Inoculum Survival and Infectivity of *Arceuthobium americanum* Infecting *Pinus banksiana* in Manitoba

by

Darren Earl Robinson

A thesis presented to the University of Manitoba in partial fulfillment of the requirements for a degree of Master of Science in the Faculty of Graduate Studies.

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Abstract

Aspects of the pathology and reproductive biology of Arceuthobium americanum Nuttal ex Engelman were examined with respect to microclimate and as they related to the inoculum potential and population dynamics of this pathogen. Fruit survival was measured over time to maturity and in successive years. Overwinter fruit survival was high in both years, averaging 94.9% and 87.5% respectively. Rates of fruit mortality increased through the following spring and summer months in both years of the study. As a result, fruit survival approximated a type I survivorship curve. Aerial shoot loss accounted for the greatest proportion of fruit mortality, ranging from 0.43 to 0.66 in the first year of the study and 0.32 to 1.00 in the following year. Shoot loss showed a significant correlation with vapour pressure deficit ($r=0.87$ in 1992/93; $r=0.92$ in 1993/94). Individual fruit loss ranged from 0.21 to 0.46 and from 0.18 to 0.61 respectively, in 1992/93 and 1993/94. Individual fruit loss showed a significant correlations with vapour pressure deficit in 1992/93 ($r=0.91$).

The infection process, including overwinter seed retention, germination, factors influencing seed mortality and successful establishment of an infection, was also examined with respect to microclimate. Overwinter seed retention was 86% and 67% over all tree and tissue ages in respective years. Seed retention was significantly lower on tissues subsequently covered by snow when no needles were

present on the inoculated tissues ($p=0.001$). Between 10% and 50% of the inoculated seeds were lost during the spring and summer following inoculation owing to wind and rain. In different tree age classes between 17.5% and 40.0% and 1.1% and 14.4% of the seeds germinated and produced appressoria respectively, in 1993 and 1994. The lower value in the second field season was attributed to the colder winter in 1993-1994. Mean daily temperatures were significantly lower ($p<0.0001$; $p<0.0001$) in January and February of 1994 than in January and February of 1993.

Tree and tissue age were tested for their impact on infection success by inoculating the 1, 2, and 3 year-old tissues of 3, 5, and 7 year-old trees, and the 1, 4, and 8 year-old tissues of 12, 17, and 22 year-old trees. The proportion of successful infections differed significantly with respect to tree ($p=0.035$) and tissue age ($p=0.042$) in the 12, 17 and 22 year old age class in 1993. Infection success ranged from 0.03 in the one year-old tissue of the 7 year-old trees to 0.35 in the eight year old tissue of the 22 year old trees. Tissue and tree age were not controlling factors in the infection process, but environmental parameters were considered to have a significant impact on infection establishment.

Seed germination in the laboratory experiments was negatively correlated with vapour pressure deficit ($p=0.0007$), ranging from 30% at 2.50 kPa to 90% at 0.6 kPa. Below 0.3 kPa and temperatures of 10C, 15C, 20C and 25C

there was a decrease in the proportion of seeds that germinated in the 1992-1993 laboratory experiments. Between 0.3 kPa and 0.0 kPa, seed germination was apparently inhibited by molding. In the 1993-1994 laboratory experiments, molding did not occur, though low values below 0.2 kPa at 10C and 0.4 kPa at 20C did result in significant reductions in seed germination ($p=0.0005$).

Acknowledgments

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As well, I must extend my thanks to my advisory committee, Dr. Jeannie Gilbert, Dr. Norm Kenkel, Dr. James Reid, and Dr. Carl Shaykewich, for their insight and criticism - all of which were constructive - as to how to approach the objectives of the research. Dr. James Reid and Dr. Leonard Hutchison are also to be thanked for their help in identifying the fungi colonizing the seeds. Furthermore, I would like to thank Dr. Ross McQueen for his help in setting up the dataloggers and his help in retrieving and organizing the microclimatic data.

I also thank the other graduate students, primarily Eun Ju Lee, Barbara Dyck, and Perry Johnson-Green, for their encouragement and eclectic brand of humor.

There are two individuals upon whom the completion of this project impinged. From the onset, my supervisor and friend, Dr. David Punter, gave unselfishly of both his time and knowledge and has imparted to me an interest in plant pathology in general. Finally, I must acknowledge the encouragement and tolerance of my wife, Tracey, who supported me in every aspect of this project.

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General Introduction

Components of Inoculum Potential in the Life Cycle of Arceuthobium americanum:

Within the context of this research, the term "inoculum potential" was defined as the energy available for infection at the surface of the host tissue to be infected (Garrett 1960). Inoculum potential is determined by the number of pathogen propagules or infective units available per unit area of host surface and the nutritional status of each propagule under a given set of environmental conditions.

The genus Arceuthobium constitutes an ecologically important group -the dwarf mistletoes - within the family Viscaceae. All are green, hemi-epiparasitic phanerograms found primarily on coniferous hosts primarily of the New World. Though able to photosynthesize some of their nutritional requirements, they are obligate parasites and rely upon their hosts for water and mineral nutrients. A. americanum Nuttal ex Engelman is a parasite of many hard pines ; its primary host in Manitoba is Pinus banksiana Lambert, commonly named jack pine.

The life cycle of this pathogen spans approximately 3 to 5 years, beginning with the dwarf mistletoe seed - the infective unit. Dwarf mistletoe seeds are forcibly discharged in the fall. To become established, they must reach branch or stem tissue of an appropriate host, as the needles are not susceptible to infection. The seed is enveloped in a hygroscopic viscin coat which serves two main

functions. First, the coat, when imbibed, becomes slippery and allows those seeds which land on needles to slide down to the base of the fascicle adjacent to susceptible branch tissue. As well, when the viscin coat dries out, it cements the seed to the substrate. This is essential as the seeds remain dormant through the fall and winter before germinating the following spring. Throughout this time, the seeds are subject to removal by rain, wind, snow, and fungal and insect attack (Wicker 1967).

Upon emerging in the spring, the radicles are subject to desiccation, representing a critical point in the dwarf mistletoe's existence (Knutson 1974). In order to colonize new hosts, the seeds must penetrate susceptible host tissue and initiate endophytic system development. Factors such as temperature and moisture stress are likely to influence the establishment of an infection and thus the inoculum potential of the parasite.

Temperature determines the rate of penetration and infection (Scharpf 1969) through mediating the rate of respiration and growth in the developing radicle and appressorium - the infective structure. As well, the moisture demand placed upon the developing radicle will determine how quickly it desiccates, thus influencing the number of viable infective units available (Scharpf 1969).

Host resistance is another factor governing the success of an obligate parasite such as A. americanum. Mechanical resistance, usually passive resistance of the bark, may

restrict a pathogen to younger, more succulent tissue. However, juveniles of Pinus ponderosa Laws. have exhibited greater levels of resistance to infection than older trees (Roth 1974). Only preliminary observations of the age-dependency of the infectivity of A. americanum on P. banksiana have been made (Dowding 1929). Though juvenile resistance to infection of Pinus contorta Dougl. var. latifolia Engelm. by A. americanum has been suggested as a factor determining the lack of infection of young stands, klendusity has not been ruled out (Wicker and Shaw 1967).

If an infection is successfully established, the endophytic system of the dwarf mistletoe will develop within the infected host tissue. Associated with the development of this system is the formation by the host of "witches' brooms", or dense clusters of branches lacking in apical dominance. Aerial mistletoe shoots emerge from the endophytic system and erupt through the bark of the infected tissues. All of the shoots produced from the same endophytic system bear either male or female flowers, so that witches' brooms are often referred to as male or female brooms.

Once the flowers mature, pollination will take place in April or May. This is soon followed by fertilization and fruit development. The fruit mature little during the summer immediately following fertilization and enter a period of dormancy in the fall and winter and resume growth the following spring. During this protracted period of

maturation, the fruit are subject to mortality, which will have an impact upon inoculum potential. Only one seed is produced per fruit, and as the seeds are the only means of spreading onto new hosts fruit mortality provides a useful measure of inoculum production.

Fruit survival through the summer months may be influenced by temperature and moisture stress. The fruit have numerous stomata and owing to the high rates of transpiration measured in dwarf mistletoes (Tocher et al. 1984), are highly susceptible to desiccation. Plant water potentials decrease in plants on sandy soils, but this has been overcome by the development of more efficient rooting systems in certain plants (Wambolt 1973). However, water supply is a critical factor of intraspecific competition in jack pine stands and is a function of plant water potential which has a role in determining transpiration rates (Yarranton and Yarranton 1975). Vapour pressure deficit has been found to be highly correlated with plant water potential (Wambolt 1973) and is considered to be a useful indicator of rates of transpiration. As a result, the impact of moisture stress on fruit mortality will serve as an important factor of the inoculum potential of A. americanum.

Dynamics of a Pathogen Population:

Epidemics may occur when host densities are high thus acting as a regulatory mechanism for the host-pathogen

association, though patterns of stable coexistence generally apply. These stable patterns are also likely to vary depending upon the reproductive attributes of the pathogen as they relate to time and the number of infective units produced (Anderson 1979).

The specificity of a pathogen with respect to the age of individuals that it infects is generally high in obligate parasites such as A. americanum. However, Hawksworth (1954) has contradicted this statement with his observation that tissue of P. contorta ranging in age from 1 to 65 years old is susceptible to infection by A. americanum. This raises questions as to the virulence of the pathogen in stands of different age groups. Does the virulence of the pathogen vary on tissues of different age groups? Does some other factor such as transmission efficiency or longevity of different mistletoe plants cause the pathogen to be more prevalent in older stands?

Stable coexistence in any host-pathogen association is maintained by aggregated distributions of parasite numbers per host, and density dependent mortality and fertility of the pathogen on individual hosts (Anderson 1979). Aggregated distributions cause the parasite burden to be concentrated on a few individuals, thus placing little stress on the host population as a whole. When highly suppressed individuals of the host population perish much of the pathogen population will perish as well. Though parasite numbers are significantly reduced, this leaves a

large number of vigorous suscepts available for colonization by the remaining parasite population.

The pattern of stable coexistence may be disrupted by changes to the pathogen's reproductive success owing to microclimatic changes. Such density-independent influences on the mortality of dwarf mistletoe fruit have received some treatment (Baranyay and Smith 1974 ; Smith and Wass 1979) in uncontrolled settings, though correlations with temperature and moisture stress have yet to be determined.

This study was designed to fill some of the deficiencies in our understanding of the relationship between meteorological conditions and the reproductive success, inoculum potential and infection capability of A. americanum on jack pine. The importance of each parameter was considered in conjunction with the population dynamics of the pathogen in a population of P. banksiana in Belair Provincial Forest in Manitoba.

Chapter One

Fruit Mortality Study

Introduction

Dwarf mistletoe spread and intensification in stands of jack pine are dependent upon fruit mortality (Geils and Mathiesen 1991), as this determines the number of infective units available in a given season. Fruit mortality has been examined with respect to losses incurred by the action of the many fungal hyperparasites of the genus Arceuthobium. Hawksworth (1972) and Gilbert (1988) have concluded that pathogens such as Wallrothiella arceuthobii (Peck) Sacc., Colletotrichum gloeosporioides Penz. sensu von Arx, and fungal associations causing resin disease, though destructive, are too inconsistently associated with dwarf mistletoes to account for most annual fruit losses. Gilbert (1988) observed that fruit mortality of A. americanum on jack pine, though lower over winter, increased through the summer months. Fruit mortality was attributed to high temperatures and low levels of moisture, though correlations were not made with either variable.

One objective of this experiment was to test the hypothesis that a correlation exists between fruit mortality and temperature and moisture stress. Annual variations in fruit mortality may be predictable based upon accurate measurements of canopy temperatures and moisture stress. A second objective was to isolate the causes of mortality and test the hypothesis that each cause is correlated with

temperature and moisture stress. If the hypotheses are substantiated, estimates of inoculum potential can be made more accurately. Other factors resulting in fruit mortality will be considered independently of and concurrently with damage caused by temperature and moisture stress. This will be necessary to ensure an accurate determination of the correlation between microclimatic and fruit mortality.

Literature Review

Fruit mortality is influenced by a number of factors, including losses owing to hyperparasitic activity. Wallrothiella arceuthobii parasitizes only the floral area of pistillate plants and developing fruits (Dowding 1931). Infected fruits are unable to complete seed development, as the apical portions of the fruit are replaced by the fungal stroma of this pathogen. Gilbert (1988) found that this pathogen caused less than 4% of the observed mortality in one year but noted that it is extremely variable from year to year.

Mortality of A. americanum fruit is also caused by resin disease. Its symptoms, which include excessive resin exudation from the resin-impregnated shoots, were first identified on A. americanum infecting lodgepole pine by Mark et al. (1976). Alternaria alternata (Fries) Keissler and Aureobasidium pullulans (de Bary) Arnaud were isolated from almost 90% of the cankers and shoots showing the symptoms of the disease (Mark et al. 1976). Gilbert (1984) isolated

Alternaria alternata from resin-free, healthy shoot tissue, and suggested that the disease may be caused by a number of different species of fungi, including A. alternata.

Though much is understood about the damage caused to A. americanum by fungal infection, fruit mortality in relation to temperature and moisture stress is not well documented in the literature. Controlled laboratory experiments have been used to determine the intensity and duration of cold required to damage A. americanum fruit (Baranyay and Smith 1974). The study showed that when mature fruit with immature seeds were treated at -3.9C for a duration of 2.3 hours, 4% of the fruit survived. A temperature treatment of -4.4C for a duration of 0.75 hours left none of the fruit intact. Hence, early frosts in summer that occur in high latitudes and the northern portions of the geographic range of A. americanum were suggested to limit its dispersal northward and to higher altitudes (Baranyay and Smith 1974).

Gilbert (1988) found that 56% of the fruit of A. americanum infecting jack pines at Belair Provincial Forest in the summer of 1984 survived through to the fall of that year. Approximately 75% of these survived overwinter, but only one third of the overwinter fruit survived to be explosively discharged. In subsequent trials, Gilbert (1988) found overwinter survival rates of 88% and 92%. However, only 29% and 15% survived, respectively, until the onset of seed discharge. It was suggested that the local weather conditions may have influenced mortality, thus emphasizing the need to

determine and analyze canopy temperature and moisture stress levels in concordance with fruit mortality.

Materials and Methods

The fruit mortality study was situated in a pure stand of jack pine, approximately 50 years of age, 100 km north of Winnipeg and 50 m west of highway 59 in Belair Provincial Forest. The stand used is located in Township 18, Range 7E, at $50^{0}35'$N latitude and $96^{0}32'$W longitude. All 106 trees bearing female brooms (ie. those bearing pistillate shoots) at eye level (approx. 1.5 m) and within a 50 m by 100 m area were tagged. The area was divided into five strata, each 50 m by 20 m. Neyman's proportional allocation was used to randomly select 44 brooms on trees from the population of infected trees based upon their distribution within the sample area.

A random sample of 300 dwarf mistletoe shoots was chosen from the 44 brooms in order to produce a life table of the pathogen over the course of two generations (from October 1992 to August 1993 and from October 1993 to August 1994). Finite rates of mortality for a 30 day time span were computed according to Krebs (1989). Fruit and shoot mortality, the presence of Wallrothiella arceuthobii and resin disease, and tree and broom mortality were all recorded during the course of the study. Mortality was assumed to have occurred if symptoms were present and the mistletoe fruit or shoots appeared moribund at the sample time immediately prior.

Sample times were irregular throughout the winter months as a previous study (Gilbert 1988) has shown that little, if any, loss occurs over winter. One sample was taken in the spring of either year, and monthly observations were made from

June to August.

A 21X Campbell Scientific datalogger (Campbell Scientific, Edmonton, Alberta) equipped with an HMP35C temperature and relative humidity probe and powered by an MSX-18 photo voltaic module (Solarex Corp., Rockville, MD) was established in the fruit mortality site. Measurements were made at a frequency of 10 seconds and averaged over hourly intervals. All microclimatic data and measurements of fruit survival and the causes of fruit mortality were analyzed using regression analysis. To determine the significance of the correlations, t-statistics were computed using a 95% level of confidence. Temperature and vapour pressure deficit were the two microclimatic factors considered in each analysis. However, when the two factors were found to be significantly correlated themselves, only vapour pressure deficit was analysed.

To confirm the presence of Wallrothiella arceuthobii, flowers and immature fruit that were recorded as being infected by the hyperparasite were taken from the field to be cultured in the laboratory. The black fungal stroma was surface sterilized using 3% H_2O_2 and then crushed between two sterile microscope slides to remove the ascospores. Single spore isolations were made into solid agar (7.5%), modified from the recipe used by Parker (1970) and containing glycine (1.0 g/1), glucose (30.0 g/1), $MgSO_4 7H_2O$ (0.5 g/1), KH_2PO_4 (1.5 g/1), and yeast extract (2.0 g/1).

Results and Discussion

Phenological Observations Relating to Fruit Survival:

i. 1992-1993 field season

Five of the brooms sampled did not produce any fruit during the course of the study and were thus excluded from the analysis. The proportions of fruit surviving at each sample time, the proportions of fruit dying between sample times and the instantaneous rates of mortality at each sample time are summarized in Table 1.

Percent live fruit retention in all five strata was consistently very high, ranging from 92.0% to 95.8% between the months of October 1992 (day 0) and December 1992 (day 63). The instantaneous rates of mortality varied from 0.066 to 0.115 at this sample time. No further fruit mortality was observed before February 1993 (day 120). When considered over all five strata, mean fruit retention over the winter months remained at 94.9% $\pm$ 7.1. Between the February and May sample times, (day 201), fruit survival ranged from 70.0% to 83.8%. Live fruit retention overall averaged 82.6% $\pm$ 16.9. The instantaneous rate of mortality ranged from 0.216 to 0.564. In June (day 235) of 1993, percent live fruit retention ranged from 63.3% to 85.4% and averaged 72.5% $\pm$ 23.2. Instantaneous rates of fruit mortality were slightly lower, ranging from 0.043 to 0.204. Fruit retention was in the range of 50.0% to 73.3% to in July 1993 (day 276). Overall fruit retention in this sampling interval was 60.3% $\pm$ 23.1. Rates of fruit mortality varied from 0.237 to 0.378. In the final sampling

Table 1. Life table for fruit of Arceuthobium americanum during the 1992/1993 field season. Finite rates of mortality were calculated based upon a standard time base of 30 days.

Age, x (days)	No. alive at start of time x	Proportion surviving at start of time interval, x	No. dying in interval, x to x+1	Proportion dying during interval, x to x+1	Finite rate of mortality over 30 day period starting at x
Stratum 1					
0	120	1.000	8	0.067	0.098
63	112	0.933	0	0.000	0.000
120	112	0.933	21	0.188	0.512
201	91	0.758	15	0.165	0.204
235	76	0.633	11	0.145	0.243
276	65	0.542	50	0.769	0.852
325	15	0.125			
Stratum 2					
0	262	1.000	20	0.076	0.110
63	242	0.924	0	0.000	0.000
120	242	0.924	20	0.083	0.369
201	222	0.847	29	0.131	0.166
235	193	0.737	27	0.140	0.237
276	166	0.634	159	0.958	0.974
325	7	0.027			
Stratum 3					
0	240	1.000	10	0.042	0.066
63	230	0.958	0	0.000	0.000
120	230	0.958	5	0.022	0.216
201	225	0.938	20	0.089	0.118
235	205	0.854	29	0.141	0.239
276	176	0.733	176	1.000	1.000
325	0	0.000			
Stratum 4					
0	387	1.000	18	0.047	0.072
63	369	0.953	0	0.000	0.000
120	369	0.953	47	0.127	0.439
201	322	0.832	28	0.087	0.116
235	294	0.760	42	0.143	0.241
276	252	0.651	194	0.770	0.852
325	58	0.150			
Stratum 5					
0	100	1.000	8	0.080	0.115
63	92	0.920	0	0.000	0.000
120	92	0.920	22	0.239	0.564
201	70	0.700	2	0.029	0.043
235	68	0.680	18	0.265	0.378
276	50	0.500	18	0.360	0.535
325	32	0.320			

interval of the 1992/1993 field season, percent live fruit retention ranged from 0.0% to 32.0%. Mean fruit retention overall was 9.3% $\pm$ 21.4 in August 1993, and instantaneous rates of mortality varied from 0.535 to 1.000.

As mortality is one of the key parameters driving population change, it is useful to summarize these data graphically. When the mean percent fruit survival was plotted against interval of observation, a 'survivorship' curve for the dwarf mistletoe fruit is developed. Figure 1 illustrates that the changes in mean fruit survival with time in each of the five sample strata approximate a type I survivorship curve (Pearl 1928), in which survival remains relatively high until the latter stages of the fruit's 'lifespan'. Similar observations on fruit survivorship have been made by Gilbert (1988) who found, in consecutive years, that fruit survival overwinter remained above 90%. Throughout the summer months, fruit survival decreased at a steady rate to approximately 83% in May, 73% in June and 60% in July. In August, just prior to seed discharge, fruit survival fell sharply to approximately 10%.

ii. 1993-1994 field season

Of the 44 selected brooms, 26 produced viable shoots and were included in the analysis. Proportions of fruit survival and mortality, and instantaneous rates of mortality are given in Table 2.

Overall fruit retention in October 1993 was 87.5% $\pm$ 26.7% and ranged from 88.2% to 100.0% between the five strata.

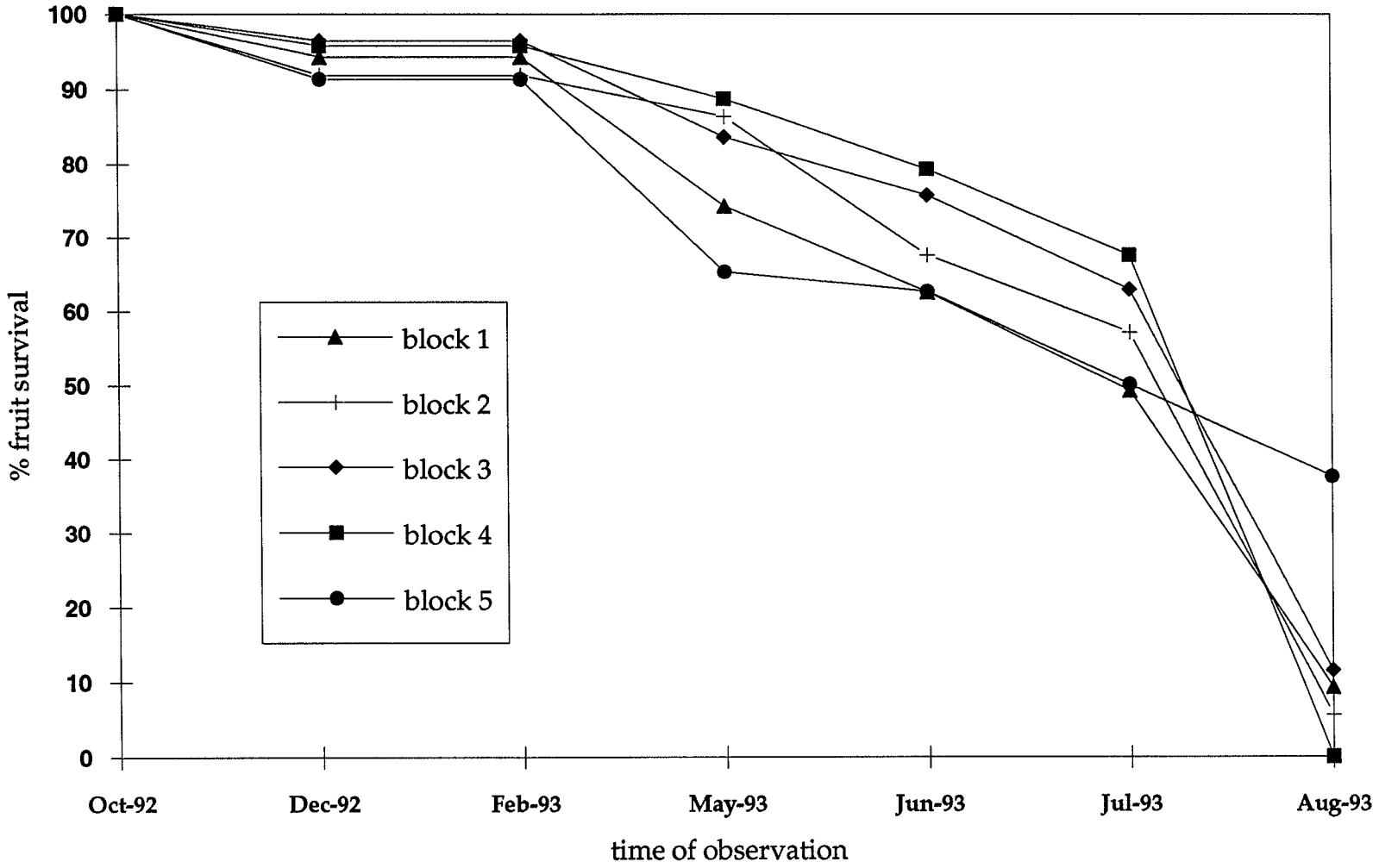


Figure 1. Mean percent fruit survival through the 1992-1993 field season in each of five strata. Mortality data were collected between October 1, 1992 and August 31, 1993.

Table 2. Life table for fruit of Arceuthobium americanum during the 1993/1994 field season. Finite rates of mortality were calculated based upon a standard time base of 30 days.

Age, x (days)	No. alive at start of time x	Proportion surviving at start of time interval, x	No. dying in interval, x to x+1	Proportion dying during interval, x to x+1	Finite rate of mortality over 30 day period starting at x
Stratum 1					
0	171	1.000	0	0.000	0.000
32	171	1.000	0	0.000	0.000
151	171	1.000	13	0.076	0.070
245	158	0.924	119	0.753	0.746
274	39	0.228	39	1.000	1.000
304	0	0.000			
Stratum 2					
0	213	1.000	25	0.117	0.134
32	188	0.883	0	0.000	0.000
151	188	0.883	18	0.096	0.088
245	170	0.798	133	0.782	0.776
274	37	0.174	37	1.000	1.000
304	0	0.000			
Stratum 3					
0	341	1.000	0	0.000	0.000
32	341	1.000	0	0.000	0.000
151	341	1.000	7	0.021	0.018
245	334	0.979	127	0.380	0.368
274	207	0.607	207	1.000	1.000
304	0	0.000			
Stratum 4					
0	376	1.000	34	0.090	0.105
32	342	0.910	0	0.000	0.000
151	342	0.910	55	0.161	0.151
245	287	0.763	190	0.662	0.653
274	97	0.258	97	1.000	1.000
304	0	0.000			
Stratum 5					
0	187	1.000	22	0.118	0.134
32	165	0.882	0	0.000	0.000
151	165	0.882	7	0.042	0.038
245	158	0.845	101	0.639	0.629
274	57	0.305	57	1.000	1.000
304	0	0.000			

Fruit mortality rates of 0.000 to 0.134 were obtained at this sample time. There was not a substantial drop in overall fruit retention between October and November of 1993. As was observed in the first year of the study, fruit loss was not observed over winter. Overall fruit survival in March 1994 (day 151) was 87.5% $\pm$ 26.7. Between March and June (day 245) of 1994, fruit survival dropped to 78.3% $\pm$ 33.0 overall. Fruit retention ranged from 76.3% to 97.9% between the five strata. The range of instantaneous mortality rates fell to 0.018 to 0.088. In July of 1994 (day 274), fruit retention averaged 31.3% $\pm$ 33.8 overall and ranged from 17.4% to 60.7% between strata. Rates of mortality increased to a range of 0.368 to 0.776. At the August 1994 sample time (day 304) no fruit remained alive. Some of the fruit were likely killed by desiccation, though seed discharge had already begun. Using data from the previous year and that of Gilbert (1988), fruit mortality may have been overestimated by as much as 10% to 30%. However, temperature and moisture stress in the latter year were higher, and it is suspected that fruit survival would have been somewhat lower.

