1.5 cm from the surface of the galls (Fig. 2.2). Both galls had several telia, some of which may have coalesced. Spore collection began after the peridia on the galls had burst. Any bark covering the aecia was carefully

removed without damaging the peridia. Released spores were sucked through the orifice of the spore trap and were impacted onto the surface of a sticky tape attached to a rotating ring. The tape was changed every day between 0930 and 0940. Although spore collection was intermittent in June 1985, a continuous collection was made between June 5 and June 21 during 1986. Spores were counted differently in 1985 and 1986. In 1985, tapes collected from spore traps were brought back to the laboratory and were divided into 24 pieces, each representing a 1 h period. The number of spores in a 30 μm wide transect was counted under a compound microscope at 100X and the total number of spores caught per hour was calculated. In 1986, a more rapid method of counting spores was used. The tapes were cut into 24 segments and placed into test tubes containing 20 mL mineral oil (Soltrol 170, Phillips 66 Co., Chicago, IL). The spores were dislodged from these segments of tape by agitating the tubes with a Vortex mixer for 2 min. The spores were allowed to settle out overnight and the supernatant and tape segments were then carefully removed. The spores that had settled to the bottom of the tubes were then resuspended with a Votex mixer in the oil that remained in the test tube. One drop of suspension was immediately removed from the test tubes and placed on a hemacytometer. The number of spores captured per hour was calculated on the basis of the hemacytometer count and the volume of oil remaining in the test tube. For both years, spore concentration was calculated by dividing the hourly spore catch by the volume of air sampled per hour.

Meteorological conditions were recorded continuously using two data loggers (CR-21 Micrologger, Campbell Scientific Inc. Logan, UT 84321). One of the dataloggers was placed near the gall; it recorded temperature and RH with a temperature and RH probe (model 201, Campbell Scientific Inc.); wind speed with an anemometer (model 014A, Met One Inc. Grants Pass, OR 97526); and leaf wetness with a circuit grid sensor (Campbell Scientific Inc). The other data logger was set in an open site about 300 m from the spore trap; it recorded sunlight with a pyranometer (model LI-200SZC, Li-Cor,

Lincoln, NE 68504) and rain with a tipping bucket rain gauge (model 2501, Sierra-Misco Inc. Berkeley, CA 94710). Weather data were recorded every minute, compiled into 0.5-h intervals, and stored on a cassette tape. The data were read from the cassette tape into a computer file, from which they were sorted and printed as graphs. The RH data were converted into vapor pressure deficit (VPD) because VPD values are more meaningful in considering atmospheric effects (Lacy and Pontius, 1983).

Because spore trap data tend to be quite variable, no attempt was made to construct a precise model to correlate spore release with environmental conditions. Instead, the minimum continuous time period, in hours, required to account for 90% of each day's total spore release was determined. This number was multiplied by 100/24 so that the time required for 90% of the spores to be released could be expressed as a percentage of the total hours in a day. The percentages of each day's total values of wind speed, light intensity, and VPD that occurred during that time period were calculated. These percentage values were compared with the time required for 90% of the spores to be released, to indicate the association between the environmental parameters and spore release. For example, an association between sunlight and spore release would be indicated if the time required for 90% of a day's spores to be released was 12 h (50% of a day) and if 80% of the day's sunlight occurred during that 12-h period.

2.2.2 Spore Release under Controlled Conditions

A gall on which the peridia were just beginning to rupture was collected and used within 1 h. The gall was tapped vigorously to remove loose spores that were produced before the beginning of the experiment. The stem below the gall was inserted into a test tube containing water and the gall surface was placed 1 cm from the orifice of a spore trap. To simulate daily fluctuations in RH, light, and wind, under controlled conditions, the spore trap and gall were placed inside an acrylic chamber (Figs. 2.3 and 2.4), which was put into an incubator. The 8-h simulated night was characterized by

still air, 92 ± 3% RH, darkness and a temperature of 18 ± 1 °C (0.17 ± 0.07 kPa VPD). During the 16-h simulated day, a mild stream of air (1.9 m . s^{-1}) was blowing across the gall into the spore trap, the RH was 30 ± 5%, the gall was receiving 0.14 kw . m^{-2} (326 μE · m^{-2} · sec^{-1}) of light, and the temperature was 24.5 ± 0.5 °C (2.16 ± 0.22 kPa VPD). The gall remained in the chamber for 2 days. At the end of this 2 day period, the gall was replaced by a fresh gall, and the experiment was repeated.

2.2.3 Spore Production under Humid and Dry Conditions

Eleven galls were collected in the field and stored at 4 °C for 1-3 days. The galls were placed in weighing boats and put in a dew chamber (Percival Manufacturing Co., Boone, IA 50036) for 8 h at 15 ± 1 °C. After removal from the dew chamber, they were exposed to the drier air of the laboratory for 1 h to catch any spores that fell from them. The galls were held directly above the weighing boats and tapped repeatedly until no more spores could be seen falling into the containers. The galls were then transferred to new weighing boats and put into an incubator for 16 h at 77 ± 5% RH and15 ± 1 °C (0.40 ± 0.11 kPa VPD). Spore production by these 11 galls was determined over two wet and two dry cycles. The RH in the dew chamber was not measured, but dew began to form on a circuit grid sensor within 30 min of its being placed inside the dew chamber. Nevertheless, there was no visible water on the surface of the galls after 8 h in the chamber. The number of spores produced during each cycle was determined by adding 3 mL of mineral oil to the weighing boats and then using a hemacytometer to determine the concentration of the resulting suspension. The galls differed in the number and size of telia present. Thus, a series of paired *t*-tests was done, based on differences in spore production per hour by the same gall during the two humid and two dry periods.

Photography was used to document the effect of alternating humid and dry conditions on spore production. A single gall was collected and stored at 4 °C for 3 days. It was then kept on the laboratory bench for 24 h, after which it was photographed and

put in the dew chamber for 9 h. Upon removal from the dew chamber it was quickly photographed and then kept on the laboratory bench for 2 more days before a final photograph was taken.

2.3 Results

2.3.1 Field Studies

The numbers of spores caught in June of 1985 and 1986 are shown in Figs. 2.5 and 2.6, respectively. Spore release, in both years, began at the end of May and continued until the beginning of July, with most spores being released during June. In 1985, some of the peridia on the galls already had ruptured before spore collection was started. In 1986, spore collection began just as the peridia were rupturing. Dry days and days with rainfall were analyzed separately. Dry days were defined as days without rain or days in which there was only a brief shower occurring during the evening, after 1800 and before 2400. Rainfall was assumed to have occurred if rainfall was recorded by the rain gauge or if leaf wetness was recorded during the daytime after 0900 and before 1800.

On dry days, there was a diurnal pattern of spore release (Figs. 2.5 and 2.6). Typically, few spores were collected before 0500 or after 2200. This was especially evident for evenings in which the temperature was below 10 °C or in which the VPD was low, such as on June 28, 1985, and on June 8 and 16 , 1986. The number of spores trapped began to increase at about 0500-1000 and peaks occurred between 0900 and 2200. Most spores were caught during the sunlight hours when temperature, wind speed, and VPD were high. The time interval during which 90% of the spores were caught was, on average, 13.9 h long (58% of a day). On average, 89, 77, and 81% of the day's total values of sunlight, wind speed, and VPD occurred during this period.

Spore release also occurred on rainy days (Figs. 2.5 and 2.6). However, the period of maximum spore release extended over a longer period than on days without rain. The

average time required for 90% spore release during rainy days was 17.3 h (72% of a day). A *t*-test indicated that this was statistically different (at $p = 0.05$) from the 13.9 h required for 90% spore release during dry days. During this 17.3 h period, 98, 90, and 88% of the days total values of sunlight, wind speed, and VPD occurred.

2.3.2 Spore Release under Controlled Conditions

There was a major spore release peak immediately after the beginning of the 2 simulated days, when the VPD was increasing, the lights were on, and a mild stream of air was blowing across the gall (Fig. 2.7). Minor peaks occurred during the 2nd day in both of the replicates. There was virtually no spore release during the simulated night.

2.3.3 Spore Production

A large number of spores were produced during the first 8 h exposure of galls to the humid conditions of the dew chamber (Fig. 2.8). Significantly fewer spores were collected during the subsequent 16 h drying period. Returning the galls to humid conditions stimulated spore production, although the number of spores collected was less than in the first humid period. Few spores were trapped during the final dry treatment.

Under ambient laboratory conditions, the aecial peridium remained intact (Fig. 2.9). After 9 h in the dew chamber, the aecium appeared swollen. The peridium was ruptured, presumably by the force of spore production, and a small mound of fresh spores was protruding from the opening that had been created (Fig. 2.10). Two days later, following return to laboratory conditions, most of the spores that had been extruded had dried up and fallen away from the telium (Fig. 2.11).

2.4. Discussion

The diurnal periodicity of fungal spore release has been reported by many workers (Kramer *et al.*, 1968; Pady *et al.*, 1969; Gregory, 1973; Ormrod *et al.*, 1984). However, there is very little information about peridermioid teliospore release in tree rusts (Powell, 1972; Peterson, 1973). Peterson (1973) reported that on dry days, spore release by *E. harknessii* reached a maximum at about 1100 and declined sharply between 1200 and 1300. We, however, found that the time of maximum spore catch was quite varible. For example in 1986, the single gall sampled by spore traps 3 and 4 showed a peak at 1900 -2000 on June 10 and a peak at 0900 -1000 on June 13 (Fig. 2.6). Furthermore, there often was a difference in the pattern of spore release obtained from two spore traps facing the same gall. This is not totally unexpected. Telia are unevenly distributed over the surface of the gall, and they mature at different times. Thus, the only generalization that can be made with confidence about the time of spore release on dry days is that spore release typically started around 0500 and peaks occurred between 0900 and 2200.

There appeared to be a relatively strong association between spore release and sunlight, wind speed, and VPD. The 13.9 h, during which 90% of the day's spores were trapped on dry days, represents only 58% of the hours in a day; yet during this same time period, 89, 77, and 81% of the day's total readings of sunlight, wind speed, and VPD values respectively, were recorded.

The laboratory experiments suggest a passive spore release mechanism that could account for these associations. The two experiments in which galls were transferred into and out of a dew chamber indicate that high humidity (low VPD) favors spore production (Figs. 2.8 - 2.11). Presumably, more spores would be produced at night, when the VPD is low and the internal water potential of the bark is high (Lopushinsky, 1975), than during the daytime. After the peridium had ruptured, the spores that had been produced would not likely be actively discharged; rather they would remain inside the telium or as a mound of fresh spores resting on the aecium (Figs. 2.9 and 2.10). By mid-

spores likely would dry out and be blown away. In our field experiment, the spore traps were placed close to the galls, thus shielding them from direct wind exposure (Fig. 2.2). Nevertheless, it is likely that daytime winds could have triggered the increase in spore release by shaking the gall or by increasing turbulent air movement between the spore traps.

This hypothesized mechanism of spore release could also explain the results obtained during simulated daytime and nighttime conditions in the growth chamber. Presumably, more spores were produced during the simulated night when the VPD was low. At the beginning of the simulated day, the spores would have dried out and been blown off the gall by the mild airstream created by the fan. Fewer spores would have been produced during the remainder of the simulated day, although spore production would have resumed during the night, resulting in the peak of spore release that occurred the next day.

