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GREENHOUSE ATMOSPHERIC ENVIRONMENT AND
GREY MOULD (BOTRYTIS CINEREA) OF
FOREST NURSERY SEEDLINGS


by

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ABSTRACT

Microclimatic data were collected in the vicinity of Douglas-fir seedlings grown in polyethylene and fibreglass-covered greenhouses operated by Canadian International Paper at Saanichton on southern Vancouver Island. Coinciding with these observations, airborne Botrytis cinerea spores were trapped above each seedling canopy.

Environmental parameters observed above and below the canopy in each greenhouse included light intensity and spectral quality, air temperature, humidity and vapour pressure deficit.

Light intensity in all wavelengths was highest in the polyethylene-covered greenhouse. Beneath each seedling canopy, light intensities in wavelengths that stimulate (300-420 nm) or inhibit (430-490 nm) B. cinerea sporulation, were too low to be effective.


A diurnal periodicity of increased spore concentrations between 04:00 and 20:00 was observed in each greenhouse. Airborne spore concentrations were significantly higher in the fibreglass greenhouse following an outbreak of grey mould.

Greenhouse covering had a minimal direct effect on grey mould of container-grown Douglas-fir seedlings. Restricted air movement created by a denser seedling canopy in the fibreglass-covered greenhouse contributed to increased humidities and the presence of longer intervals when conditions were ideal for spore germination. This is


considered to be the most important control of the occurrence of grey mould on Douglas-fir seedlings.

After its establishment, grey mould intensified within the fibreglass greenhouse. Increased airborne spore densities coincided with the application of overhead irrigation water. Multiple regression analysis was made using LOG_{10} of spore density versus climatic elements in the fibreglass greenhouse for September and October during those times the irrigation water was on. Canopy humidity and ambient vapour pressure deficit were included in the model. Ambient vapour pressure deficit was the most influential factor with regard to increased spore density.


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CHAPTER 1

INTRODUCTION

Environment influences plant disease in many ways. Adverse conditions may predispose a host or prolong the stage when it is susceptible to disease. Development of a disease is limited to the time when the environment favours both the host and the pathogen. As a corollary, climate significantly affects disease development, spread, and survival. Epidemiological investigations of plant disease therefore, require measurement of microclimatic factors in the plant's vicinity. Knowing the microclimate associated with a disease increases our understanding of the biological relationship between pathogen and host and also provides an opportunity for better disease management. Quantification of the microclimate associated with such environments often enables the modification of cultural practices used in disease management such as more accurate timing and application of fungicides.

Development of new culture techniques can create new environments that favour new diseases. Increased emphasis on reforestation in British Columbia has seen the development of a new seedling production technology where seedlings are grown in small containers, often in greenhouses. The main advantage of this cultural system is reduced time to produce a plantable seedling (faster growth) and more efficient planting at the reforestation site. This new technology, however, has seen a change in the types of diseases affecting tree seedlings i.e., from soil-borne in bareroot (field-grown) nurseries to shoot diseases in containers. Grey mould, caused by Botrytis cinerea (Fries) Persoon,

has become one of the most important impediments to production of containerized, conifer seedlings (Sutherland and Van Eerden, 1980).

Presently, grey mould control is achieved mainly by the use of fungicides. The increasing concerns about, and restrictions on, fungicide use make it imperative that cultural controls be developed. Better understanding of the environmental factors associated with the B. cinerea disease cycle in greenhouses is a necessary first step for developing cultural controls. Also, insight gained about the disease is important because previous work has mainly been done either in the laboratory or on field crops.

The significance of grey mould on container seedlings and the importance of its associated microclimate was brought to the attention of the author and the University of Victoria, Department of Geography, by Dr. J.R. Sutherland, Senior Research Scientist and Project Leader, Regeneration Pests, of the Canadian Forestry Service, Pacific Forestry Center (P.F.C.).

A container nursery at Saanichton, operated by Canadian International Paper Inc. (C.I.P.), was chosen as the study site. Grey mould is an annual problem there and the nursery was easily accessible. Moreover, seedlings there are grown in two different kinds of greenhouse. It was felt that information gathered there would have practical significance and also contribute to our understanding of microclimatology and plant disease.

Problem Definition

In British Columbia, the Ministry of Forests (BCMF) operates 10 nurseries, nine of which have container growing facilities. In 1984, BCMF nursery production was 110.1 million seedlings. Private growers produced another 38.8 million seedlings (B.C. Ministry of Forests 1983-84). Since 1981, container seedlings have accounted for more than half of the Province's annual seedling production. Of the total seedlings produced in 1984, about 98 million were container grown (Figure 1).

Although there are numerous types of containers (Tinus and McDonald, 1979), the preferred system in British Columbia is the BC/CFS styroblock (Johnson, 1981) which was developed in 1969-70. Thus many of the important diseases have been identified and appropriate management practices established (Sutherland and Van Eerden, 1980; Sutherland et al., 1981). Most of the diseases reported on local container seedlings have previously been reported from bareroot nurseries. However, the relative incidence of disease has altered in that shoot diseases are more important in containers than in bareroot nurseries where soil-borne root diseases and damping off predominate. In container nurseries the microenvironment is such that the relative importance of certain pests has changed rather than disappeared.

One disease responsible for significant losses of container seedlings is grey mould (Sutherland et al., 1981). In British Columbia, Douglas-fir (Pseudotsuga menziesii) and western hemlock (Tsuga heterophylla) are more susceptible to the disease than are lodgepole

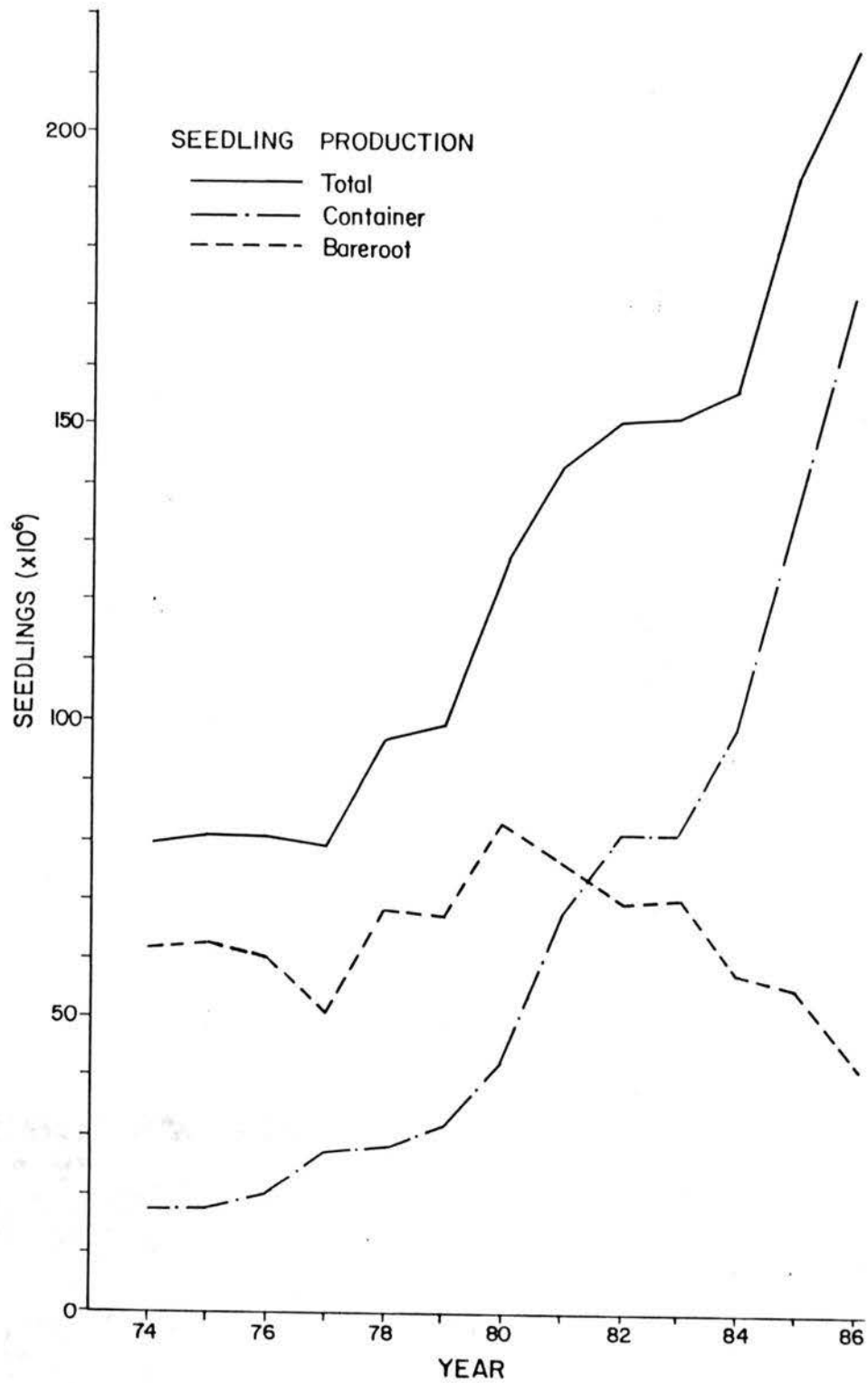


Figure 1. Total, bareroot and container-grown seedling production in British Columbia from 1974 to 1986 (C. Koistra, pers. comm. 1).

pine (Pinus cortorta) and spruces (Picea spp.) (Figure 2). Initial symptoms include a watery moulding and killing of lower needles. This spreads upward, killing needles and sometimes woody portions of the shoot. Seedlings may be killed outright but more often develop an overall lack of vigour which predisposes them to other diseases or reduces their survival after outplanting. Also, grey mould can be carried over and affect cold-stored seedlings (i.e., the disease becomes a storage mould).

Grey mould is thought to develop on individual seedlings and then spread to form greyish brown patches of mycelium (vegetative growth) which often coalesce. Subsequent spread occurs by mycelial growth or by the release and dissemination of air-borne spores (propagative units of a fungus, analogous to seeds). For initial infection to occur, primary inoculum must enter the greenhouse, e.g., as airborne spores, by the introduction of infected extraneous organic material, sclerotia (survival structures), or the overwintering of mycelium on organic debris. Once introduced, the pathogen may remain latent until environmental conditions favour growth and conidia (asexual spore) production.

Conidia production, release and dispersal may all initially occur on infected vegetation outside the nursery. This did not constitute a problem when primary seedling production was in bareroot beds. However, changes in growing technique have created a greenhouse microclimate that appears to favour grey mould.

Once inside the greenhouse, inoculum is in an environment conducive to infection, disease establishment and its subsequent spread.

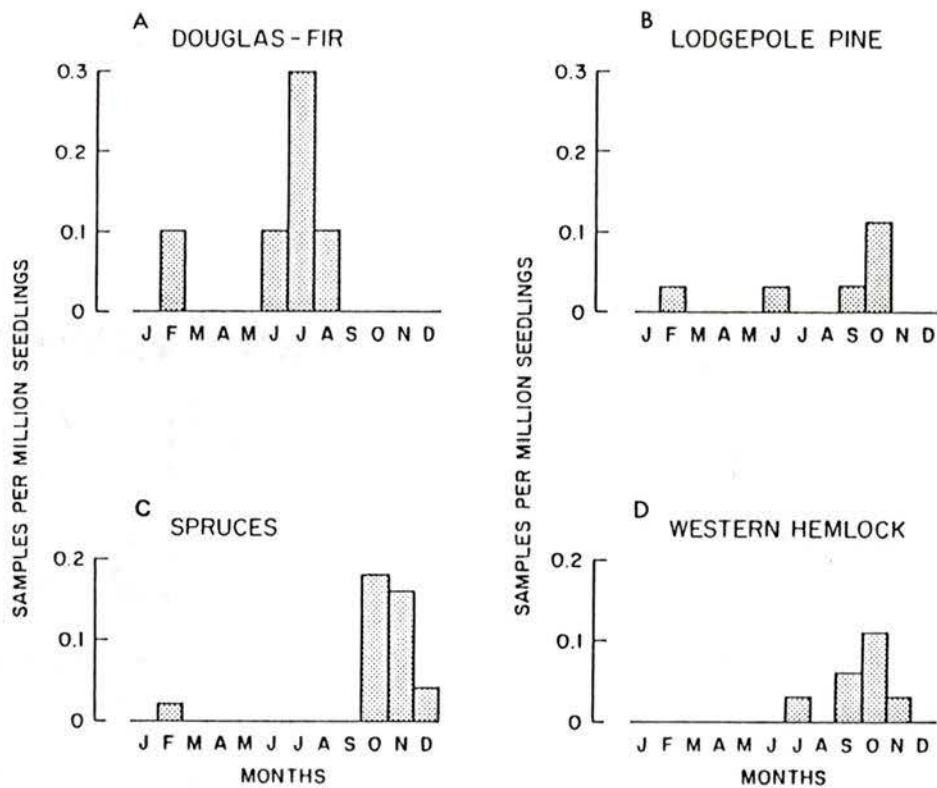


Figure 2. Samples per million seedlings produced, of *Botrytis*-affected (A) Douglas-fir, (B) Lodgepole pine, (C) Spruces and (D) Western hemlock, received by the Canadian Forestry Service per month (1976-80) (Sutherland *et al.*, 1981).