The survivorship curve plotted for the 1993-1994 study year was similar to that plotted in the previous year, and also approximated a type I curve (see Figure 2). Survival dropped from 100% to 87% in November 1993 and did not change between November of 1993 and March of 1994. By May of 1994, fruit survival averaged approximately 78%. By July, fruit survival had dropped to 31%, owing to aerial shoot loss and

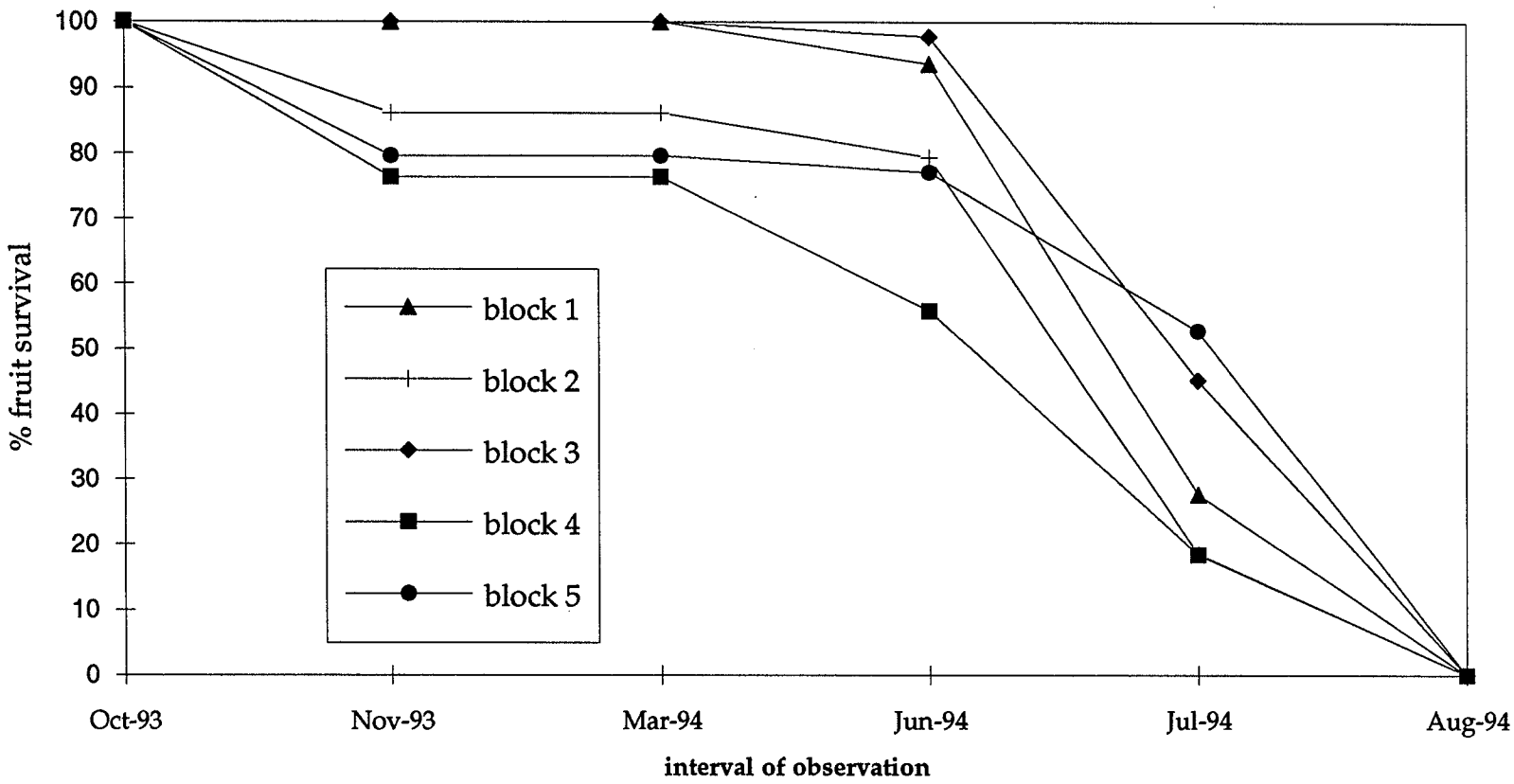


Figure 2. Mean percent fruit survival through the 1993-1993 field season in each of five strata. Observations were made between October 1, 1993 and August 31, 1994.

individual fruit loss. In August of 1994, no fruit, dead or alive, were found on any of the sampled brooms.

The survivorship data indicated that the population of A. americanum in Bel Air Provincial forest is underlined by a pre-determined and repetitive pattern of birth and mortality. Lotka (1922) has shown that such populations approach a stable age distribution. Differential equations based upon the innate capacity for increase and the initial population size may thus be used to describe the rate of change in such a population. The basis for this equation has already been established (Lotka 1922):

$$dN/dt = r_m N$$

The innate capacity for increase (r_m) exists under a given set of environmental conditions, and may thus only be usefully discussed with respect to environmental parameters. Furthermore, in a host-pathogen complex, the net rate of reproduction of the parasite population is also modified by the success of infection (Anderson 1979). Both the transmission efficiency of the pathogen and the death rate of the infective stages modify the ability of a pathogen to successfully establish an infection. Each of these factors determines the degree of suppression of a pathogen in a host population, and will be discussed as they relate to the present study.

It is of interest that the variation between sampling intervals was much greater in the 1993-1994 field season than the previous year. Some of the increase in variation was

likely owing to the smaller sample size in the latter year (n=26 brooms as opposed to n=39). However, it was observed that many of the trees which did produce viable inoculum at the onset of the experiment appeared to be somewhat less vigorous by its conclusion. Changes included browning of needles or a complete absence of needles in portions of the crown and a reduction in the number of viable dwarf mistletoe shoots produced in a given area on the broom. It is postulated that the heavy transpiration stress dwarf mistletoes impose upon their hosts caused these changes, and thus may have been responsible for reduction in the number of brooms able to sustain aerial shoots. Host vigour, then, may also account for a significant portion of the observed variation in fruit survival between brooms within a block.

These data suggest that the population of A. americanum is likely to become aggregated or clumped as inoculum production is unequally dispersed over the sample range. Anderson (1979) has defined this as a characteristic of stable coexistence in any host-parasite association as a lower proportion of the host population becomes infected, though each individual host harbours a greater proportion of the parasite population. Such observations support the finding that most of the spread and build-up of dwarf mistletoe is effected by a relatively small proportion of the dwarf mistletoe population (Scharpf and Parmeter 1982). These authors observed that fruit production varied markedly depending upon the vigour of an infected branch and the age of

an infection. The findings of the present study and those of Scharpf and Parmeter (1982) may explain the slow rates of dwarf mistletoe build-up.

Correlations Between Fruit Mortality Rates and Microclimate:

i. 1992-1993 field season:

In order to examine the association between microclimate and instantaneous rates of fruit mortality, the change in mortality rates over time was plotted against vapour pressure deficit (Figure 3). Pearson product-moments were calculated to determine the correlation between mortality rates and vapour pressure deficit. Vapour pressure deficit showed a positive, but insignificant, correlation with mean percent fruit survival ($r=0.38$; $t=0.919$; $0.50 < p < 0.20$). The correlation indicates that levels of evapotranspiration, and hence water loss, may not have been influenced as much by vapour pressure deficit as by other microclimatic variables such as temperature. Vapour pressure deficit was rarely greater than 1.0 kPa during this period, ranging from a minimum of 0.1 kPa to a maximum of 2.2 kPa.

ii. 1993-1994 field season:

In the second year of the study, instantaneous mortality rates showed a significant correlation with vapour pressure deficit ($r=0.97$; $t=7.980$; $p < 0.05$; Figure 4). Vapour pressure deficit during this time reached maxima of 1.5 kPa and 4.4 kPa in July and August respectively. However, a good

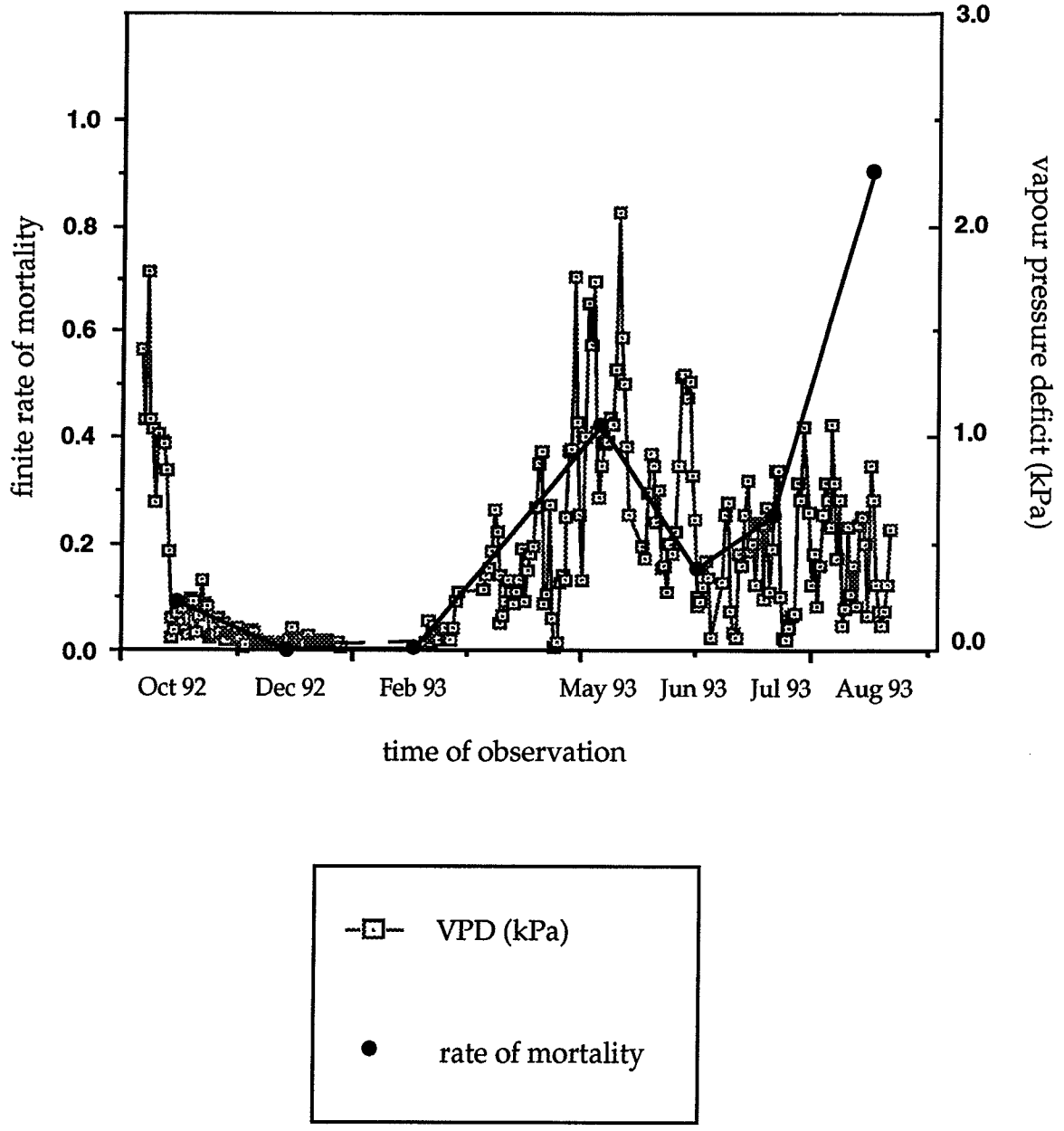


Figure 3. The change in instantaneous finite rate of fruit mortality with time during the 1992-1993 field season and its correlation with mean daily vapour pressure deficit (r=0.38). The data are based upon a sample size of n=39 brooms.

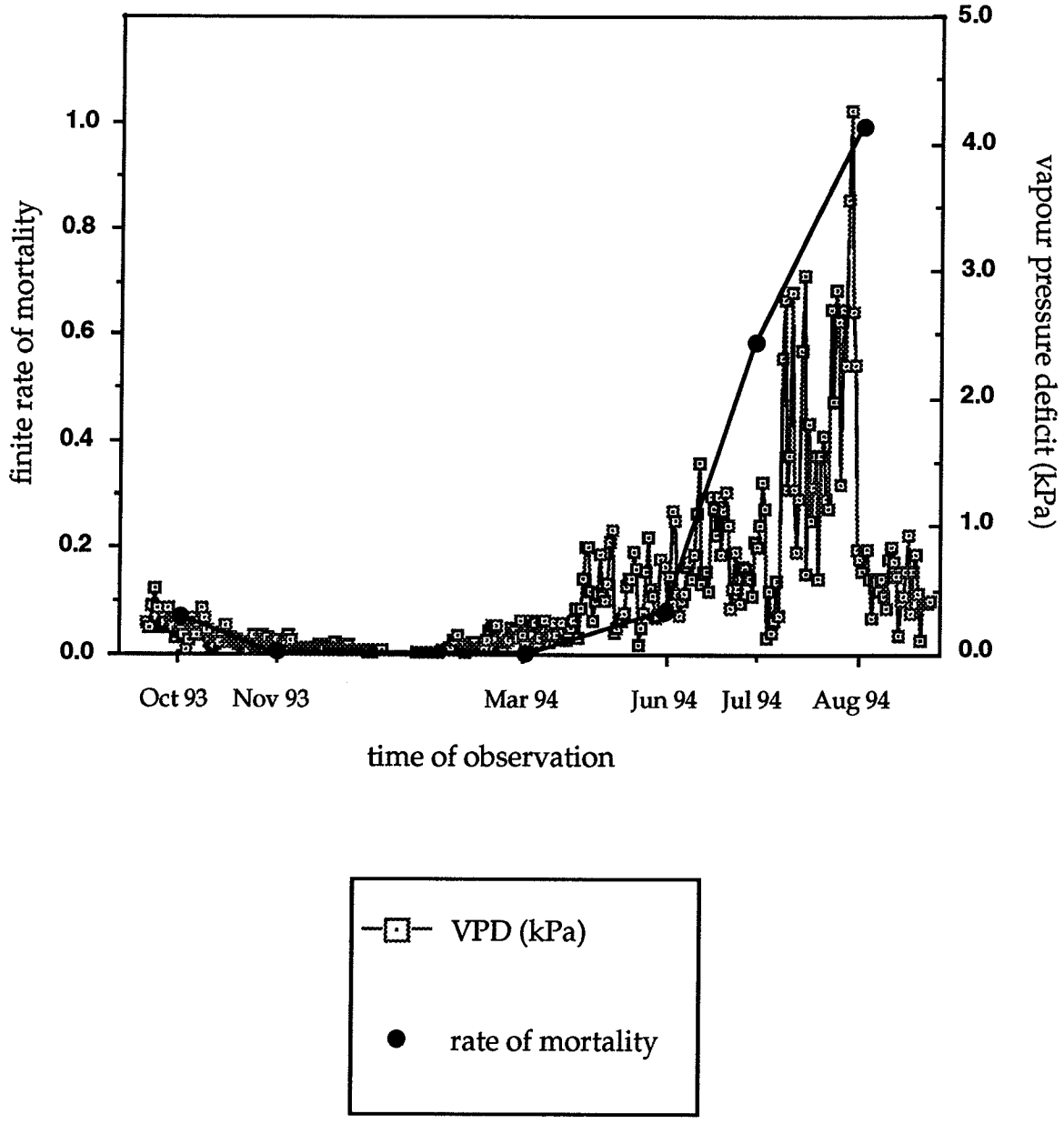


Figure 4. **The change in instantaneous finite rate of fruit mortality with time during the 1993-1994 field season and its correlation with mean daily vapour pressure deficit (r=0.97). The data are based upon a sample size of n=26 brooms.**

deal of the observed fruit loss occurred before vapour pressure deficits exceeded 1.5 kPa.

The correlations indicated that periods of relatively high moisture stress were accompanied by high rates of fruit mortality, while in years during which conditions are less stressful other factors play a more important role. The innate capacity for increase (r_m) is likely to be influenced by factors other than temperature and moisture stress. Those factors responsible for fruit mortality may provide a useful qualitative indication of the r_m value of A. americanum.

Factors Responsible for Fruit Mortality:

i. 1992-1993 field season:

To accompany the analysis of fruit survival, those factors which were thought to have the greatest impact upon fruit mortality, including temperature and vapour pressure deficit, were examined. Pearson's product-moments were calculated for each of the following variables : mean daily temperature, mean daily vapour pressure deficit, mortality owing to Wallrothiella arceuthobii , resin disease, individual fruit loss and aerial shoot mortality (Table 3).

Owing to the fact that temperature and vapour pressure were significantly correlated ($r=0.90$; $t=4.616$; $p < 0.01$), only vapour pressure deficit was correlated with each of the factors responsible for fruit mortality.

Wallrothiella arceuthobii accounted for very low proportions of the total fruit mortality, ranging from 0.00 +

Table 3. Pearson product moment matrix indicating correlations between factors responsible for fruit mortality and environmental variables in the 1992/93 field season.

	WAL	RES	IND	SHO	TEM	VPD
WAL	**1.00**					
RES	**0.40**	**1.00**				
IND	**0.58**	**0.26**	**1.00**			
SHO	**0.69**	**0.61**	**0.75**	**1.00**		
TEM	**0.54**	**0.41**	**0.78**	**0.95**		
VPD	**0.55**	**0.47**	**0.91**	**0.87**	**0.90**	**1.00**

WAL - fruit loss owing to fruit infection by Wallrothiella arceuthobii

RES - fruit loss owing to the fungal complex which causes resin disease

IND - individual fruit loss

SHO - fruit loss owing to mortality of aerial shoots

TEM - mean daily temperature (C)

VPD - mean daily vapour pressure deficit (kPa)

0.00 in the winter months to 0.22 $\pm$ 0.06 in May (Figure 5). The insignificant correlation with vapour pressure deficit ($r=0.55$; $t=1.743$; $0.20 < p < 0.10$) indicated that fruit loss owing to infection by this pathogen is difficult to predict using vapour pressure deficit alone. This moderate redundancy is an indication of the sporadic nature of the disease on a yearly basis and its propensity for occurrence in moist, shady environments (Dowding 1929).

The range of loss owing to resin disease was 0.00 $\pm$ 0.00 overwinter to 0.09 $\pm$ 0.03 in May of 1993. Mortality caused by resin disease was insignificantly correlated with vapour pressure deficit ($r=0.47$; $t=1.191$; $0.50 < p < 0.20$; Figure 6). As this disease caused very low proportions of mortality, it was concluded to have little impact on the inoculum potential of A. americanum.

Individual fruit mortality ranged from 0.00 $\pm$ 0.00 during the winter months to 0.46 $\pm$ 0.11 in June 1993. Vapour pressure deficit decreased after reaching a maximum daily value of 2.2 kPa in June. As a result, vapour pressure deficit and individual fruit loss showed a significant correlation ($r=0.91$; $t=4.908$; $p < 0.01$; Figure 7). Vapour pressure deficit, as a measure of moisture stress or evaporative demand, appears to be a useful indicator of fruit mortality. Individual fruit loss accounted for a significant proportion of overall fruit mortality and therefore is an appropriate factor to consider in predicting the inoculum potential of this plant pathogen.

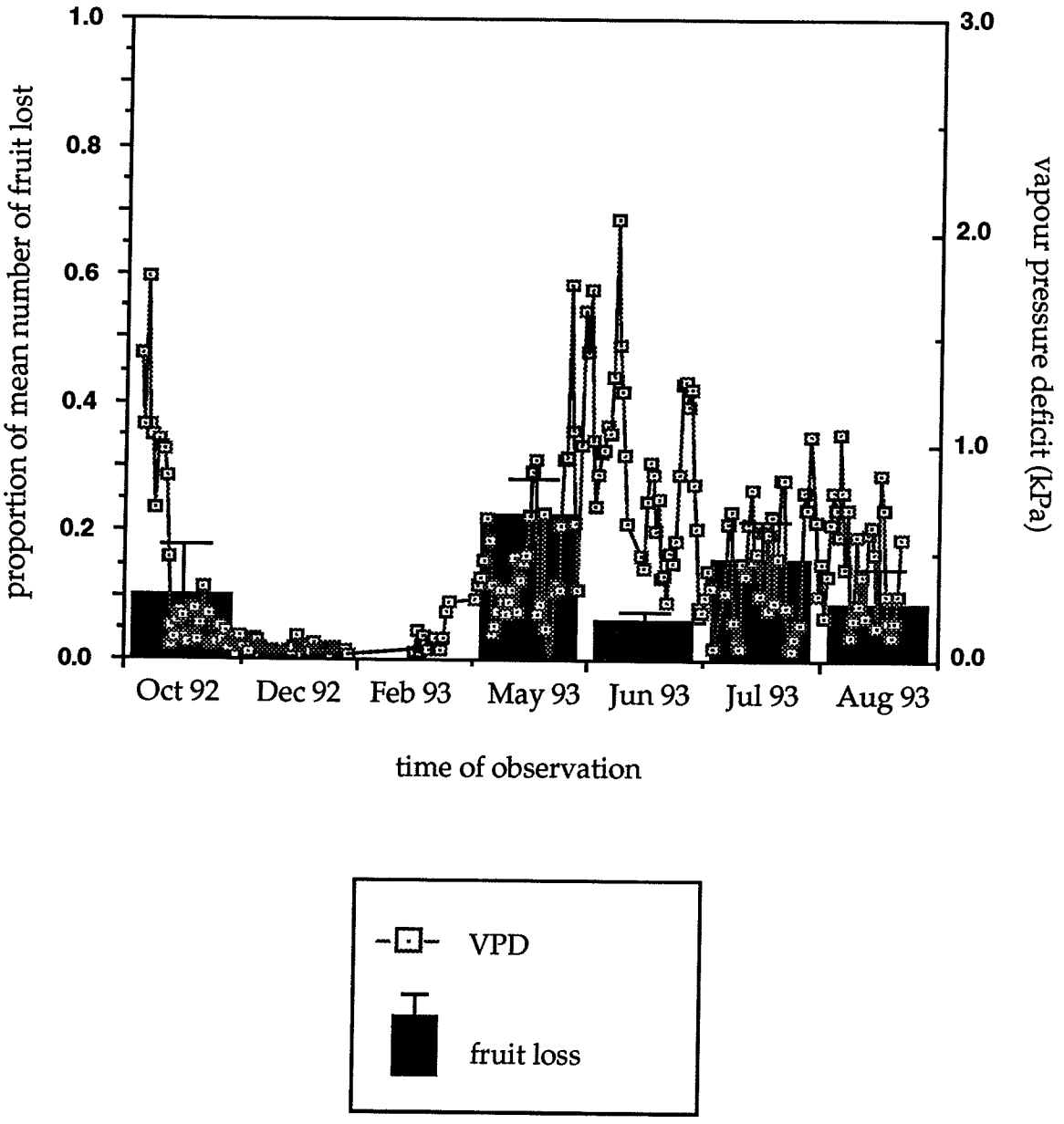


Figure 5. Proportion of mean fruit loss per broom as a result of infection by the hyperparasite, Wallrothiella arceuthobii, and its correlation with vapour pressure deficit (r=0.55). Mortality data for the 1992/93 field season and are based upon a sample size of n=39 brooms ; the vertical bars indicate 1 standard deviation of the mean.

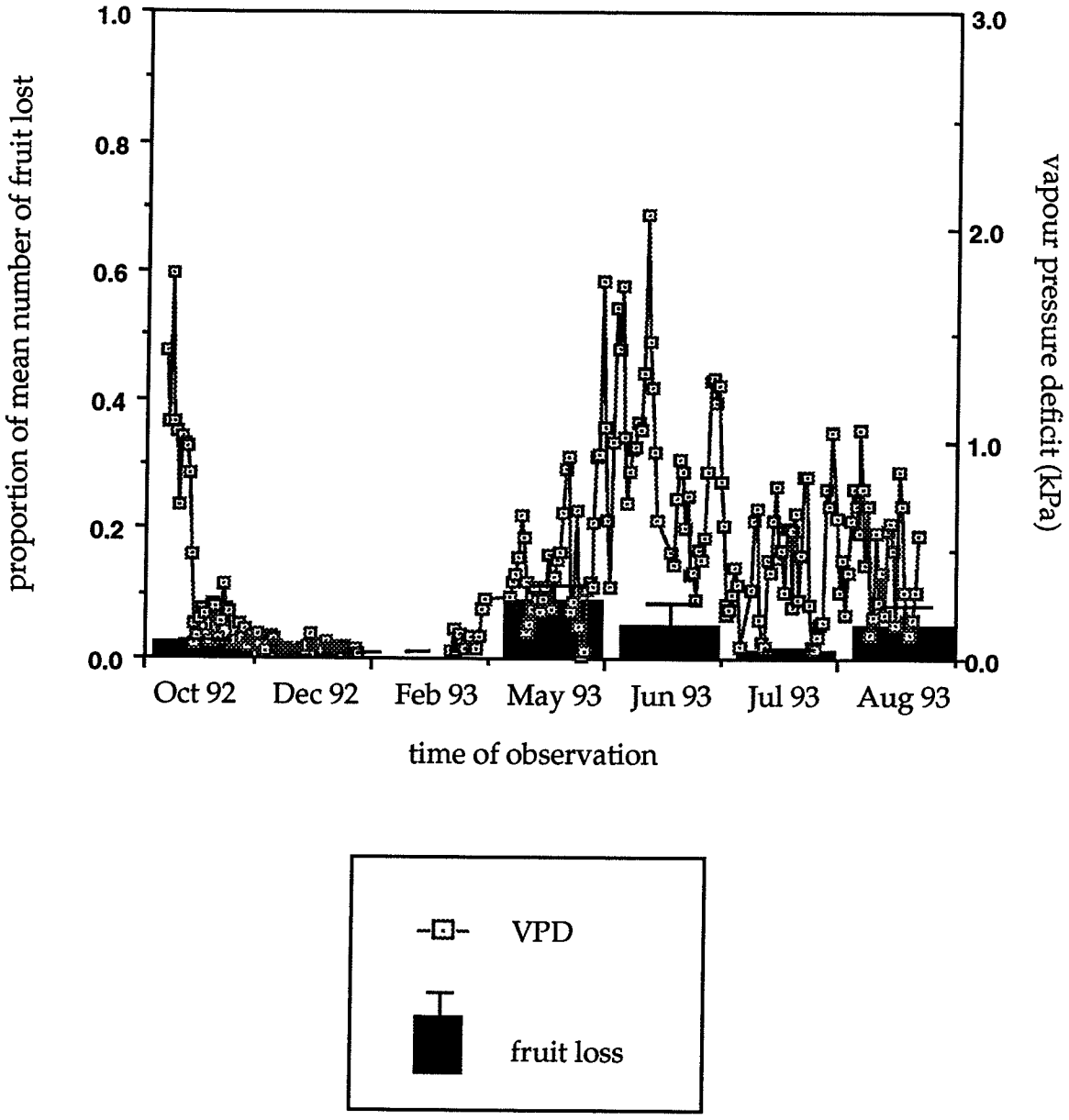


Figure 6. The proportion of mean fruit loss per broom owing to infection by the fungal complex which causes resin disease and its correlation with vapour pressure deficit (r=0.47). These data are given as proportions and are based upon a sample size of n=39 brooms ; the vertical bars indicate 1 standard deviation of the mean.