On rainy days, spore release also followed a diurnal pattern, with most spores being released during the daylight hours (Figs. 2.5 and 2.6). However, the period of maximum spore release occurred over a significantly longer time interval than was the case during dry days. One possible explanation for this is that during rainy days, a longer time period may have been necessary for the spores to dry out and be dispersed. Secondly, spore release occasionally occurred at night, presumably in response to rainfall, as seen on June 27, 1985, and June 15, 1986.

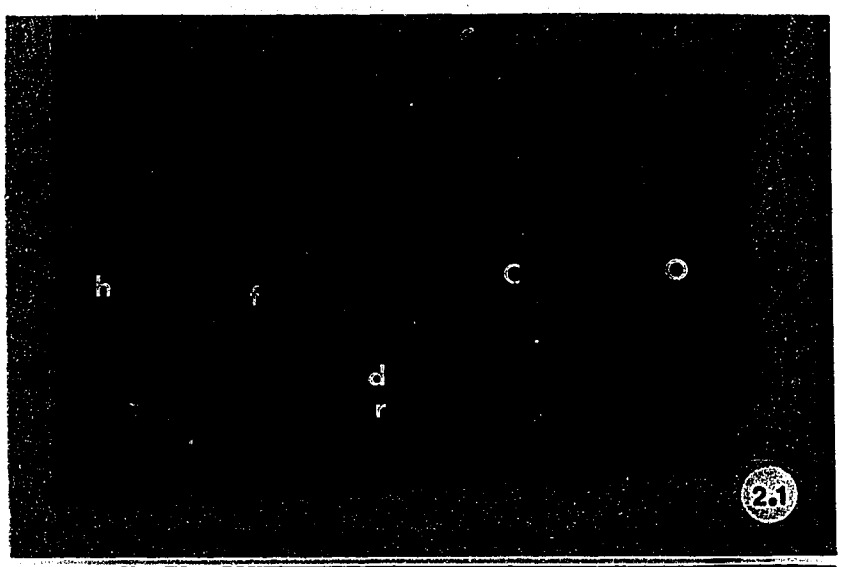
Rainfall may play a role in triggering the initial release of large numbers of spores at the beginning of the season. The first major flush of spore production in 1986 occurred on June 6, following 3 rainy days. Powell (1972) observed the same phenomenon in his study of comandra blister rust on lodgepole pine. Because spore release data was not available for the beginning of the season in 1985, it is not possible to determine whether there was a similar association of rainfall, and the initial release of large numbers of spores that year.

whether there was a similar association of rainfall, and the initial release of large numbers of spores that year.

Nevertheless, it was not possible to find a consistant or exact association between rainfall and spore release. Spore release was measured on an hourly basis and this may be too long an interval to permit exact correlation with the onset of rainfall. In any event, it was not possible to determine precisely when rainfall began. The rain gauge recorded rainfall only after 1 mm of rain had accumulated. However, the circuit grid sensor, which would have recorded the onset of rain, would also have recorded dew or rains that were so light that they might not have been important in spore release.

In summary, we have provided strong evidence that spore release by *E. harknessii* is passive and occurs following the production of spores under conditions of low VPD. Based on this, it has been possible to provide an explanation for the diurnal periodicity in spore release that occurs on dry days. Spore release on rainy days appears to be more complex, and additional laboratory simulation and field studies with more precise measurement of environmental parameters and spore release are necessary.

FIGS. 2.1-2.2. Arrangement and design of spore traps used in the field. Fig. 2.1. Top view of a 24-h impaction spore trap, with the sealed lid removed to show the electrical fan (f), clock (c), resin disc (d), acrylic ring (r), air intake orifice (o), and exhaust hole (h). A 3.5 cm wide strip of masking tape coated with a thin layer of silicon grease was placed on the acrylic ring. Scale bar represents 5 cm. Fig. 2.2. Two impaction spore traps (st) were connected to 12-V battery (b). A single sporulating branch gall was located between the traps. A data logger (m) was placed nearby to record weather data. An anemometer (a) was located in front of the traps. The relative humidity and temperature sensors were enclosed in a shelter (s) and the leaf wetness sensor was located near the data logger. Scale bar represents 20 cm.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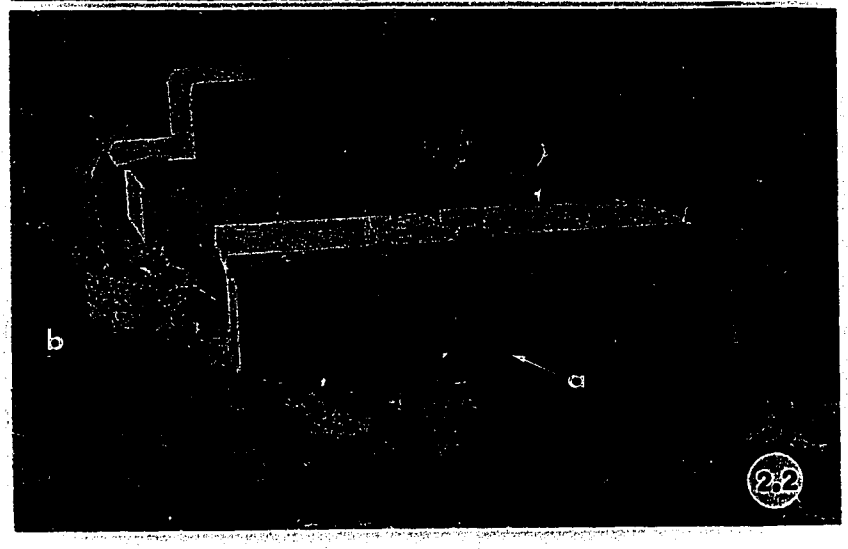
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2.2

FIGS. 2.3-2.4. Apparatus for simulating diurnal fluctuations of light, RH, and wind. Fig. 2.3. The acrylic chamber was placed inside an incubator kept at 24.5 ± 0.5 °C and 2.16 ± 0.22 kPa VPD (30 ± 5% RH). During the simulated night, the incubator was dark, and the top of the chamber was closed. The humidifier (h) was turned on for 1 h and then set to come on for twelve 1-min intervals per hour to maintain 0.17 ± 0.07 kPa VPD (92 ± 3% RH) in the chamber. Air moved from the spore trap (st) through the connecting tube (ct), in the direction of the arrow, and back into the chamber through a funnel (f). During the simulated day, the lights in the incubator were on. The top of the chamber was opened, and the humidifier was turn off to permit the RH to decrease. Scale bar represents 20 cm. Fig. 2.4. Close-up view of part of the chamber. The reentering air from the connecting tubing was diverted away from the gall (g), by the rubber tubing (rt). During the simulated day, but not during the simulated night an electrical fan (ef) generated a mild airstream across the gall. Scale bar represents 5 cm.

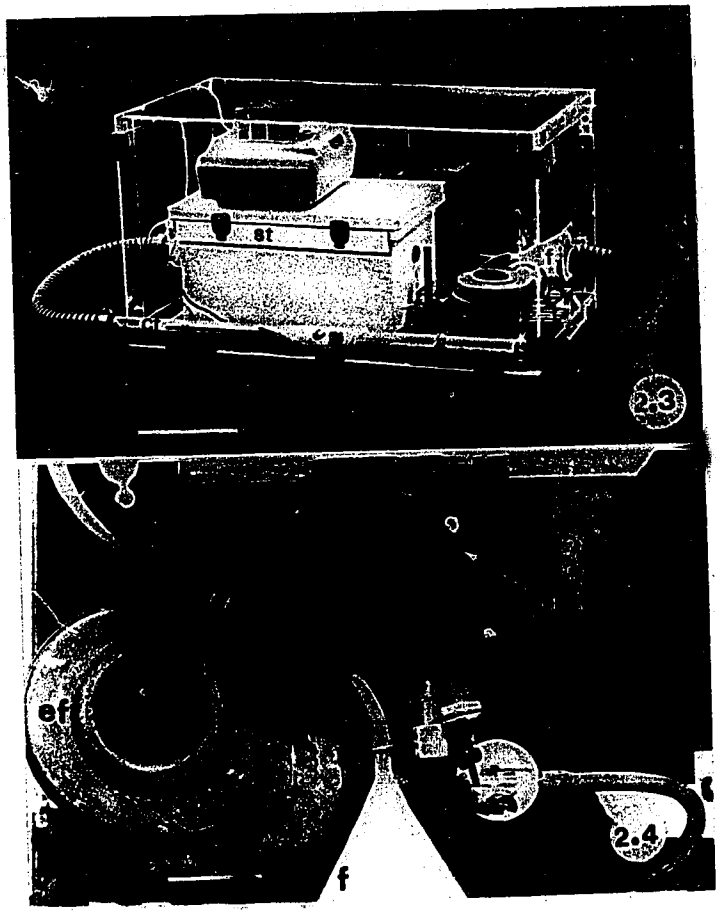

2-3
ef
2-4
f

FIG. 2.5. Changes in spore concentration ($x10^4 . m^{-3}$) of *E. harknessii* in relation to climatic conditions in Hinton, Alta., during June 1985. Spore traps 1 and 2 faced one gall and traps 3 and 4 faced a second gall. Both galls were approximatley 20-40 cm aboveground in a lodgepole pine stand. a-f, maxima, not shown in graphs (a = 268, b = 89, c = 195, d = 290, e = 240, f = 1.5 mm rain); r, rainy day; *, interrupted data as a result of mechanical breakdown.