Thus, the primary objective of the study was to characterize the greenhouse environment and examine its effect upon grey mould. To achieve this goal a research programme was designed with four objectives, i.e., (i) to determine if the greenhouse climate is favourable for grey mould disease outbreaks; (ii) to determine levels of climatic elements related to or precursors of outbreaks; (iii) to find if a disease cycle is self-contained within the greenhouse or relies upon outside inoculum; and (iv) investigate whether open-sided, plastic-covered or open-sided, fibreglass-covered greenhouses are most favourable for the disease. Prior to addressing these objectives however, an understanding of the biology of the disease and the effects of microclimate is necessary.

CHAPTER 2

GREY MOULD

Taxonomy

Grey mould of container-grown seedlings is caused by the fungus Botrytis cinerea (Fries) Persoon. Botrytis cinerea belongs to the form-genus Botrytis. The type-species B. cinerea is the asexual stage of Botryotinia fuckeliana Buchw. of the Sclerotiniaceae, one of the largest families of the inoperculate Discomycetes (Alexopoulos and Mims, 1979). Jarvis (1977) and Trolinger (1983) give further details on the fungus' taxonomy.

Biology

Life Cycle

The sexual stage (i.e., B. fuckeliana) of Botrytis cinerea is rarely encountered. The genetic connection between Botryotinia spp. and Botrytis spp. was not demonstrated until 1949 although the genus Botrytis, erected in 1729, was one of the first genera of fungi described (Jarvis, 1977).

Botrytis cinerea is a ubiquitous fungus that becomes established and survives on dead organic matter (i.e., it is a facultative saprophyte). Under appropriate environmental conditions it can parasitize living plants. This is how the disease is thought to operate in container nurseries. Here the pathogen likely becomes established on dead (e.g., fertilizer burned) or senescent needles and then spreads to

living tissues. A disease cycle for B. cinerea is shown in Figure 3. Following infection, the pathogen may remain latent until conditions favour disease development and aggressive pathogenicity. Damage occurs from mid- to late-summer onward, after the seedling canopy closes. This, and the senescing of the lower needles appears to create a suitable microenvironment for the disease.

Climatic influences on plant disease development are well recognized (Coakley, 1985; Foister, 1946; Meredith, 1973; Sutton et al., 1984). Once greenhouse seedlings become susceptible and inoculum is present, environmental conditions in the canopy determine whether infection and a subsequent epidemic can occur. Host morphology, which could affect susceptibility, sufficient concentrations of viable inoculum, and favourable climatic conditions will determine disease severity. Once the pathogen is established, the occurrence of a disease epidemic depends upon a favourable environment and the maintenance of host susceptibility.

Host Susceptibility

As a facultative saprophyte, B. cinerea usually becomes established on dead or moribund tissues and infection often occurs if moribund host tissue is present (Jarvis, 1977). Numerous factors that physically damage plants permit easy infection, e.g. insect damage, sun scald, pollutants, bacteria and other fungi (Jarvis in Coley-Smith et al., 1980). On conifer seedlings, predisposition to grey mould is often attributed to fertilizer burn (Yalpani, 1979). Canopy closure,

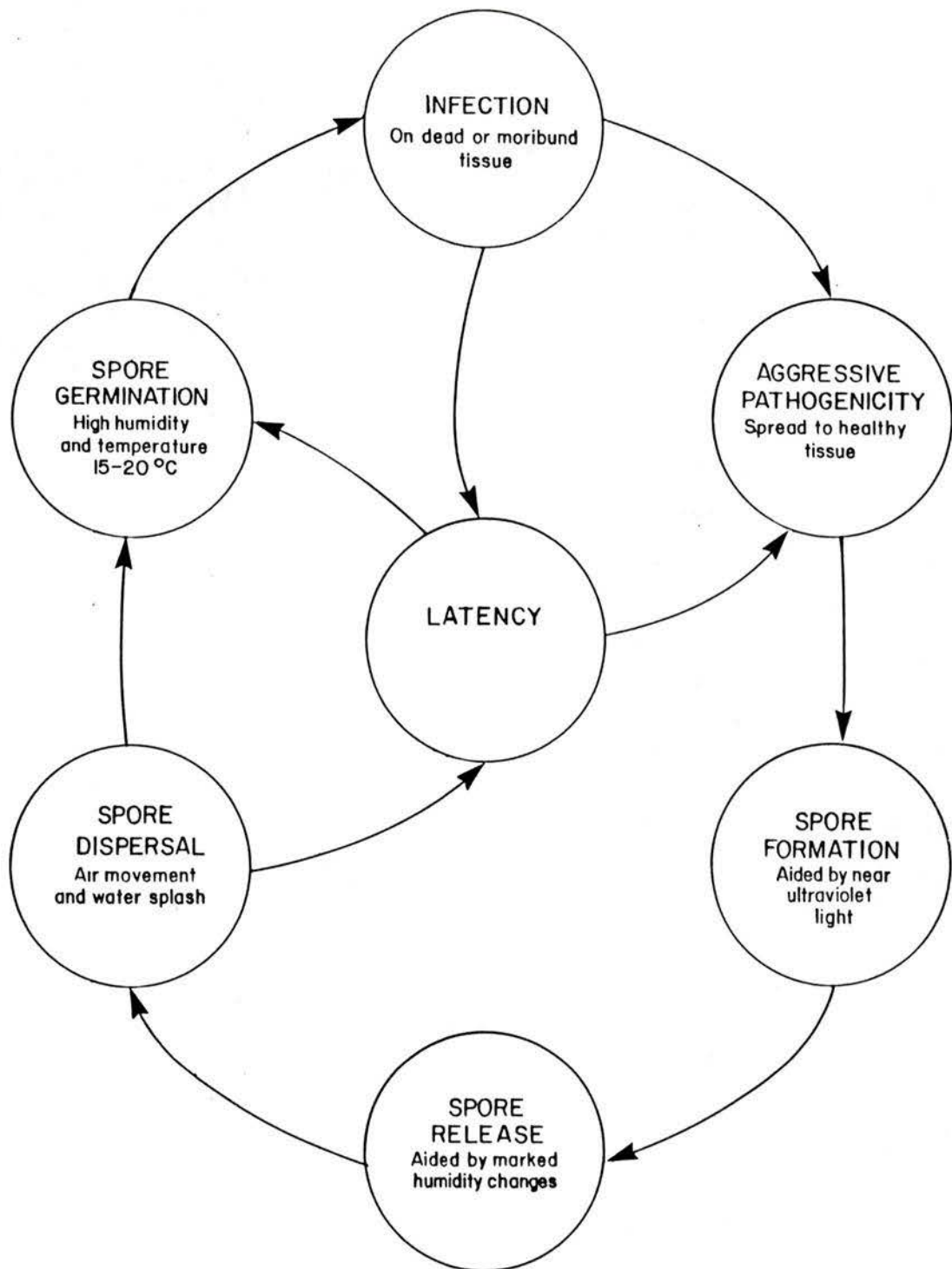


Figure 3. The life cycle of *Botrytis cinerea* (Adapted from Coley-Smith et al., 1980).

resulting in reduced light intensities and decreased air movement, contribute to senescence of the lower needles. These conditions, resulting from fast-growing, succulent shoots, also predispose seedlings to grey mould.

Besides wounds or dead and moribund tissue, host water status can significantly affect predisposition as high tissue water content leads to increased cell permeability and decreased osmotic pressure (Herling cited in Jarvis, 1977), conditions which facilitate germ tube or mycelial penetration of healthy tissue. Increased host water content can cause swelling and splitting of tissues providing infection courts and it also assures increased opportunities for stomatal penetration by mycelium or spore germ tubes.

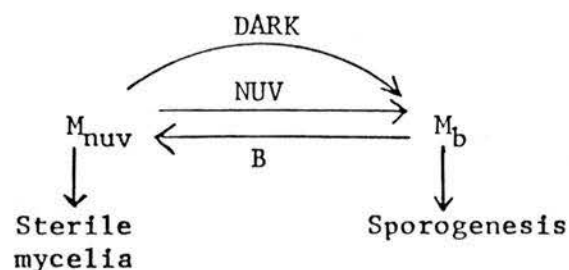
Mycelial infection is essentially the same as that from germ tubes. However, the mycelium usually has a nutrient-providing saprophytic base (Jarvis, 1977). The inoculum potential of mycelium is much greater than that of germinating conidia and is less dependent upon the environment. Therefore, following initial infection of dead or senescent tissue and when environmental conditions are unfavourable for spore germination, succulent seedlings become susceptible to infection via mycelium.

Light

In many fungi, sporulation is a photochemical response (Leach, 1962). Light affects various growth processes depending upon its wavelength, although B. cinerea can germinate and grow in the dark.

Tan and Epton (1973) determined that growth of B. cinerea mycelium was inhibited by continuous near-ultraviolet (NUV) irradiation. Red light had the same influence (Rabinovitz-Sereni, cited in Jarvis, 1977). Investigations by the author (unpubl.) on the effects of NUV on B. cinerea isolates from Douglas-fir seedlings showed that dark did not affect mycelial growth.

Light has a significant ability to enhance sporulation of B. cinerea. Tan and Epton (1973) showed that B. cinerea has a photo-receptive response. The 300-420 nanometer bandwidth was the most conducive to spore production. A single 1 min. exposure to NUV light (300-420 nm) at $151 \mu\text{W}/\text{cm}^2$ during the most responsive phase induced sporulation. Repeated reversibility of sporulation by NUV and blue (B) light has also been demonstrated (Tan, 1974a). Maximum sporulation was found with at least $150 \mu\text{W}/\text{cm}^2$ of NUV (300-420 nm). Following this, maximum inhibition occurred with at least $250 \mu\text{W}/\text{cm}^2$ of B (430-490 nm). The fungus responds to the last irradiation regime to which it has been subjected. When both critical wavelengths occur simultaneously, the higher intensity regime prevails. Tan (1974b) proposed the following mechanism of mycochrome (a hypothetical receptor pigment):



The form of mycochrome M_b , effective for sporulation is converted to M_{NUV} , the form not active for sporulation. Irradiation of the M_{NUV} by NUV converts it back to M_b . Besides this, M_{NUV} or some intermediate in the step $M_b \rightarrow M_{\text{NUV}}$ may be unstable and over time, revert back to the M_b even though the last radiation regime may not have been that of NUV. The hypothesis that M_{NUV} may revert back to M_b , helps explain the formation of some conidia when B. cinerea is exposed to full sunlight. Although sunlight is invariably stronger in the B component of the spectrum than in NUV, the dark night period between exposures may allow sufficient time for any M_{NUV} present to revert back to M_b .

Hyre (1972) found B. cinerea sporulation on intact geranium leaves to be nearly as profuse in darkness as under light containing the spore inducing NUV. He cautioned that results obtained in controlled environments on culture media might not mimic field observations. However, the use of ultraviolet-absorbing film to control in vitro sporulation of B. cinerea by Hite (1973) also controls grey mould on greenhouse-grown tomatoes and cucumbers (Honda et al., 1977).

Some Botrytis isolates sporulate in the dark or on enriched culture media (Hite cited in Hite, 1973). Also B. cinerea sporulation can be significantly increased by exposing the fungus to NUV. Managing the quality of light in greenhouses would not completely control grey mould, but it may reduce the amount of inoculum.

The apparent importance of light as an environment agent affecting B. cinerea is clear. Therefore, light was investigated with regard to

its possible role in grey mould development on greenhouse-grown conifers.

Temperature

Temperature affects all stages of fungus disease epidemics (Foister, 1946). It generally acts in concert with humidity and other than the fungicidal effect of excessively high temperatures, it likely does not limit grey mould spread. However, it is important that the right temperature and humidity prevail when the fungus is at the appropriate physiological age.

Favourable moisture conditions for spores, combined with optimum germination temperatures, determines the success of initial infection by conidia. Infection can then be very rapid (Jarvis in Coley-Smith et al., 1980). Fifteen hours of temperatures between 15 and 20°C and saturated humidity is sufficient for B. cinerea conidia to germinate and infect grapes (Bulit et al., 1970). Hunter et al. (1972) obtained strong positive correlations between the incidence of B. cinerea infection of Macadamia flower racemes and the hours per week the leaves remained wet at temperatures of 18-22°C. At 18°C, less time was required than at 18-22°C. Disease incidence was depressed above 22°C.

Trolinger (1983) examined the effects of temperature on grey mould severity on potted zinnias. Inoculation of the plant was with dry conidia, followed by incubation at 8-30°C with an average low/high for the range of 14/21°C. Disease was most severe in the 6-30°C range where the average low/high was 15/22°C.

Hennebert and Giles (cited in Jarvis, 1977) found 20°C to be optimum for spore germination. Based upon epidemiological studies, e.g., Trolinger (1983), Hunter et al. (1972) and Bullit et al. (1970), 15-20°C appears to be optimum for spore germination.