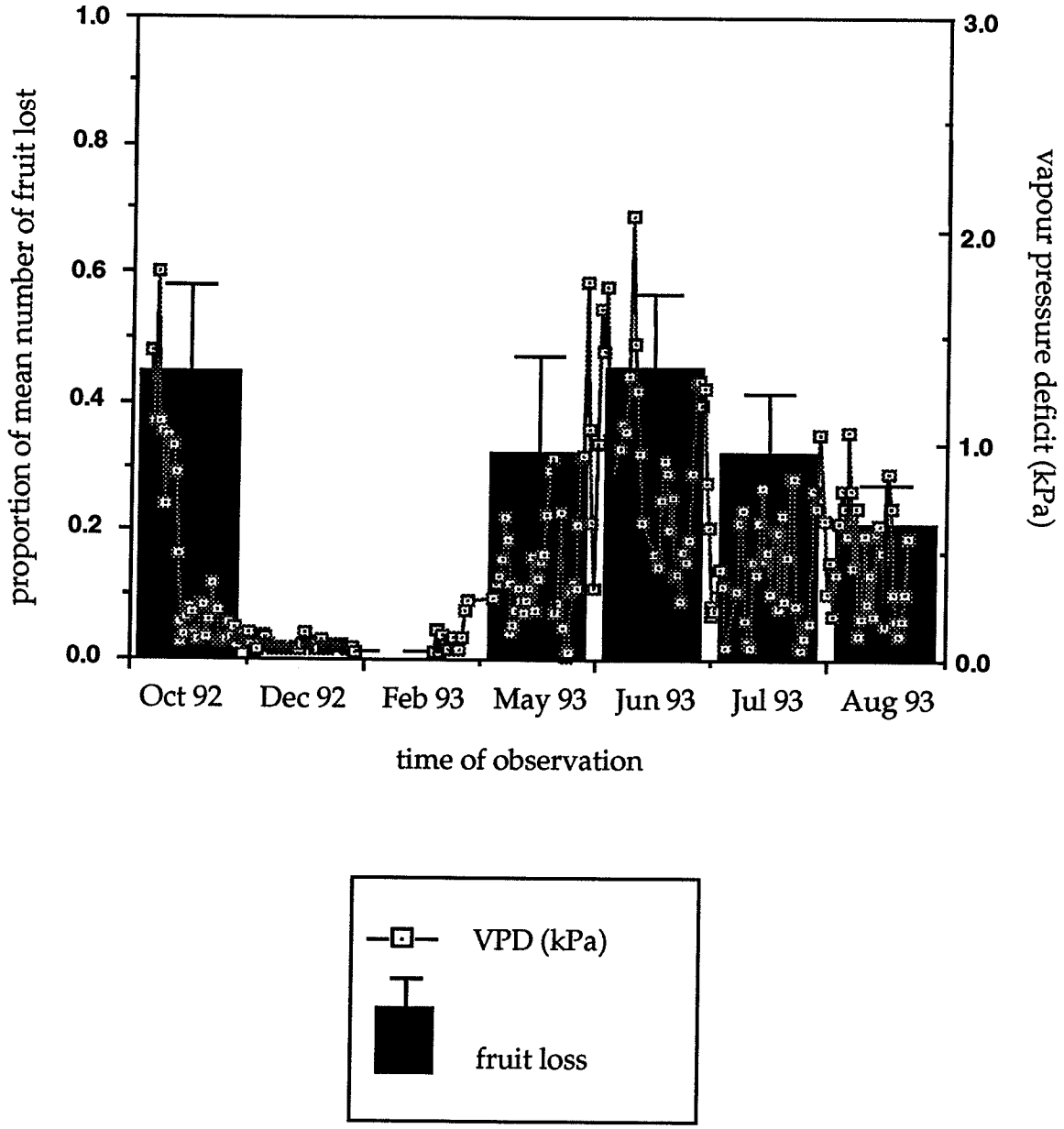


Figure 7. **The proportion of individual fruit loss per broom and its correlation with vapour pressure deficit (r=0.91). Mortality data are for the 1992/93 field season and are based upon a sample size of n=39 brooms ; the vertical bars indicate 1 standard deviation of the mean.**

Aerial shoot loss had the greatest impact on fruit mortality. With the exception of the winter months when fruit mortality was not observed, proportions of between 0.44 $\pm$ 0.16 to 0.66 $\pm$ 0.10 of the overall loss resulted from shoot mortality. Vapour pressure deficit showed a similarly significant, positive correlation ($r=0.87$; 3.946 ; $p < 0.05$; Figure 8) with fruit mortality owing to aerial shoot loss. These correlations may provide evidence for the influence of evapotranspiration on the physiology of A. americanum. Tocher et. al (1984) have demonstrated that rates of transpiration of water-stressed dwarf mistletoe plants may increase threefold over those at field capacity.

ii. 1993-1994 field season:

Resin disease did not account for any fruit mortality in the 1993-1994 field season. The correlations between the microclimatic parameters and the causes of fruit mortality in the 1993-1994 field season are given in Table 4. Once again, as temperature and vapour pressure deficit were significantly correlated ($r=0.91$; $t=4.390$; $p < 0.05$) and thus not independent of one another, only vapour pressure deficit was used in further analysis.

Wallrothiella arceuthobii accounted for less than 7% of the observed mortality in the 1993-1994 field season and was not significantly correlated with vapour pressure deficit ($r=-0.06$; $t=0.120$; $p > 0.50$). The warmer, drier conditions (Figure 9) in this field season were even less likely to

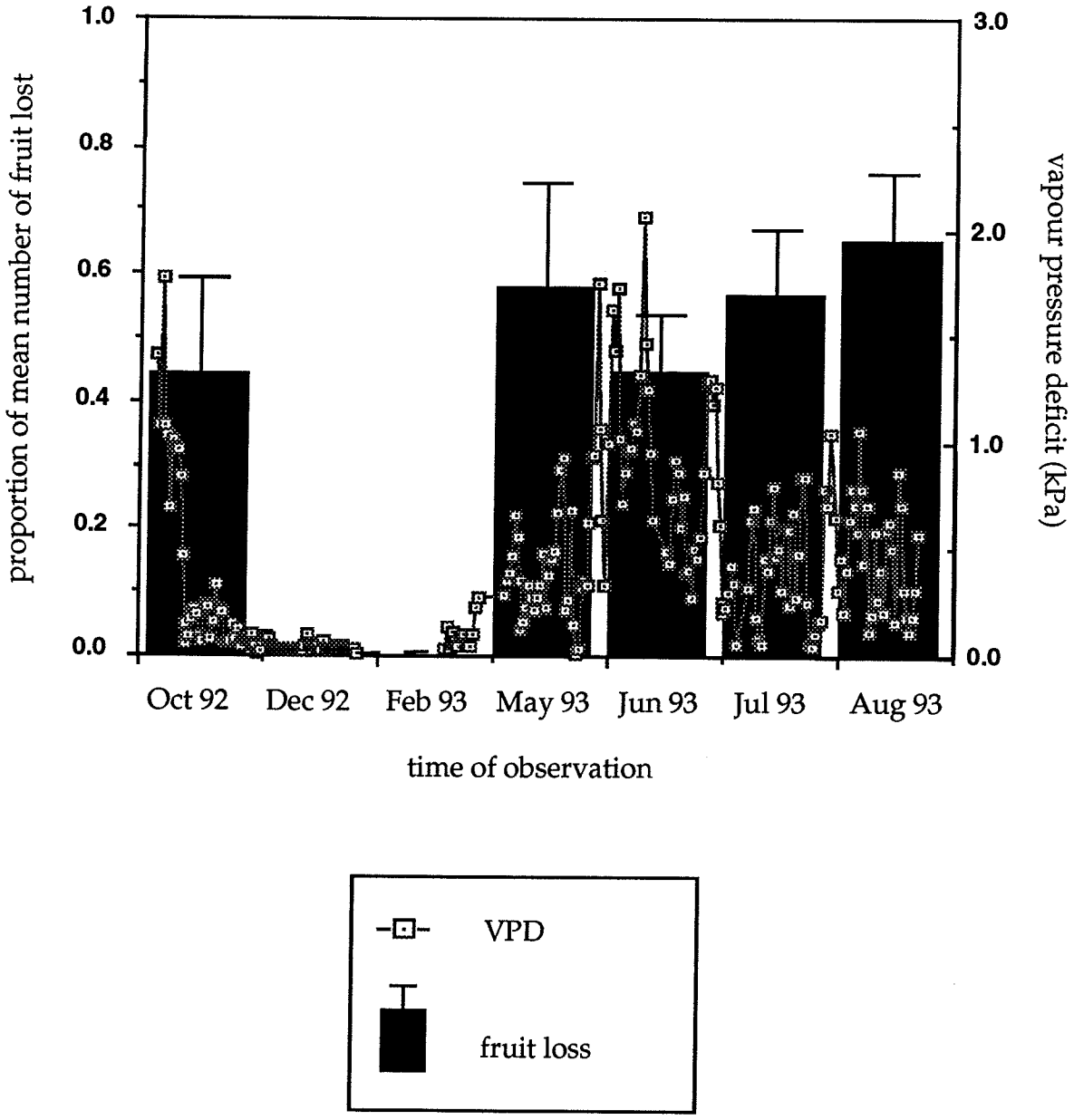


Figure 8. **Proportion of mean fruit loss per broom as a result of aerial shoot loss and its correlation with vapour pressure deficit (r=0.87). Mortality data are for the 1992/93 field season and are based upon a sample size of n=39 brooms ; the vertical bars indicate 1 standard deviation of the mean.**

Table 4. Pearson product-moment matrix indicating correlations between factors responsible for fruit mortality and environmental variables during the 1993-1994 field season.

	WAL	IND	SHO	TEM	VPD
WAL	**1.00**				
IND	**-0.05**	**1.00**			
SHO	**-0.03**	**0.46**	**1.00**		
TEM	**0.03**	**0.50**	**0.83**		
VPD	**-0.06**	**0.56**	**0.92**	**0.91**	**1.00**

WAL - fruit loss owing to fruit infection by Wallrothiella arceuthobii

IND - individual fruit loss

SHO - fruit loss owing to mortality of aerial shoots

TEM - mean daily temperature (C)

VPD - mean daily vapour pressure deficit (kPa)

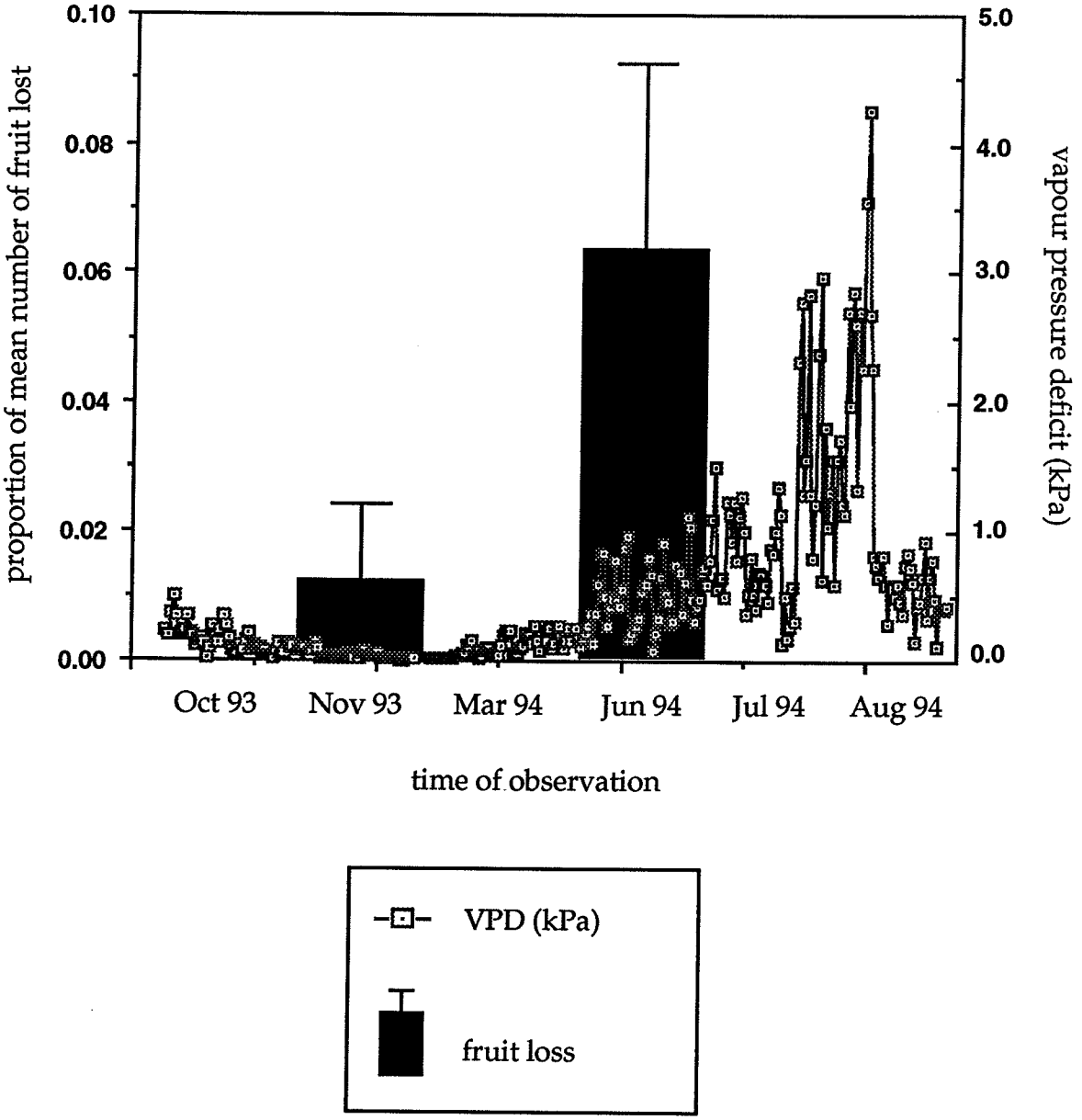


Figure 9. **The mean proportion of fruit lost per broom owing to infection by the hyperparasite Wallrothiella arceuthobii and its correlation with vapour pressure deficit (r=-0.06). Mortality data are for the 1993/94 field season and are based upon a sample size of n=26 brooms ; the vertical bars indicate 1 standard deviation of the mean.**

promote the spread of this fungus than in the previous field season.

In either year, fruit mortality caused by this pathogen was greatest in the summer months. In the 1992-1993 field season, fruit mortality owing to infection by W. arceuthobii was greatest in May of 1993. Kuijt (1969) observed that the development of infected fruit was extremely variable in the second growing season, such that some fruit may survive until just prior to seed discharge. It is possible that the warmer and drier conditions of the 1993-1994 field season hindered the growth of the mycelium of W. arceuthobii, resulting in the apparent delay. The fungus infects the pistillate flowers in the spring, prior to or following fertilization depending upon the environmental conditions existing at the time. Wicker and Shaw (1968) suggested that its rate of development may depend upon the timing of pollination and infection.

Individual fruit mortality was insignificantly correlated with vapour pressure deficit ($r=0.56$; $t=1.352$; $0.50 < p < 0.20$; Figure 10). The proportion of fruit loss ranged from 0.00 ± 0.00 overwinter to 0.61 ± 0.23 in July of 1994. Individual fruit loss was low in August (0.18 ± 0.09), apparently a reflection of the large amount of aerial shoot loss recorded during this sampling interval. The transpiration demands may have been sufficient to cause more aerial shoot mortality than individual fruit mortality by desiccating entire shoots.

Aerial shoot loss ranged from 0.00 ± 0.00 overwinter to

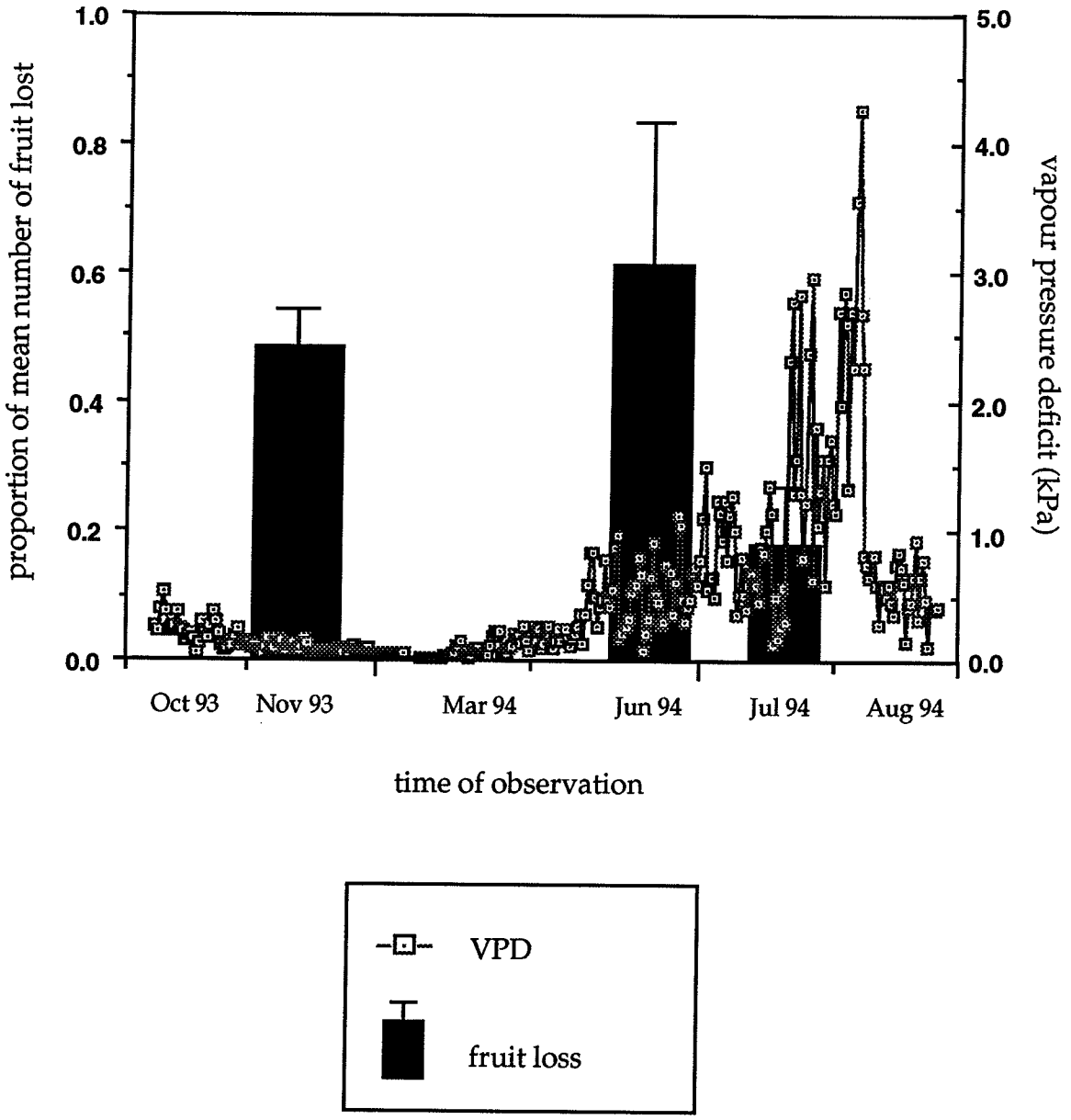


Figure 10. The mean proportion of individual fruit loss per broom and its correlation with vapour pressure deficit (r=0.56). Mortality data are for the 1993/94 field season and are based upon a sample size of n=26 brooms ; the vertical bars indicate 1 standard deviation of the mean.

0.82 $\pm$ 0.09 in August 1994. The implication that evaporative demands placed a strong enough stress upon the dwarf mistletoe plants to cause entire shoots to die is supported by the significant correlation between aerial shoot loss and vapour pressure deficit ($r=0.92$; $t=4.695$; $p < 0.01$; Figure 11).

Seed discharge had already begun by the time the observation was made in August of 1994. When seeds were collected for the inoculation study, pieces of dwarf mistletoe shoots were occasionally found in the seed traps. As a result, seed discharge may have accounted for some of the observed aerial shoot loss.

Aerial shoot loss significantly depressed the innate capacity for increase of the jack pine dwarf mistletoe. Temperature and vapour pressure deficit, both significant in determining evaporative stress, are also important factors governing the temporal fluctuations of the population of A. americanum in the sample area.

The fruit survival data are indicative of a steady-state equilibrium between the host and parasite, in which the parasite population is aggregated within its host population. However, stable associations may become disrupted by changes in transmission efficiency owing to fruit mortality (Anderson 1979). This occurs because the innate capacity for increase described by Lotka (1922) is specific for a given set of environmental conditions, the result being that fixed age schedules of birth and death may be altered during periods of seasonal change. However, a physiologically pre-determined

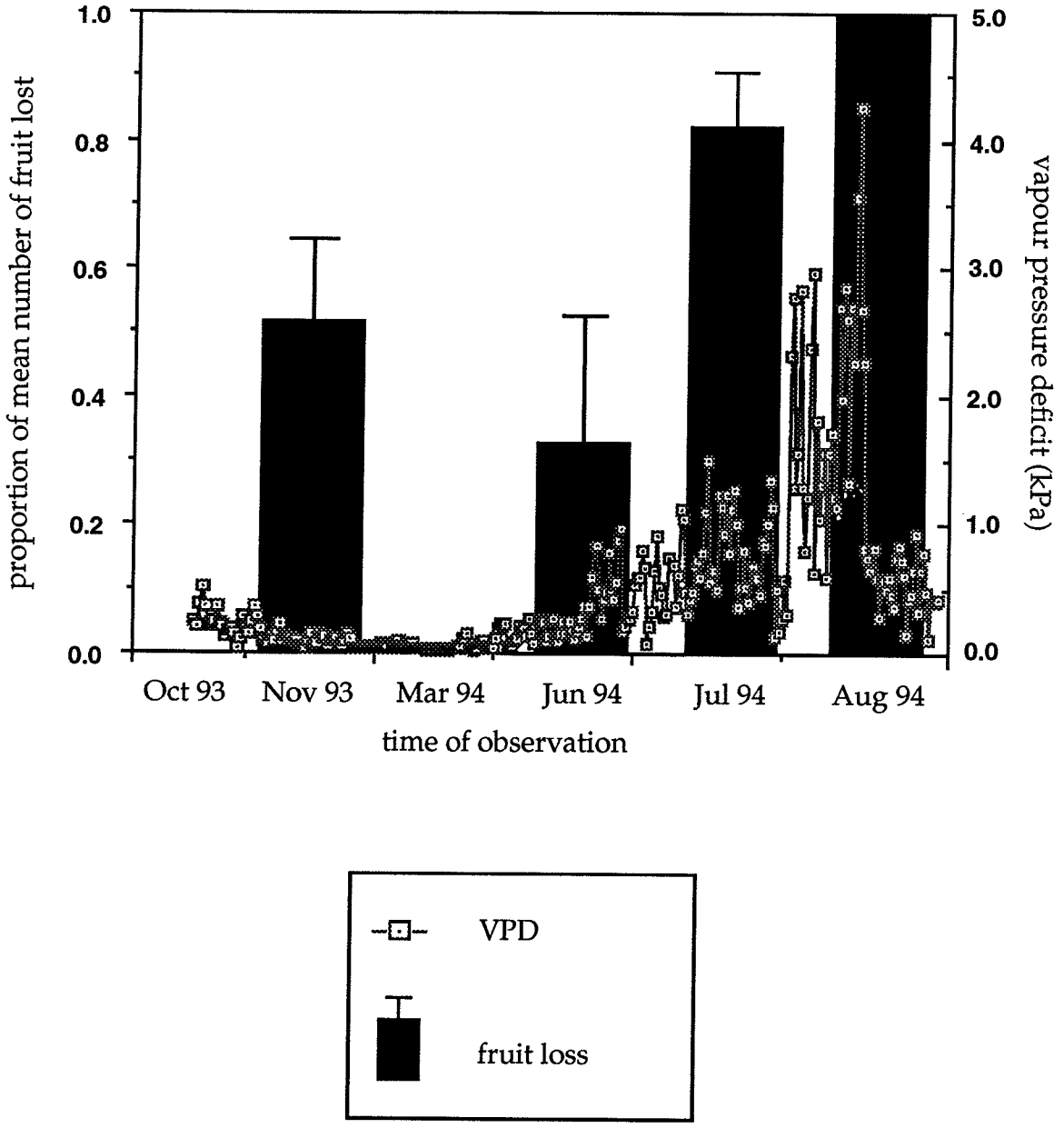


Figure 11. The proportion of mean fruit loss per broom owing to shoot loss and its correlation with vapour pressure deficit (r=0.92). Mortality data are for the 1993/94 field season and are based upon a sample size of n=26 brooms ; the vertical bars indicate 1 standard deviation of the mean.

pattern of mortality is most likely responsible for the rates of mortality observed in the population of A. americanum infecting P. banksiana in Belair Provincial Forest.

Chapter Two

Inoculation Study

Introduction

Under natural conditions, lodgepole pines up to 15 years of age have been observed to grow adjacent to mature stands infected with dwarf mistletoe without becoming infected themselves (Dowding 1929). The explanations offered are juvenile resistance or klendusic factors, such as low available surface area or removal of seeds by snow (Dowding 1929, Wicker 1967, Wicker and Shaw 1967).

Overwinter losses of between 50% and 65% have been observed for seeds of A. campylopodum (Engelm.) Gill on Pinus ponderosa Laws. (Roth 1959) and A. americanum on P. contorta (Wicker 1967). It was suggested than the hygroscopic viscin coat imbibed water upon snow melt, making the seeds slippery enough to be dislodged from host tissue. However, Shaw and Loopstra (1991) found that overwinter seed retention of A. tsugense (Rosendahl) G.N. Jones on Tsuga heterophylla (Raf.) Sarg. averaged 79% over three field seasons.

Postwinter seed retention is affected by wind and rain, insect attack and fungal pathogens. Between 29% and 40% of the postwinter losses of A. americanum seed observed in one study were attributed to wind and rain (Wicker 1967). An additional 9% to 17% of the loss was a result of insects and fungi. Overwinter and postwinter losses are significant factors determining the inoculum potential of A. americanum.

Throughout the germination process, the radicle and

appressorium of dwarf mistletoe seeds are affected by a number of factors which may inhibit completion of their development. Typically, pathogen populations lose their dormancy at irregular intervals such that, on average, the probability of mortality at any one time is reduced (Dimond and Horsfall 1965). However, the onset of germination of Arceuthobium spp. seed is considered to be controlled by temperature (Scharpf 1970).

Gill and Hawksworth (1961) have suggested that an optimum range of 15C to 20C exists for germination of most temperate zone dwarf mistletoes. Temperature and moisture stress are both important indicators of the potential for seed desiccation, and the inoculum potential of any given pathogen is partially dependent upon the external environmental conditions present at the time of penetration (Agrios 1979).

Weir (1918) suggested that A. vaginatum (Willd.) Presl. could not infect ponderosa pine tissue greater than three years of age owing to the formation of cork. He found that by removing the cork layer that tissue as old as 7 years of age could be infected (Weir 1918). In contrast, Hawksworth (1954) found that branch and stem tissue of lodgepole pines over 60 years of age can be infected by A. americanum. He also found that infections in tissues greater than five years of age were common in both dominant and suppressed trees. Hawksworth (1954) suggested that the thinness of lodgepole pine bark may be a factor enabling A. americanum to infect older tissue of P. contorta.

The observations made by Weir (1918), Dowding (1929) and Hawksworth (1954) raise the question as to how tissue and tree age influence the success of infection in the closely related species, P. banksiana.

The frequency at which infections become established may vary with climate and year. Smith and Wass (1979) found that mean infection percentages for A. americanum on lodgepole pine in British Columbia varied significantly between the mild, coastal climate (27.3%) and the colder, drier, interior climate (16.3%). In one of the study years, following a winter during which both minimum and mean monthly temperatures were significantly below normal at the interior site, no infection was observed (Smith and Wass 1979).