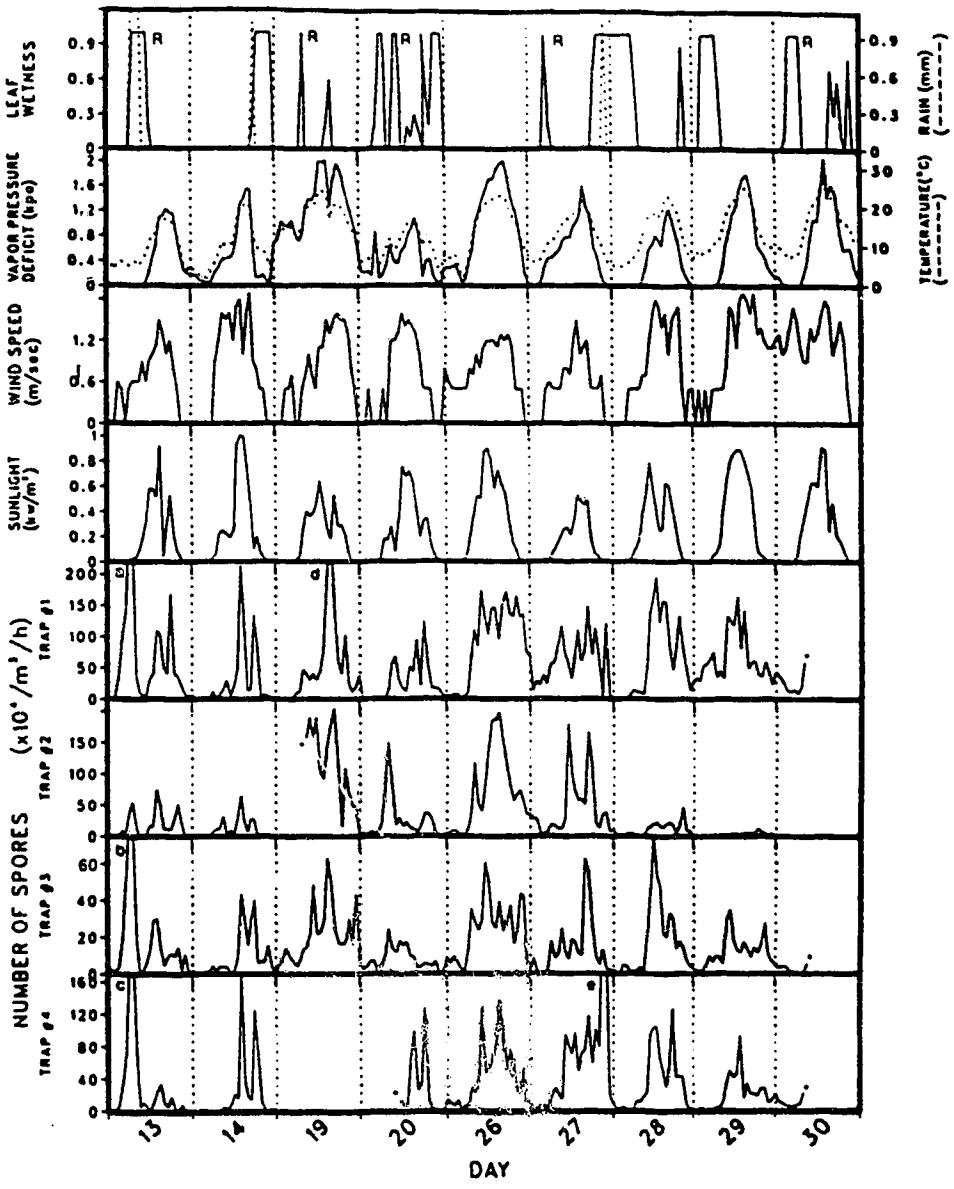

LEAF WETNESS
R
RAIN (mm)
VAPOR PRESSURE DEFICIT (kpa)
TEMPERATURE(°C)
WIND SPEED (m/sec)
SUNLIGHT (kw/m²)
NUMBER OF SPORES (x10⁴/m³/h)
TRAP #1
TRAP #2
TRAP #3
TRAP #4
DAY
13
14
19
20
26
27
28
29
30

FIG. 2.6. Changes in spore concentration ($x10^4 . m^{-3}$) of *E. harknessii* in relation to climatic conditions in Hinton, Alta., during June 1986. Spore traps 1 and 2 faced one gall at ground level and traps 3 and 4 faced a second gall at 20-40 cm aboveground in a lodgepole pine stand. a-j, maxima, not shown in graphs (a = 137, b = 82, c = 181, d = 90, e = 133, f = 303, g = 180; h = 19 mm, i = 19 mm, j = 16 mm rain) ; r, rainy day.

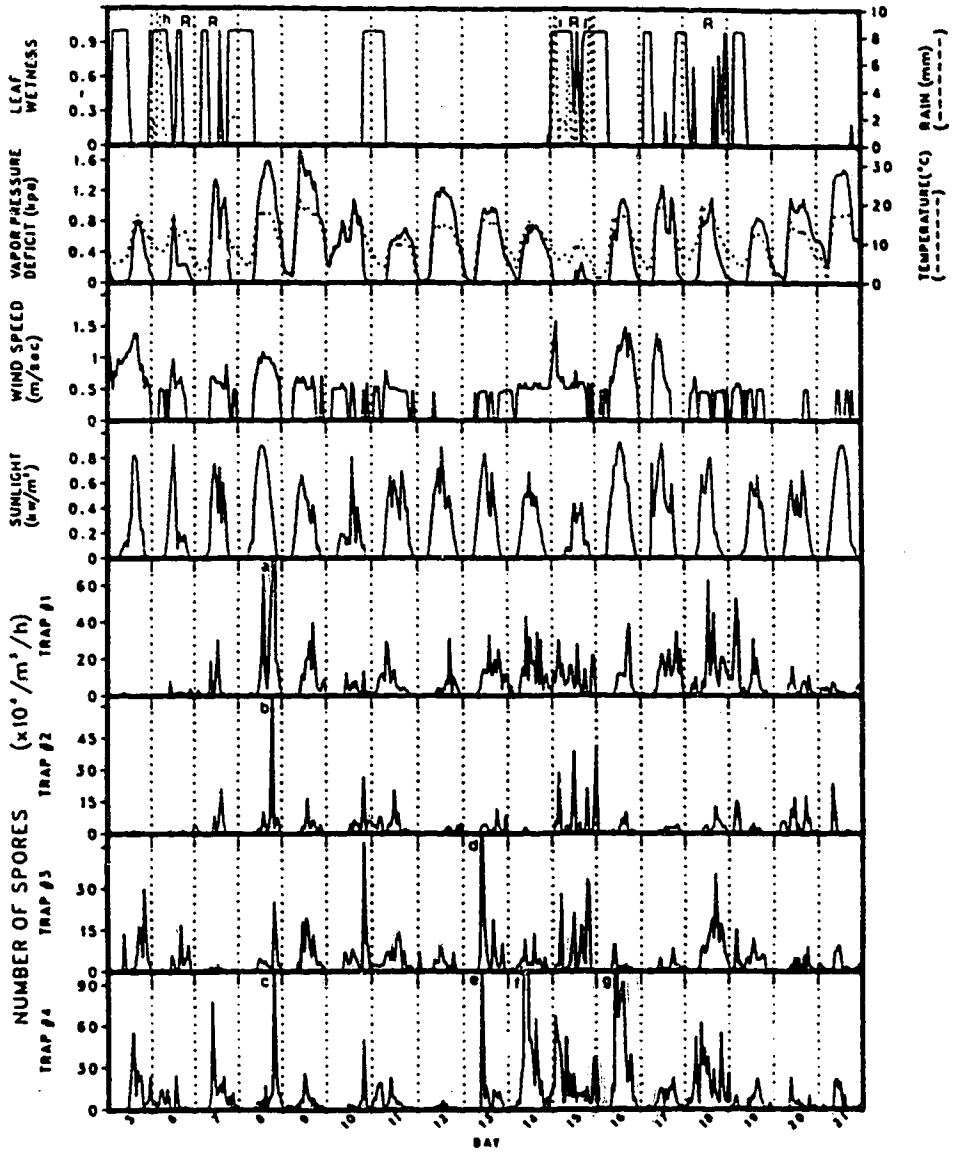

LEAF WETNESS
VAPOR PRESSURE DEFICIT (kpa)
WIND SPEED (m/sec)
SUNLIGHT (kw/m²)
NUMBER OF SPORES (x10³/m³/h)
TRAP #1
TRAP #2
TRAP #3
TRAP #4
RAIN (mm)
TEMPERATURE(°C)
DAY

FIG. 2.7. Spore release by *E. harknessii* in response to changes of VPD, exposure to fluorescent light, and airspeed. A single gall was placed in front of the spore trap at the beginning of day 1. After 2 days it was replaced by a fresh gall that remained in place for 2 more days.