Higher temperatures seem to be optimum for mycelial growth. Trolinger (1983) found mycelial growth on culture media occurred between 4-36°C, with 16-28°C being optimum. Jarvis (1962) cites 20°C as the optimum temperature for mycelial growth. Hennebert and Giles (cited in Jarvis, 1977) found 30°C to be optimum for mycelial growth as the fungus became dependent upon external nutrients. The inoculum potential of mycelium is greater than that of conidia because mycelium is less dependent upon the external environment, e.g. lower moisture (Jarvis, 1977). Compared to conidia, mycelium grow over a wider temperature range and tolerate higher temperatures. Following mycelial growth, conidiation and sclerotia formation can be induced by decreasing the temperature. Temperatures favourable for B. cinerea mycelial growth generally inhibit sclerotial production and vice versa (Trolinger, 1983).

Considering the effect of temperature on spore formation, spore germination and mycelial growth, it appears that temperatures in the 15-20°C range are the most conducive to grey mould epidemics.

Moisture

Humidity and temperature act jointly in affecting fungus-caused plant diseases. Atmospheric moisture, usually expressed as relative

humidity (RH), is temperature dependent. Together, temperature and humidity determine initial infection from spores and the transition from latent infections to aggressive lesions (Coley-Smith et al., 1980).

Most Botrytis species sporulate best in less than saturated atmospheres where conidiophores are short and bear numerous spores that are easily liberated (Paul, 1929). Paul (1929) noted that sporulation diminished under high humidity while sclerotia formation prevailed. It is after spores form that humidity affects grey mould epidemics.

Jarvis (1962) correlated B. cinerea spore release and dispersal in a raspberry plantation, with a rapid rise or fall of RH in the 65-85% range. This was attributed to hygroscopic twisting of the conidiophores induced by the rapid humidity changes. He found that spore dispersal had two daily peaks. The first, in the morning, was associated with falling RH during dew evaporation and atmospheric warming. The other, in the afternoon, was related to dew fall. Corbaz, cited in Coley-Smith et al. (1980), found similar patterns of dispersal in grey mould affected grape plantations.

McCoy and Dimock (1971) suggest that liberation of B. cinerea conidia from necrotic Chrysanthemum morifolium requires free water. This supports Jarvis' (1962) results that B. cinerea conidia are passively released by the action of rain. Rain splash affects the release and dispersal of many fungus spores (Meredith, 1973). This is true for both "slime-spored" fungi and certain "dry-spored" fungi such as Botrytis (Jarvis, 1962).

The significance of humidity upon grey mould is reflected by its dominance in epidemic forecasting models, e.g., Herve and Moysan (1967), who used an empirical graphical method of forecasting based upon the hours per day when RH exceeds 90%. Any model forecasting epidemics based upon conidial formation and dispersal however, has little relevance where conditions for infection do not follow (Coley-Smith et al., 1980). Spore germination significantly affects grey mould.

Spore germination is very dependent upon the presence of free water, rather than a water saturated atmosphere (Foister, 1946). Coley-Smith et al. (1980), state, "Relative humidity and availability of free water on the surface of plants are often thought of as the most important single factors influencing infection by species of Botrytis." At the Western Ornamental Disease meeting in Portland, Oregon in January, 1985, it was reported that B. cinerea conidia would germinate only with free water and not at RH's as high as 98%.

Not all researchers agree, however, that B. cinerea conidia require free water to germinate. Yarwood (1950) found that the water content of B. cinerea conidia was only 17% of their fresh weight but, when at equilibrium with the atmosphere, a high proportion of this water was hygroscopic. Snow (1949) found a lag period before B. cinerea conidia germinated when exposed to atmospheres of different RH. At RH's of less than 93%, germination did not occur. Jarvis (1977) interpreted this lag phase as a period during which hygroscopic water is being imbibed from the atmosphere. Nelson (1951) found the

length of time free water was available to be unimportant for germination as long as RH was over 94%. The length of the wet period became significant when humidities dropped below 94%.

Humidity, temperature, and liquid water are closely related. Small temperature changes can cause condensation to occur from near saturated and saturated air. Therefore, a high potential for infection exists during times of abundant inoculum, high humidities ($\geq 98\%$) and optimum temperatures (15-20°C) for spore germination.

CHAPTER 3

MATERIALS AND METHODS

Study Site

To determine the effect of covering material (polyethylene vs. fibreglass) on greenhouse microclimate, studies were made in 1985 at two greenhouses operated by Canadian International Paper Inc. (C.I.P.) at Saanichton (48°36'10"N; 123°24'30"W), southern Vancouver Island. One greenhouse, designated MAIN (5200 m² area) was covered with corrugated fibreglass panels. The other, designated HOOP (500 m² area) was covered with a single layer of polyethylene. Both had their side walls removed during the observation period to provide ventilation once the seedlings were established.

The seedlings in each greenhouse were Douglas-fir (Pseudotsuga menziesii), grown in styroblock containers (type PSB313 160) on benches 1 m above the floor. Seeds in the MAIN house were sown on March 25 and in the HOOP house on April 4. The seedling canopy was fully closed in the MAIN house by June 15 and in the HOOP house one month later.

Data Collection

Climatic Variables

The following environmental parameters were recorded: light intensity and spectral quality, air temperature, air movement, humidity parameters and a relative measure of foliage wetness. Except for light spectral analysis, all environmental parameters were recorded on data

loggers (model CR21, Campbell Scientific Canada Corp., Edmonton, Alberta T5E 2P4).

Light. Light readings were taken in each greenhouse using a microprocessor-based spectroradiometer (model LI-1800, Portable Spectroradiometer, LI-COR Instruments Inc., Lincoln, NE). Observations were taken outside, and above and below the canopy in each greenhouse on June 18, July 17, August 22 and September 24. Radiation flux density (energy per unit time per unit area, Wm^{-2}) was recorded in two nanometer increments over the 300-1100 nm wavelength interval. Light curves of radiation flux density versus wavelength were then constructed for this spectral band. Following this, radiation flux density in the critical wavelength bands, 300-420 nm and 430-490 nm, was determined via integration over the wavelength interval. These values could then be related for possible associations with spore production or inhibition.

Air temperature. Air temperature above and within the seedling canopy was recorded. The ambient air temperature, also used to correct the humidity sensor, was measured by a shielded, beaded thermistor set 25 cm above the seedlings. The temperature in the canopy was measured by a beaded thermistor also used as the dry bulb temperature for foliage humidity calculations.

Ambient air temperature was recorded every 30 min. as an average over the previous 30 min. Canopy temperature, recorded every 30 min., was sensed by the dry bulb probe used in an aspirated psychrometer. Accurate measurements for humidity calculations could not be made

unless the psychrometer fan operated for 2 min. Therefore, the recorded temperature was a single value taken on the half hour rather than the mean of the previous 30 min.

The input range of the thermistors used was -40 to 60°C with curve fit errors of less than 2%. All thermistors were calibrated to within 0.5°C of each other. The dry bulb thermistor measuring canopy temperature was housed in a plastic tube, covered with reflective tape to avoid radiational heating and water damage. The thermistor for recording ambient temperature was within a shielded humidity sensor and the entire unit was housed in a plastic tube covered with reflective tape.

Air movement. To obtain a general knowledge of the air movement within the greenhouses, simple cup anemometers (model Casella Sensitive Anemometer, C.F. Casella and Co. Ltd., London) were set 0.5 m over the seedling canopies. The Casella anemometer was chosen for its low starting threshold of 0.23 m/s. Wind speed was recorded in m/s, every 30 min. as the mean of the previous 30 min.

Humidity. Atmospheric relative humidity was monitored with a humidity sensor (Model PL2-200, Micromet Systems Inc., Vancouver, B.C. V5Z 1L7) mounted 25 cm above the canopy. Forced ventilation was not needed as the sensor was referenced to the thermistor used for ambient air temperature. RH was recorded every 30 min. as the mean of the previous 30 min.

Spot checks on the accuracy of each humidity sensor were made using an Assmann aspirated psychrometer which showed that the Micromet sensors consistently overestimated RH in the higher humidity ranges,

i.e. $\geq 95\%$ RH. A corrective algorithm based on simple linear regression was developed for each sensor using the Assmann psychrometer RH as the dependent variable and the Micromet RH as the independent variable (Appendix I). The sample size was limited to 10 because the best curve fit was obtained with the dependent variable in the 60-100% range. For each sensor a logarithmic relationship produced the smallest standard error of estimate. The algorithm used to correct RH in the MAIN greenhouse was: $RH \text{ (corrected)} = -83.99 + 35.92 \text{ Log } RH \text{ (observed)}$. In the HOOP greenhouse it was: $RH \text{ (corrected)} = -143.54 + 47.08 \text{ Log } RH \text{ (observed)}$.

An aspirated psychrometer was used to monitor wet and dry bulb temperature in each seedling canopy. Each sensor consisted of a beaded thermistor mounted in a radiation-shielded tube attached to a fan. Aspiration was for 2 min. prior to recording wet and dry bulb temperatures every 30 min. These measurements were not means but rather samples recorded each half hour. The wet bulb thermistor was kept moist by a close fitting wick from a reservoir attached to the shielding tube. The wet and dry bulb thermistors of each psychrometer were mounted 5 cm above the styroblock surface and 10 cm from the edge of the block. This arrangement avoided moving seedlings and disrupting the canopy.

A source of error common to each psychrometer occurred at very high relative humidities. If a sample were taken while the overhead irrigation was on or immediately afterwards, moisture could be drawn onto the dry bulb thermistor. This gave a dry bulb lower than wet bulb

reading, resulting in an RH > 100%. Spot readings of RH within the canopy were made using an Assmann psychrometer. The wet and dry bulb Assmann temperatures were regressed on the wet and dry bulb thermistor temperatures (Appendix II). The relationships were all linear with r^2 values of > 0.9 in each case. A two-tailed t test was used to test each regression coefficient at $P = 0.01$. Because each relationship was linear with slopes that were not significantly different from unity, no corrective algorithms were developed. Relative humidity > 100% was only observed occasionally and at times with visible moisture present and the actual RH assumed to be 100%. Therefore, if the canopy RH was observed to be greater than 100%, final canopy RH was calculated as being 100%.

Leaf wetness. To measure foliage surface moisture, leaf wetness sensors were placed in the seedling canopies in each greenhouse. The sensors consisted of flat plates with electrodes printed on them in the form of interlocking fingers. Each sensor was covered with white latex paint which served as a moisture-absorbing medium and increased the sensitivity to initial dew. Changes in resistance between the electrodes, due to the moisture content of the painted surface, provided a relative measure of the degree of wetness. Data were recorded in relative units every 30 min., with 0 being completely dry and 1 being wet. Evaporation of the fertilizer solutions applied to the seedlings via the overhead irrigation system caused a rapid buildup of electrolytes on the surface of the sensors. This severely limited the use of

the data. It was later decided that the leaf wetness information was unreliable.

Host Morphology

Seedling morphology directly influences canopy microclimate. Additionally, seedling shoot form can often predispose seedlings to secondary grey mould infection, e.g., succulent (non-woody) tissues appear to favour active parasitism (J.R. Sutherland, pers. comm.²). Thus, seedling heights and water status were determined at 2-week intervals from age class 8 to age class 28 weeks. Samples consisted of 20 random seedlings from each greenhouse. For each seedling, height, root collar diameter, shoot fresh weight and dry weight, and root fresh weight and dry weight were recorded and the means determined for each parameter. As an indicator of succulence, mean shoot water content was expressed as a function of mean root collar area (Appendix III). These data were later examined for between-greenhouse differences of seedling morphology and related to grey mould incidence.

Airborne Spore Concentration

Atmospheric spore density was measured using inertial type spore traps which draw air through a jet or tube and the airborne microbes are deposited on a sticky surface. After counting the spores trapped in a given time, spore density estimates are made.

The spore traps (model FTS Spore Recording Trap, Forest Technology Systems, Victoria, B.C.) were similar in design to the Burkard trap, as

described by Gadoury and MacHardy (1983). Each trap was placed at the climatic data collection site, and set 25 cm above the canopy. These traps each sampled 10 L of air per min. Using the method outlined by May in Gregory and Monteith (1967), trap efficiency was estimated to be 80%. Traps were operated in each greenhouse from the beginning of August until the end of October. A spore trap was also operated just outside the greenhouses from the middle of August until the end of October. It was mounted 1.5 m above the ground and was the same model as used in the greenhouses.

A Hirst spore trap (Hirst, 1953) and a box type trap (Powell, 1967) were used in a greenhouse at the Pacific Forestry Centre (PFC), where seedlings had been inoculated with B. cinerea as part of another unrelated experiment. Spore data obtained at PFC were used to confirm observations made at the C.I.P. location.

Data Analysis

Climatic data were automatically recorded on the half-hour.

It was anticipated that recording how close the atmosphere was to being saturated, in absolute terms rather than with a ratio like RH, would more closely relate to B. cinerea epidemiology. Therefore, an output programme from each data logger calculated ambient vapour pressure and vapour pressure deficit in kilopascals. These were recorded every 30 min. as the mean of the previous 30 min.