Using seeds of A. campylopodum, Scharpf (1969) tested the effect of constant temperature on the rate of penetration and infection in young Digger (Pinus sabiniana Dougl.) and Monterey (P. radiata D. Don.) pines. Scharpf (1969) found that penetration was greatest at 16C (59%) and decreased to 0% at temperatures greater than 21C. An outdoor control which averaged 13C gave an infection percentage of 66%. Scharpf (1969) concluded this to be an indication of favourable influence of fluctuating diurnal conditions or gradual long term changes in temperature.

The conditions under which pines may become infected by dwarf mistletoes are of great importance to forest managers. This study was principally designed to test the hypothesis jack pines under 15 years of age young are not susceptible to

infection by A. americanum. The secondary objective was to examine the effect of klendusic and microclimatic factors on the infection process.

Materials and Methods

Overwinter and Postwinter Seed Retention, and Seed Germination

Stands of trees aged 3, 5, 7, 12, 17 and 22 were chosen for inoculation in Belair Provincial Forest as shown in Figure 12. Four blocks of 50 trees were staked out for each of the tree age classes in 1992 ; three such blocks were staked out in 1993 as fewer seeds were collected in the latter year. A grid was superimposed over each block, and a random number table was used to select ten trees - or replicates - from each block of 50 trees.

In the first three tree age classes, on each of the randomly selected replicates, the 1st, 2nd, and 3rd year tissues to be inoculated were marked with a grease pen. In the latter three tree age classes, the 1st, 4th and 8th year tissues were marked on each replicate. In 1992, there were : 6 tree ages, 4 blocks per tree age, 10 trees (replicates) per block, and 3 tissue ages inoculated per tree, or 6 * 4 * 10 * 3 = 720 seeds. In 1993, there were : 6 tree ages, 3 blocks per tree age, 10 replicates per block, and 3 tissue ages inoculated per replication, or 6 * 3 * 10 * 3 = 540 seeds.

Inoculum was trapped from three separate seed sources in Belair Provincial Park from dwarf mistletoe-infected stands of P. banksiana. Cheesecloth was laid out in approximately 20 m strips on the ground, 6 m to 10 m away from the trees bearing discharging fruit. As well, pieces of cheesecloth were wrapped loosely around brooms bearing mature fruit. Seeds were placed into water to allow the viscin coats to become

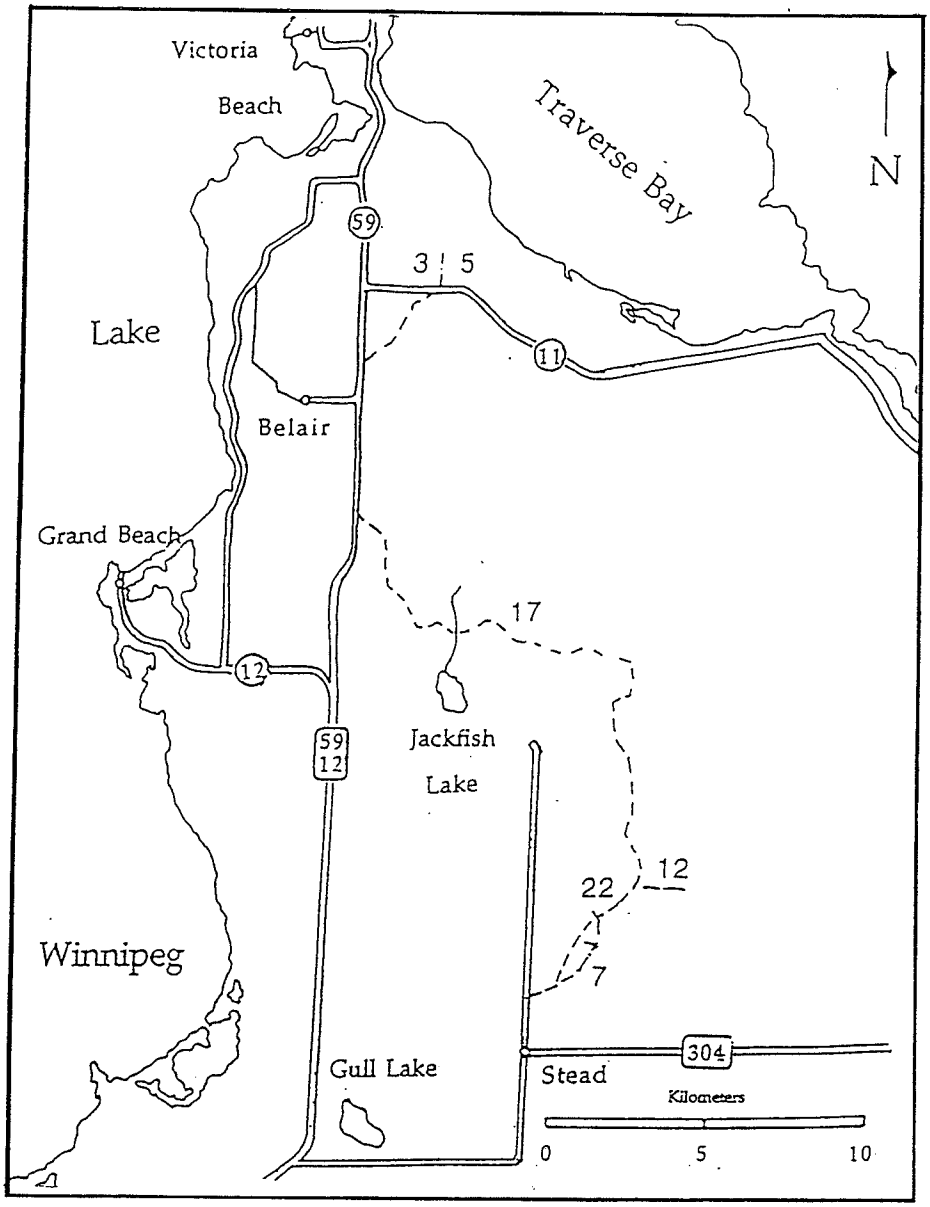


Figure 12. Map of the six Pinus banksiana stands used in the inoculation study. The numerals indicate the stand ages - 3, 5, 7, 12, 17, 22 years old.

fully imbibed and immediately inoculated onto the branches of the randomly selected trees.

The seeds were monitored over winter until late summer or early fall just prior to harvesting. Overwinter retention, germination, appressorial development, molding, insect damage and summer retention were recorded at various times to determine their respective effect upon the destiny of the inoculum.

A CR10 datalogger with an HMP35C temperature and relative humidity probe (Campbell Scientific Corp., Edmonton, Alberta) was set up in the 7 year old plots. Measurements of temperature, relative humidity and vapour pressure deficit were made every 10 seconds and averaged every hour. Regression analysis provided correlations between rates of germination and temperature.

Infection Success

All inoculated branches were harvested in September, one year after inoculation to assess the extent of infection. Tissues that were not prepared immediately for sectioning were marked and placed in a cold storage chamber. Seeds that appeared mouldy in the field were cultured on tap water agar (2%). Fungi were identified to genera with the aid of Drs. J. Reid and L. Hutchison.

Prior to sectioning, lengths of branches bearing seeds were soaked for 48 hours in 5ml distilled water to soften the tissue and infiltrated in a 5ml 1:1 mixture of distilled water

and mucilage for approximately three to four days. Twenty-micron sections were cut through the region of penetration using hand cryotome and stained with 0.01% ethidium bromide. The sections were mounted in glycerin:ethanol (1:1) on clean glass slides and examined for the presence of the chromocentric nuclei of the endophytic system of the pathogen using a Nikon Optiphot Biological Microscope. The microscope was fitted with an episcopic fluorescence attachment (Nippon Kogaku, Tokyo, Japan), an IP410-485 exciter filter and a 515W absorption filter.

Infection success data were calculated as proportions, so in order to equalize variances and render the data normal the arcsin square root transformation was applied. Two factor ANOVA (tissue and tree age) was used to determine the influence of tissue and tree age on the successful establishment of an infection. Duncan's MRT indicated significant differences in infection success between tissue and tree age treatment combinations.

Seed Viability

Seed viability was tested using 2,3,5 - triphenyl tetrazolium chloride (2,3,5-TTC) (Scharpf and Parmeter 1962). In each year, three lots of 20 seeds were presoaked in distilled water at 22C for 24 hours. The seeds were bisected longitudinally, placed in a sterile petri dish, and submerged in 1% aqueous 2,3,5-TTC at 22C in the dark for 72 hours. Red staining in the embryo was used to indicate viability. A

paired t-statistic was calculated to determine if a significant difference in mean seed viability occurred between the two years of the study.

Overwinter Seed Retention as a Function of Height of Inoculation

Four blocks of 50 trees aged 7 years were staked out in 1992. Ten replicates, or trees, were randomly selected per block as in the first experiment of the inoculation study. Tissues aged 1, 2, and 3 years were marked at a height of 1.5 m and 0.25 m on each of the randomly selected 10 replicates. In total, 240 seeds were placed on marked tissues in September 1992 (4 blocks * 10 replicates/block * 3 tissue ages/replicate * 2 heights/replicate = 240 seeds). The seeds were assessed for overwinter retention in April 1993.

The mean proportions of seeds remaining over winter were transformed using the arcsin square root function, and analysed using 2-factor ANOVA (height and tissue age). The data were separated using Duncan's MRT. Owing to a lack of seeds in the 1993/1994 field season, this experiment was not repeated.

Results and Discussion

Overwinter Seed Retention:

i. 1992-1993 field season:

Rates of seed removal for the 1992-1993 field season are given in Figure 13 (a-f). Appendix 3 contains the life table data concerning seed removal in the 1992-1993 field season. Overwinter seed retention between September 1992 (day 0) and April 1993 (day 231) ranged from 77.8% in the 5 year-old age group to 92.9% in the 7 year-old age group. As a result, finite rates of seed removal were relatively low, ranging from 0.009 in the 7 year-old trees to 0.032 in the 5 year old trees.

ii. 1993-1994 field season:

Finite rates of seed removal for the 1993-1994 field season are given in Figure 14 (a-f), while life table data are in Appendix 4. Overwinter seed retention in each of the tree age classes inoculated was lower than in the previous year. Seed retention between September 1993 and May 1994 ranged from 48.9% in the 12-year old age class to 86.7% in the 7 year-old trees. Corresponding rates of seed removal varied from 0.018 in the 7 year-olds to 0.085 in the 12 year-old trees. Though comparable, rates of seed loss were higher for each age class than in the previous field season.

Rates and proportions of overwinter seed retention in this study were comparable to those of others (Smith 1974, Smith and Wass 1979, Shaw and Loopstra 1991). Shaw and

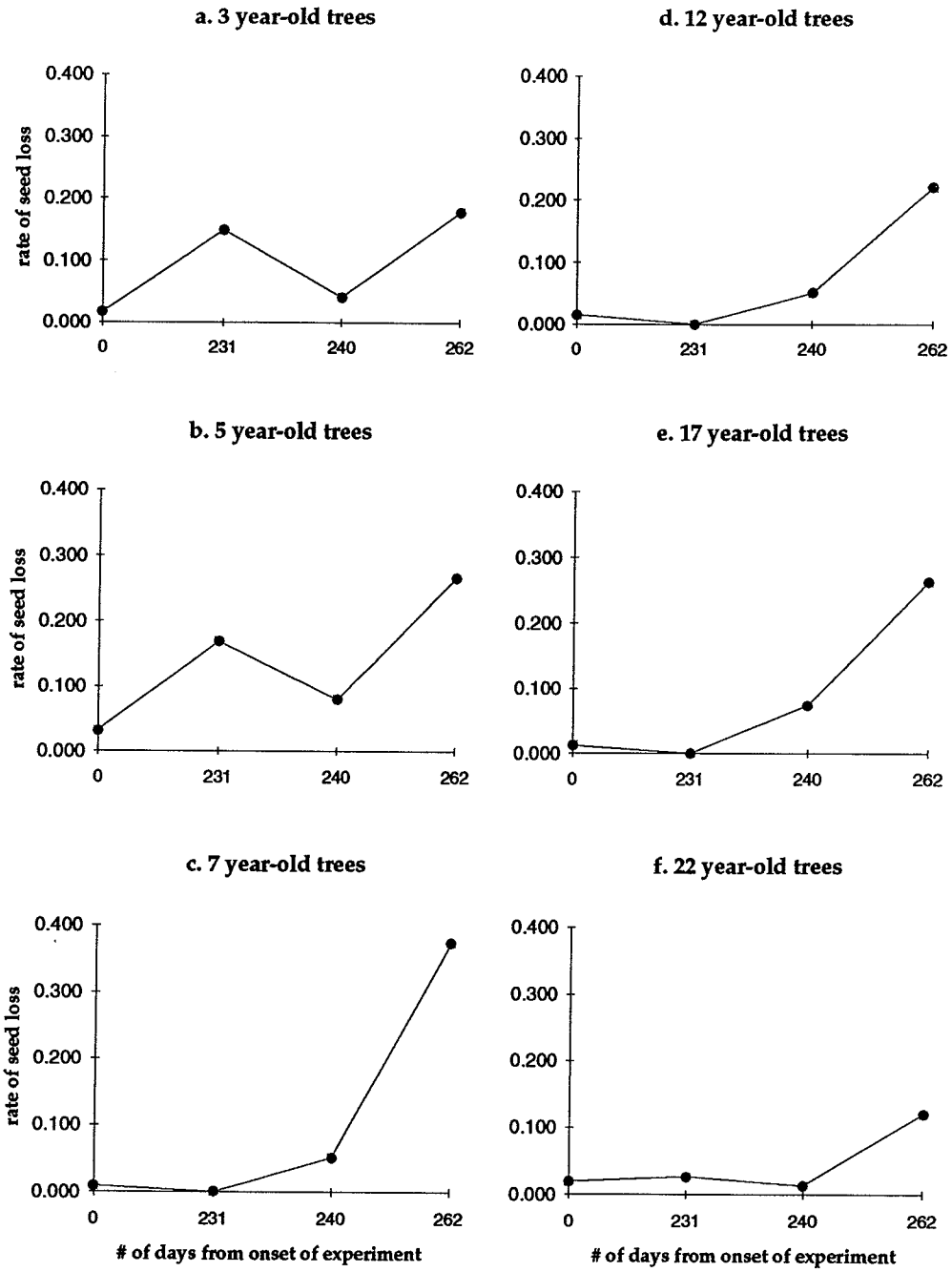


Figure 13 (a-f). Instantaneous rates of seed loss as a function of time in the six tree age categories inoculated in 1992. Data are based upon a sample size of n=120 seeds per age class placed on 4 blocks on 3 ages of tissue with 10 replicates - or 30 seeds per block.

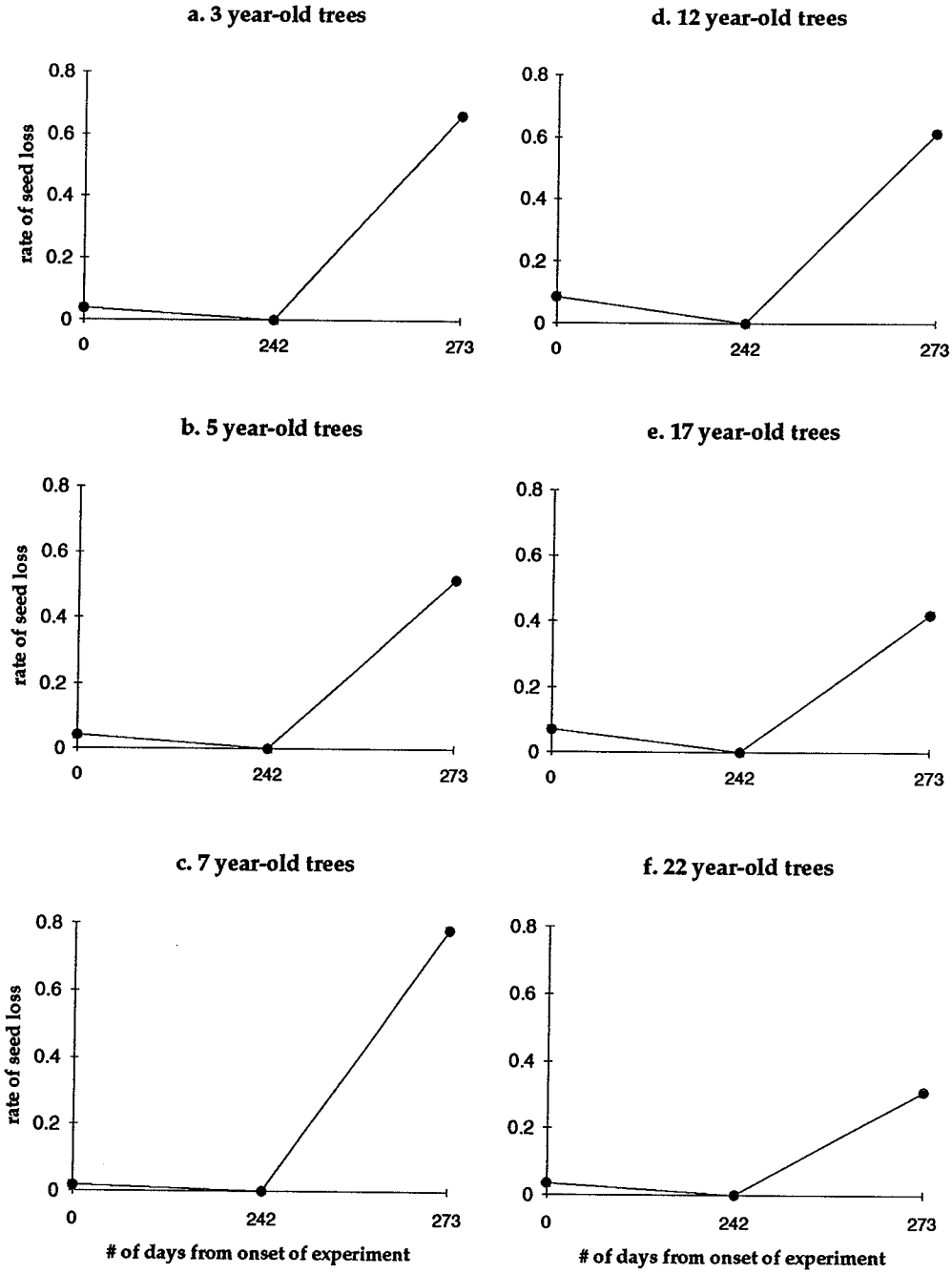


Figure 14 (a-f). Instantaneous rates of seed loss as a function of time in the six tree age categories inoculated in 1993. Data are based upon a sample size of n=90 seeds per age class placed on 3 blocks on 3 ages of tissue with 10 replicates - or 30 seeds per block.

Loopstra (1991), Smith (1974) have observed that mean overwinter retention of A. tsugense seeds on twigs of western hemlock of 79% and 96% respectively. Smith and Wass (1979) found overwinter retention of A. americanum seeds on lodgepole pine to vary between 82% and 94% in successive years.

Despite minor differences in percent retention between tree age classes there was a substantial loss of seeds over winter in both years. However, this was not consistent within age classes, for the 12 and 17 year-old trees showed very high retention in 1992/1993 (88.3%) and the lowest retention in 1993/1994 (48.9%). These observations may help to explain the annual variation in the number of infections established within and between tree age classes.

Postwinter Seed Retention:

i. 1992-1993 field season:

Following the period of winter dormancy seeds are subject to a number of factors which may remove or damage them prior to germination. In this study, postwinter loss was caused by wind, rain, insect attack and fungal molds. Between day 231 (April 1993) and day 240 (May 1993), seed retention fell from 87.4% to 82.4% in the 3 year-old trees and from 77.8% to 72.6% in the 5 year-old trees (Figure 13 ; Appendix 3). Finite rates of removal at day 231 were 0.150 and 0.170, respectively. Low or no loss was observed in each of the other age classes.

Rates of seed removal ranged from 0.013 in the 22 year-

old stands to 0.079 in the 5 year-old trees at day 240 (May 1993). Between day 240 and day 262 (June 1993), the range of reduction in seed retention was 92.9% to 89.4% in the 7 year-olds to 72.6% to 68.4% in the 5 year-old age class.

The greatest rates of seed removal were observed in July 1993 at day 320. Finite rates of removal varied from 0.119 in the 22 year-old trees to 0.373 in the 7 year-olds. As a result, rates of retention fell to 65.8% in the 22 year-olds and 36.3% in the 7 year-olds.

An examination of the factors which may cause postwinter loss (Figure 15) revealed that fungal moulding caused losses of 2.5% to 8.3% of seeds between April 1993 and July 1993. Insect attack had less impact upon seed retention and caused 0.8% to 7.5% of the seed loss between the different age classes. The remainder of the loss, between 10.8% and 48.3%, was likely owing to the effect of wind and rain.

Postwinter loss owing to wind and rain (28.8% overall) was greater than overwinter loss (12.2% overall), loss owing to moulding (5.6% overall) and loss owing to insect attack (3.5% overall). Overall seed retention was 49.9%.

ii. 1993-1994 field season:

No seeds were lost in any of the tree age categories between May 1994 (day 242) and June 1994 (day 273). In the 22 year-old plots, seed retention ranged from 75.6% to 52.2% between days 273 and 303 (July 1994 - Appendix 4), corresponding to the lowest rate of seed loss - 0.309 (Figure

tree age	3	5	7	12	17	22
molding damage	7	6	3	8	10	6
insect attack	1	3	3	3	9	6
overwinter loss	13	26	8	14	11	16
postwinter loss	32	40	58	32	33	13

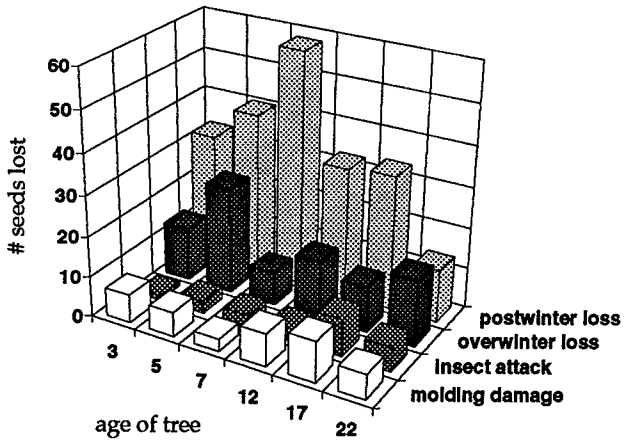


Figure 15. The number of seeds lost in 1992/93 as a result of molding by fungi, insect attack, overwinter loss and post-winter loss. The data are based upon a sample size of n=120 seeds inoculated per tree age category.

14). The rate of seed loss was greatest in the 7 year-old age class (0.782), as seed retention dropped from 86.7% to 18.9% between days 273 and 303.

Moulding was a relatively minor cause of seed loss (Figure 16), reducing numbers of seeds by 1.1% to 12.2% during the spring and summer of 1994. Insect attack also accounted for a small portion, between 2.2% and 13.3%, of the seed loss. Wind and rain were likely responsible for the remainder of the summer loss, between 2.2% and 47.8%.

When a comparison of the two field seasons is made, a general trend with respect to finite rates of seed loss is apparent. Rates of seed loss ranged between 0.010 and 0.085 overwinter and remained low - in most instances - until July. It is hypothesized that losses were a cumulative effect of wind, rain, snow, and insect and fungal attack. As well, desiccation is thought to have played a factor as much of the losses were observed between June and July of both years when moisture demands are greatest.

These data provide some insight into the reductions in inoculum potential that are effected by seed loss and the various factors which act to bring about seed removal. In 1993 and 1994 approximately 50% and 30% of the seeds, respectively, remained where they were placed on susceptible host tissue. Postwinter seed loss was 28.8% and 22.2% in the first and second years of the study respectively, while overwinter loss was 12.2% and 30.6% in the two years. Seed loss as a result of fungal attack was 5.6% and 8.9%

tree age	3	5	7	12	17	22
molding damage	7	11	1	9	11	9
insect attack	5	6	12	2	7	10
overwinter loss	15	26	17	46	39	22
postwinter loss	41	14	43	16	4	2

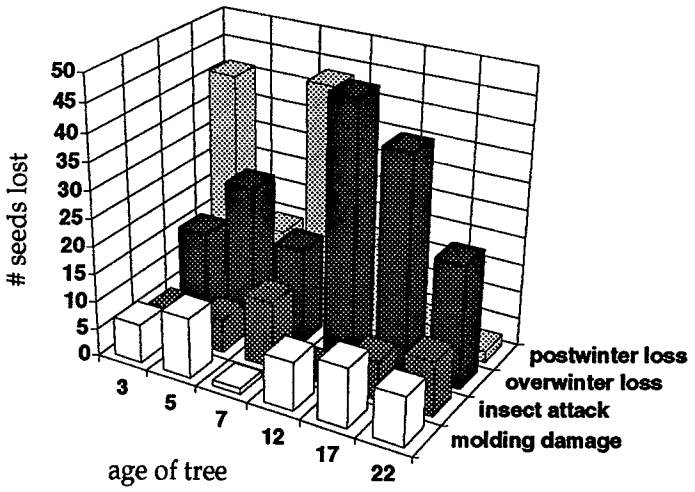


Figure 16. The number of seeds lost in 1993/94 as a result of molding by fungi, insect attack, overwinter loss and post-winter loss. The data are based upon a sample size of n=90 seeds inoculated per tree age category.

respectively, while insect damage was 3.5% and 7.8%.

It is hypothesized that low mean daily temperatures in January and February of 1994 caused high overwinter seed losses. In January and February (Figures 17 and 18) mean daily temperatures were significantly lower ($t=15.114$, $p<0.0001$; $t=7.169$, $p<0.0001$) in 1994 (-24.7C, -19.3C) than in 1993 (-17.7C, -15.6C).

Seed Germination:

i. 1992-1993 field study:

The data for seed germination are presented in Figure 19 (a-f). Seed germination was not observed until May 1993 (day 240) and continued through June (day 262) and July (day 320) - see Appendix 5. An increase was observed between May and June in each tree age class, ranging from 21.0% to 25.7% in the 7 year-old trees and from 54.9% to 60.4% in the 22 year-old age class. Rates of germination were 0.247 and 0.662 for the 7 and 22 year-olds respectively.

At day 262, rates of seed germination ranged from 0.143 in the 7 year-old trees to 0.381 in the 22 year-olds. Some of seeds which had germinated were lost, thus causing the apparent decrease in seed germination rates observed in Figure 19 (a-f).

Finite rates of germination increased between April and June of 1993 and decreased slightly in July. The observed trend in seed germination may be an indication that the seeds are subject to desiccation during the warm, dry summer months.