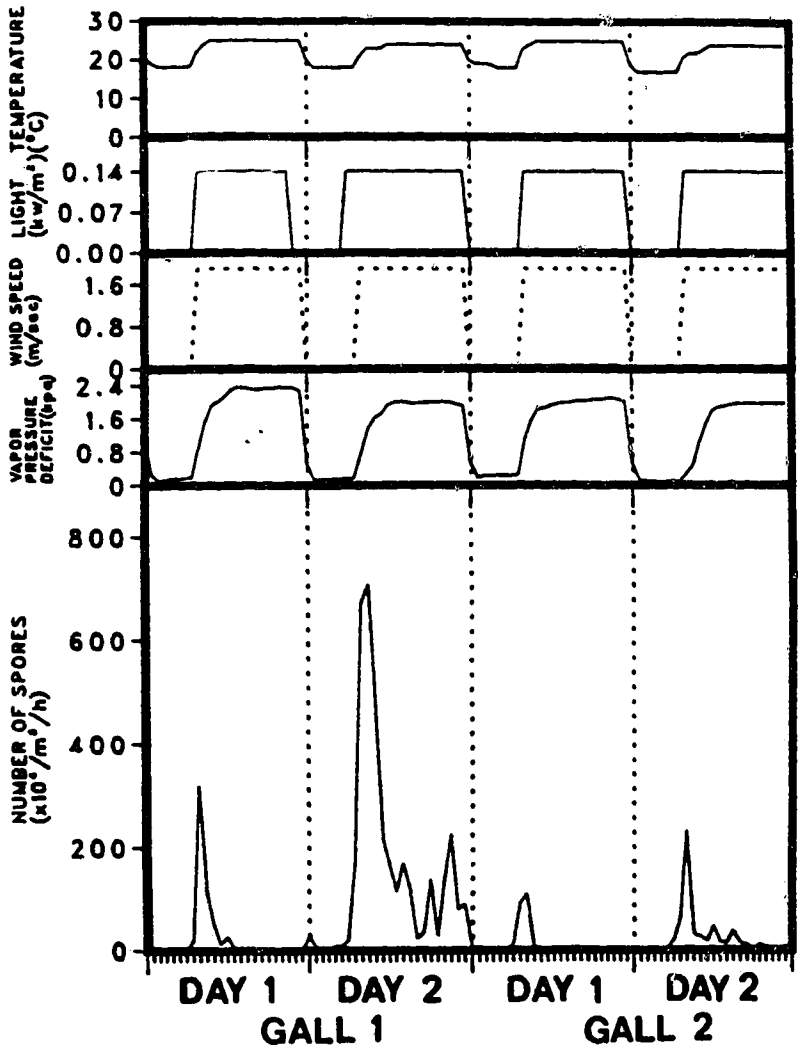
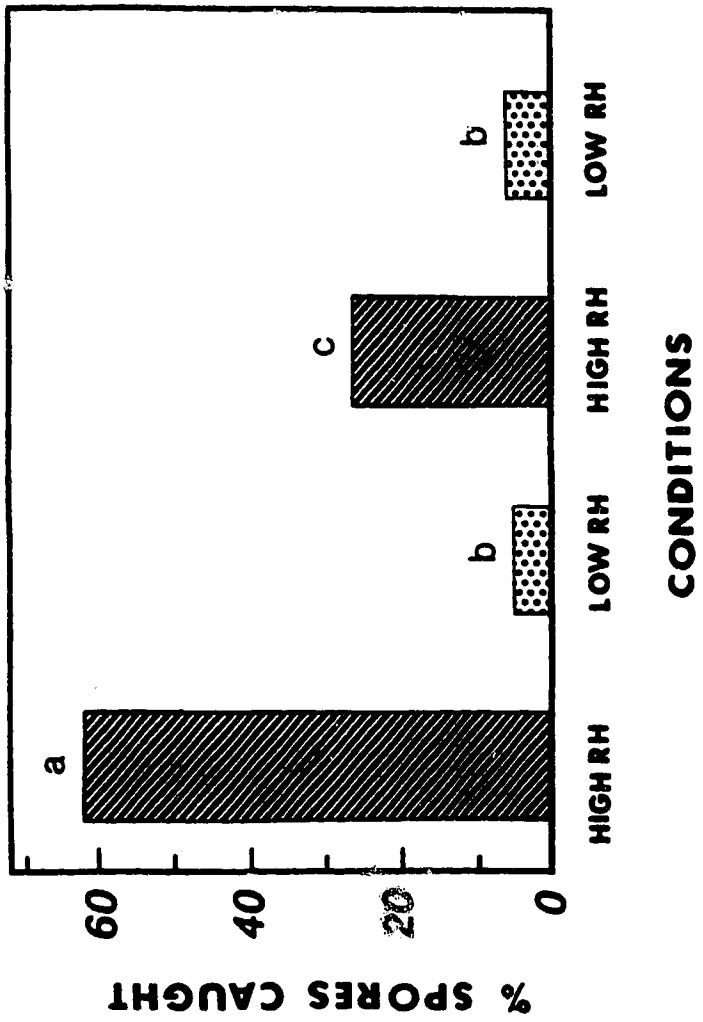
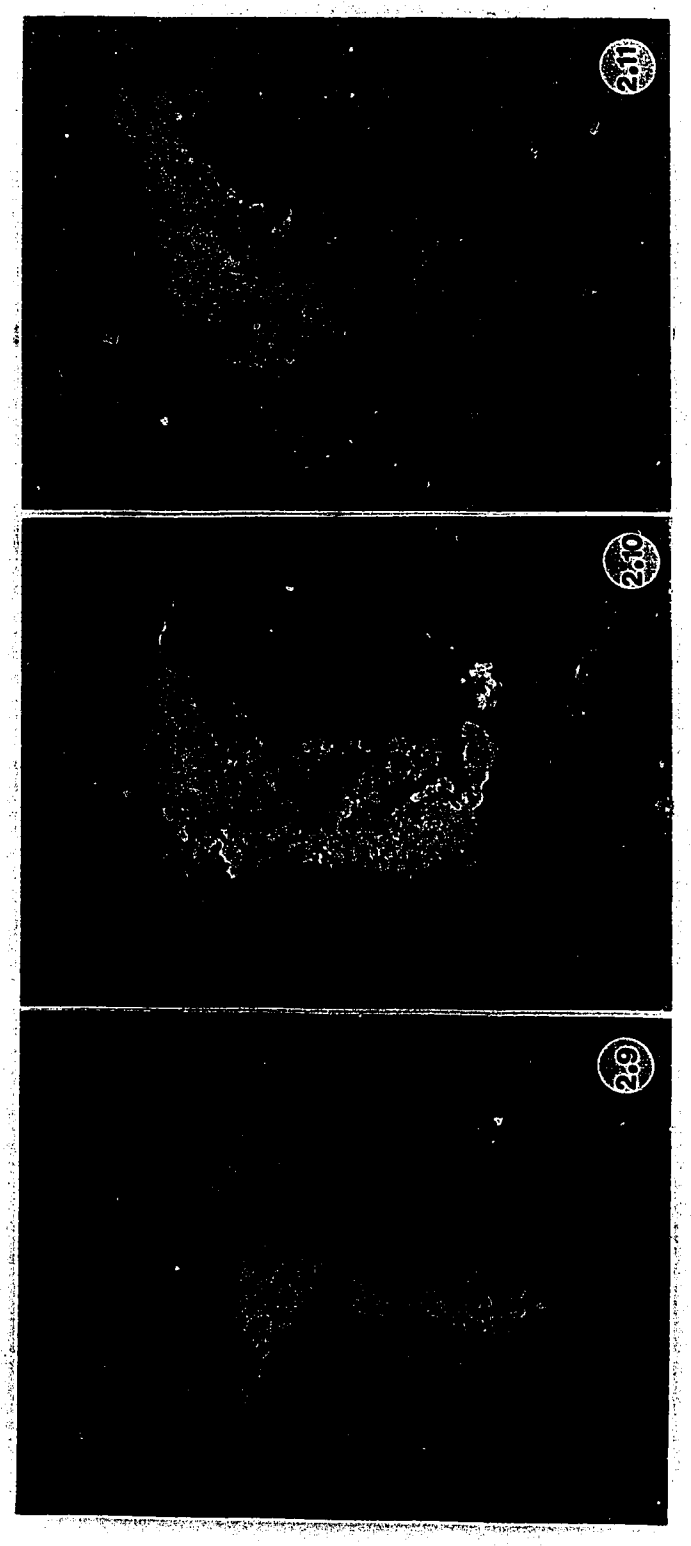

TEMPERATURE (°C)
30
20
10
0
LIGHT (kw/m²)
0.14
0.07
0.00
WIND SPEED (m/sec)
1.6
0.8
0
VAPOR PRESSURE DEFICIT(kpa)
2.4
1.6
0.8
0
NUMBER OF SPORES (x10³/m³/h)
800
600
400
200
0
DAY 1
DAY 2
DAY 1
DAY 2
GALL 1
GALL 2

FIG. 2.8. Spore production under conditions of low and high VPD. Galls were exposed to 0.0 kPa VPD (100% RH) for 8 h and then exposed to high VPD (0.40 ± 0.11 kPa) (77 ± 5% RH) for 16 h. The procedure was then repeated. For each of the 11 galls, the percentage of the total number of spores produced in each of the four periods was determined. Period percentage values were then averaged over the 11 galls to produce the values shown. Bars with the same letter are not significantly different at $p = 0.05$ in paired *t*-tests of spore production per hour.


% SPORES CAUGHT
0
20
40
60
a
b
c
b
HIGH RH
LOW RH
HIGH RH
LOW RH
CONDITIONS

FIGS. 2.9-2.11. The process of peridial rupture in *E. harknessii*. Fig. 2.9. Telium with intact peridium after 24 h on a laboratory bench. x20. Fig. 2.10. After 9 h in a dew chamber, the telium has expanded. The peridium has ruptured, and masses of fresh spores have been partially extruded from the openings. x20. Fig. 2.11. After an additional 2 days on the laboratory bench, many of the spores had dried up and fallen away, thus making the openings in the peridium more conspicuous. The peridium was rather rigid, and did not recurve to cover the opening that has been created. x20.


2.11
2.10
2.9

2.5 References

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Chapter 3. Survival of *Endocronartium harknessii* Peridermioid Teliospores in a Simulated Airborne State[1]

3.1 Introduction

Western gall rust, caused by *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka, was first reported in 1876 (Nelson, 1971), and now is considered to be one of the most important stem rusts of hard pines in western Canada and the United States (Dietrich *et al.*, 1985; Ziller, 1974). Because of its importance, there has been considerable interest in screening or breeding trees for resistance to this disease (Merrill *et al.*, 1986; Old *et al.*, 1986). The fungus produces peridermioid teliospores (Hiratsuka, 1969) that are released in greatest numbers during the daytime when temperature, wind speed, and solar radiation are high, and relative humidity (RH) is low (Chang and Blenis, 1986; Chang *et al.*, 1989). *Endocronartium harknessii* teliospores are similar in size to the urediniospores of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn. (Cummins, 1971; Meinecke, 1916), which is known to spread long distances (Stakman and Harrar, 1957). If *E. harknessii* spores could remain viable in an airborne state for some time, it is possible that trees selected for resistance to local spores would be liable to infection from distant inoculum sources. Given the long life cycle of trees, this would have considerable implications for the long term stability of resistance to this disease.

Currently, however, there is virtually no data to indicate the probable longevity of *E. harknessii* in an airborne state. Nor is there much indication of how survival would be affected by such factors as RH, temperature, or sunlight, which influence the longevity of other fungal spores (Hwang, 1942; Maddison and Manners, 1972; Rotem *et*

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[1]A version of this paper has been published in Can. J. Bot. 67: 928-932., 1989.

al., 1985; Tisserat and Kuntz, 1983). Most studies of *E. harknessii* survival have focussed on methods for preserving spores, and have been conducted at temperatures lower than those that likely would be encountered during spore dispersal (Lightle, 1955; Mielke, 1955; Nelson, 1971). Survival studies have been conducted at higher temperatures with some other tree rusts, although these experiments generally examined the viability of masses of spores in vials or *in situ* on aecia rather than in an airborne state. Longevity of *Cronartium quercuum* (Berk.) Miyabe ex Shirai aeciospores, which are morphologically similar to the teliospores of *E. harknessii*, decreased with increasing temperature. At 4 °C, survival was 63% after 4 months; at 20 °C, 17% of the spores were alive after 4 weeks; and at 36 °C the spores survived only a few hours (Nighswander and Patton, 1965). Germinability of spores removed from aecia of *C. comandrae* Pk. was not clearly related to diurnal fluctuations of temperature and RH, although cool, humid conditions were associated with high germinability, and rainfall during the previous day was associated with low germinability (Powell, 1974). Because of the lack of data on the survival of *E. harknessii* spores and because of the significance of spore survival in light of attempts to develop resistance to this pathogen, we attempted to determine the longevity of *E. harknessii* spores in a simulated airborne state. Our specific objectives were to (*i*) determine the effects of temperature and RH on spore survival of *E. harknessii*, and (*ii*) to determine the longevity of *E. harknessii* spores out of doors during daylight hours.

Materials and methods

Endocronartium harknessii spores were collected from freshly sporulating galls on lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) trees located near Hinton, Alta., by knocking the galls and catching the spores in a cardboard box held beneath the gall. Large pieces of debris were discarded and the spores were transferred to 20-mL

vials and kept at 5 °C in a sealed desiccator containing $CaCl_2$. The next day the spores were passed through a 75 µm mesh sieve, put into 2-mL "Nunc Cryotubes" (Kamstrup, DK 4000 Roskilde, Denmark), and stored at -5 °C for 1-5 days. The tubes were transported to the laboratory in an ice chest, and stored in liquid nitrogen for about 1 year. Fresh spores, used in some experiments, were collected from lodgepole pines on the campus of the University of Alberta and were used immediately after sieving.

A microthread technique, originally designed by May and Druett (1968) and later modified by Tisserat and Kuntz (1983), was used to maintain the spores in a simulated airborne state by impacting them onto spider webs or fine plastic strands that were attached to rectangular frames. These frames consisted of two pieces of electrical wire (3 mm diameter and 6 cm long) that were kept 3 cm apart by mounting the ends of the wire into plastic strips (4 x 1.5 x 0.1 cm) (Fig. 3.1). The frames were sterilized in 70% ethanol and dried in acetone before the microthreads were attached to them. Spiders (*Achaearanea tepidariorun* Koch) were maintained in 300-mL glass jars and fed fruit flies (*Drosophila* sp.). To attach a web to a frame, a spider was placed on one of the wires of the frame, and then dislodged by tapping the frame. As the spider fell, the filament that it produced was wound around the wires. During winter, when spiders could not be maintained, a single filament (30 µm diameter) from a strand of dental floss was wound around each frame and held in place by glue.