Data recorded by the data loggers were stored on standard cassette tapes and later removed using an interface (model C20, Campbell

Scientific Canada Corp., Edmonton, Alberta T5E 2P4), with a Digital Professional 350 (DEC 350) microcomputer at PFC. Data were then transferred (via computer tape) to the University of Victoria where analysis was made using the IBM 3083 BX mainframe computer.

Spores caught in the FTS traps were impacted on a strip of Melinex tape (Burkard Manufacturing Ltd., Rickmansworth, Hertfordshire, England) coated with a thin film of silicone grease and mounted on a rotating drum placed in the trap. The traps in each greenhouse were set for one drum rotation every 24 h. The trap outside the greenhouses sampled over every seven days. Following removal from the traps, each tape was dissected for microscopic examination. Because many hourly segments of tape observed contained no B. cinerea conidia, the daily greenhouse tapes were dissected into six 4 h periods as follows: 00:00-04:00, 04:00-08:00, 08:00-12:00, 12:00-16:00, 16:00-20:00, 20:00-24:00. The outside, 7-day tapes were divided into 12 h periods; 08:00-20:00 and 20:00 until 08:00 the following morning.

Each segment of tape was mounted in polyvinyl alcohol (Elvanol) on glass slides and a microscope (400X) was used to count the B. cinerea conidia. Subsequently, these data were used to calculate spores per m³ per 4 h period (Appendix IV). The spore counts were then entered into computer files.

To determine the viability of trapped B. cinerea conidia, segments of exposed tape from each greenhouse were incubated periodically throughout the season. Tapes placed on wet filter paper in petri

plates were kept at 18°C for three days. The presence of germ tubes were evidence of spore viability.

CHAPTER 4

RESULTS

Climatic Variables

Light

Spectrum analyses (300-1100 nm) made at noon on June 18, July 17, August 22 and September 24 showed a greater overall transmissivity for the polyethylene-covered HOOP house than for the fibreglass-covered MAIN house (Figure 4). Wavelengths conducive (300-420 nm) and inhibitory (430-490 nm) to B. cinerea sporulation were also more readily transmitted through the HOOP house covering. Spectral (300-1100 nm) readings were also taken at the base of the seedling canopies (Figure 5). Light intensity above the canopy (Figure 4) was higher inside the HOOP house than in the MAIN house. However, from July 17 onwards, following complete canopy-closure in each greenhouse, below-canopy radiation flux densities were small for wavelengths less than 700 nm.

Table 1 shows the integrated values of radiation flux density between specific wavelengths. In each of the specified bandwidths, radiation flux densities, above and below the canopy, were higher in the HOOP house than the MAIN house. On the four days when samples were taken, the polyethylene-covered HOOP house was transmitting 20% more of the widest spectral bandwidth (300-1100 nm) to the top of the seedling canopy than was the fibreglass-covered MAIN house. The HOOP house was more efficient at transmitting NUV light (300-420 nm) than the MAIN house. In this bandwidth, light intensities reaching the top of the

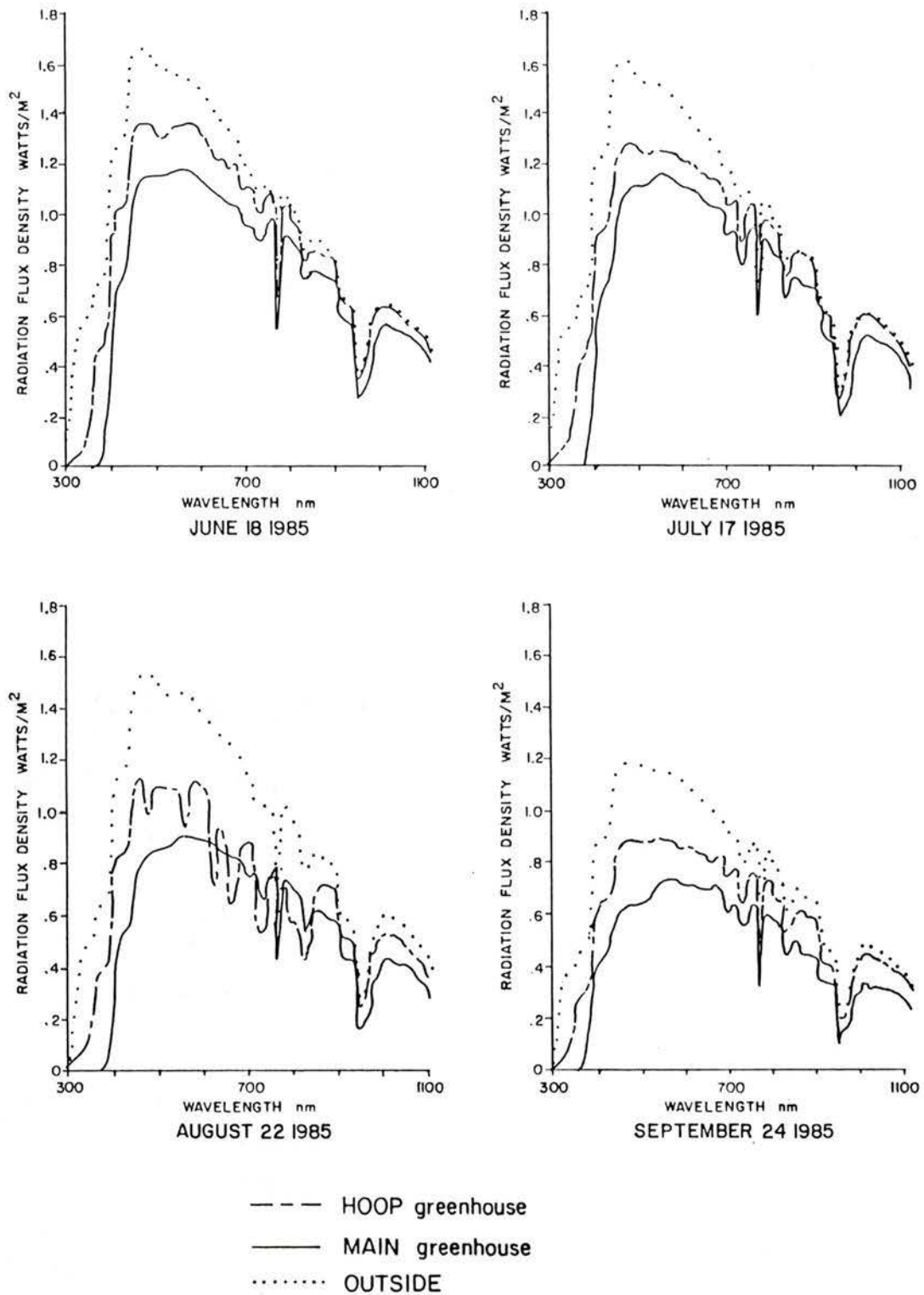


Figure 4. Radiation flux density outside and above the seedling canopy inside the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

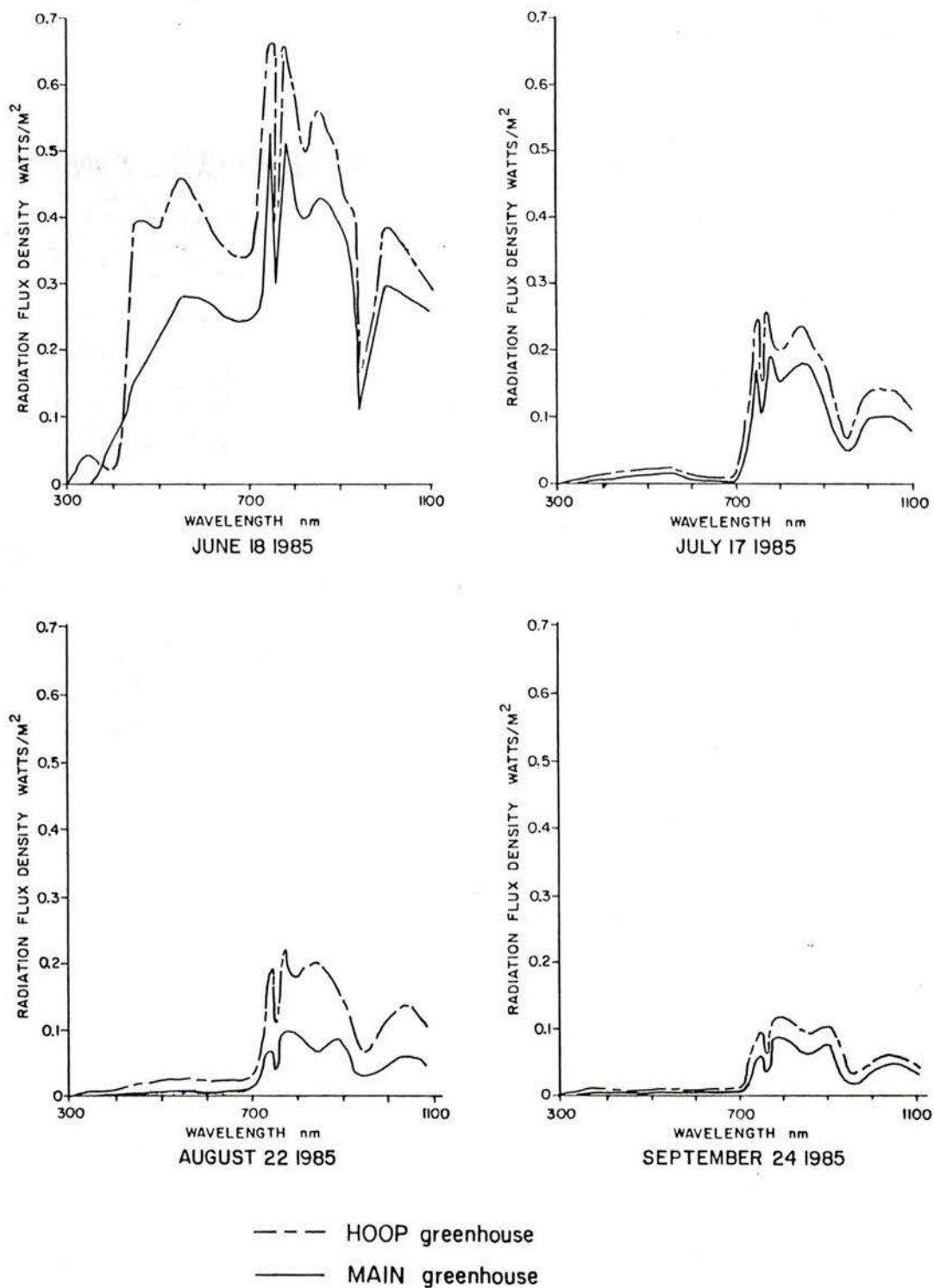


Figure 5. Radiation flux density below the seedling canopy inside the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

Table 1. Radiation flux densities between specific wavelengths above and below the seedling canopies in the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

Date	Bandwidth	Radiation Flux Density $\mu\text{W}/\text{cm}^2$				
		Outside	Above Canopy		Below Canopy	
			HOOP	MAIN	HOOP	MAIN
June 18		74,320	64,400	54,580	30,370	20,710
July 17	300-	68,460	57,740	50,170	8,097	5,350
Aug. 22	1100 nm	66,910	47,470	40,680	5,614	2,656
Sep. 24		53,870	42,060	32,090	2,685	1,805
June 18		7,529	4,058	1,709	1,368	230
July 17	300-	6,872	3,656	1,539	49	14.7
Aug. 22	420 nm	--*	3,361	1,174	31	0.49
Sep. 24		4,891	2,643	913	4	0.1
June 18		8,832	7,193	5,995	2,420	1,033
July 17	430-	8,187	6,460	5,482	88	41
Aug. 22	490 nm	--*	5,642	4,212	56	3.5
Sep. 24		6,224	4,559	3,130	5	1.1

* Integration not available

canopy in the HOOP house averaged 2.6 times those in the MAIN house. Light transmission in the 430-490 nm bandwidth through the polyethylene-covered HOOP house was 13% greater than through the fibreglass covering of the MAIN house.

On diseased seedlings, B. cinerea sporulates beneath the seedling canopy, i.e., on the lowermost needles. Thus, the intensities of NUV and B light (that affect sporulation) in this region are important. In the HOOP house, NUV and B intensities were much greater below the canopy than they were in the MAIN. This is a reflection of both the amount of light at the top of the canopy as well as canopy height and density.

Air Temperature

Paired comparison t-tests were used to examine the daily mean ambient and canopy temperatures between greenhouses. During July and October, ambient temperatures in the MAIN house were significantly higher* than those in the HOOP house (Appendix V). Ambient temperatures in the greenhouses were not significantly different during August and September. Canopy temperatures in the two greenhouses were not significantly different during July and August (Appendix VI). However, during September and October, the daily mean canopy temperatures in the MAIN house were significantly higher than those in the HOOP house.

* P = 0.05 unless otherwise stated.

The highest mean difference between daily mean canopy temperatures occurred in September when temperature for the MAIN house canopy averaged 1.1°C higher than that in the HOOP house. For other times when the MAIN house ambient and canopy temperatures were significantly higher than those in the HOOP house, mean temperature differences were all less than 1.1°C. Less variability in temperature and thus lower standard deviations at these times, resulted in lower mean differences being significant at $P = 0.05$.