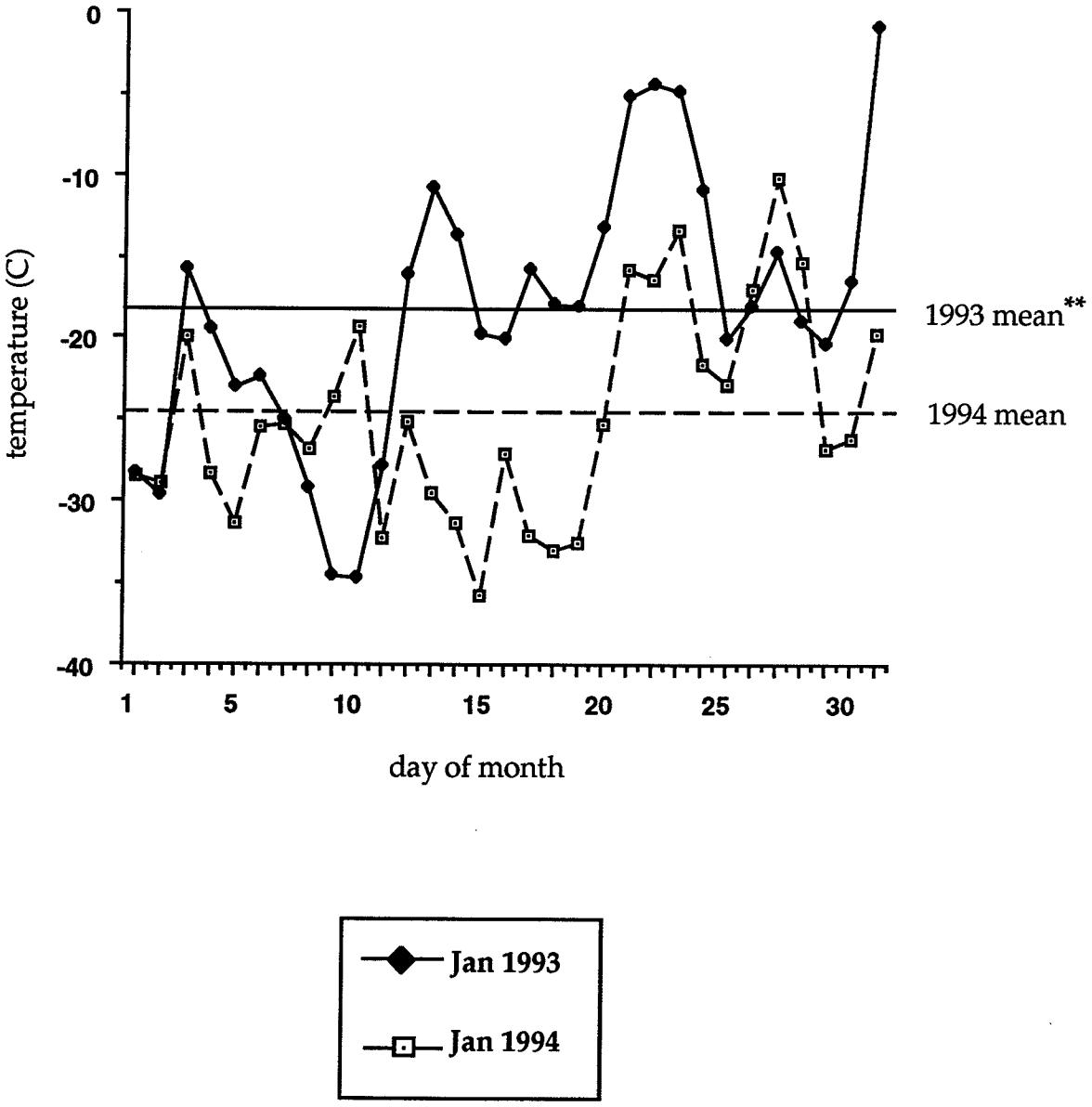


Figure 17. Mean daily temperature for January of 1993 and 1994. The average temperature was -17.7 C in 1993 and -24.7 C in 1994. **- Indicates significant difference at $p < 0.0001$.

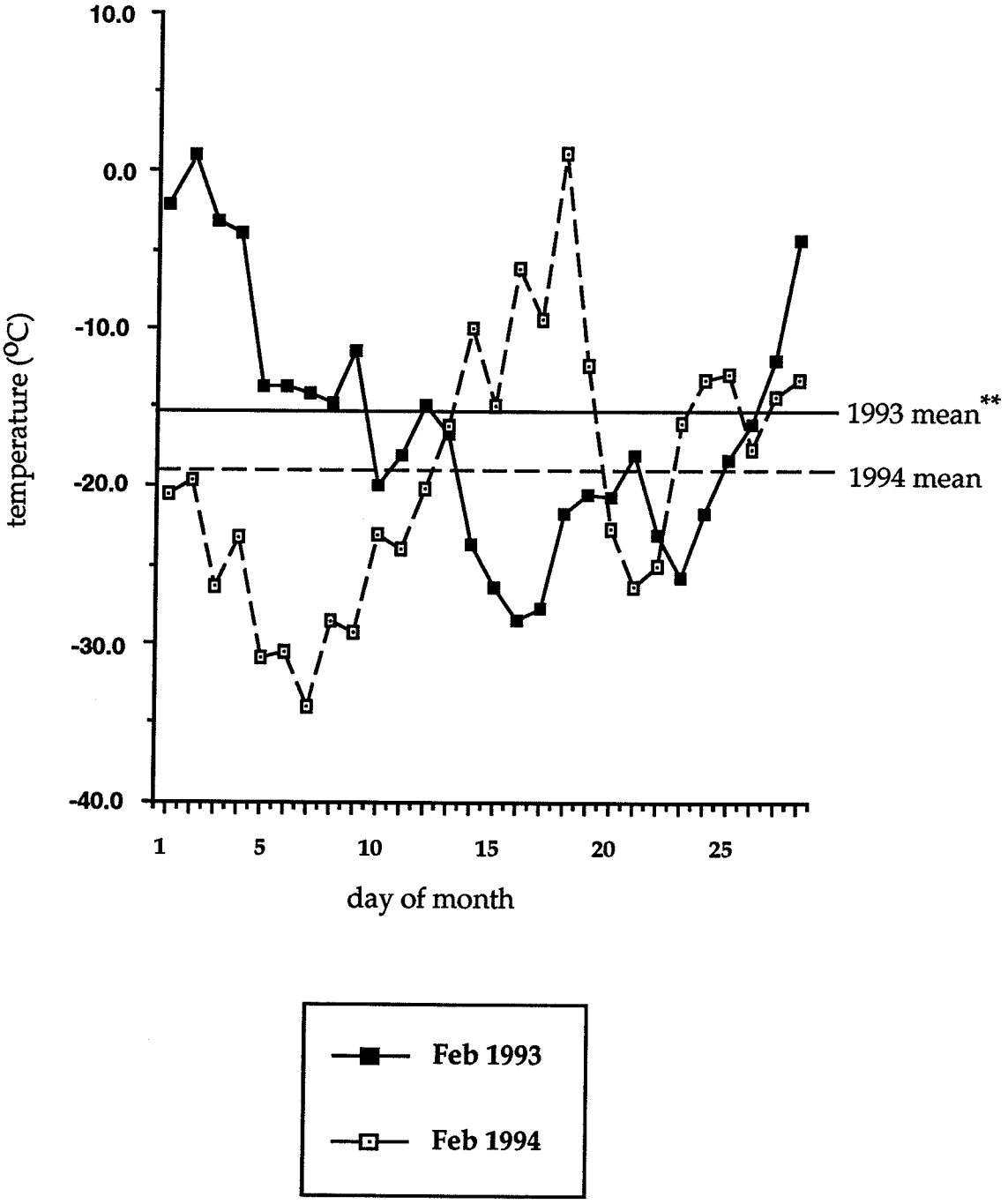


Figure 18. Mean daily temperature for February of 1993 and 1994. The average temperature was -15.6 C in 1993 and -19.3 C in 1994. **- Indicates significant difference at $p < 0.0001$.

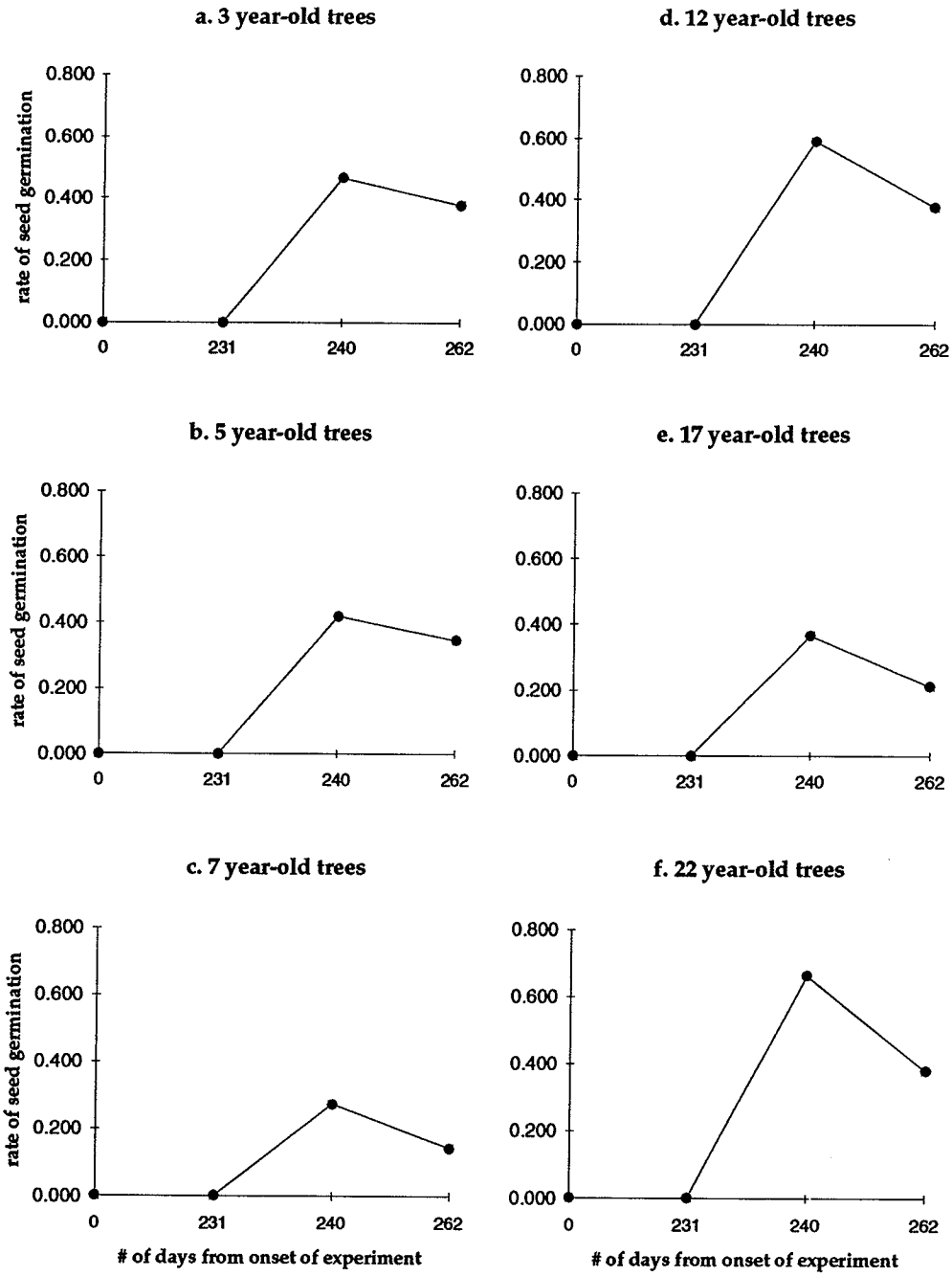


Figure 19 (a-f). Instantaneous rates of seed germination as a function of time in the six tree age categories inoculated in 1992. Data are based upon a sample size of n=120 seeds per age class on 4 blocks on 3 ages of tissue with 10 replicates - or 30 seeds per block.

In 1993, temperatures reached daily maxima of 27C for an extended period between June 15 and June 30 (appendix 8e).

Rates of seed germination were insignificantly correlated with temperature on a linear scale ($r=0.84$; $t=2.189$; $0.20 < p < 0.10$). It is hypothesized that the high temperatures observed in June 1993 were damaging to seeds and may have resulted in some seed loss through desiccation. This is substantiated by the finding that seed germination for most members of the Arceuthobium genus seed germination has an optimal range of 15C to 20C (Gill and Hawksworth 1961).

ii. 1993-1994 field season:

Germination was not observed until July 1994 (day 303). Rates of germination data ranged from 0.031 in the 12 year-old age class to 0.175 in the 17 year-old blocks (Figure 20 (a-f)). Seed germination ranged from 5.9% in the 12 year-old trees to 31.0% in the 17 year-old trees (Appendix 6).

The correlation between germination and temperature ($r=0.48$; $t=0.774$; $p > 0.50$) was insignificant. The cold winter of 1994 may have reduced seed viability and thus seed germination relative to that of 1993. Smith and Wass (1979) have observed a similar phenomenon in A. americanum on lodgepole pine in the interior of British Columbia following a winter in which the mean monthly temperature in January dropped below -18C, significantly lower than the average monthly mean temperature. Williams et al. (1972) have suggested that low temperatures are the probable factor are

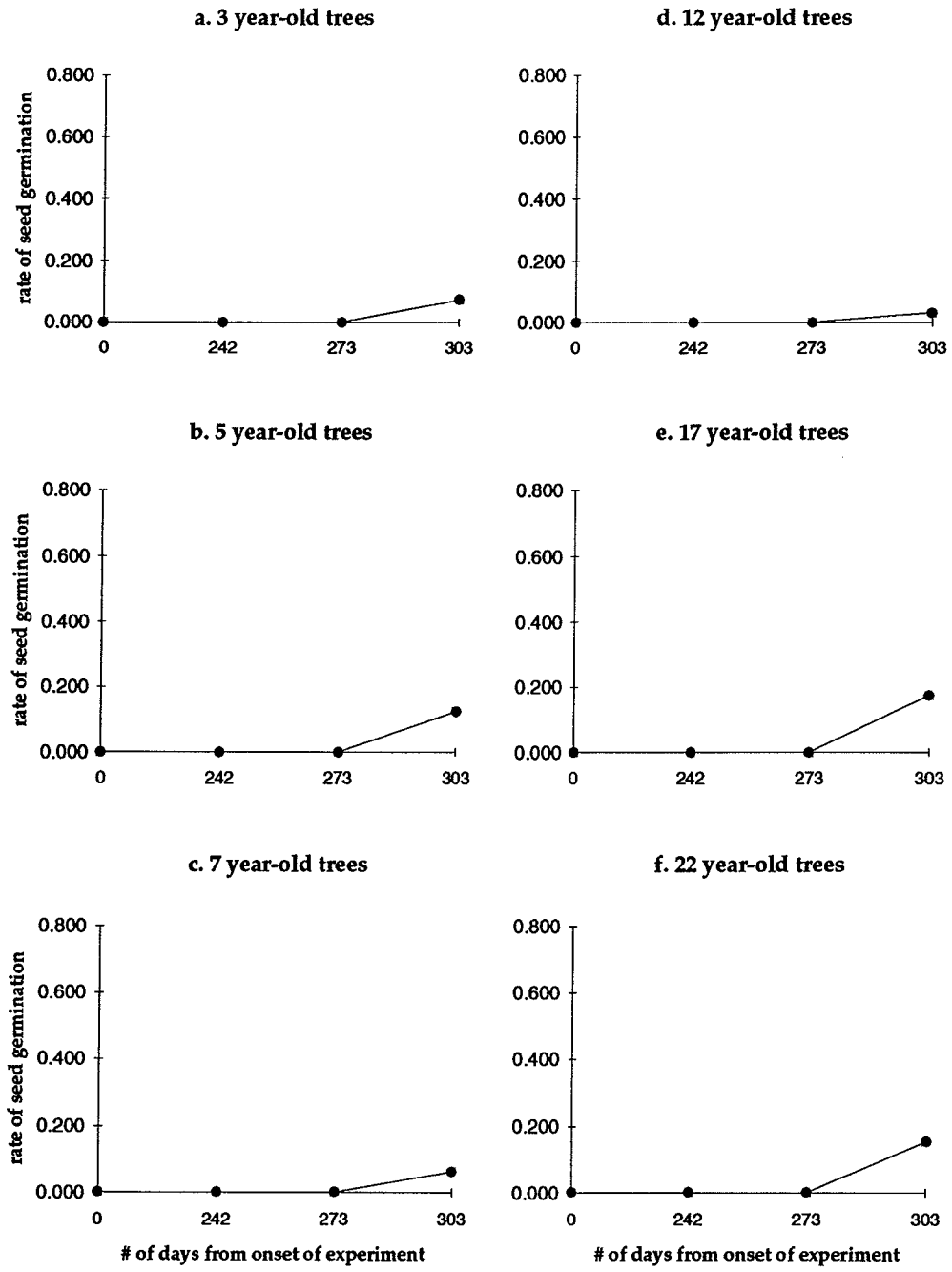


Figure 20 (a-f). Instantaneous rates of seed germination as a function of time in the six tree age categories inoculated in 1993. Data are based upon a sample size of n=90 seeds per age class on 3 blocks on 3 ages of tissue with 10 replicates - or 30 seeds per block.

the probable factor limiting the elevational spread of A. americanum on P. contorta in the central Rocky Mountains.

The results of this study suggest that in seasons when average daily winter temperatures are mild enough not to reduce seed viability, seed germination may correspond to changes in spring and summer temperatures. As dwarf mistletoe seeds lack a true seed coat, evapotranspiration is likely to place stress on them and result in seed desiccation.

The germination process represents a critical stage in the lifecycle of A. americanum as the seeds must germinate in order to infect a new host and continue the lifecycle. Estimates of seed interception have been provided by Hawksworth (1965), and approximate 40% of those seeds expelled. In this study, approximately 50% and 30% of seeds remained on susceptible tissue until the time that germination and appressorial development occurred in 1993 and 1994 respectively. Overall rates of germination varied widely in the two consecutive years ; 27.4% in the 1992-1993 field season and 6.5% in the 1993-1994 field season. Using Hawksworth's (1965) data on interception, the estimated rates of infection success vary from 11.1% (40% * 27.4%) to 2.6% (40% * 6.5%) under different conditions of temperature, moisture stress, wind, rain, moulding and insect attack in the absence of effective mechanisms of host resistance.

Typical rates of infection success from other studies agree with this and generally vary between 2% and 16% (Livingston and Blanchette 1986, Baker et al. 1981, Scharpf

and Parmeter 1982, and Shaw and Loopstra 1991). In each of these studies, though, rates of infection were much more constant between years. Data from the present investigation provide some support for the possibility of 'wave years' of infection which may be responsible for the aggregated distribution of A. americanum on jack pine in Belair Provincial Forest.

Infection Success:

i. 1992-1993 field season:

Figure 21 (a-b) gives the proportion of successful infections as a function of tree and tissue age in the 1992-1993 field season. In the 3, 5 and 7 year-old tree age classes, the proportion of successful infections ranged from 0.05 in the 7 year-old age class on the three year-old tissue to 0.26 in the 3 year-old age class on the three year-old tissue (Figure 21a). The average proportion of infections established in these three age classes was 0.13. From the analysis of variance (Appendix 9a), neither tissue age ($p=0.145$) nor tree age ($p=0.126$) explained any of the observed variation in infection success.

The proportion of infections established in the 12, 17 and 22 year-old age classes ranged from 0.02 on the 1 year-old tissue of the 17 year-old age trees to 0.35 on the 8 year-old tissue of the 22 year-old trees (Figure 21b). The average proportion of infections successfully established was 0.15. From the analysis of variance, tree age ($F=3.790$, $p=0.035$;

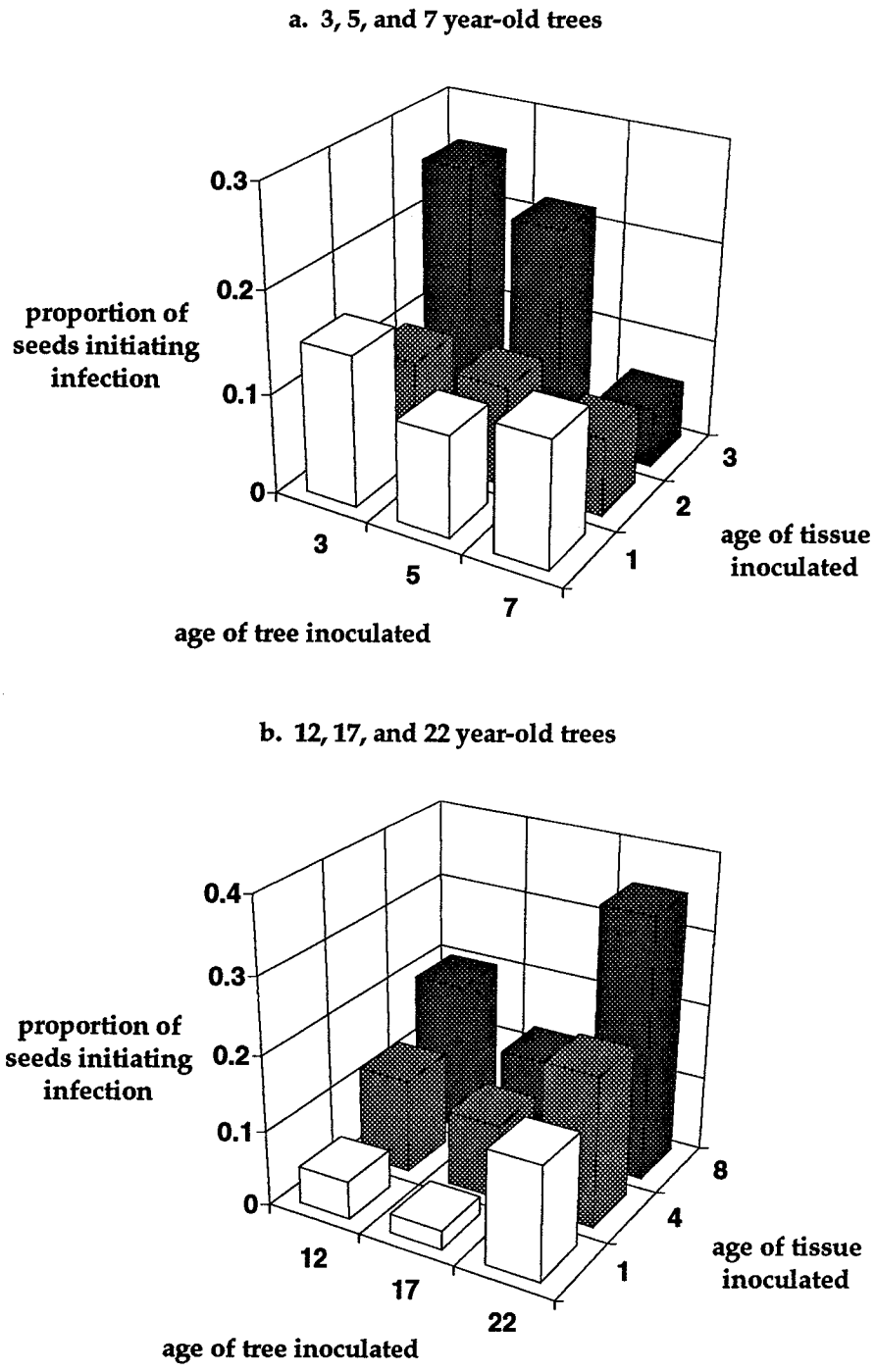


Figure 21. The proportion of successful infections established as a function of tree and tissue age in 1993. These data are based upon a sample size of n=40 inoculations per treatment combination.

Appendix 9b) and tissue age (F=3.571, p=0.042) accounted for a significant proportion of the observed variation. The trend in the data is an increase in the proportion of infections established with increasing tissue age in each tree age class.

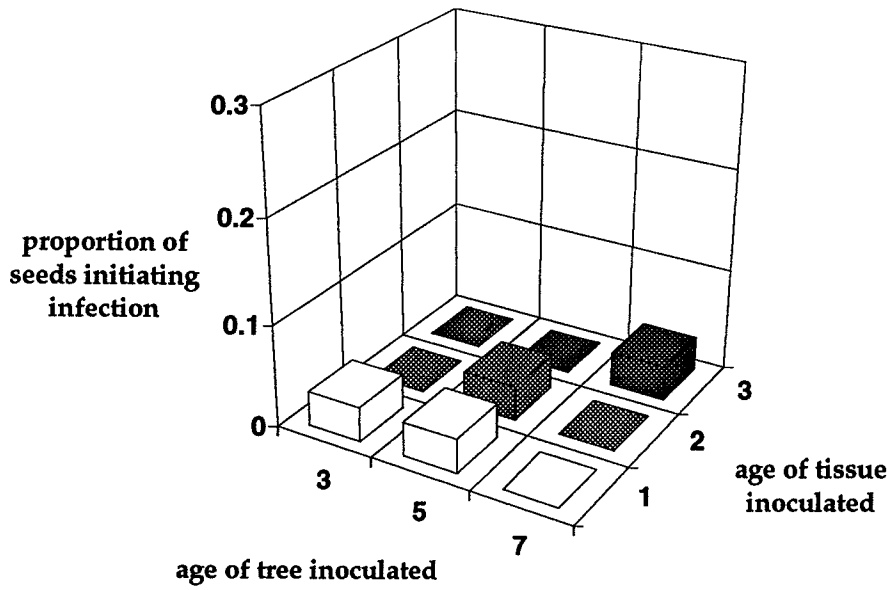
ii. 1993-1994 field season:

The proportions of seeds which established a successful infection in the 1993-1994 field season are given in Figure 22 (a-b). In the 3, 5 and 7 year-old age classes, the proportion of infections ranged from 0.00 to 0.03. The average proportion of infections established in these three age groups was 0.02 (Figure 22a). Analysis of variance confirmed that neither tree age (p=0.781) nor tissue age (p=0.781) accounted for a significant proportion of the observed variation (see Appendix 10a).

The observed proportions of successful infections ranged from 0.00 to 0.13 in the 12, 17 and 22 year-old trees. The average proportion of inoculations which established an infection was 0.04 (Figure 22b). Again, analysis of variance indicated that neither tree (p=0.564) nor tissue age (p=0.564) explained a significant proportion of the observed variation (Appendix 10b).

It is apparent from this experiment that neither tree nor tissue age has a significant impact upon the successful establishment of A. americanum on P. banksiana. However, the 1992-1993 data provide some evidence for increasing frequency of infection with increasing tissue age in the 12, 17 and 22

a. 3, 5, and 7 year-old trees



b. 12, 17, and 22 year-old trees

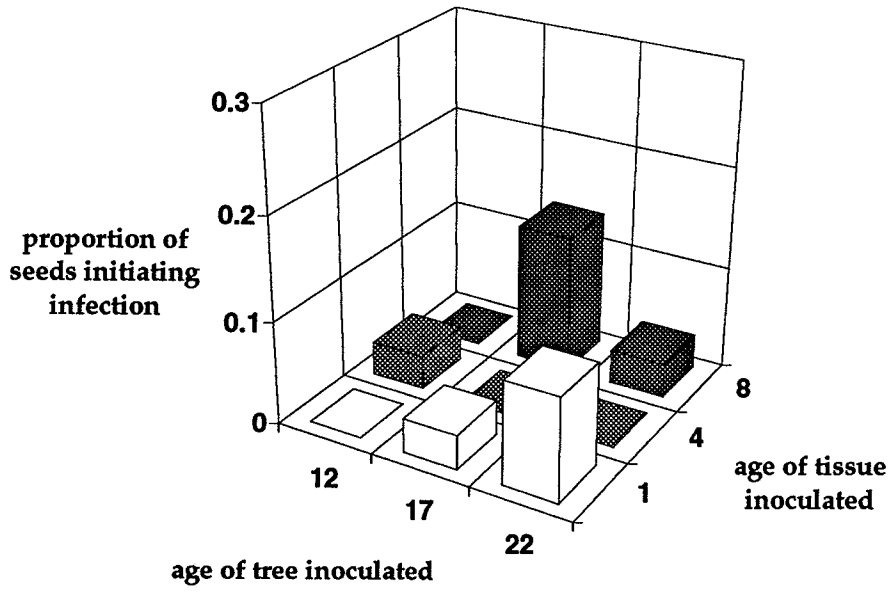


Figure 22. The proportion of successful infections established as a function of tree age tissue age in 1994. These data are based upon a sample size of n=30 inoculations per treatment combination.

year-old age categories. This is explained by the fact that seed retention on the older tissues of these age classes was higher than on the younger tissues. It is possible that the effect of the crown on reducing incoming solar radiation to the older portions of the branches would reduce moisture stress on seeds placed older tissues. If the crown does provide some protection to the seeds, then the increased retention with tissue age in the 12, 17 and 22 year-olds may result in greater rates of germination and thus infection.