Prior to starting an experiment, stored spores were given heat shock by placing the vials of spores in a 40 °C water bath for 5 min (Cunningham, 1973). The spores were rehydrated for 12 h at 15 °C by placing them into a Petri dish that was floating on water inside a sealed desiccator. To apply the spores to the microthreads, 3 mg of spores was thoroughly mixed with 97 mg of talc and placed on a sheet of aluminum foil. The foil was held inside of a 68 x 42 x 76 cm cabinet, 50 cm above the frames that had been placed on the floor of the cabinet. The spores were dispersed by a puff of air, and the cabinet door was closed for 5 min to permit the spores to settle onto the frames.

Spore viability after the specific treatments was assessed by pressing the frames onto Petri plates containing 1.5% water agar. The microthreads were cut from the frames with a razor blade and the frames were removed, leaving the microthreads and spores on the agar surface. After an incubation period of 24 h at 22 °C in the dark, percentage germination of 100 spores per plate was determined. Spores were considered germinated if they had produced germ tubes equal to the length of the spores.

3.2.1 Effect of relative humidity and temperature on spore survival

Twenty spore-bearing frames were mounted on styrofoam blocks inside six 6 L or 9 L desiccators (Fig. 3.1). Two desiccators were put into each of three unlighted incubators at 6, 15, and 24°C. Glycerine solutions with refractive indices of 1.3463 and 1.4529 had been added to the desiccators to produce RHs of 39 and 98%, respectively, over the range of temperatures (American Society for Testing and Materials, 1980). The resultant vapor pressure deficits (VPD) would have varied between 0.570 and 1.820 kPa at 39% RH and between 0.019 and 0.060 kPa at 98% RH. After incubation periods of 1, 2, 4, 8, and 16 days, four frames were randomly removed from each desiccator and placed onto the plates. The experiment was conducted five times with stored spores and four times with fresh spores. Germination at each time was expressed as a percentage of germination at time 0, which ranged from 76 to 86% (average = 81%) over the nine replicates.

Repeated-measures of analysis of variance, which was suggested as a method to determine the effects of different treatments on disease progression over time (Madden 1986), were used to determine if RH and temperature had a significant effect on spore survival. For this analysis, replicates were considered to be nested within spore type (fresh vs. stored). Each replicate served as a block in which temperature was the main plot, and RH was the subplot. For each combination of temperature and RH, repeated measures of spore survival were taken over time. In determining the F values of time,

and the interactions of time with temperature and RH, conservative critical values of F were used. This is recommended if the correlation of two successive measurements over time is likely to be greater than the correlation of measurements that are more widely separated over time (Gill, 1978). To determine whether the survival curves for the separate RH and temperature combinations had similar shapes, third-order polynomials were used to partition the linear, quadratic, and cubic effects of time on spore survival for each of the separate RH and temperature combinations.

3.2.2 Survival of spores exposed to sunlight

Eighteen frames, wound with spider web and bearing fresh spores, were placed out of doors, inside each of two shelters (Fig. 3.2). The shelters permitted the spores to be exposed to sunlight at all times, but helped prevent the spores from being dislodged by wind. Nevertheless, spores tended to be lost over time and completely removed by rain. Therefore to ensure adequate spore numbers at each sampling time, the experiment was performed only on clear days, between 09:30-19:30 in 1985 and 08:30-20:30 in 1986, with three frames being randomly sampled at 2-h intervals. The experiment was conducted three times in 1985 and six times in 1986. During four of the trials in 1986, a temperature and RH probe (model 201, Campbell Scientific Inc., Logan, UT), and pyranometer (model LI-200SZC, Li-Cor Inc., Lincoln, NE), were placed near the frames and connected to a datalogger (CR-21 Micrologger, Campbell Scientific Inc.). RH and temperature data for the remaining trials were obtained from a meteorological station located 2 km away, and sunlight data were obtained from a meteorological station located 30 km away. Environmental conditions at these meteorological stations appeared similar to those at the experimental site; temperature, RH, and sunlight data from those stations were similar to those obtained from the datalogger for the 4 days in which it was in operation.

Table B-2

Higher Order Thematic Description of N's Experiences in Obtaining Health Care for her Daughter

Thematic Clusters	Generalized Descriptions
1. Physician characteristics (Excerpt numbers 1,9, 10, 12, 13, 14, 30, 31, 32, 33, 35, 36, 40, 41)	Helpful physicians have been those who are perceived as caring for the child and family, being available to the family, supportive of the mother's concerns. The presence of mental retardation in the child does not affect treatment prescribed; treatment is not based on ability or social status.. The physician expresses confidence in the mother's caring ability, is aware of the needs of the family and is knowledgable about community resources. He/she is at ease with the child and has skills that encourage cooperation. Physicians are dedicated individuals, confident of their abilities and able to put the child at ease. They possess good communication skills, are knowledgable and familiar with mental retardation.
2. Adolescent characteristics. (Excerpt numbers 2, 6, 7, 8, 23,)	Generally, the child is healthy now as compared to infancy when associated health problems required hospitalizations. As a child, M. was fearful of hospital situations resulting in uncooperative and uncontrolled behavior outbursts. Adolescence has diminished this fear but has brought new problems associated with sexual maturity, including birth control, sexual abuse and providing appropriate socialization experiences.
3. Mother's behavior (Exerpt numbers 2, 3, 21, 27)	Mother needed to shop around or look for physicians and other health care providers who could work with the child. Mother learned to use referrals from other individuals and acquired advocacy skills. Mother believes in preventative medicine.
4. Health care provision requires cooperation between parents and providers.(Excerpt numbers 4, 11).	Health care providers have worked together in a cooperative manner assisted by their location in the same hospital. Thus service provision has been coordinated
5. The local health units have not been supportive (Excerpt numbers 17, 18)	Interactions with the health unit staff left the mother discouraged. Information given by health unit staff did not seem to be current and differed from that obtained from other professionals with whom the mother was in contact.

(table continues)

3.3 Results and discussion

3.3.1 Effect of temperature and relative humidity on spore survival.

Spore survival was favored by low RH and low temperature. At both 98 and 39% RH, and at all time periods, percentage of spore survival decreased with increasing temperature. Similarly, at all temperatures and times, excepting the combination of 24 °C and 8 days, survival was less at 98% than at 39% RH. Repeated-measures of ANOVA indicated that these temperature and RH effects were significant and that the interaction between them was not (Table 3.1). The analysis also showed that there was no significant difference between stored and fresh spores, indicating that the liquid nitrogen storage did not adversely affect subsequent spore survival. The time effect was significant, as expected, as were all of the interactions with time.

The survival curves appear to have a different shape at high RH than at low RH (Fig. 3.3). The three curves at 98% RH have an inverse J shape, the curves at 39% RH and 6 and 15 °C appear linear, and the curve at 39% RH and 24 °C appears linear over the first 8 days. The apparent slope change of the latter curve after 8 days probably results from a levelling off because of the death of most spores by 8 days. The apparent difference in shape was confirmed by determining the linear, quadratic and cubic effects of time on survival: linear and quadratic effects were significant for the three curves at 98% RH, whereas only linear effects were significant for the curves at 39% RH and 6 and 15 °C, and for the first 8 days at 39% RH and 24 °C. This suggests that RH, in addition to affecting the numbers of surviving spores, also affects the pattern of spore survival over time.

3.3.2 Survival of spores exposed to sunlight.

Spore viability declined over time, in almost direct proportion to the duration of exposure (Fig. 3.4). Regression of percentage spore survival against time yielded r^2

values of 0.63-0.98 and residuals that appeared to be randomly distributed around the regression line. Plotting the slope of the survival versus time curves, for the individual days, against environmental conditions (Fig. 3.5), indicated that spore viability tended to decrease most rapidly on those days with the highest temperature and sunlight. Although high RH adversely affected spore survival in darkness (Fig. 3.3), at moderate RH values (<50%), increasing RH favored survival in sunlight (Fig. 3.5). It is possible that the enzymes responsible for photoreactivation (Nasim and James, 1978) are more active at moderate RH than at low RH.

This experiment was not designed to determine the role of sunlight on survival. However, the rapid death of spores placed out of doors, compared with spores kept in the darkness in the first experiment, suggests that sunlight is important in determining spore longevity. For example, an average of 91% of the spores remained viable after 1 day at 39% RH and 24 °C in darkness (Fig. 3.3), whereas an average of only 33% of the spores placed outside were alive after 12 h at RH values of 21-49% and temperature values of 14-31°C (Figs. 3.4 and 3.5).

Although the viability of spores incubated out of doors decreased quickly, survivability of these spores may be adequate to permit long distance dispersal of the pathogen. The majority of the spores of *E. harknessii* are released during the daylight hours (Chang and Blenis, 1986; Chang *et al.*, 1989). Based on the results shown in Fig. 3.4, 33% or more of these spores likely would last until nightfall. Data from the *in vitro* studies (Fig. 3.3) suggests that spore survival at night would be quite high if the temperature was 15 °C or lower, as typically was the case in one area where *E. harknessii* was common (Chang *et al.*, 1989). Given that 1 g of spores represents approximately 10^8 spores (K. -F. Chang, unpublished data), it would seem that a large number of spores could last at least 1 and perhaps 2 days. During a 1-day period, fungal spores may move from several hundred to 2000 km (Aylor, 1986; Aylor *et al.*, 1982; Close *et al.*, 1978). Furthermore, survival of *E. harknessii* under sunlight seems

comparable to that of *P. graminis* f. sp. *tritici*, which is well known for its ability to spread long distances (Hwang, 1942; Maddison and Manners, 1972) and *Uromyces phaseoli* (Reb.) Wint. var. *typica* Arthur, which has been considered to be relatively resistant to high temperatures (Rotem *et al.*, 1985) and to be more resistant than spores of *P. striiformis* West. (Hwang, 1942) (Table 3.2). In summary, our results indicated that (*i*) survival of *E. harknessii* spores was adversely affected by high temperature and high RH, (*ii*) about two-thirds of the spores exposed to sunlight on clear summer days had died after 12 h of exposure, and (*iii*) *E. harknessii* survived relatively well out of doors in a simulated airborne state and thus presumably could be capable of long distance spread.

Fig. 3.1. Apparatus for exposing spores to constant RH and temperature in a simulated airborne state. Frames were mounted on styrofoam inside a desiccator in which glycerine solutions had been placed to maintain 39 or 98% RH. Fourteen of the 20 frames had been removed for sampling.

Fig. 3.2. Apparatus for exposing spores to sunlight. Photograph shows six frames mounted on a wooden support inside a shelter constructed from iron stands, nylon netting, and paper. During the actual experiment, three such wooden supports were placed inside the shelter.