Diurnal patterns of ambient and canopy temperature are shown in Figures 6 and 7. Despite the differences between greenhouses indicated earlier, the same daily pattern was evident both above and within the canopy for each greenhouse. Minimum daily mean temperatures occurred both above and within the canopy in each greenhouse between 04:00 and 08:00 and temperatures were highest during the afternoon, about 16:00. Mean canopy temperatures in the HOOP house exceeded those of the MAIN house during the day. During the evening, HOOP house canopy temperatures fell below those in the MAIN. Thus, the HOOP house had a greater diurnal range.

Figure 8 shows the seasonal ambient and canopy air temperature. Highest weekly mean temperatures occurred between the third and fourth week (July 14-21). Weekly mean ambient and canopy temperatures in both greenhouses stayed below 15°C from between the middle of the eighth (August 20) and middle of the ninth week (August 27) onwards. From July 1 to August 27, the weekly mean temperatures in each greenhouse were optimum for B. cinerea spore germination (15-20°C). Daily mean

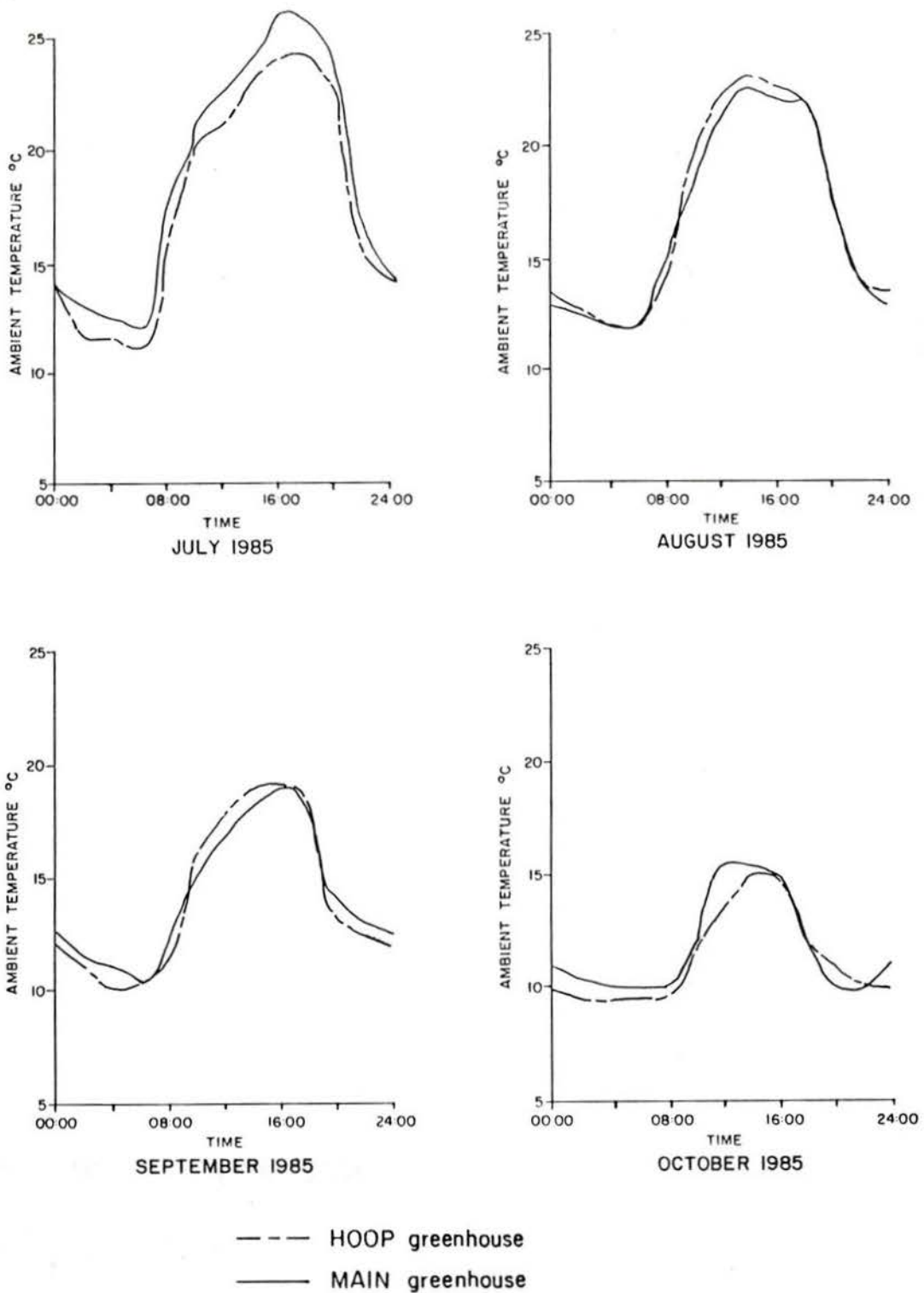


Figure 6. Ambient temperature ($^{\circ}\text{C}$) inside the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

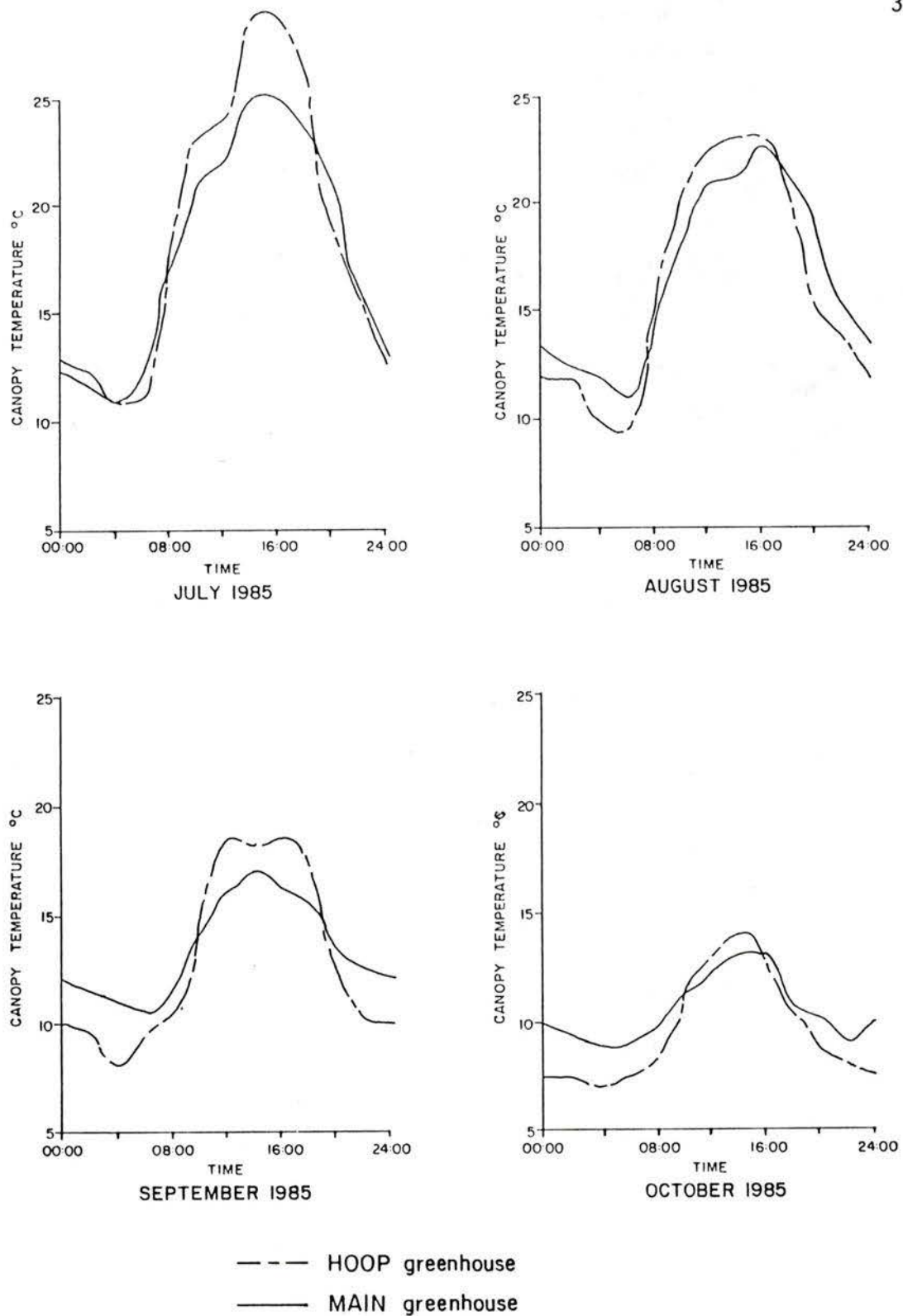


Figure 7. Canopy temperature (C°)inside the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

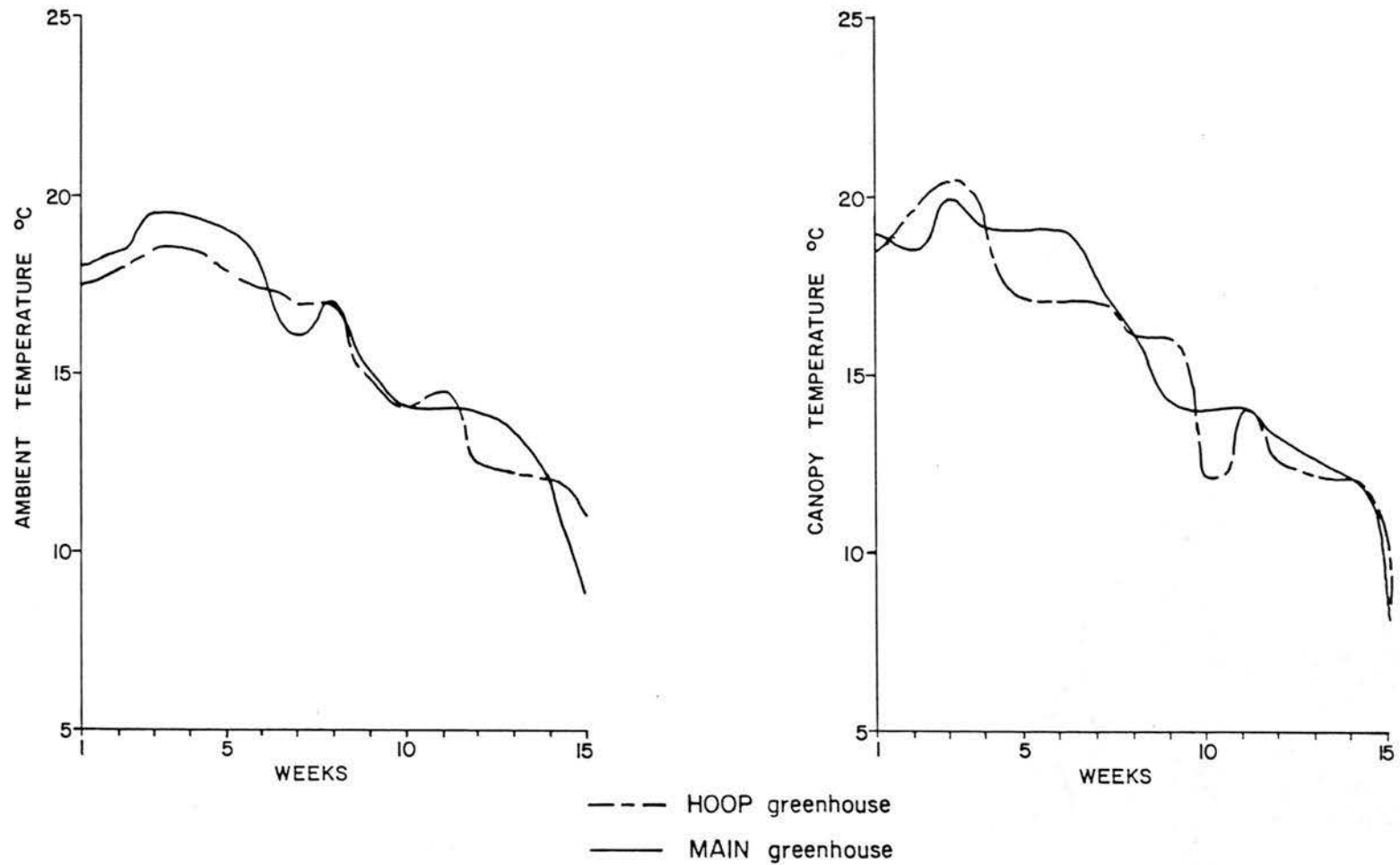


Figure 8. Seasonal ambient and canopy temperatures (C°) inside the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses from July 1 onwards.

canopy temperatures were optimum for about 16 h per day during July and 15 h per day in August, while in September, daily mean canopy temperatures fell within this range for about 8 h per day (Figure 7).