The proportion of infections established in the 1992-1993 field season for the 3, 5 and 7 year-olds was 13% and that for the 12, 17 and 22 year-olds was 15%. In the following year proportions of infections established were 2% for the 3, 5 and 7 year-old age classes and 4% for the 12, 17 and 22 year-old age classes. These are slightly higher than the estimated values of 11.1% and 2.6% previously mentioned in the seed germination discussion, likely owing to the fact that in the inoculation study all inoculations were made at the base of the needles. Natural emplacement of dwarf mistletoe seeds often results in seeds landing on needles (Hawksworth 1965) from which they are more easily removed than from the bark of the twigs (Shaw and Loopstra 1991).

The conclusion from the data is that neither tree age or tissue age is a primary determinant of the ability of A. americanum to cause infection. Other factors, such as seed interception, retention and germination are more significant in determining the successful establishment of an infection.

The observation that trees under 15 years of age may grow adjacent to heavily infected mature stands (Dowding 1929) without becoming infected is best explained by klendusic factors. One factor is the relatively small surface area available for inoculation (Wicker and Shaw 1967), and another is the likelihood that more seeds will be intercepted by needles from which they are more likely to be dislodged.

Seed Viability:

In 1992 an average of 93.3% of the seeds showed a positive response to the 2,3,5-triphenyl tetrazolium chloride (TTC) test. In 1993 an average of 88.3% showed a positive response. The t-statistic for paired means ($t=1.732$, $p=0.113$) indicated that the yearly averages were not significantly different from one another. These data suggest that any significant difference in terms of seed germination was not likely to be caused by differences in seed viability.

Overwinter seed retention as a function of height of inoculation:

Seed retention as a function of the height of inoculation is presented in Figure 23. At a height of 1.5m, seed retention was 95% $\pm$ 5% on the 1 year-old tissue, 92% $\pm$ 5% on the 2 year-old tissue, and 87% $\pm$ 6% on the 3 year-old tissue. On tissues inoculated at a height of 0.25m, seed retention varied from 66% $\pm$ 12% on the 1 year-old tissue, 70% $\pm$ 8% on the 2 year-old tissue, and 37% $\pm$ 7% on the 3 year-old tissue.

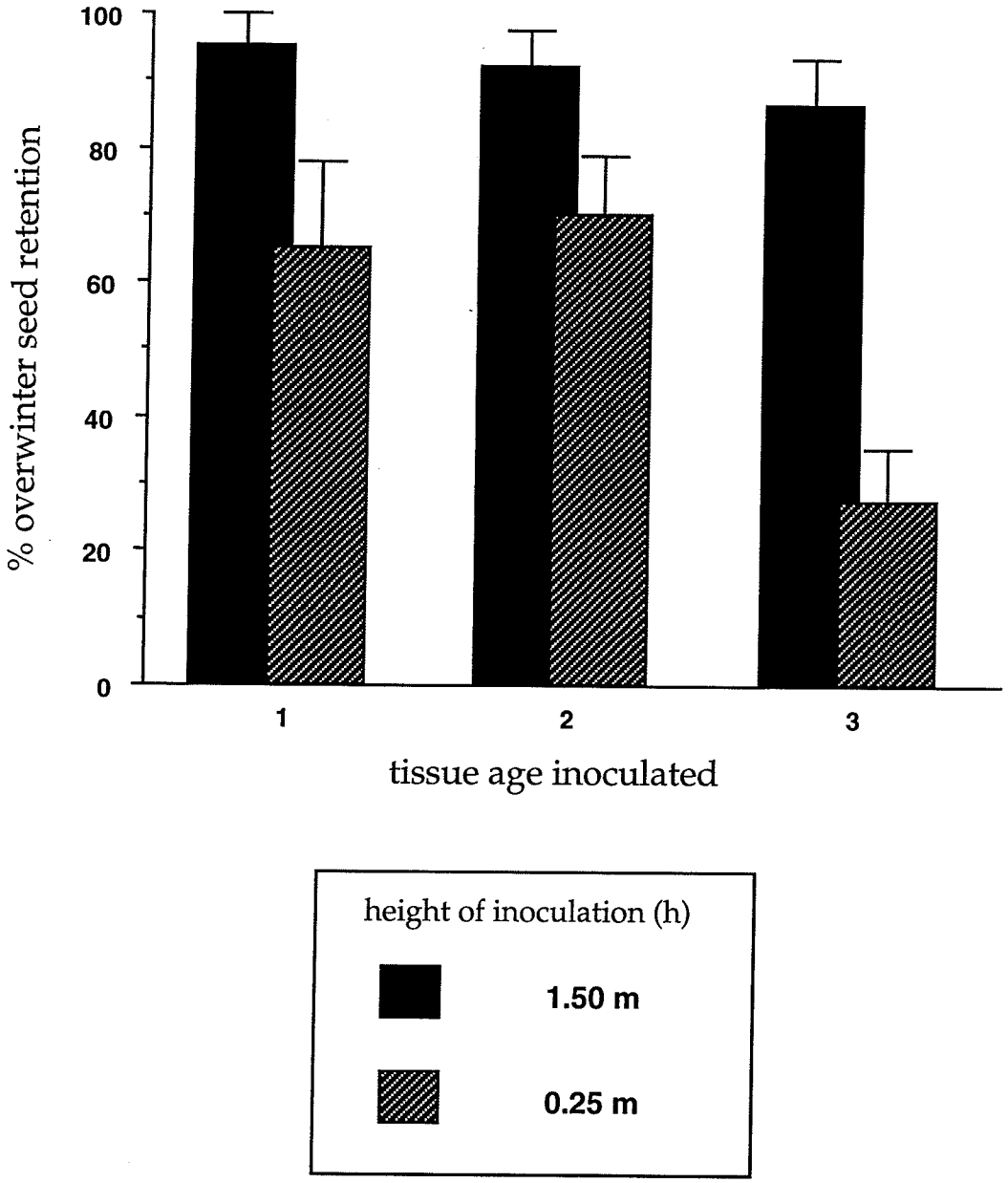


Figure 23. Mean overwinter seed retention at different combinations of height (h=1.50m, 0.25m) and tissue age (1, 2, 3 year old tissue). The data are based upon a sample size of n=40 seeds per treatment combination. The error bars indicate 1 standard deviation of the mean.

Analysis of variance indicated that both the height of inoculation ($F=52.831$, $p<0.0001$; Appendix 7) accounted for a significant proportion of the variation in overwinter seed retention. As well, the analysis indicated that the interaction between the two variables was responsible for a significant proportion of the observed variation ($F=6.380$, $p=0.003$). Significantly more seeds inoculated above the snowline were retained than those inoculated below, regardless of the age of tissue serving as a substrate. However, when the age of inoculated tissue was also considered, the 3 year-old tissue below the snow line lost significantly more seeds than any other treatment combination.

The effect of height of inoculation may be explained by the action of snow (Wicker 1967). If the hygroscopic viscin coat became hydrated, perhaps during snowmelt, a dwarf mistletoe seed might be dislodged more easily from its substrate. Either the adherence of the swollen viscid layer to the melting snow or shaking by wind or animals might cause this. However, this experiment also indicated that another factor was important in determining the effect of snow on seed removal. On the twigs that had been inoculated at 0.25 m, only the 3 year-old tissue lacked needle fascicles, which would likely act as anchoring sites for the dwarf mistletoe seeds. It is hypothesized that the absence of anchoring sites resulted in a further significant decrease in seed retention in addition to removal by snow.

Chapter Three

In Vitro Study of Seed Germination

Introduction

Observations of germination rates for a number of species of the genus Arceuthobium have been reported in the literature. The roles of temperature, relative humidity, and storage time have all been rigorously investigated under controlled laboratory conditions. Despite all the data that exist, none have been gathered or considered in conjunction with field studies of germination and infection success.

In order for a dwarf mistletoe seed to germinate, it must first be viable. Beckman and Roth (1968) have found that the viability of samples of A. campylopodum seeds maintained at 1.5C and 34-75% humidity remained significantly greater than those stored at higher temperatures. Scharpf (1970) observed that seed viability in A. abietinum (Engelm.) Gill was in seeds stored at 2C in the dark at a relative humidity of 93%. Both Scharpf and Parmeter (1962) and Wicker (1962) found dwarf mistletoe seed viability, as tested with 2,3,5-TTC to be greater than actual germination under a number of treatment combinations of temperature and relative humidity.

A study of seed germination of A. pusillum Peck found percent germination rates of 60% with seed stored at -10C and rates of 47% with seed stored at 4C (Livingston and Blanchette 1986). Incubated seeds also developed longer radicles after having been stored at -10C. Knutson (1984) suggested that germination tests would only give satisfactory results if they

were carried out under optimal conditions for germination. He implied that these optimal conditions have yet to be elucidated.

A. americanum which encounters extreme seasonal temperatures in the more northerly portions of its geographic range probably requires a cold treatment to initiate seed germination. Knutson (1974) observed that maintaining A. campylopodum seed at 2C for a few days and then incubating at 15C for 3 days gave only 10% to 20% germination. When the length of storage was increased to 80 to 100 days, percent germination increased to 70% to 80%. Beckman and Roth (1968), however, determined that a dormancy period of 5 to 6 months for A. campylopodum could be interrupted following a prechilling treatment. Scharpf (1970) found no such requirements of A. tsugense, but this species is typically found in more moderate climates along the Pacific coast.

Moisture requirements vary depending upon the species in question. While some mistletoes may germinate in dry air (Lamont and Perry 1977), most require some moisture to germinate. Certain species, such as A. pusillum will germinate at high relative humidities over 90%, but better in the presence of free water (Bonga 1972). Internal seed moisture content has been found to vary between the different species (Scharpf 1970) and may thus account for the variable moisture optima for germination.

All members of the genus Arceuthobium can germinate within a temperature range of 0C to 30C (Gill and Hawksworth

1961), with an optimum range of 15C to 30C. The optimum range is generally realized in spring and summer. Beckman and Roth (1968) found that the optimum range for germination of A. campylopodum was 17C to 19C. Germination at these temperatures was significantly higher than at 5C and 26C. They also examined the influence of alternating temperatures to account for diurnal fluctuations. Germination did not significantly vary between a 12 hour : 12 hour temperature treatment of 5C and 15C and a constant treatment of 19C. A significant decrease in germination was observed when the seeds were exposed to alternating temperatures of 10C and 30C (Beckman and Roth 1968).

The objective of this study was to allow two important environmental parameters, temperature and moisture stress, to be evaluated for their influence upon the germination of A. americanum seed under controlled conditions. The purpose of this was to isolate the interactive effect that these two factors might have upon germination in the field study which followed the process of inoculation through to infection. Germination success in the field is an important variable which limits the inoculum potential of a pathogen. Understanding how certain environmental variables such as temperature and moisture stress influence the inoculum potential of A. americanum will increase our knowledge of the epidemiology of this important pathogen.

Materials and Methods

Seeds of A. americanum were collected at three separate sites of mature dwarf mistletoe-infected jack pines in Belair Provincial Forest. Cheesecloth was laid out in approximately 20 m strips on the ground, 6 m to 10 m away from the trees bearing discharging fruit. As well, pieces of cheesecloth were wrapped loosely around brooms bearing mature fruit. Temperature and relative humidity were recorded in one of the areas prior to and during the period of discharge using a 21X datalogger (Campbell Scientific, Edmonton, Alberta) with an HMP35C temperature and relative humidity probe.

All seeds collected were combined, stored at 0C, and suspended over a saturated solution of NaCl to prevent seed desiccation and reduction in viability (Knutson 1974).

Dry P_2O_5 powder, saturated salt solutions of LiCl, K_2CO_3, and NaCl and distilled water were placed in Mason jars to provide relative humidity levels of 0%, 15%, 45%, 75% and 100% respectively. The jars were left in culture cabinets set at 5C, 10C, 15C, 20C, and 25C to allow them to equilibrate for one week prior to beginning the experiment (Winston and Bates 1960). The jars served as incubators to control the level of moisture stress.

Seeds were surface sterilized with 3% H_2O_2 for 30 minutes and rinsed in two washes of distilled water. Immediately prior to placing the seeds in the incubators, the viability of 60 seeds (3 trials with 20 seeds per trial) was tested with 2, 3, 5-triphenyltetrazolium chloride (see Materials and Methods

- Chapter Two). Two lots of 10 seeds were then placed on to sterilized Whatman's (No. 1) filter paper in petri dishes and suspended in the prepared seed incubators. Seeds were observed for seven weeks.

The number of seeds which germinated, indicated by the development of a bright red radicle at least 3 mm in length, and the number of ungerminated seeds (molded and not molded) were recorded. Data were analyzed for the effect of temperature and relative humidity upon germination using 2-factor ANOVA.

In 1992, many seeds incubated under the lowest level of moisture stress (suspended over distilled water) became covered in molds despite the initial surface sterilization. Fewer of these seeds germinated than those subjected to the next lowest stress. In order to resolve the confounding of the effect of moisture stress and moulding on germination, the tests were repeated the following year. In 1993, saturated solutions of NaCl, KBr, $ZnSO_4 7H_2O$ and distilled water provided a narrower range of relative humidity closer to saturation (75%, 85%, 95% and 100%). Owing to the small number of seeds collected this year, only two temperatures were tested: 10C and 20C. The procedures used for the collection, storage, preparation, viability testing, observation and data analysis were duplicated exactly as in the previous year.

Results and Discussion:

Seed Germination Under Controlled Conditions of Moisture Stress and Temperature:

i. 1992 laboratory experiments:

Seed germination data for the five temperatures tested are given in Figure 24 (a-e). At 5C, seed germination increased from 0.35 ± 0.07 at 1.0 kPa to 0.70 ± 0.00 at 0.5 kPa and then fell to 0.60 ± 0.00 at 0.0 kPa. At 10C, germination increased from 0.30 ± 0.00 to 0.70 ± 0.00 at 0.3 kPa and then dropped sharply to 0.40 ± 0.00 at 0.0 kPa. Seed germination at 15C rose from 0.35 ± 0.07 at 2.0 kPa to 0.75 ± 0.07 at 1.0 kPa and then decreased to 0.55 ± 0.07 at 0.0 kPa. At 20C, the highest level of germination was observed, ranging from 0.60 ± 0.00 at 2.4 kPa to 0.90 ± 0.00 at 0.6 kPa and then decreasing to 0.55 ± 0.07 at 0.0 kPa. Germination ranged from 0.30 ± 0.00 at 3.50 kPa to 0.70 ± 0.00 at 0.9 kPa and dropped to 0.00 ± 0.00 at 0.0 kPa at 25C.

Though seed germination was greatest at 20C, temperature did not account for a significant proportion of the observed variability. However, vapour pressure deficit did account for a significant proportion of the observed variability in the data ($F=6.877$, $p=0.0007$; Appendix 11). At all temperatures tested, seed germination followed a similar linear trend of increase as vapour pressure deficit decreased until some threshold level - approximately 0.5 kPa - after which there was a decrease in germination. A portion of this decrease at

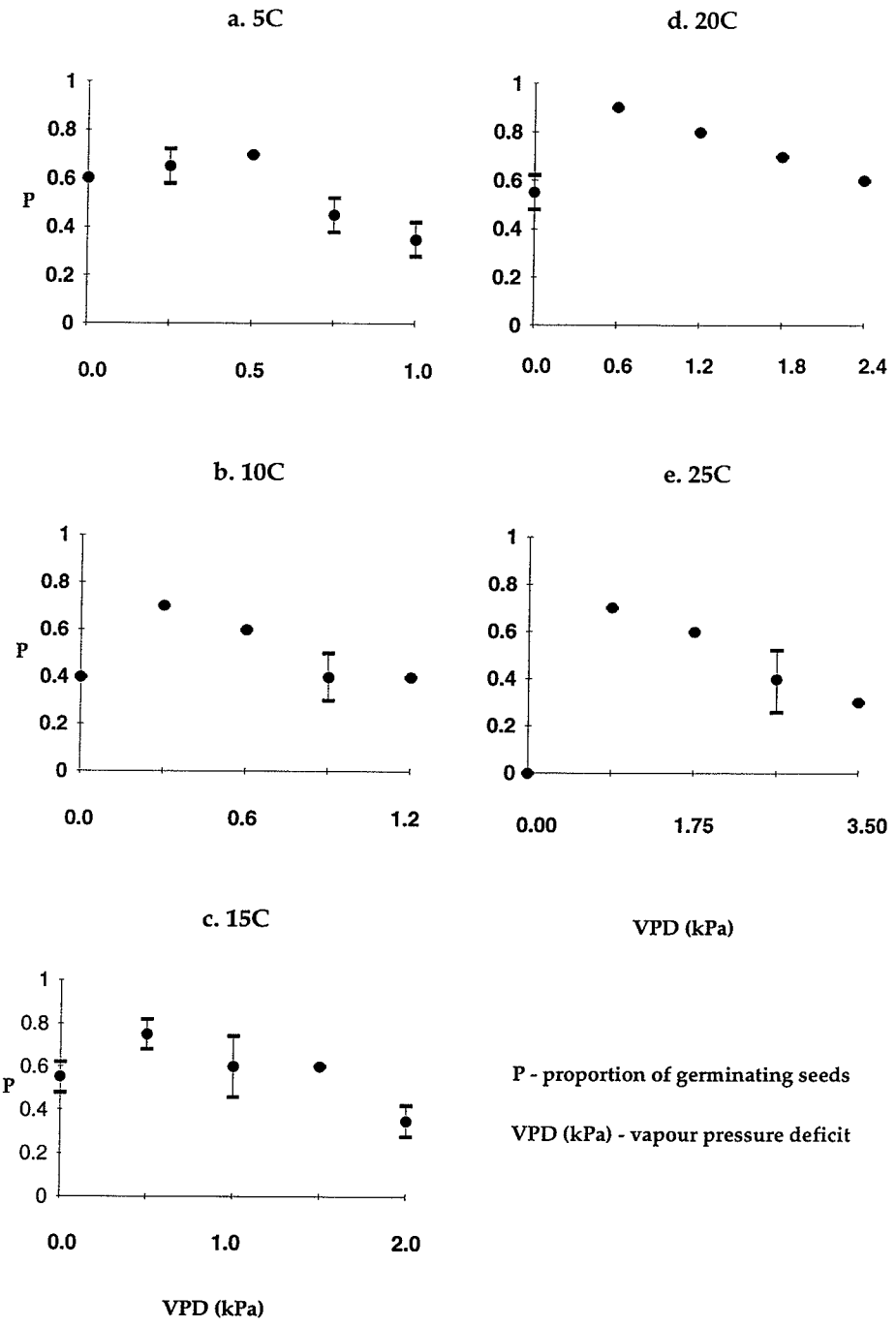


Figure 24 (a-e). Seed germination as a function of vapour pressure deficit at the five temperatures tested (5C, 10C, 15C, 20C, 25C). Data are based upon a sample size of n=10 seeds in each of two replicates. The data are expressed as the mean proportion of seeds which germinated per replicate ; the vertical bars indicate 1 standard deviation of the mean.

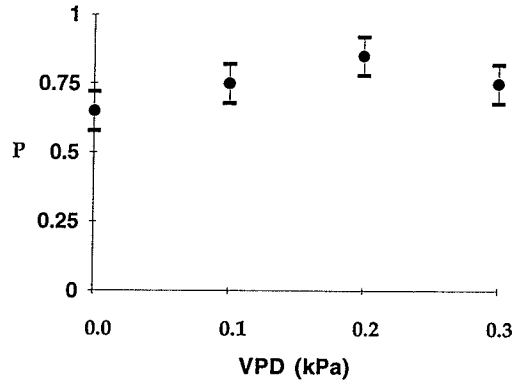
0.0 kPa was possibly attributable to fungal colonization, which covered a large portion of the seeds that did not germinate. However, at 5C, no moulding was found at 0.0 kPa and there was only a slight depression of germination. Other causes of inhibition may have been an accumulation of carbon dioxide in the incubation chambers. The chambers were closed and maintained in the dark, thus allowing for respiration and the production of carbon dioxide.

ii. 1993 laboratory experiments:

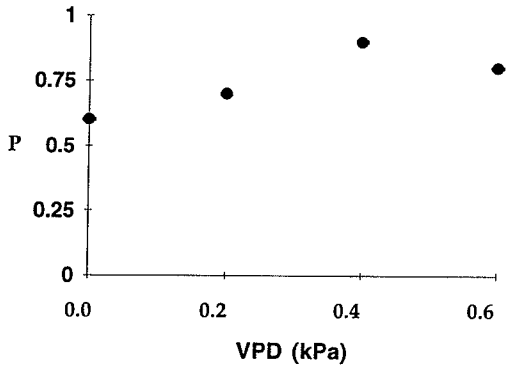
The 1993 germination data are given in Figure 25 (a-b). At 10C, germination ranged from 0.75 $\pm$ 0.07 at 0.3 kPa to 0.85 $\pm$ 0.07 at 0.2 kPa and then fell to 0.65 $\pm$ 0.07 at 0.0 kPa. Germination, though slightly higher at 20C, followed a similar trend, rising from 0.80 $\pm$ 0.00 at 0.6 kPa to 0.90 $\pm$ 0.00 at 0.4 kPa and eventually falling to 0.60 $\pm$ 0.00 at 0.0 kPa. No moulding was observed at either temperature. Vapour pressure deficit was the only factor accounting for a significant portion of the observed variation ($F=19.667$, $p=0.0005$; Appendix 12).

Although optimum conditions of moisture stress vary somewhat with temperature, the overall optimum conditions for germination for A. americanum lie between 15C and 20C and 0.2 kPa and 0.6 kPa. Germination falls off at higher temperatures, presumably owing to desiccation, and at low levels of moisture stress, perhaps because of the inhibitory influence of water vapour in the air on oxygen uptake by the

a. 10C



b. 20C



P - mean proportion of seeds which germinated at each treatment level.

Figure 25 (a-b). Seed germination as a function of vapour pressure deficit at 10C and 20C in the 1993 laboratory experiments. Data are based upon a sample size of n=10 seeds replicated twice per treatment combination ; the vertical bars indicate 1 standard deviation of the mean.

seeds. Another explanation may be that the moisture content of the air around the seed was much higher than that within the seed, resulting in high rates of imbibition. At high rates of imbibition, leakage of cellular solutes occurs owing to phase transitions in the phospholipid bilayer (Crowe et al. 1989). This leakage of solutes upsets cellular composition, pH and may interfere with the organization of the membrane enough to result in cell death.

In order to explain the role of fungal colonization, which, though an important determinant of germination in the 1992 experiments, was absent in the 1993 experiments, an examination of the microclimatic data was made. Immediately prior to seed discharge between 25 August 1992 and 31 August 1992, temperature ranged from approximately 10.0C to 13.0C and averaged 12.4C, while vapour pressure deficit ranged from 0.10 kPa to 0.40 kPa and averaged 0.23 kPa. In the following year, temperature ranged from 17.0C to 21.0C and averaged 17.8C (Figure 26 a-b). Meanwhile, vapour pressure deficit ranged from 0.20 kPa to 0.65 kPa and averaged 0.47 kPa. Using t-tests to compare the environmental data, conditions were found to be significantly cooler ($t=-11.285$, $p<0.0001$) and significantly moister ($t=-5.424$, $p<0.0001$) in August 1992. These conditions are likely to have been more conducive to a build-up of fungal spores which then infected some of the dwarf mistletoe seeds at such a depth that they were not eliminated by surface sterilization.

Beckman and Roth (1968) determined that an optimum

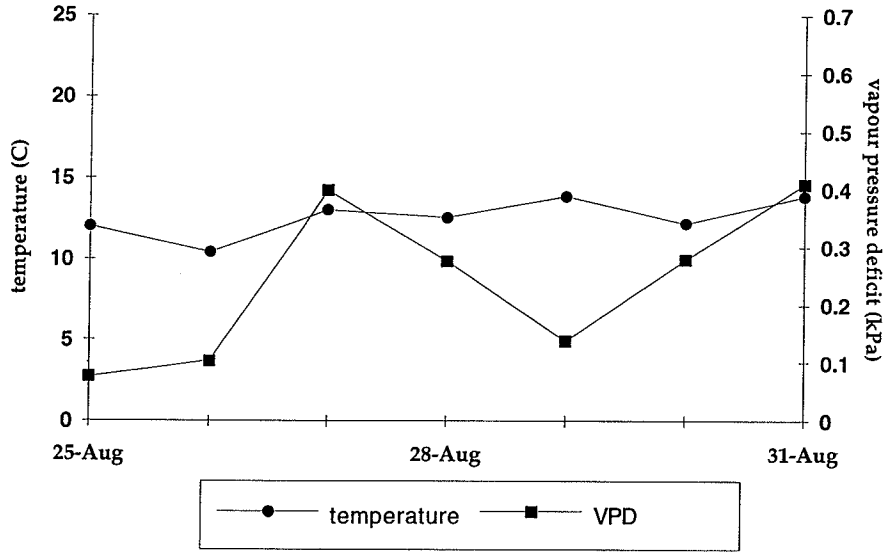


Figure 26a. Temperature and vapour pressure deficit profiles immediately prior to seed discharge in 1992. The data are expressed as daily means.

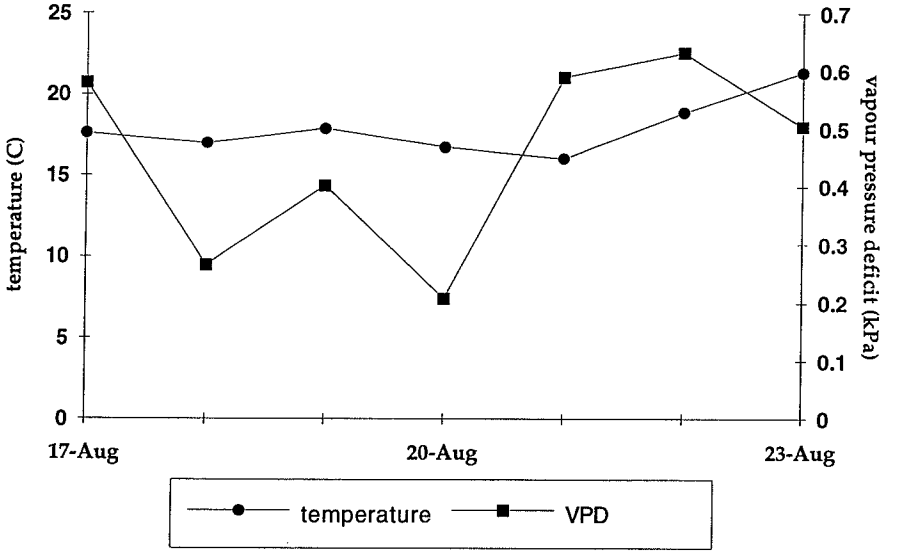


Figure 26b. Temperature and vapour pressure deficit profiles immediately prior to seed discharge in 1993. The data are expressed as daily means.

temperature of 15C to 20C exists for most dwarf mistletoes, though this does not account for the daily temperature fluctuations that the seeds are exposed to under natural conditions. Despite this fact, Beckman and Roth (1968) did not find that day-night temperature fluctuations had a significant impact on germination. As a result, the *in vitro* experiments provide a useful means of evaluating the impact of temperature and moisture stress on seed germination under natural conditions.

Chapter Four

General Discussion and Summary:

The view that plant pathogens "rampage" through a host population and then suddenly disappear is misguided, but perpetuated because of the difficulty of recognizing their presence until an epidemic occurs. Patterns of stable coexistence typically occur when host population densities are high, though epidemics occur when environmental conditions are favourable for the pathogen. This cyclic phenomenon is apparent in the long term trends of the better studied diseases of animals and man (Anderson 1979).

However, disease cycles do vary among host-parasite combinations, depending on such attributes of the host and pathogen as reproductive rate and generation time, host response to infection, and virulence of the pathogen (Augspurger 1988). Brief outbreaks, as well as long-term associations of stable coexistence play important roles in the population dynamics of the host-parasite association. The potential for an organism to regulate or suppress host population growth is measured by its impact upon host survival, reproduction, competitive fitness, and susceptibility to predation (Anderson 1979).