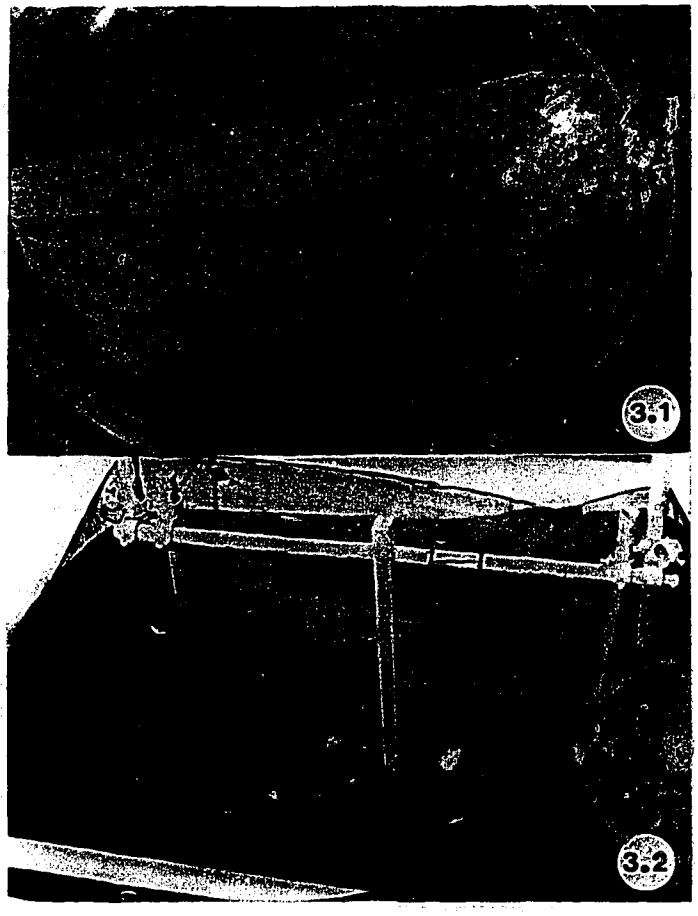
3.1
3.2

Fig. 3.3. Survival of spores of *Endocronartium harknessii* incubated at 39 and 98% RH and 6, 15, and 24°C for 1, 2, 4, 8, and 16 days in darkness. Vertical lines represent standard deviations, based on nine replicates.

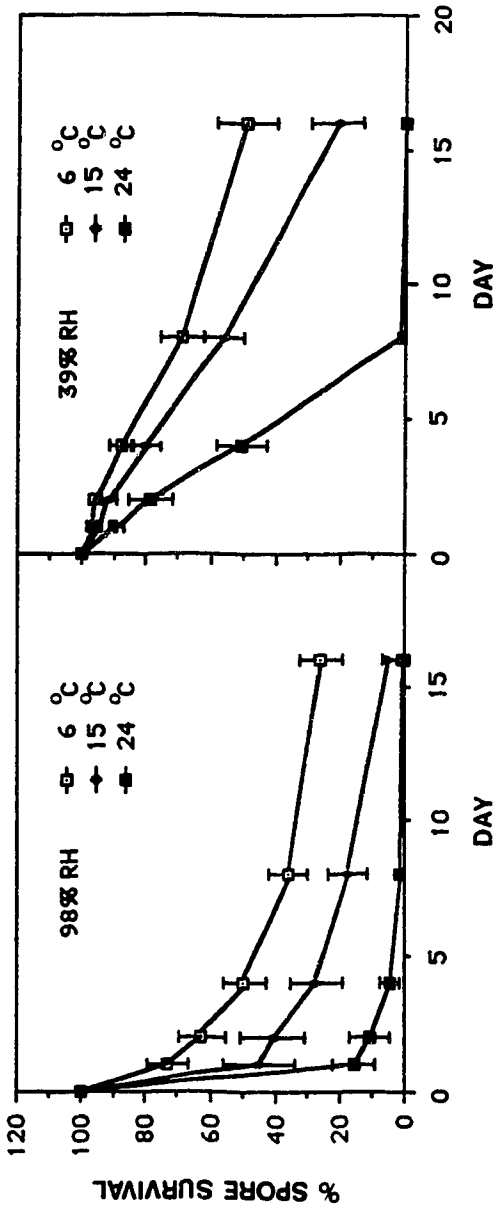
% SPORE SURVIVAL
120
100
80
60
40
20
0
98% RH
6 °C
15 °C
24 °C
0
5
10
15
DAY
39% RH
6 °C
15 °C
24 °C
0
5
10
15
20
DAY

Fig. 3.4. Survival of spores of *Endocronartium harknessii* exposed to full sunlight on clear days from 08:30 to 20:30. Vertical lines represent two standard deviations, based on nine replicates, except for the12-h exposure, which was based on six replicates.

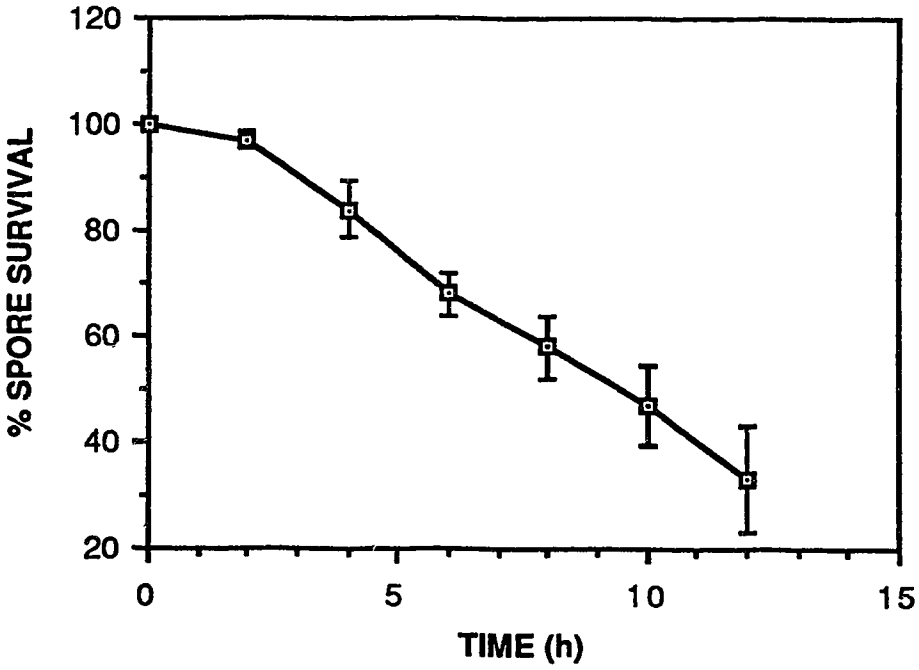
120
100
80
60
40
20
% SPORE SURVIVAL
0
5
10
15
TIME (h)

Fig. 3.5. The relationship between temperature, RH, VPD, and sunlight, and the slopes of nine survival curves obtained by placing spores out of doors and regressing percent survival against time. The environmental data for the days with slopes of -8.15, -3.73, -3.47, -3.94, and -5.03 were obtained from a remote weather station. The curves were fitted by hand, ignoring the slope values of -8.15 for which the environmental readings were aberrant. The slopes of value -8.15, -3.73, and -3.47 were calculated on the basis of survival over 10 h; the remainder were based on survival over 12 h.

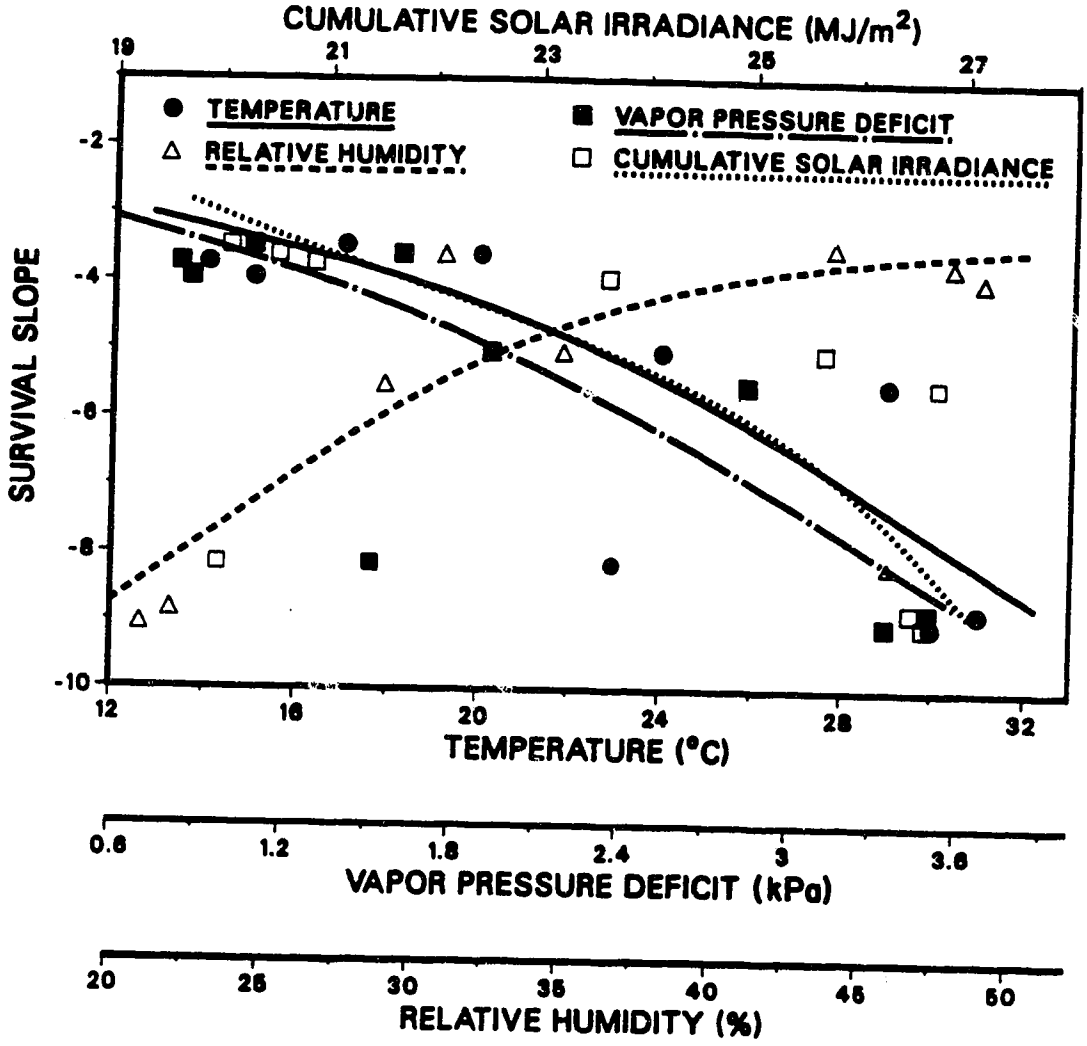
CUMULATIVE SOLAR IRRADIANCE (MJ/m²)
19
21
23
25
27
TEMPERATURE
RELATIVE HUMIDITY
VAPOR PRESSURE DEFICIT
CUMULATIVE SOLAR IRRADIANCE
SURVIVAL SLOPE
-2
-4
-6
-8
-10
12
16
20
24
28
32
TEMPERATURE (°C)
0.6
1.2
1.8
2.4
3
3.6
VAPOR PRESSURE DEFICIT (kPa)
20
25
30
35
40
45
50
RELATIVE HUMIDITY (%)

Table 3.1. Analysis of variance table showing the effects of spore freshness (fresh vs. stored), temperature (T), relative humidity (RH), and time (TI) on the survival of *Endocronartium harknessii* spores

Source	Degrees of freedom	Sum of squares	Mean square	F-ratio
Blocks (B)				
Spore freshness	1	25287.8	25287.8	4.2^{ns}
Replicate within spores	7	41906.7	5986.7	
Main Plot				
T	2	154507.4	77253.7	82.8*
Error = B x T	16	14928.6	933.0	
Subplot				
RH	1	244864.0	244864.0	67.8*
T x RH	2	7220.3	3610.2	2.2^{ns}
Error = B x (RH+RH x T)	24	39,154.5	1631.4	
Sub-subplot (repeated measures)				
TI	3 (1)[a]	125949.7	41983.2	204.2*
TI x T	6 (2)	8138.4	1356.4	6.6*
TI x RH	3 (1)	19776.1	6592.0	32.1*
TI x T x RH	6 (2)	16492.9	2748.8	13.4*
Error = B x (TI+TI x RH+ TI x T+TI x T x RH)	144 (48)	29603.4	205.6	

a Numbers in parentheses indicate the degrees of freedom used in the F-test (Gill 1978).