Air Movement

Air movement above the seedling canopies in each greenhouse was insignificant. Most observations were below the anemometer's threshold speed of 0.23 m/s. A maximum recorded air speed of 0.40 m/s occurred in the MAIN house on July 8. Air movement was visually observed to be most active beneath the seedling growing benches, within about 1 m of the floor and about 2 m below each greenhouse roof. The anemometers location 0.5 m above the seedling canopy was not in the region of maximum airflow within the greenhouse and thus the results are not indicative of the air movement at other locations.

Humidity

Paired t-tests were used to compare the differences in daily mean ambient and canopy RH's between greenhouses. During July, August, September and October, the MAIN house ambient RH's were significantly higher than those in the HOOP house (Appendix VII). During July, the MAIN house daily mean canopy RH was significantly greater than that of the HOOP house. However, during August, September and October, there were no significant differences in daily mean canopy RH's between the two greenhouses.

The greatest mean differences between daily mean RH values above and within the canopies of the two greenhouses occurred in July, when mean differences of 17.8% above and 11.2% RH within the canopies were observed.

During August, September and October when the ambient RH's were significantly higher in the MAIN house, variability between the two greenhouses was low. Thus, mean differences of less than 3% RH between greenhouses were significant. For these three months, when the canopy in each greenhouse was fully closed, no significant differences were detected in daily mean canopy RH's.

Canopy humidity is important for grey mould establishment. Although canopy RH in the MAIN house during July was significantly higher than in the HOOP house, no differences in canopy RH occurred between the greenhouses during the next three months. Table 2 gives the frequency of periods when moisture and temperature conditions within the seedling canopies were optimum for Botrytis germination (RH > 98% and temperature 15-20°C). A dramatic difference in the mean hours per day that optimum conditions for spore germination existed coincided with a grey mould outbreak in the MAIN house, but not in the HOOP house.

Diurnal patterns of ambient and canopy RH are shown in Figures 9 and 10. Ambient humidities in each greenhouse were highest during the late evening and early morning. Relative humidity was lowest about 16:00, coinciding with maximum ambient temperature. Overall daily ambient RH increased from July to October (Figure 9) while diurnal

Table 2. Mean hours per day from July 1 to October 31 when environmental conditions (canopy RH $>$ 98% and temperatures of 15 to 20°C) were ideal for Botrytis cinerea spore germination in the MAIN and HOOP greenhouses.

Month	Greenhouse	
	MAIN Hours	HOOP Hours
July	3.8	0.24
August	2.0	0.2
September	1.0	0.03
October	0.03	0

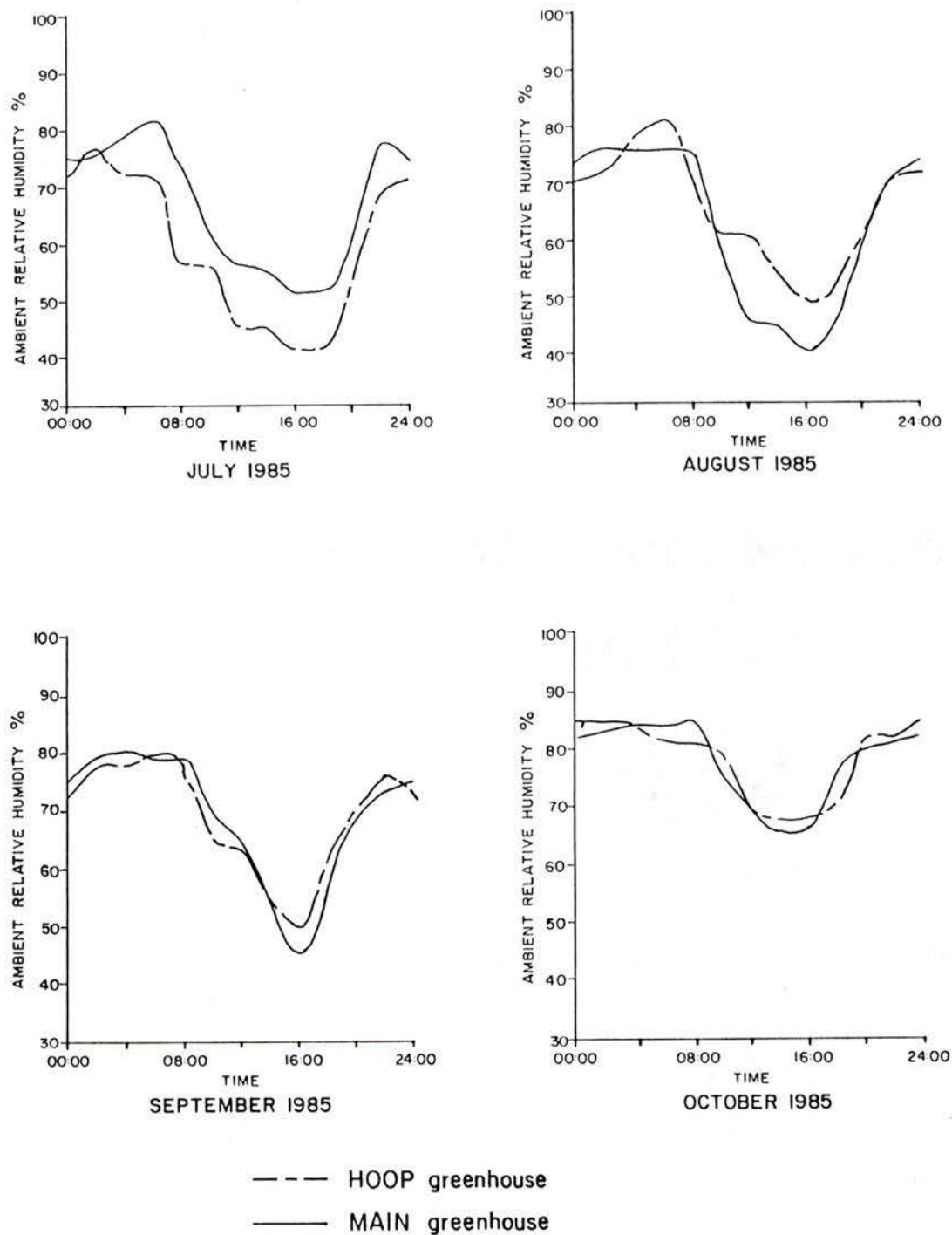


Figure 9. Ambient relative humidity inside the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

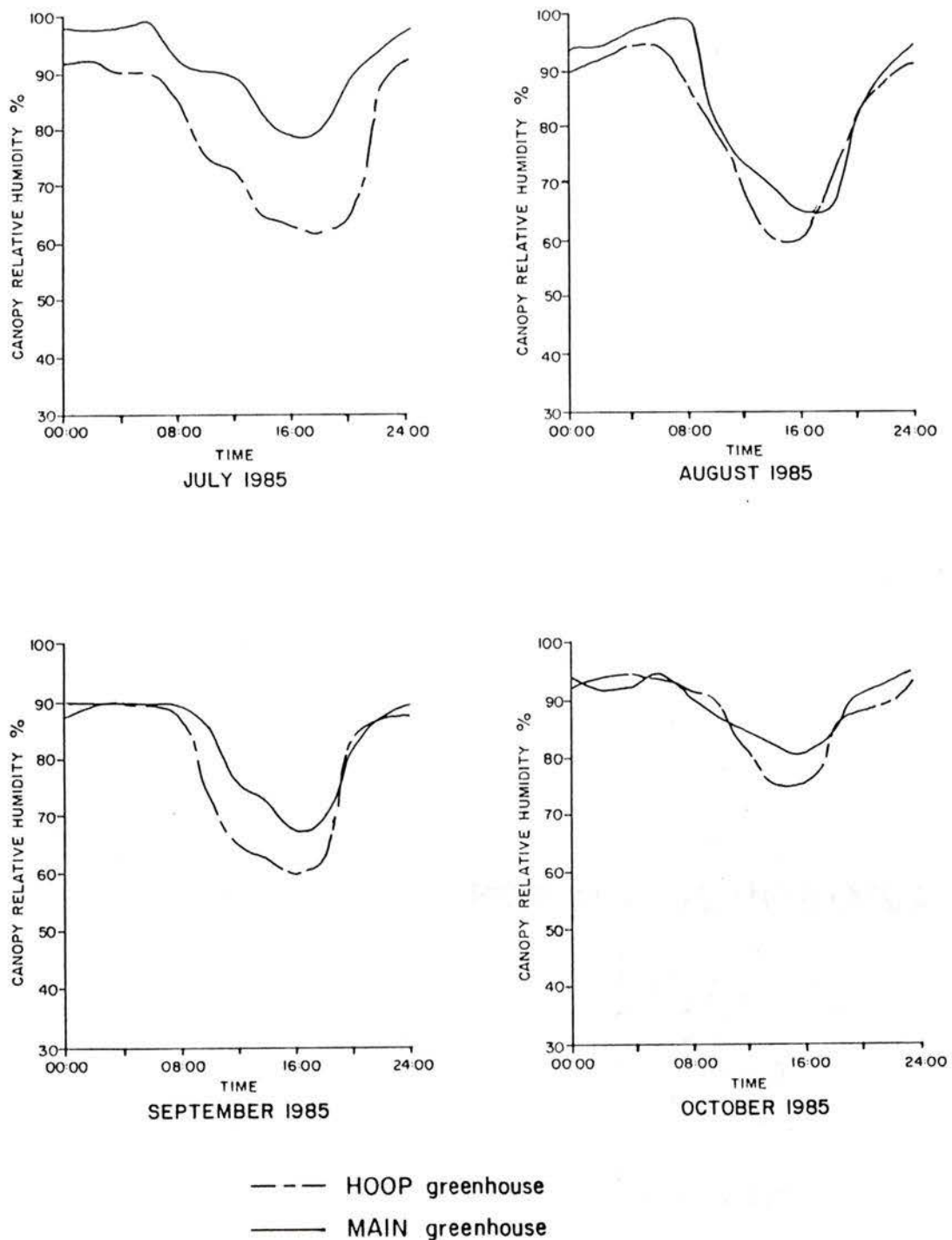


Figure 10. Canopy relative humidity inside the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

fluctuation of ambient RH decreased toward the end of the growing season (October). The seasonal decline in daily ambient RH variability coincided with a decline in daily ambient air temperature (Figure 6). Besides being generally higher than ambient humidities, canopy humidities showed less daily variation (Figure 10).

Daily patterns of ambient and canopy vapour pressure deficit (VPD) closely followed those of temperature and RH. Ambient and canopy VPD's in each greenhouse were lowest during the late evening and early morning, coinciding with minimum temperatures and maximum RH's. Maximum ambient and canopy VPD's in each house occurred about 16:00 daily, coinciding with maximum temperatures and minimum RH's. Ambient and canopy VPD in each greenhouse varied much more than did RH. Daily mean VPD coefficients of variation were 3.5 to 6.5 times greater than coefficients of variation for daily mean RH.

Host Shoot Growth and Water Content

Seedling height in each greenhouse is shown as a function of age class (Figure 11). In the MAIN house, seedling shoot growth was continuous until age class 17 weeks, when it averaged 35 cm. No further shoot growth occurred through September and October in the MAIN house. In the HOOP house, seedling shoot growth ceased at 20 cm (age class 16 weeks), 43% less than their counterparts in the MAIN house.