Some insights into the reproductive potential of A. americanum may be gathered from this study. Fruit survival tends to be limited to approximately 10% by the time seed discharge occurs, owing primarily to individual fruit loss or

the death of aerial shoots. Both of these factors exhibited a aignificant linear correlationa with mean daily vapour pressure deficit. Transpiration rates of the dwarf mistletoe plants are known to exceed those of their hosts by many times under conditions of water stress (Tocher et al. 1984).

Despite the high mortality rates of the fruit during the months of June, July and August, isophasic growth of the endophytic system permits A. americanum to produce systemic infections. This increases the surface area over which dwarf mistletoe shoots may erupt from the surface of infected branches, thus amplifying the potential for inoculum production.

Epidemics of the pathogen will be difficult to predict unless conditions which lead to high rates of infection, parasite survival, and parasite reproduction are known (Augspurger 1988). This study elucidated some of the factors which may account for the variability in infection success and parasite mortality. Moisture stress, as measured by vapour pressure deficit, showed a strong linear correlation with aerial shoot loss ($r=0.87$ in 1992-1993; $r=0.92$ in 1993-1994), the primary determinant of fruit mortality in either year. Temperature also proved to be an important indicator of aerial shoot loss ($r=0.95$ in 1992-1993; and $r=0.83$ in 1993-1994). From these observations, one would expect the pathogen to thrive in years with slightly cooler summers with low evaporative demands. If so, there is likely to be a tension between the conditions that would support an epidemic of A.

americanum and those that would support an increase in the population of Wallrothiella arceuthobii. Research into this tension will provide more insight into the suitability of this hyperparasite as a means for biological control of A. americanum.

Persistent infections are caused by pathogens such as A. americanum, which does not invoke a permanent immunity response so that the parasite population is harbored continuously (Anderson 1979). In persistent infections, any process which results in aggregated distributions of parasite numbers on a given host incites a stable host-pathogen association. This occurs because fewer hosts harbor greater proportions of the parasite population thus restricting host mortality to those which are heavily infected. The net effect is that the entire host population is only slightly depressed when compared to a similar host population in the absence of infection.

The variability that was observed between the different blocks and years in fruit survivorship indicated that A. americanum is aggregated throughout its host's range in the area studied, suggesting stable coexistence. However, any changes in environmental parameters that tend to alter the reproductive success of the pathogen, such as temperature or vapour pressure deficit, may disrupt this stable association (Anderson 1979). As was noted earlier, only 39 and 26 of the 44 randomly selected brooms in the fruit mortality study area produced aerial shoots. This represents a considerable

reduction in reproductive capacity over the entire population of the pathogen and may indicate that the association is destabilizing. In order to determine with accuracy the state of the association, it would be necessary to ascertain the number of new infections occurring in the study site.

The severity of the direct effect of a parasite on its host depends upon the physiological burden placed on that host. In the case of Arceuthobium americanum this may be measured by the number of aerial shoots present and producing inoculum, as this will give an estimate of the transpiration demand placed upon a given host. Fisher (1975) has found that dwarf mistletoes typically transpire at four times the rate of their hosts. Under conditions of water stress, Tocher et al. (1984) have found that A. americanum may transpire forty times the rate of Pinus contorta, thus placing a major stress on its host with respect to water loss. This may explain why aerial shoot loss increased significantly in the summer months and showed strong correlations with vapour pressure deficit.

Infection success showed a strong dependency upon the environmental conditions that exist throughout the entire, protracted infection process, which includes inoculation, overwinter retention, germination and penetration. Hawksworth (1965) determined that approximately 40% of all those seeds produced in a stand of lodgepole pine infected with A. americanum may be intercepted by nearby neighbors. This, together with the fact that seeds are explosively discharged as far as 18 meters from the source provides a useful

indication of the potential for spread in a given year (Gilbert 1988).

Wicker (1967) examining seed density as a klendusic factor of infection, found that overwinter retention of seeds deposited below the snowline was significantly less than those above. This was suggested as one of the factors enabling younger trees to avoid infection. Overwinter retention in the present study was consistently above 80% and 65%, respectively in 1992-1993 and 1993-1994, and did not show a significant reduction in the younger age classes where seeds might be subject to removal by snow. Furthermore, this study revealed that overwinter retention below the snow line was significantly reduced, especially when needles were absent from the inoculated tissue.

Inoculum potential of A. americanum is also reduced through colonization of seeds by fungi. Molds, such as Alternaria spp., Aureobasidium spp., and Epicoccum spp. which have been isolated from non-germinating A. americanum seeds between the months of April and June, accounted for 6%-12% of the overall seed loss (Wicker 1967). Similarly low percentages of loss (1%-8%) owing to molding were observed in this study. However, many of the fungi isolated in this study, including unidentified species of Alternaria, Cladosporium, Penicillium, and Trichoderma, are weak parasites or saprophytes. It is possible that the seeds which did not germinate and supported fungal growth may simply represent the non-viable portion of seeds that exist in any plant

population. As a result of the low percentages of seeds colonized by fungi and their weakly parasitic or saprophytic nature, molding was considered to have only a slight impact on the inoculum potential of A. americanum in this study.

Insect attack also accounted for a small portion of the seed loss, less than 10% overall and similar to that observed by Wicker (1967). However, when seed damage was recorded, it was similar in all cases. The seeds appeared to have been rasped open, and the embryo and endosperm sucked out through the opening. The damage was consistent with that typically produced by members of the Thripidae, a group of small - 0.05 - 0.15 cm in length - fairly ubiquitous insects, many of which are obligate seed herbivores (Kozlowski 1972). These insects often have mouth parts that are adapted for rasping, piercing and sucking. One thrips, Frankliniella hawksworthii, has been found associated with dwarf mistletoes, though it typically feeds on the flowers and aerial shoots. These observations indicate that there is a need for further study to elucidate the agents responsible, and their potential for biological control of A. americanum.

Post winter loss, caused by wind and rain, was responsible for between 28.8% and 22.2% of the overall loss observed. When overwinter loss was high, as in the second year of the study, post winter loss was somewhat reduced. However, there was an interaction between the age of tree inoculated and the cause of loss, possibly an indication that micro-environmental differences between the selected sites

were responsible. In both years, for example, post winter losses were lower in the 22 year-old age category than in the other age groups inoculated.

Age and composition of a plant community are likely to influence microclimate (Vaartaja 1954), especially in the case of an older and more closed canopy, by reducing wind velocity and incoming solar radiation. As wind was suggested to be responsible for much of the post winter losses observed by Wicker (1967), it is reasonable to consider that a more densely packed canopy could reduce the amount of inoculum lost. As well, shading might lessen the evaporative demand placed on the dwarf mistletoe seeds, thus making them less subject to desiccation during the summer months. Seed germination in the field studies provided some evidence for these hypotheses. In either year, seed germination was greatest in the 22 year old age group - reaching a maximum of 50% in 1992-1993 and 16.7% in 1993-1994. Shading, texture and the microtopography of the substrate have all been found to have a significant impact upon the microclimate of the site of germination (Harper et al. 1965), and may have accounted for the differences observed between the different age classes inoculated. As a result, it is concluded that microclimatic factors are useful indicators of the components of inoculum potential, but variables such as stand age and density will modify their impact.

The possibility that overwinter temperatures significantly influence the viability of dwarf mistletoe seeds

(Smith and Wass 1979) may explain the differences in correlation between germination and microclimate observed between years. In the first year, germination in the field showed a strong linear correlation with summer temperature (r=0.80) and vapour pressure deficit (r=0.80). However, in the 1993-1994 study, germination exhibited a moderate correlation with temperature (r=0.44) and none with vapour pressure deficit (r=0.16).

The *in vitro* study of germination eliminated the possibility of confounding seed viability at the time of inoculation with the effect of cold winter temperatures on seed survival. At the same levels of temperature and moisture stress germination showed no differences between years. For example, the proportion of seeds which germinated at 20oC and 0.6 kPa was 0.9 and 0.8 respectively in the 1992-1993 and 1993-1994 laboratory test. The proportion of seeds was 0.75 in either year under a treatment regime of 10C and 0.3 kPa. This was further substantiated by the 2,3,5-TTC test which indicated no significant difference in seed viability between the two years. These data suggest that seed viability prior to exposure to overwinter temperatures did not vary significantly between either year.

The inoculation study was established to determine the age dependency of the infectivity of A. americanum on Pinus banksiana. The dependency of the infectivity of a pathogen upon the age or size of its host may cause the host population's age structure to shift. The hypothesis was that

susceptibility to infection in P. banksiana would increase with increasing tree age and decrease with increasing tissue age. The latter hypothesis was made on the basis of the greater thickness of the bark in older tissues. Tainter (1970) has found that the primary haustorium of A. pusillum was occasionally unable to penetrate the tanniniferous cells. These cells are found in the phellogen layer of older tissue, and in black spruce may offer a form of passive mechanical resistance to infection by A. pusillum. It has been reported that stands of lodgepole pine under fifteen years of age often grow adjacent to mature infected stands without becoming infected themselves (Dowding 1929). Conversely, Roth (1974) has reported the possibility of juvenile susceptibility in ponderosa pine. Muir (1972) discovered that the number of infections of A. americanum in lodgepole pine increased exponentially in trees aged 16 to 23 years. Prior to this age range, the rate of increase was significantly lower and thus suggested that control treatments be applied to a stand prior to its reaching 16 years of age. These findings were attributed to two factors: juvenile resistance and available surface for inoculation.

In the 1992-1993 trials, though no significant trends with increasing tissue age occurred in the 3, 5, and 7 year old analysis, a significant trend was found for the 12, 17, and 22 year old trees. Infection success increased significantly with increasing tissue age in the latter age groups. Seed retention was higher on the older tissue ages,

possibly because they were relatively more protected from wind and rain that may dislodge the seeds. The longevity and growth of radicles are also influenced by temperature. Scharpf (1969) found that maximum penetration occurred at 13C and decreased at higher temperatures because the increasing temperatures reduced the survival of the radicle and appressorium. The denser crowns of older trees could reduce incoming solar radiation and temperatures enough to significantly reduce seed desiccation. However, no significant trends with respect to infection success in different tissue and tree ages were observed in the 1993-1994 field season. This may have been as a result of the very low winter temperatures to which the seeds were exposed in the latter field season.

The observation that juvenile resistance was not apparent in this study may have some significance in relation to the fire ecology of Pinus banksiana. Typically following a fire, even-aged stands of jack pines develop on sandy glacial deposits (Yarranton and Yarranton 1970). Dowding (1970), Alexander and Hawksworth (1975) and Smith and Baranyay (1970) have suggested that fires might determine the distribution of A. americanum in jack pines in Alberta. Where undergrowth is dense, wildfires killed most of those trees infected with dwarf mistletoe, virtually eliminating it. The pathogen persisted primarily on sparsely vegetated sandy ridges (Dowding 1929).

Since these observations were made, fire prevention has

become more effective, allowing stands to survive longer. Infected areas which were once sanitized by burns now become much more intensely infected, in turn increasing the flammability of stands and the intensity of wildfires when they do occur (Smith and Baranyay 1970). Juvenile resistance may never have had a chance to evolve owing to the cycling of wildfires which, in the past, prevented the build-up of dwarf mistletoe populations.

There was no evidence for the presence of juvenile resistance to A. americanum in the stands of P. banksiana inoculated in this study. However, a number of other factors were found to control the infection process. Overwinter losses accounted for a large reduction in seed retention in both year of the study, thus representing a substantial influence on the inoculum potential of A. americanum. As well, postwinter losses owing to wind and rain accounted for a sizable proportion of the overall seed loss, and were also considered to have a significant impact on the inoculum potential of this pathogen. Other factors such as fungal colonization and insect attack had a minor impact on seed retention. However, more study into the environmental conditions which predispose the seeds to these types of damage may provide a useful estimate of their potential for biological control. Because of the long delay between establishment and readily detectable symptom expression there is a need to examine plantations carefully from early stages to provide detection and facilitate sanitation, the only

practical and economical control measure.

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Appendix 1. Mean percent fruit survival through the 1992-1993 field season averaged over each of the five blocks. Standard deviations are provided for each sampling interval.

sampling interval	Block 1		Block 2		Block 3		Block 4		Block 5	
	mean	*stdev*	*mean*	*stdev*	*mean*	*stdev*	*mean*	*stdev*	*mean*	*stdev*
Oct-92	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
Dec-92	94.3	5.6	91.9	6.6	96.5	5.8	95.8	7.8	91.4	14.9
Feb-93	94.3	5.6	91.9	6.6	96.5	5.8	95.8	7.8	91.4	14.9
May-93	74.3	13.6	86.4	11.5	83.7	16.8	88.8	18.2	65.4	19.7
Jun-93	62.6	17.7	67.7	20.0	75.8	27.5	79.4	22.4	62.7	21.7
Jul-93	49.4	21.9	57.2	18.9	63.0	25.8	67.7	23.4	50.2	18.0
Aug-93	9.3	22.7	5.6	13.6	11.5	25.1	0.0	0.0	37.7	33.4

Appendix 2. Mean percent fruit survival through the 1993-1994 field season averaged over each of the five blocks. Standard deviations are provided for each sampling interval.

sampling interval	Block 1		Block 2		Block 3		Block 4		Block 5	
	mean	*stdev*	*mean*	*stdev*	*mean*	*stdev*	*mean*	*stdev*	*mean*	*stdev*
Oct-93	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0	0.0
Nov-93	100.0	0.0	86.2	16.9	100.0	0.0	76.4	36.6	79.6	40.2
Mar-94	100.0	0.0	86.2	16.9	100.0	0.0	76.4	36.6	79.6	40.2
May-94	93.6	5.1	79.5	18.8	97.9	3.7	55.9	45.1	77.1	38.7
Jul-94	27.6	18.6	18.5	14.7	45.3	50.3	18.4	22.0	52.8	43.8
Aug-94	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Appendix 3. Life table for retention and removal of seed of Arceuthobium americanum in the 1992/1993 field season.

Age, x (days)	No. seeds at start of age interval, x	Proportion of seeds present at start of interval, x	No. seeds removed in interval, x to x+1	Proportion of seeds removed in interval, x to x+1	Finite rate of seed removal over 30 days from x
3 year-olds					
0	119	1.000	15	0.126	0.017
231	104	0.874	6	0.058	0.150
240	98	0.824	3	0.031	0.042
262	95	0.798	30	0.316	0.178
320	65	0.546			
5 year-olds					
0	117	1.000	26	0.222	0.032
231	91	0.778	6	0.066	0.170
240	85	0.726	5	0.059	0.079
262	80	0.684	36	0.450	0.266
320	44	0.376			
7 year-olds					
0	113	1.000	8	0.071	0.009
231	105	0.929	0	0.000	0.000
240	105	0.929	4	0.038	0.052
262	101	0.894	60	0.594	0.373
320	41	0.363			
12 year-olds					
0	120	1.000	14	0.117	0.016
231	106	0.883	0	0.000	0.000
240	106	0.883	4	0.038	0.051
262	102	0.850	39	0.382	0.221
320	63	0.525			
17 year-olds					
0	120	1.000	11	0.092	0.012
231	109	0.908	0	0.000	0.000
240	109	0.908	6	0.055	0.074
262	103	0.858	46	0.447	0.264
320	57	0.475			
22 year-olds					
0	120	1.000	17	0.142	0.020
231	103	0.858	1	0.010	0.026
240	102	0.850	1	0.010	0.013
262	101	0.842	22	0.218	0.119
320	79	0.658			

Appendix 4. Life table for retention and removal of seeds of Arceuthobium americanum in the 1993/1994 field season.

Age, x (days)	No. seeds at start of age interval, x	Proportion of seeds present at start of interval, x	No. seeds removed in interval, x to x+1	Proportion of seeds removed in interval, x to x+1	Finite rate of seed removal over 30 days from x
3 year-olds					
0	90	1.000	25	0.278	0.040
242	65	0.722	0	0.000	0.000
273	65	0.722	43	0.662	0.662
303	22	0.244			
5 year-olds					
0	90	1.000	26	0.289	0.041
242	64	0.711	0	0.000	0.000
273	64	0.711	33	0.516	0.516
303	31	0.344			
7 year-olds					
0	90	1.000	12	0.133	0.018
242	78	0.867	0	0.000	0.000
273	78	0.867	61	0.782	0.782
303	17	0.189			
12 year-olds					
0	90	1.000	46	0.511	0.085
242	44	0.489	0	0.000	0.000
273	44	0.489	27	0.614	0.614
303	17	0.189			
17 year-olds					
0	90	1.000	40	0.444	0.070
242	50	0.556	0	0.000	0.000
273	50	0.556	21	0.420	0.420
303	29	0.322			
22 year-olds					
0	90	1.000	22	0.244	0.034
242	68	0.756	0	0.000	0.000
273	68	0.756	21	0.309	0.309
303	47	0.522			

Appendix 5. Life table for germination of seeds of Arceuthobium americanum in the 1992/1993 field season.

Age, x (days)	No. seeds present at start of interval x	Total no. of germinating seeds at time x	Proportion of seeds germinatec in interval, x to x+1	Finite rate of seed germn over 30 days from x
3 year-olds				
0	119	0	0.000	0.000
231	104	0	0.000	0.000
240	98	36	0.367	0.464
262	95	57	0.600	0.377
320	65	36		
5 year-olds				
0	117	0	0.000	0.000
231	91	0	0.000	0.000
240	85	28	0.329	0.420
262	80	45	0.563	0.348
320	44	30		
7 year-olds				
0	113	0	0.000	0.000
231	105	0	0.000	0.000
240	105	22	0.210	0.274
262	101	26	0.257	0.143
320	41	23		
12 year-olds				
0	120	0	0.000	0.000
231	106	0	0.000	0.000
240	106	51	0.481	0.591
262	102	61	0.598	0.376
320	63	39		
17 year-olds				
0	120	0	0.000	0.000
231	109	0	0.000	0.000
240	109	31	0.284	0.366
262	103	38	0.369	0.212
320	57	21		
22 year-olds				
0	120	0	0.000	0.000
231	103	0	0.000	0.000
240	102	56	0.549	0.662
262	101	61	0.604	0.381
320	79	107 48		

Appendix 6. Life table for germination of seeds of Arceuthobium americanum in the 1993/1994 field season.

Age, x (days)	No. seeds present at start of interval x	Total no. of germinating seeds at time x	Proportion of seeds germinated in interval, x to x+1	Finite rate of seed germn over 30 days from x
3 year-olds				
0	90	0	0.000	0.000
242	65	0	0.000	0.000
273	65	0	0.000	0.000
303	22	3	0.136	0.073
5 year-olds				
0	90	0	0.000	0.000
242	64	0	0.000	0.000
273	64	0	0.000	0.000
303	31	7	0.226	0.124
7 year-olds				
0	90	0	0.000	0.000
242	78	0	0.000	0.000
273	78	0	0.000	0.000
303	17	2	0.118	0.063
12 year-olds				
0	90	0	0.000	0.000
242	44	0	0.000	0.000
273	44	0	0.000	0.000
303	17	1	0.059	0.031
17 year-olds				
0	90	0	0.000	0.000
242	50	0	0.000	0.000
273	50	0	0.000	0.000
303	29	9	0.310	0.175
22 year-olds				
0	90	0	0.000	0.000
242	68	0	0.000	0.000
273	68	0	0.000	0.000
303	47	13	0.277	0.154

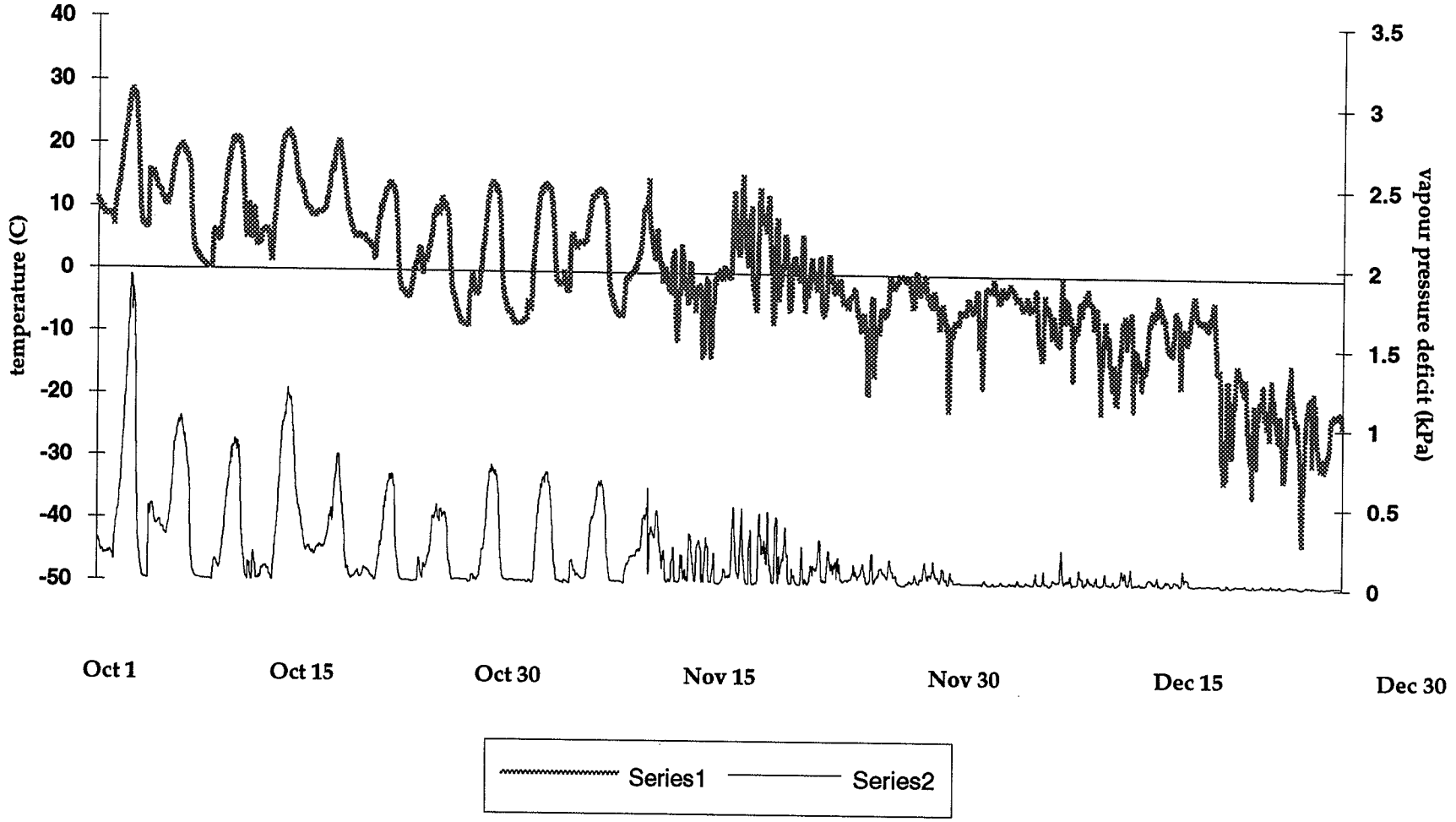
Appendix 7. Analysis of variance for the effect of tissue age and height of inoculation on overwinter seed retention. The experiment was conducted only in the 1992-1993 inoculation trial.

dependent variable - overwinter seed retention
independent variables - tissue age and height of inoculation

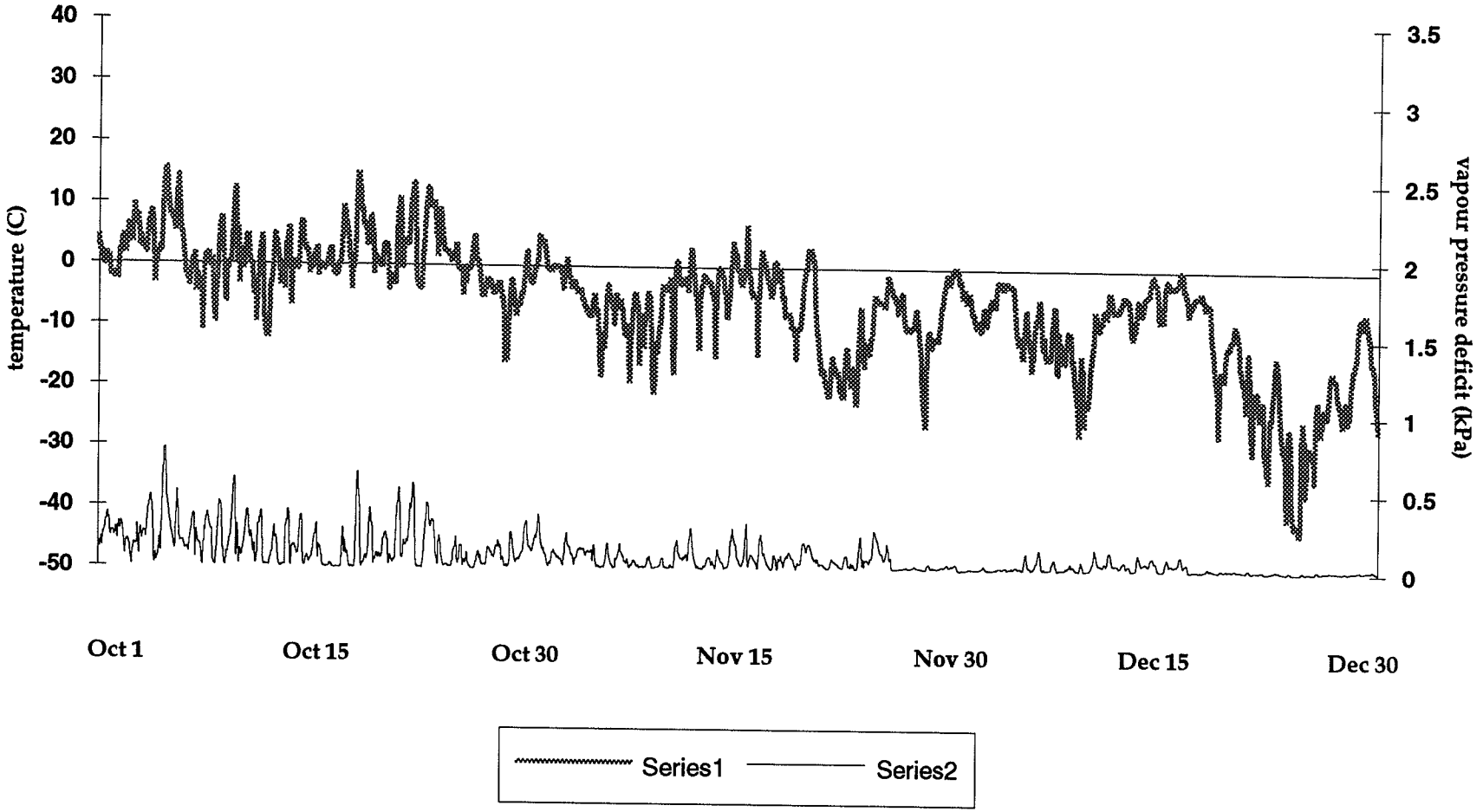
* - statistically significant at alpha = 0.05

Source of Variation	*Sum of Squares*	*degrees of freedom*	*Mean Sum of Squares*	*F-calculated*	*P-value**	*F-crit*
tissue age	2.509	2	1.255	8.11	0.001	3.07
height	8.172	1	8.172	52.831	< 0.0001	3.92
Interaction	1.974	2	0.987	6.38	0.003	3.07
Within	13.921	90	0.155			
Total	26.576	95				

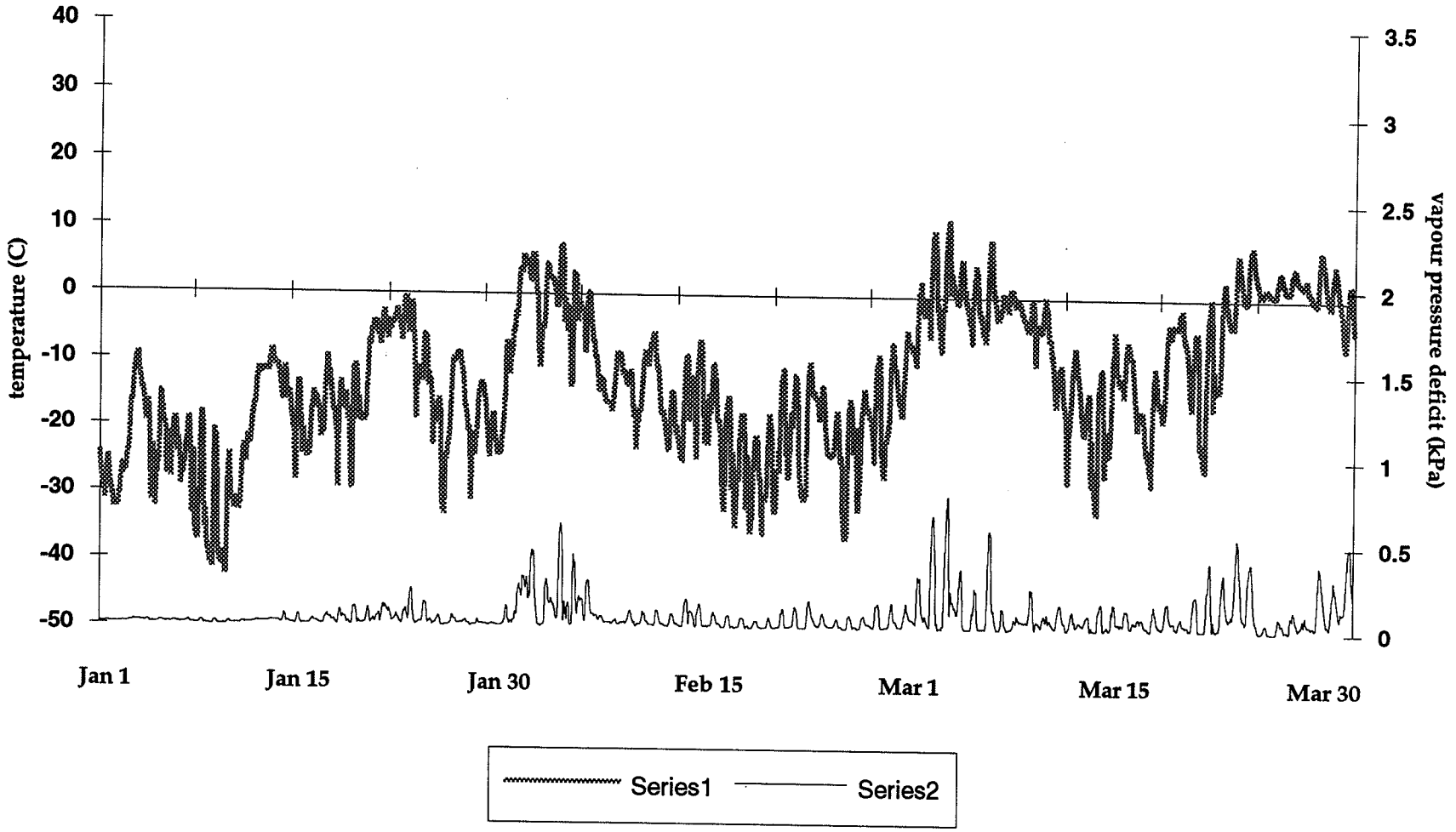
Appendix 8a. Mean hourly temperature (series 1) and vapour pressure deficit (series 2) in the inoculation study area throughout the months of October, November and December - 1992.