* Significant at P = 0.01.

^{ns} Not significant at P = 0.05.

Table 3.2. A comparison of the outdoor survival of the peridermioid teliospores of *Endocronartium harknessii* with the urediniospores of three other rusts

Species	%survival	Duration of experiment (hrs+min)	Temperature (°C)	Light intensity	Reference
P. graminis f. sp. *tritici*	55	8+00	12-26	'high' [b]	Hwang 1942
E. harknessii	47	8+00	14-31	19.5 MJ/m^2 [c]	
U. phaseoli	35	8+00	34.8 [a]	20.1 MJ/m^2 [c]	Rotem *et al.* 1985
P. graminis f. sp. *tritici*	20	9+00	27-42	'high' [b]	Hwang 1942
P. striiformis	8	8+20	9-22	clear, hazy [d]	Maddison and Manners 1972
P. striiformis	0.15	9+00	10-25	hazy [e]	Maddison and Manners 1972

[a] Average temperature during experiment; the other temperatures are ranges.

[b] Results reported from the University of Minnesota, St. Paul.

[c] Total amount of sunlight.

[d] Experiment was carried out on 27 March 1968 in Southampton, England (latitude 50.9°N, 65 m above sea level). Sky was initially clear and became hazy after period of time.

[e] Experiment was conducted on 1 June 1968 in Southampton, England.

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Chapter 4. Dispersal and Disease Gradients of Western Gall Rust[1]

4.1 INTRODUCTION

Western gall rust, caused by *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka (Anamorph, *Peridermium harknessii* J. P. Moore (Arthur and Cummins, 1962; Epstein and Buurlage, 1988)) is one of the most serious diseases of hard pines, including lodgepole pine (van der Kamp, 1988), jack pine (Dietrich *et al.*, 1985), and ponderosa pine (Walla and Tuskan, 1987) in North America. The pathogen has no alternate hosts and peridermioid teliospores produced on pines are capable of infecting other pines (Hiratsuka, 1969). *E. harknessii* causes galls, which typically have little impact if they occur on branches, but which can kill a tree if they occur on the main stem (Chang *et al.*, 1989; Gross, 1983). Growing seedlings in nurseries and planting them on harvested sites may become more common as forest management becomes more intensive. There is a risk that young trees may become infected by gall rust inoculum originating from nearby infected trees both in the nursery and on the planting site. For that reason, it is important to know how far the spores are capable of being dispersed in order to determine if there is a critical distance beyond which inoculum levels and infection frequencies would be tolerable.

Spore dispersal gradients may result in disease gradients if the spores are viable and are deposited on susceptible hosts in the presence of environmental conditions that are favorable for infection (Gregory, 1982; McCartney and Fitt, 1987). To date there is little information available on dispersal and infection gradients of western gall rust. Few infections were present among ponderosa pines growing further than 90 m from

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[1]A version of this chapter has been submitted to **Plant Disease** (1990) for publication.

infected trees in Nebraska (Peterson, 1973), and infected jack pine seedlings were "virtually nonexistent" beyond 274 m from an inoculum source in a Manitoba study (Carlson, 1969). However, there was no detailed information about dispersal or infection gradients in either study. Infection gradients have been described for sweetfern blister rust and fusiform rust, caused by *Cronartium comptoniae* Arth (Van Arsdel, 1961) and *C. quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Schmidt *et al.*, 1982), respectively. Both species produce aeciospores that are morphologically similar to the aeciospores of *E. harknessii*. The number of *C. comptoniae* spores decreased significantly beyond 27 m from an inoculum source on one plot but numbers remained relatively constant over 10 m on a second plot. No infected sweetfern (*Comptonia peregrina* (L.) Coult.) plants were found beyond 30 m from an inoculum source (Smeltzer and French, 1981). For *C. quercuum* f. sp. *fusiforme*, aeciospore counts and water oak (*Quercus nigra* L.) seedling infection decreased by approximately 87% and 90%, respectively, over a distance of 3.1 to 152.4 m. Nevertheless, there were still 0.9 infections/cm^2 leaf at the furthest distance. The authors predicted that even at 400 m from a heavily infected pine stand, enough oaks would be infected to result in 10% infection among pines growing among the oak (Schmidt, 1982).

Spore concentration will vary with distance from the spore source, but meteorological factors also may be important (Gregory, 1968; 1982). Rainfall, relative humidity (RH), and temperature could influence spore production and release (Chang *et al.*, 1989). Wind speed, which tends to increase with increasing height above ground (Bergen, 1971), and wind orientation, likely would affect spore numbers at different directions from a source (Fitt and McCartney, 1986). Turbulence also may play an important role both in local and long distance dispersal of spores (Robert, 1962). The size of openings in forest canopies may influence rust incidence; an inverse relationship existed between opening size and the incidence of *C. ribicola* (Van Arsdel, 1961) and *E. harknessii* (Blenis and Bernier, 1986). This relationship may have been

caused by the effect of opening size on micrometeorological conditions, wind patterns, and wind speed (Bergen, 1971; Blenis and Bernier, 1986; Van Arsdel, 1961).

The objectives of this project were to (i) determine the relationship between distance from a heavily infected lodgepole pine stand and spore concentration and seedling infection in a young adjacent stand, and (ii) determine the effect of environmental factors on spore concentration and spore concentration gradients.

4.2 MATERIALS AND METHODS

The experiments were conducted in a cut block located near Hinton, Alberta, at an elevation of about 1,280 m which had been scarified in 1977. The block was bounded on the west by approximately 20-year-old lodgepole pine trees (Fig. 4.1). Eighty one percent of the 20-year-old trees were infected, with an average of seven galls per infected tree, based on a sample of 200 trees randomly selected within 15 m of the stand edge.

A total of 12 rotorod samplers (U-rod type, Ted Brown Associates, Los Altos Hills, CA 94002) were mounted on wooden poles 0.5 m above ground in two parallel rows at right angles to the infected stand, at distances of 2, 5, 10, 20, 40, and 80 m from the stand edge (Fig. 4.2). Sporulating galls on seedlings in the cut block that were growing within 100 m of a rotorod were wrapped with masking tape (3.5 cm in width) to prevent background contamination. The samplers were 2.5 m apart at the 2 and 5 m locations, to ensure that they were equally distant from the nearest trees with galls at the stand edge, and 5 m apart at all other locations. The samplers were powered by car batteries and operated from 0920 to 1120 hr each day for 16 days in 1985 and 17 days in 1986. Spores were impacted onto silicon grease coated polystyrene rods (5.0 x 0.16 x 0.1 cm) mounted on the samplers. Spore numbers were determined at a magnification of 100x over a 0.01 cm x 5 cm area of the rods and the counts were converted to spores/m^3 of sampled air (Table 4.1) according to the formula:

spore concentration = total spores caught in two polystyrene rods · (total volume of sampled air)$^{-1}$

where total volume (m^3) of sampled air = collecting area (0.05 cm^2) · swing diameter (8.6 cm) · RPM (2400) · time (120 min) · π · (10^6 cm^3/m^3)$^{-1}$.

A datalogger (CR-21 Micrologger, Campbell Scientific Inc. Logan, UT 84321) was placed in the cut block, 40 m from the eastern edge of the infected stand (Fig. 4.2). The datalogger recorded temperature and RH with a temperature and RH probe (Model 201, Campbell Scientific Inc.); wind speed and direction with a cup anemometer (model 014A, Met One Inc. Grants Pass, OR 95726) and a wind vane (model 024A, Met One Inc.); solar radiation with a LI-COR pyranometer (model LI-200S, Lincoln, NE 68504); and rainfall with a tipping bucket rain gauge (model 2501, Sierra-Misco Inc. Berkeley, CA 94710). The environmental data (Table 4.2) were recorded every minute and compiled into 30 min intervals. The average wind speed data were partitioned into eight wind direction sectors (north to north north east, north north east to east, etc.), and from these data the average easterly and westerly wind speed components were calculated trigonometrically. The spore concentration data for each separate day were fitted to the power law model (Gregory, 1968; 1982), which assumes that the number of spores caught or infections produced (y) is inversely proportional to some power of the distance from the inoculum source (x). This model can be expressed as:

$y = a \cdot x^b$ or $\ln(y) = \ln(a) + b \cdot \ln(x)$,

where a = a constant, b = the slope of the spore dispersal or the infection gradient. Stepwise multiple regression was then used to express the slopes of the spore dispersal gradients as a function of the following six environmental conditions: westerly wind component, easterly wind component, total wind, sunlight, temperature, and RH. Regression was also used to relate the total number of spores caught during each 2 hr period against the six environmental factors.

For disease gradient determination, seven transects were established perpendicular to the stand edge. Each transect consisted of eight 5 m x 15 m rectangular plots which were centered 2.5, 10, 20, 40, 60, 80, 100, and 120 m from the stand edge, with their long axis parallel to that edge. The numbers of healthy and infected trees in each plot were counted. The infection frequency data was weighted according to the total number of seedlings present in each plot and was then fit to the linear form of the power law by linear regression.

4.3 RESULTS AND DISCUSSION

Spore concentration, averaged over 33 days, decreased sharply from 115 spores/m^3 at 2 m to 67 spores/m^3 at 5 m from the stand (Table 4.1). Beyond 5 m, the reduction in inoculum concentration was gradual, and at 80 m, the average concentration was 27 spores/m^3.

It is possible that air flowing through the spore-rich layer of air mixed turbulently at the stand edge with the faster moving layer of spore-poor air flowing over the source stand. The spore-rich air layer close to the stand edge was sampled, thus resulting in the detection of a high concentration of spores in those areas. The decrease in spore concentration at further distances from the source may have been due to the same number of spores being suspended in a large volume of air and some spore deposition occurring over those distances (van der Kamp, personal communication).

When the spore dispersal data (Table 4.1) were analyzed separately by days, the data for 25 of the 33 days fit the power law with a r^2 value of 0.70 or more. Stepwise multiple regression of the slopes of these 25 spore dispersal gradients against the environmental parameters (Table 4.2) yielded the following equation:

$$y = -6.1 + 0.057 \cdot w,$$

where y = slope of the dispersal gradient and w = average wind speed from all directions. The equation was significant at $P = 0.005$, with a r^2 value of 0.29. These results indicated that the spore dispersal gradient would flatten with increasing wind speed, perhaps because spores would tend to be concentrated near the source on calm days and spread over a wider area on windy days. The low r^2 value, however, indicates that there was considerable variability in slope values that could not be accounted for by the measured environmental parameters.