Figure 12 shows the water content of seedling shoots as a function of age class. Throughout the growing season, MAIN house seedlings had about 30% more water per mm^2 of root collar area than those of the HOOP

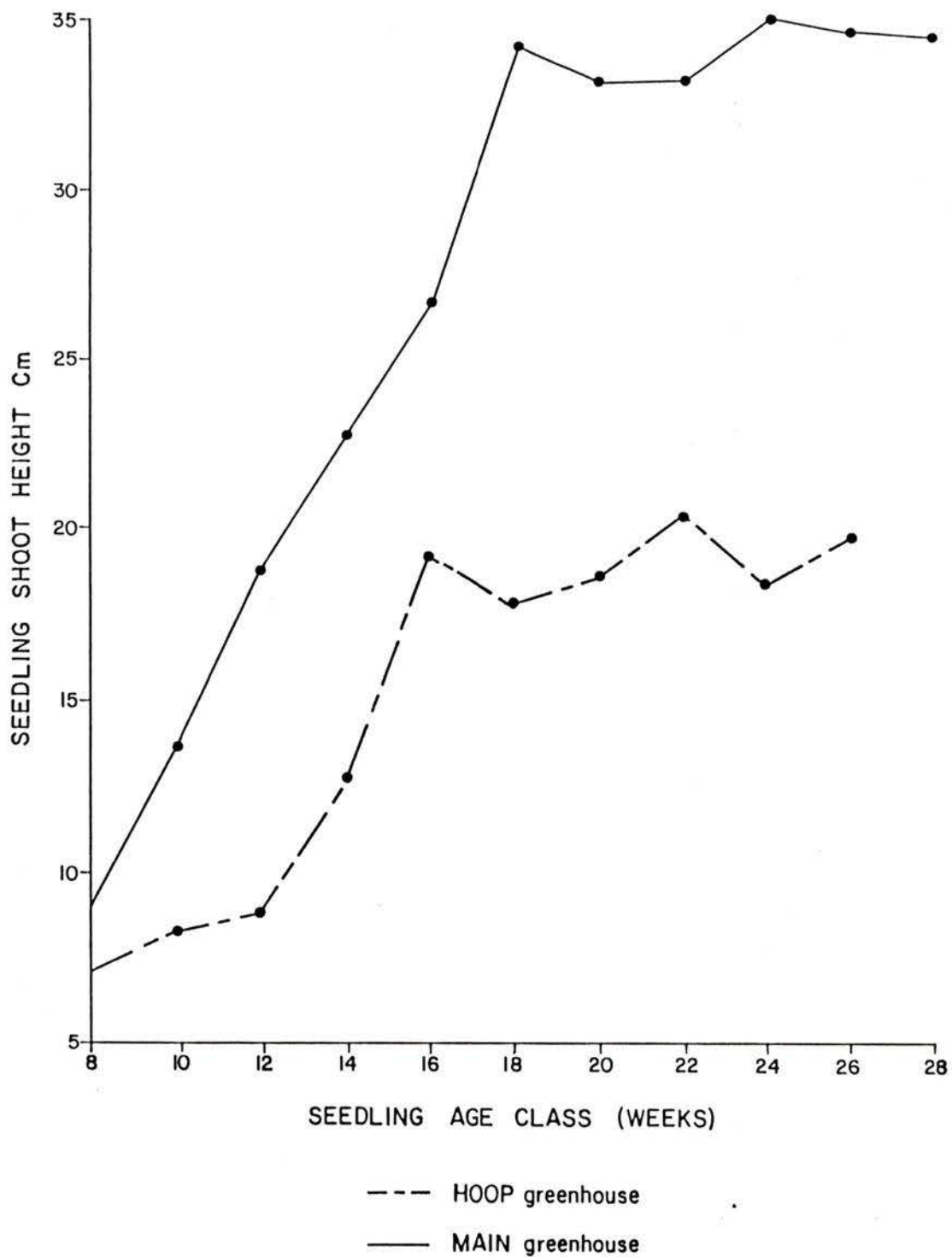


Figure 11. Seedling shoot height versus age class (weeks from sowing date) for the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

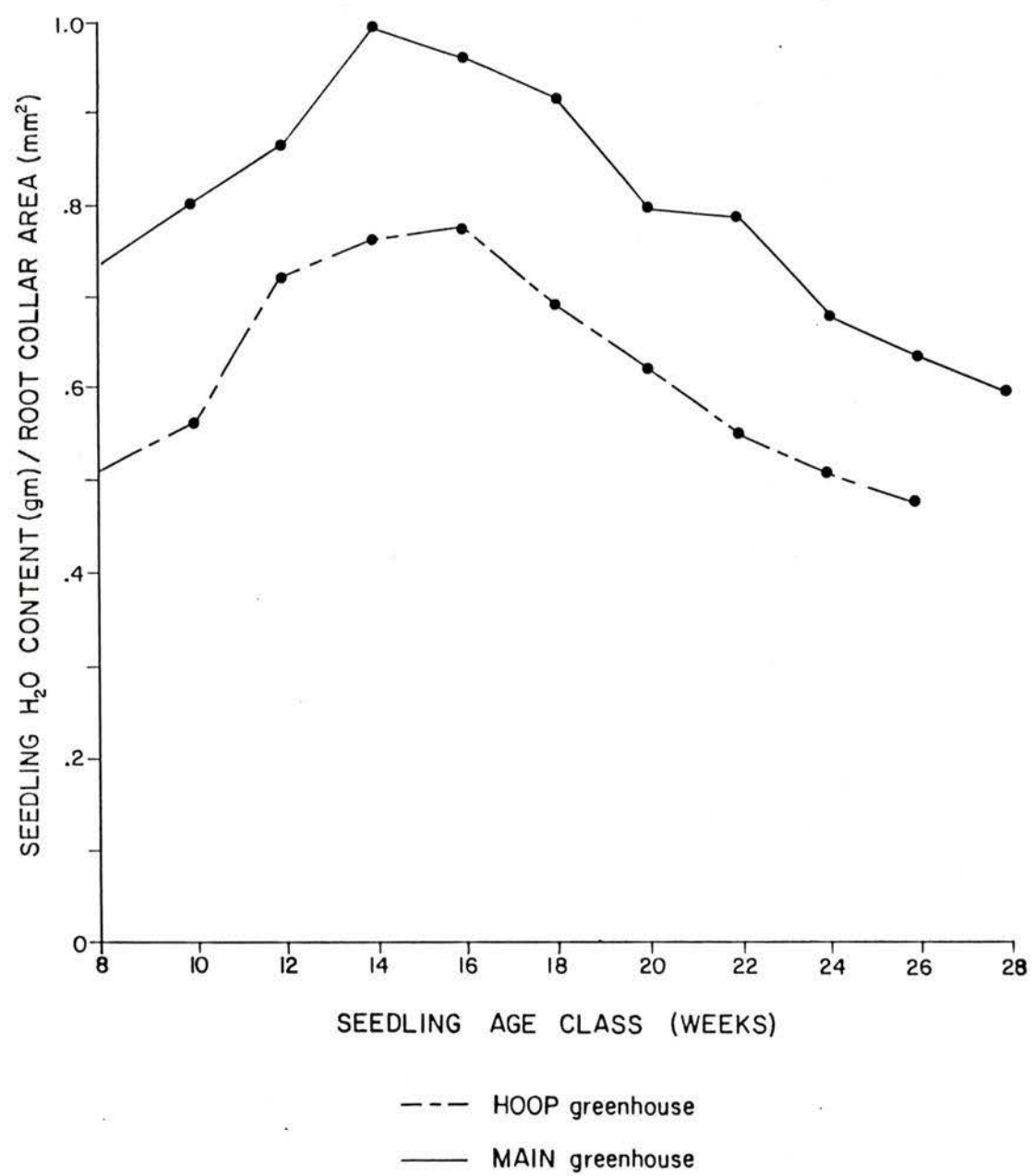


Figure 12. Seedling water content (gm) as a function of root collar area (mm²) versus age class for the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses.

house. Seedlings were most succulent at age class 14 weeks in the MAIN house and at 16 weeks in the HOOP house.

Airborne Spore Concentration

Paired t-tests were used to compare the significance of differences in average concentrations of airborne spores in the two greenhouses during six, 4 h periods (00:00-04:00, 04:00-08:00, 08:00-12:00, 12:00-16:00, 16:00-20:00, 20:00-24:00) in August, September and October. During August, there were no significant* differences in the airborne spore concentrations within the two greenhouses (Appendix IX). This held true for all of the daily 4 h periods.

During September and October, spore densities in the MAIN house were much greater than those in the HOOP house from 04:00-08:00, 08:00-12:00 and 12:00-16:00. Because of the high standard deviations for the counts in the two greenhouses at these times however, the differences in the spore numbers were significant only at $P = 0.2$ (Appendix X).

During October, MAIN house airborne spore numbers were greater than the HOOP house from periods 04:00-08:00, 08:00-12:00 and 12:00-16:00 (Appendix X). Large variations in the mean spore densities made it necessary to perform the t-tests at $P = 0.2$ to detect statistically significant differences between greenhouses.

* $P = 0.05$ unless otherwise stated.

Diurnal patterns of airborne spore density are shown in Figure 13. Maximum densities occurred during the daytime in each greenhouse during all three months. In August, mean spore densities in each greenhouse were highest between 08:00 and 12:00. Small difference in spore density between the two greenhouses, confirmed by the t-tests, is readily apparent. During September and October, a large difference occurred in mean atmospheric spore densities between greenhouses (Figure 13). Spore densities were higher in the MAIN house than in the HOOP house, between 04:00 and 20:00.

Regression analysis. Pearson product-moment correlation coefficients were determined for climatic variables and the LOG_{10} of the airborne spore density (LSPS) for each greenhouse (Table 3). Correlations and regressions were poor when untransformed spore numbers were used. Thus a LOG_{10} transformation was used to correct for heterogeneity of variance of the dependent variable (spore numbers). In each greenhouse, correlations were higher between ambient conditions and LSPS than between canopy microclimate and LSPS. Higher correlations between the climatic variables and LSPS were found for the MAIN house, with the best correlations being during September and October. The best correlations between the independent variables and LSPS occurred in the MAIN greenhouse during September and October and during those periods when the irrigation was on. The highest correlation coefficients were between LSPS and ambient climatic variables.

Forward, stepwise, multiple regression analysis (MRA) was used to regress LSPS on the climatic factors observed only during the periods

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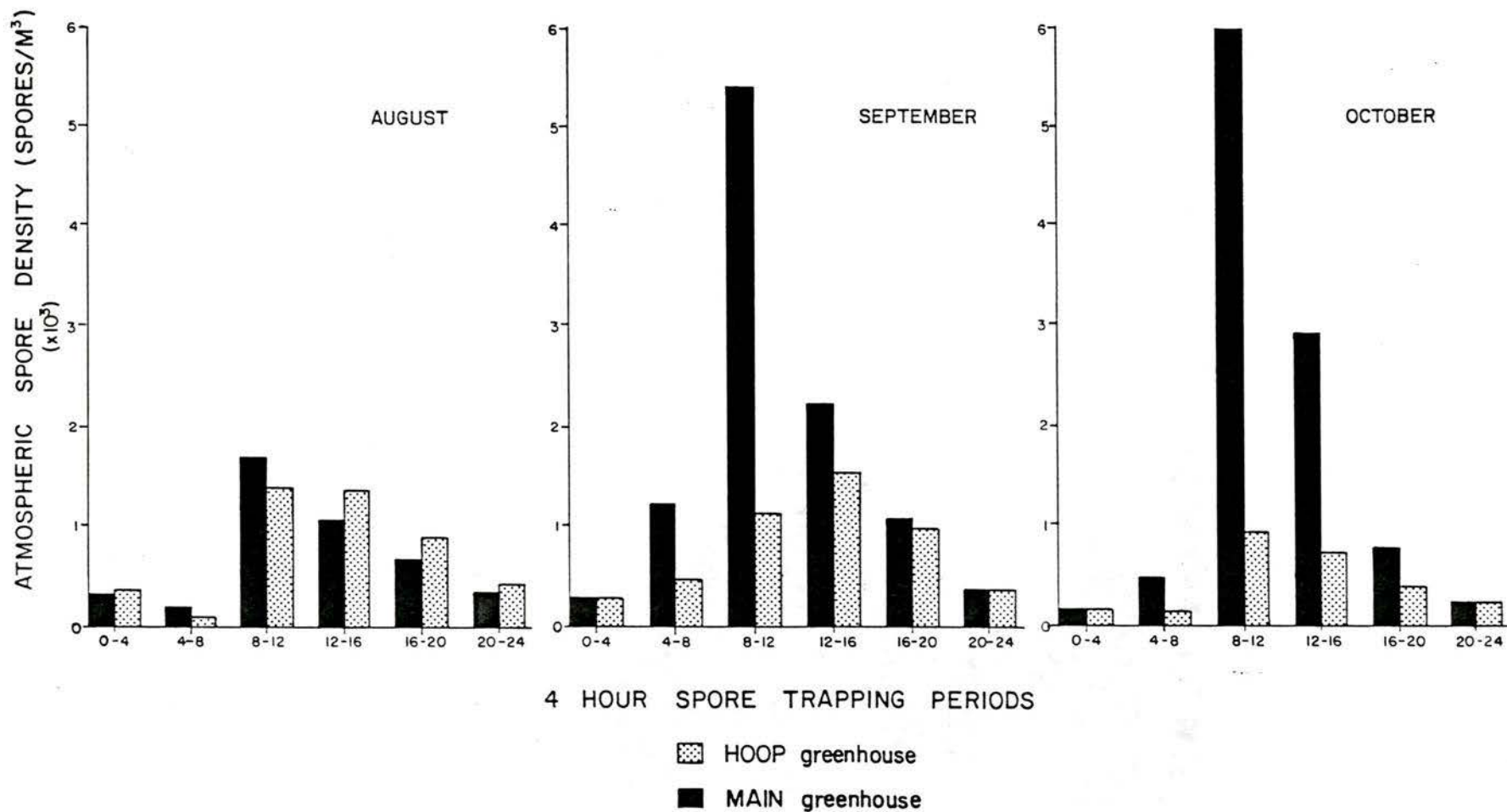


Figure 13. Diurnal pattern of atmospheric spore density inside the HOOP (polyethylene-covered) and MAIN (fibreglass-covered) greenhouses from August to October.

Table 3. Pearson product-moment correlation coefficients between LOG_{10} of airborne Botrytis cinerea spore densities and climatic variables.