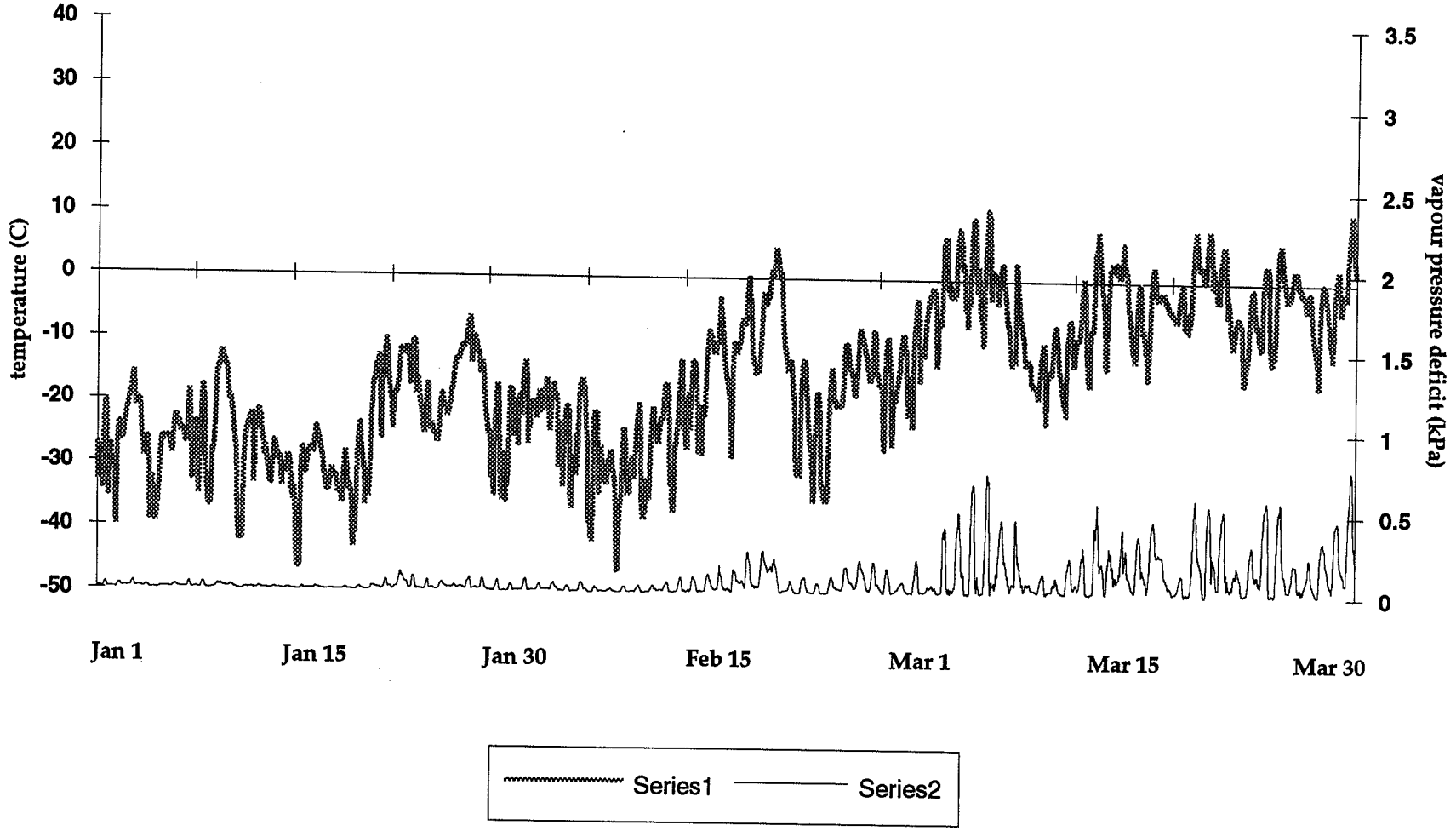
Appendix 8b. Mean hourly temperature (series 1) and vapour pressure deficit (series 2) in the inoculation study area throughout the months of October, November and December - 1993.



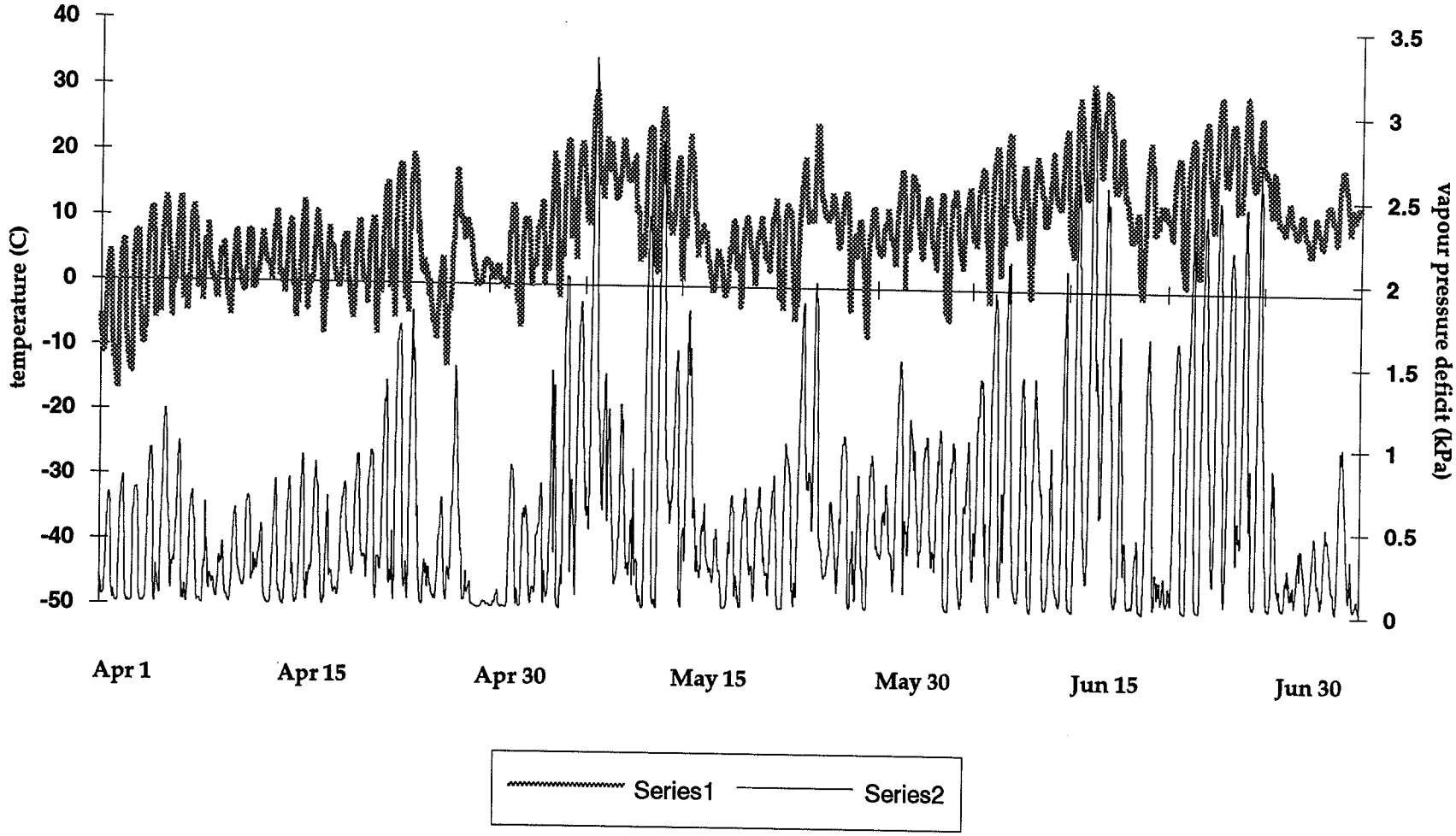
Appendix 8c. Mean hourly temperature (series 1) and vapour pressure deficit (series 2) in the inoculation study area throughout the months of January, February, and March - 1993.



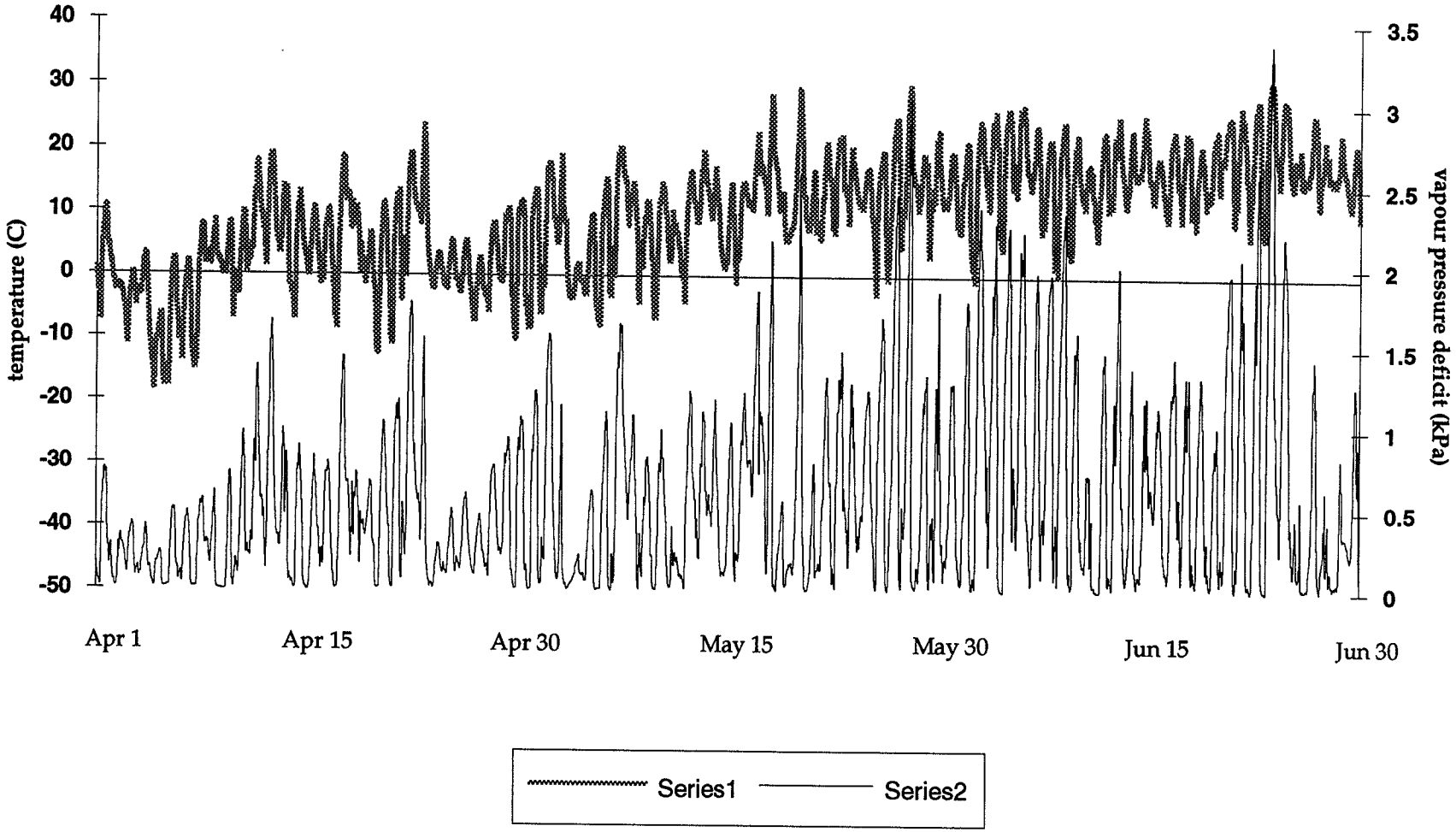
Appendix 8d. Mean hourly temperature (series 1) and vapour pressure deficit (series 2) in the inoculation study area throughout the months of January, February and March - 1994.



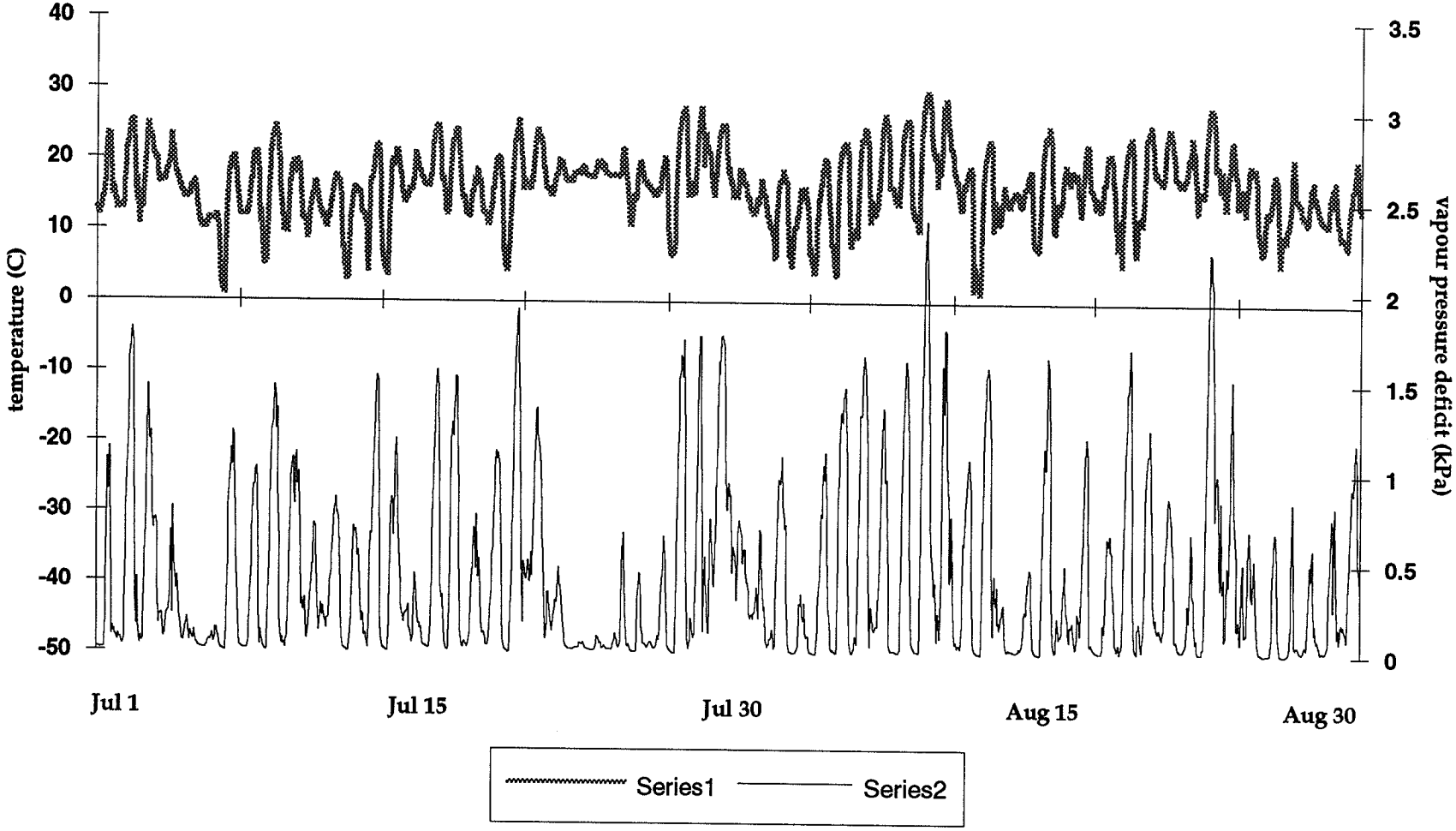
Appendix 8e. Mean hourly temperature (series 1) and vapour pressure deficit (series 2) in the inoculation study area throughout the months of April, May and June - 1993.



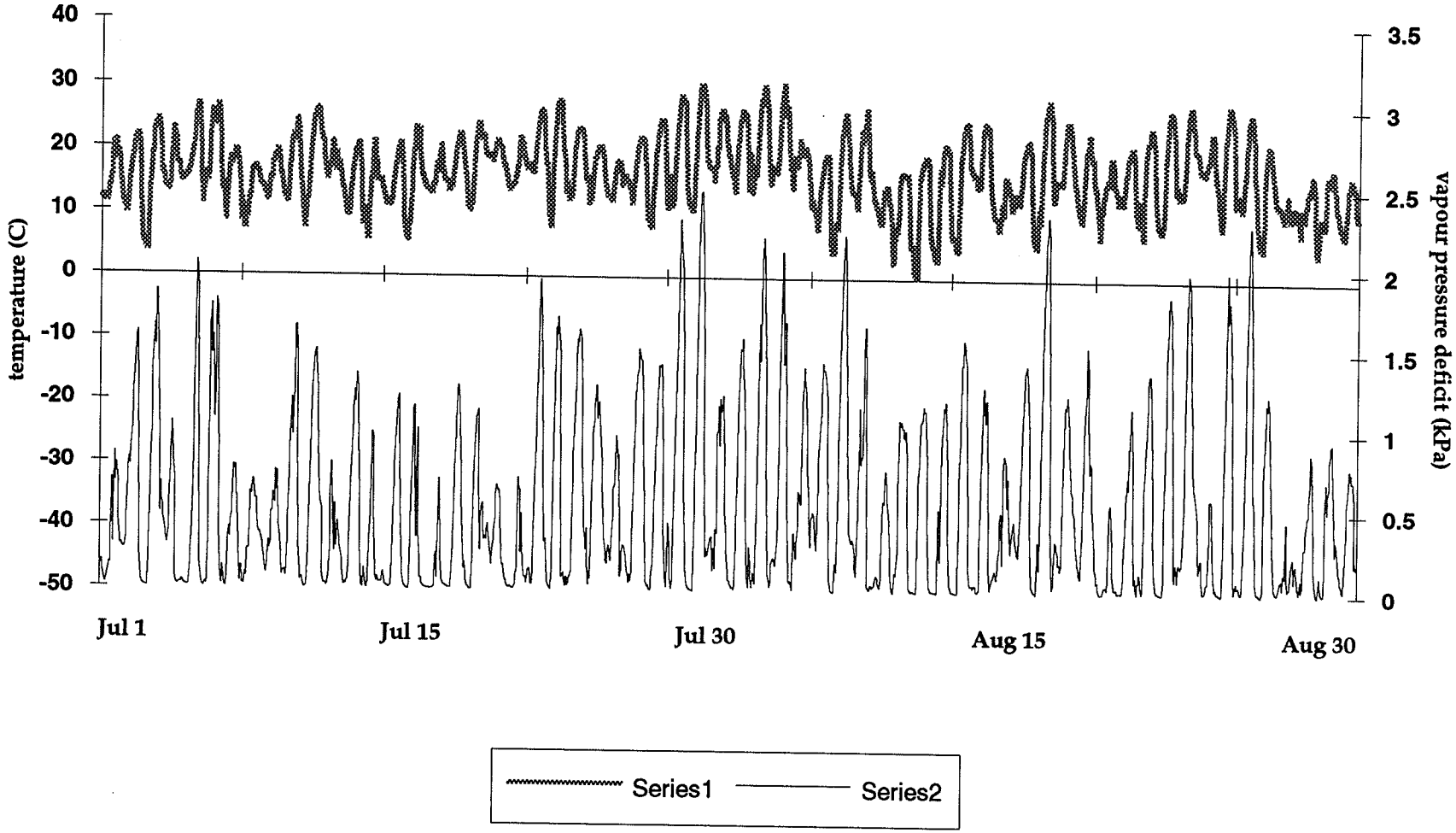
Appendix 8f. Mean hourly temperature (series 1) and vapour pressure deficit (series 2) in the inoculation study area throughout the months of April, May and June - 1994.



Appendix 8g. Mean hourly temprature (series 1) and vapour pressure deficit in the inoculation study area throughout the months of July and August - 1993.



Appendix 8h. Mean hourly temperautre (series 1) and vapour pressure deficit (series 2) in the inoculation study area throughout the months of July and August - 1994.



Appendix 9a. Analysis of variance for the effect of tree age (3, 5, 7) and tissue age (1, 2, 3) on the proportion of successfully established infections in the 1992-1993 field season.

dependent variable - proportion of infections established
independent variables - tree age and tissue age

* - statistically significant at alpha = 0.05

Source of Variation	*Sum of Squares*	*degrees of freedom*	*Mean Sum of Squares*	*F*	*P-value**	*F crit*
tissue age	0.041	2	0.020	2.079	0.145	3.354
tree age	0.044	2	0.022	2.234	0.126	3.354
Interaction	0.058	4	0.015	1.481	0.236	2.728
Within	0.265	27	0.010			
Total	0.409	35				

Appendix 9b. Analysis of variance for the effect of tree age (12, 17, 22) and tissue age (1, 4, 8) on the proportion of successfully established infections in the 1992-1993 field season.

dependent variable - proportion of infections established
independent variables - tree age and tissue age

* - statistically significant at alpha = 0.05

Source of Variation	*Sum of squares*	*degrees of freedon*	*Mean Sum of Squares*	*F*	*P-value**	*F crit*
tissue age	0.136	2	0.068	3.571	0.042	3.354
tree age	0.144	2	0.072	3.790	0.035	3.354
Interaction	0.018	4	0.004	0.234	0.917	2.728
Within	0.513	27	0.019			
Total	0.810	35				

Appendix 10a. Analysis of variance for the effect of tree age (3, 5, 7) and tissue age (1, 2, 3) upon the successful establishment of an infection in the 1993-1994 inoculation trials.

dependent variable - successful establishment of an infection
independent variables - tree and tissue age

* - statistically significant at alpha = 0.05

Source of Variation	*Sum of Squares*	*degrees of freedom*	*Mean Sum of Squares*	*F*	*P-value**	*F crit*
tissue age	0.001	2	0.000	0.250	0.781	3.555
tree age	0.001	2	0.000	0.250	0.781	3.555
Interaction	0.006	4	0.001	1.000	0.433	2.928
Within	0.027	18	0.001			
Total	0.034	26				

Appendix 10b. Analysis of variance for the effect of tree age (12, 17, 22) and tissue age (1, 4, 8) upon the successful establishment of an infection in the 1993-1994 inoculation trials.

dependent variable - successful establishment of an infection
independent variables - tree and tissue age

* - statistically significant at alpha = 0.05

Source of Variation	*Sum of Squares*	*degrees of freedom*	*Mean Sum of Squares*	*F*	*P-value**	*F crit*
tissue age	0.010	2	0.005	0.591	0.564	3.555
tree age	0.010	2	0.005	0.591	0.564	3.555
Interaction	0.037	4	0.009	1.136	0.371	2.928
Within	0.147	18	0.008			
Total	0.203	26				

Appendix 11. Analysis of variance for the effect of vapour pressure deficit and temperature upon the proportion of seeds which germinated in the 1992 laboratory trials.

dependent variable - seed germination
independent variables - temperature and vapour pressure deficit

* - statistically significant at alpha = 0.05

Source of Variation	*Sum of Squares*	*degrees of freedom*	*Mean Sum of Squares*	*F*	*P-value**	*F crit*
temperature	0.119	4	0.030	1.049	0.402	2.759
VPD	0.781	4	0.195	6.877	0.001	2.759
Interaction	0.133	16	0.008	0.292	0.993	2.069
Within	0.710	25	0.028			
Total	1.743	49				

Appendix 12. Analysis of variance for the effect of temperature and vapour pressure deficit upon seed germination in the 1993 laboratory trials.

dependent variable - seed germination
independent variables - temperature and vapour pressure deficit

* - statistically significant at alpha = 0.05

Source of Variation	*Sum of Squares*	*degrees of freedom*	*Mean Sum of Squares*	*F*	*P-value**	*F crit*
VPD	14.750	3	4.917	19.667	0.000	4.066
temperature	0.250	1	0.250	1.000	0.347	5.318
Interaction	0.750	3	0.250	1.000	0.441	4.066
Within	2.000	8	0.250			
Total	17.750	15				