There was considerable day-to-day variability in the number of spores caught (Table 4.1). Stepwise multiple regression of the total number of spores caught during each 2 hr period, averaged over all distances, against the environmental factors, yielded the following equation:

$$y = -279.6 + 276.0 \cdot ww$$

where y = the spore concentration value and ww = the westerly wind speed component in meters per second. The equation was significant at $P = 0.0006$, with a r^2 value of 0.31. These results indicated that increasingly greater spore concentrations were associated with increasing strength of the wind blowing in a westerly direction over the infected trees and into the cut block. Nevertheless, the rather low r^2 value indicates that a large amount of the day-to-day variation in spore capture could not be explained by the measured environmental parameters. This is not too surprising, because the number of captured spores is a function of spore production, spore release, and spore transport, and there is no simple relation between these factors and environmental conditions. For example, our work has shown that there is considerable variation in spore release within and among days (Table 1). In the absence of rainfall, spores produced under conditions of high RH during the evening may be released during the subsequent day as the spores dry out and are blown away by wind (Chang *et al.*, 1989).

Although RH, temperature, and sunlight effects did not account for much of the observed variability in spore deposition, these factors may influence the formation of

infection gradients by affecting spore survival (Chang and Blenis, 1989), germination and penetration (Nelson, 1971). For example, high RH and temperature, as well as bright sunlight, had a negative effect on spore viability of *E. harknessii*, and under such conditions, few spores would likely survive for more than 2 days (Chang and Blenis, 1989).

Percentage seedling infection between 2.5 m and 122.5 m varied from 0 to 12.0% (average = 4.2%). Although 4.2% infection might not cause economic loss in a naturally regenerated stand with many seedlings, such an infection level could be significant if there was further intensification of the disease as a result of secondary spread among the seedlings. Although 7.7% infection occurring at 2.5 m from the inoculum source was greater than the infection levels at the other distances ($\chi^2 = 2.86$, $P = 0.1$), no gradient could be detected when the infection data was fitted to the power law ($r^2 = 0.0005$, $P = 0.87$). Several possible factors may have been responsible for the lack of an infection gradient. First, although there were no spore samplers present further than 80 m from the infection stand, the rather flat spore dispersal gradient between 10 and 80 m suggests that there might not have been large differences in spore concentration between 10 and 122.5 m. Secondly, some secondary spread may have occurred among the regenerating seedlings, thus flattening the infection gradient (Gregory, 1968; 1982). Thirdly, variability in environmental conditions for infection may have outweighed the effect of inoculum dose.

In summary, our results indicated that: (*i*) spore concentration at 0.5 m above ground level decreased sharply between 2 and 5 m from an infected stand, and more gradually between 10 and 80 m from that stand, (*ii*) spore concentrations and the slopes of spore dispersal gradients were significantly affected by wind values, although there was considerable variation that could not be explained by the measured environmental parameters, and (*iii*) seedling infection between 0 and 122.5 m from the stand edge

averaged 4.2%, with 7.7% infection occurring 0-5 m from the edge, and no infection gradient being detectable.

Fig. 4.1. Map of the experimental site, showing the location of the rotorods, infection plots and surrounding area.

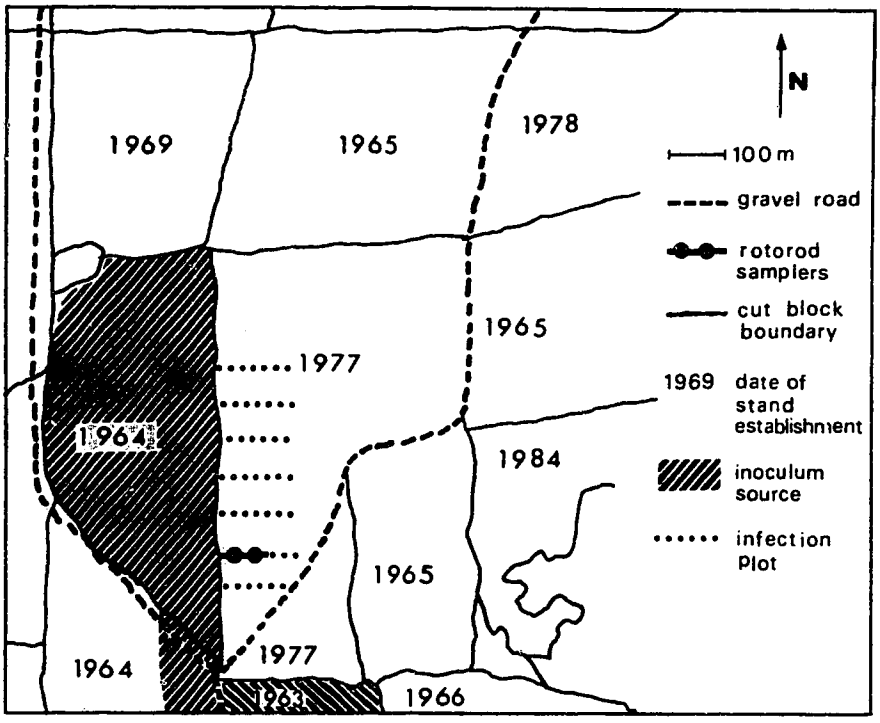
1969
1965
1978
1965
1977
1964
1984
1965
1964
1977
1963
1966
N
100 m
gravel road
rotorod samplers
cut block boundary
1969 date of stand establishment
inoculum source
infection Plot

Fig. 4.2. Detailed map of the experimental site showing the location of the inoculum source, 12 rotorod samplers, and datalogger. Seedling infection was examined in the eight rectangular plots.

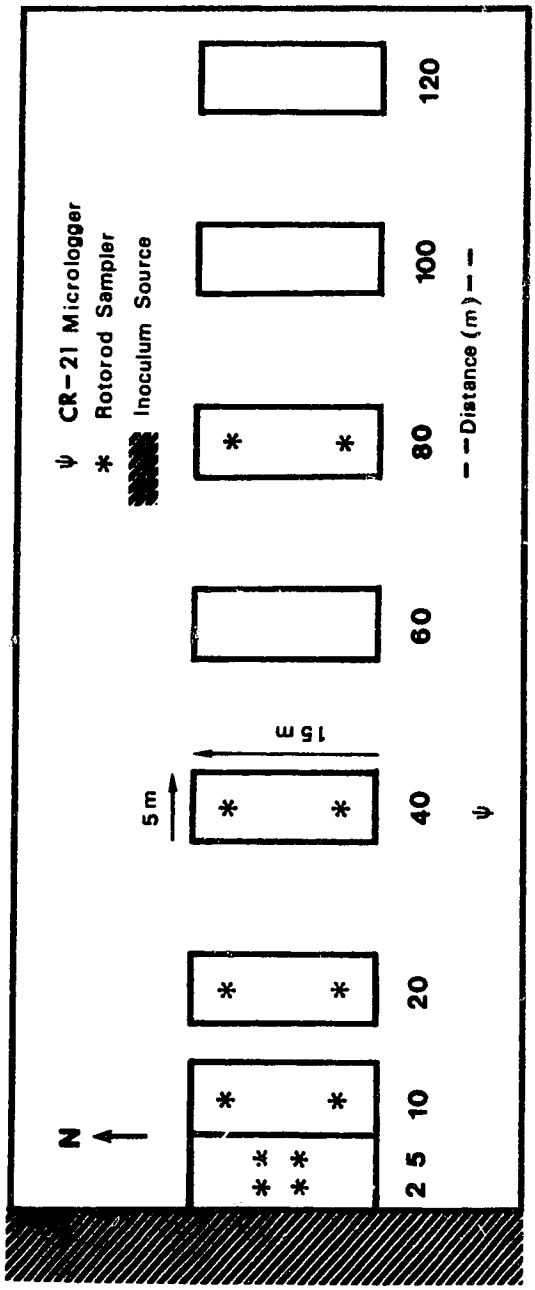
ψ CR-21 Micrologger
* Rotorod Sampler
Inoculum Source
Distance (m)
N
5 m
15 m
2 5 10 20 40 60 80 100 120

Table 4.1. Slopes (b), correlation coefficients (r^2), and the average number of peridermioid teliospores (spores/m^3 air) caught at various distances from the eastern edge of an infected lodgepole pine stand in June, 1985 and June, 1986

Sampling date	Distance (m)						Total	Power law	
	2	5	10	20	40	80		r^2	b
1985									
5	33	13	12	7	5	3	73	0.63	-0.56
6	343	142	81	74	81	42	763	0.74	-0.46
7	63	22	13	14	13	11	136	0.54	-0.39
10	94	85	47	45	36	23	330	0.81	-0.39
11	44	24	18	11	10	7	114	0.82	-0.50
12	152	74	37	39	36	18	356	0.74	-0.52
13	25	18	9	11	8	2	73	0.81	-0.58
14	60	27	19	19	16	7	148	0.73	-0.46
20	93	56	36	35	31	21	272	0.84	-0.36
21	19	14	13	11	8	6	71	0.51	-0.32
25	330	107	87	78	44	31	677	0.82	-0.57
26	208	82	45	34	26	18	413	0.87	-0.62
27	31	20	12	8	8	8	87	0.75	-0.38
28	20	14	10	11	12	7	74	0.35	-0.23
29	30	15	10	11	5	3	74	0.89	-0.59
30	17	13	7	10	7	5	59	0.82	-0.33
1986									
6	66	42	26	13	10	6	163	0.86	-0.74
7	55	35	26	26	28	15	185	0.73	-0.29
8	534	316	[illegible]	194	174	100	1548	0.88	-0.40
9	93	[illegible]	[illegible]	32	22	11	238	0.91	-0.51
10	34	[illegible]	[illegible]	18	12	9	103	0.79	-0.34
11	957	[illegible]	[illegible]	37	511	384	3678	0.78	-0.23
12	40	[illegible]	[illegible]	5	13	8	120	0.95	-0.41
13	[illegible]	[illegible]	[illegible]	[illegible]	6	6	43	0.64	-0.13
14	[illegible]	[illegible]	[illegible]	[illegible]	10	7	62	0.73	-0.18
15	[illegible]	[illegible]	[illegible]	[illegible]	8	5	62	0.59	-0.32
16	[illegible]	[illegible]	[illegible]	[illegible]	11	9	82	0.83	-0.22
17	[illegible]	[illegible]	[illegible]	[illegible]	11	9	65	-	-
18	[illegible]	[illegible]	[illegible]	[illegible]	7	7	64	0.61	-0.16
19	11[illegible]	[illegible]	[illegible]	61	56	37	439	0.96	-0.30
20	87	[illegible]	58	24	24	16	218	0.87	-0.41
21	128	[illegible]	70	52	47	36	426	0.96	-0.35
22	28	24	19	14	12	4	101	0.78	-0.50
Average	115	67	51	44	40	27			