Climatic Variables	HOOP		MAIN			
	August-October		August-October		September-October	
	All times	Irrigation on only	All times	Irrigation on only	All times	Irrigation on only
Canopy temperature	0.44*	-0.40	0.30*	-0.53*	0.34*	-0.55*
Ambient temperature	0.49*	-0.26	0.33*	-0.58*	0.38*	-0.71*
Canopy humidity	-0.27*	0.13	-0.11*	-0.06	-0.08	0.27
Ambient humidity	-0.39*	0.11	-0.23*	0.62*	-0.23*	0.78*
Canopy vapour pressure	0.43*	-0.39	0.28*	-0.54*	0.35*	-0.57*
Ambient vapour pressure	0.47*	-0.26	0.30*	-0.59*	0.37*	-0.72*
Canopy vapour pressure deficit	0.24*	-0.12	0.13*	0.07	0.13*	-0.29
Ambient vapour pressure deficit	0.35*	0.19	0.17*	-0.63*	-0.24*	-0.76*

* P = 0.05

when the irrigation was on in each greenhouse. The best parameters (independent variables) for use in a multiple regression model were canopy humidity (CH) and ambient vapour pressure deficit (AVD).

In the MAIN greenhouse during September and October, an R^2 value of 0.65 and a residual standard error of 0.27 were obtained using the multiple regression equation: $LSPS = 2.48 + 0.02 CH - 0.09 AVD$. Because a grey mould epidemic was observed in the MAIN house during September and October, spore and climatic data for these months were used in the MRA. Sample size was limited to 12. Despite this however, increased evidence that LSPS is a function of CH and AVD can be seen in Table 4. A decrease in residual standard error and an increase in R^2 can be seen moving from the HOOP house, to the MAIN house from August to October, and to the MAIN house during September and October. The F value for the regression model was 8.21 ($P = 0.01$). The regression coefficient for CH was significantly different from zero at only $P = 0.2$ while the coefficient for AVD was significant at $P = 0.01$. Figure 14 shows that the actual LSPS values for each observation all lie within the 95% confidence intervals of the values predicted using the regression equation.

The multiple regression analysis is consistent with the correlations in that it indicates humidity is closely related to spore concentrations. Also, the regression coefficient for AVD (the most important in the model) is different from zero at the greatest significance level. As with the correlations, a parameter of the ambient

rather than canopy conditions appears to be most readily associated with spore concentrations.

Table 4. Regression coefficients, residual standard errors and coefficients of multiple determination (R^2) for three multiple regression equations in which LOG_{10} of airborne *Botrytis cinerea* spores are predicted using canopy humidity (CH) and ambient vapour pressure deficit (AVD) from August to October in the HOOP greenhouse and from August to October and September to October in the MAIN greenhouse.

	GREENHOUSE		
	HOOP	MAIN	
	August-October	August-October	September-October
Regression constant	3.04	5.54	2.48
Regression coefficients			
CH	0.0001	-0.01	0.02
AVD	0.01	-0.10	-0.09
Residual standard error	0.61	0.53	0.27
R^2	0.04	0.42	0.65

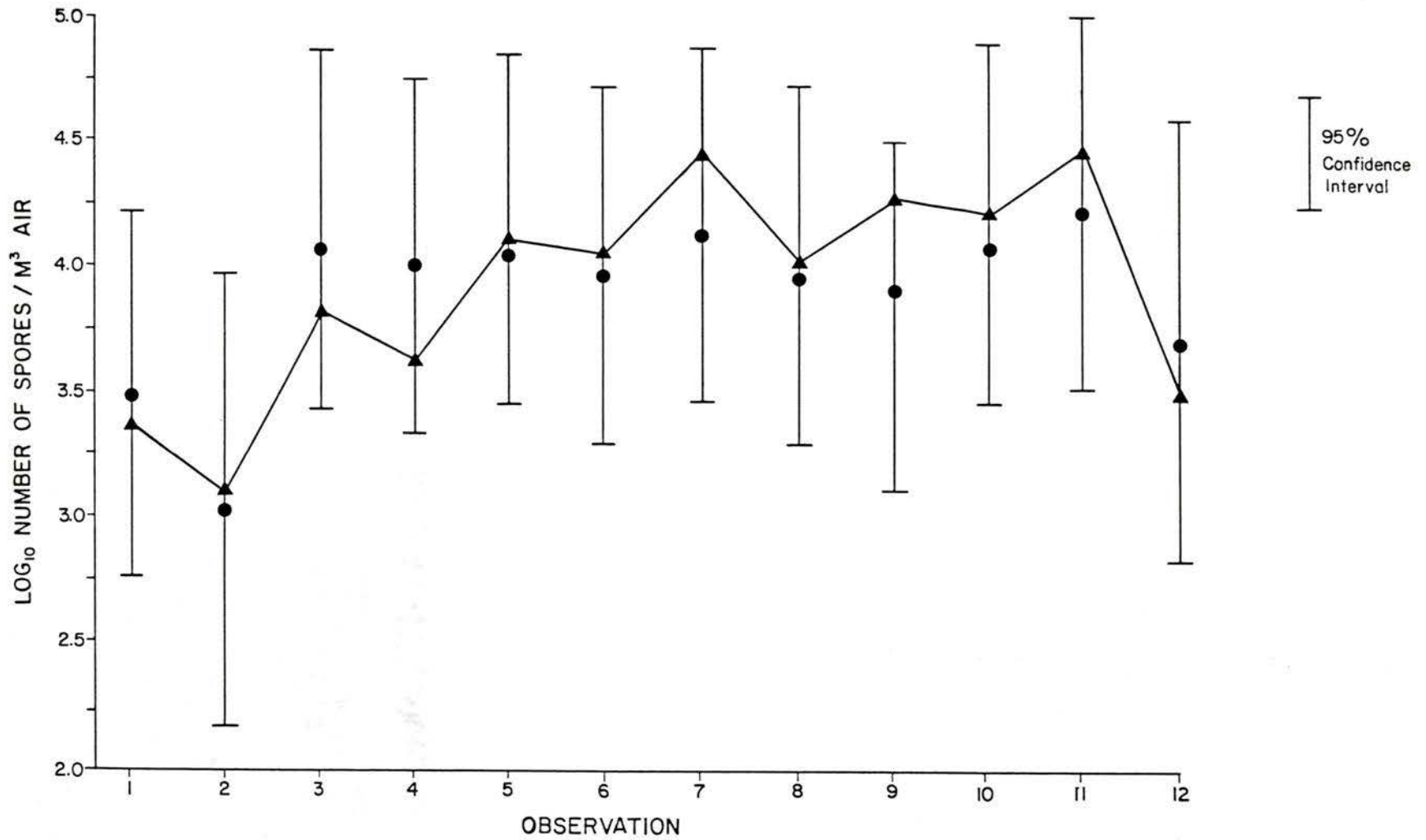


Figure 14. Actual (▲) and predicted (●) values for airborne *Botrytis cinerea* spore concentrations in the MAIN (fibreglass-covered) greenhouse during September and October.

CHAPTER 5

DISCUSSION

Climatic Variables

Light

The polyethylene-covered HOOP house transmitted light more readily in all bandwidths than did the fibreglass-covered MAIN house (Figures 4 and 5 and Table 1). Corrugated fibreglass has a lower transmittance than ultraviolet-stabilized polyethylene (Walker and Slack, 1970). Also, a large percentage of the light transmitted through fibreglass is diffuse. In 1985, the HOOP house was covered with a layer of new 100 μm , ultraviolet-stabilized polyethylene. The MAIN greenhouse fibreglass panels were installed in 1981. Variations of degradation in the coverings due to age differences and debris buildup plus differences in the transmission properties of the coverings account for the observed differences.

The mean intensity of ultraviolet (NUV) light (300-420 nm), which promotes B. cinerea sporulation (Tan and Epton, 1973), was 2.6 times greater above the canopy in the HOOP house than in the MAIN house. This suggests that the HOOP house environment was more favourable for grey mould sporulation than the MAIN house was. However, when present, B. cinerea sporulates at the bottom of the seedling canopy. Here, radiation intensities were a function of both the amount of light at the top of the canopy, and canopy height and density. Near ultraviolet radiation in this region was most intense in the HOOP house. From July

17 onwards the radiation flux densities beneath each canopy were much lower than the minimums of $150 \mu\text{W}/\text{cm}^2$ NUV found to promote B. cinerea sporulation (Tan, 1974a) and $250 \mu\text{W}/\text{cm}^2$ B light found to reverse induction (Table 1).

Although NUV light was readily transmitted through each greenhouse roof, intensities at each canopy base were reduced to levels below those affecting sporogenesis or its reversal (Tan, 1974a). Apparently light is not very significant in the epidemiology of grey mould of Douglas-fir container seedlings. Intensities of NUV could be reduced above the canopy using ultraviolet-absorbing film (UVA film) as suggested by Honda et al. (1977). These studies examined the control of grey mould on greenhouse cucumber and tomato by inhibiting sporulation. Here, B. cinerea occurs on fruit at the top of the canopy, unlike grey mould of container-grown Douglas-fir where sporulation occurs in the canopy. Thus, the use of UVA film would not likely be effective for controlling grey mould on greenhouse-grown Douglas-fir.

Air Temperature

The ambient air temperature in the MAIN greenhouse was significantly higher ($P = 0.05$) than that in the HOOP house during July and October, but temperatures were not significantly different in the two houses during August and September. Canopy temperature was significantly higher ($P = 0.05$) in the MAIN greenhouse during September and October, but not earlier in July and August. Although ambient and canopy temperature differences between greenhouses differed significantly

($P = 0.05$) during certain months, the greatest of these differences was only 1.1°C , thus temperature was likely of little importance in grey mould development. During these months when temperature differences between greenhouses were apparent, less day-to-day variability and thus, low standard deviations, accounted for their significance at the 0.05 level.

Monthly diurnal changes in ambient temperature (Figure 6) followed the same pattern for each greenhouse with daily maximums occurring about 16:00 and minimums between 04:00 and 08:00. Diurnal variation in canopy temperature also followed the same pattern in each greenhouse. However, canopy temperature in the HOOP house exceeded that of the MAIN during the day and fell below that of the MAIN canopy at night (Figure 7). The number of hours per day that HOOP canopy temperature exceeded that of the MAIN decreased from July through to October. The phenomena of higher daytime canopy temperatures can be attributed to the greater overall transmissivity of the HOOP house covering and thus, better penetration of solar radiation. Also, the sparser canopy in the HOOP house allowed better shortwave radiation penetration and subsequent increased warming. As the density of the canopy in the HOOP house increased (as the trees grew), there was a decrease in the number of hours per day its temperature remained above that of the MAIN house canopy.

Despite the above differences, temperatures between July 1 and September 31 were often optimum for B. cinerea spore germination both above and within the canopies in both greenhouses.

Humidity

During July, August, September and October, ambient RH in the MAIN greenhouse was significantly higher than in the HOOP ($P=0.05$). However, only during July was this difference of practical importance. There was low variability in the ambient RH's of the two houses, thus differences of 3% RH or less were statistically significant during August, September and October. This same pattern of small variation between greenhouses was observed with canopy RH. At $P = 0.05$, the RH in the canopy in the MAIN house was significantly higher than in the HOOP house during July. No significant differences were observed for the next three months.

Biologically important differences in ambient and canopy RH between the greenhouses were only observed during July. From August 1 onwards, with the seedling canopy completely closed in each greenhouse, differences in canopy RH were of little practical significance.

Diurnal patterns of ambient and canopy RH (Figures 9 and 10) closely followed those of ambient and canopy temperature, reflecting the temperature dependence of RH. The higher humidities in the canopy, i.e., compared to ambient humidities, likely result from a combination of seedling transpiration, their slowness to lose trapped irrigation water and decreased air movement within (below) the canopy. Relatively constant rates of transpiration, and a wet canopy for long periods, would also result in less daily fluctuation in canopy RH.

Ambient and canopy vapour pressure deficit (VPD) closely followed daily patterns of temperature and RH. However, daily variations in VPD

were much greater, with coefficients of variation 3.5 to 6.5 times those of ambient and canopy RH. Systems that equilibrate to the humidity of the air, such as the water content of porous materials are most appropriately related to RH (Unwin, 1980). Here the relationships at equilibrium are nearly independent of temperature. Small temperature changes can make small relative changes to RH. However, VPD is often more appropriate for dynamic situations where noticeably significant biological events (e.g., sporulation) often follow small humidity changes.

For a given temperature change, greater relative change in VPD occurs than for RH. Thus, the coefficients of variation were greater for daily mean VPD than for RH.

Table 2 shows the mean hours per day for each month that conditions were optimum for B. cinerea spore germination (RH \geq 98% and temperature 15-20°C). These conditions are more important than temperature or humidity values alone because they indicate relative differences between greenhouses when conditions were ideal for grey mould infection (establishment by spores). By September 1, grey mould was well established in the MAIN greenhouse and it was monitored for the remainder of the observation period. No grey mould was seen in the HOOP house at any time. Clearly a much greater frequency of hours per day with ideal spore germination conditions occurred each month in the MAIN house than did in the HOOP house. This is explained by the higher midday canopy RH's encountered in the MAIN house (Figure 10).

Infection via B. cinerea conidia can be very rapid (Hennebert and Gilles cited in Coley-Smith et al., 1980). With optimum conditions, Hennebert and Gilles found that conidia germinated on ripe strawberries within 90 min. of inoculation. Nelson (1951), working with grapes, found that infection of wet leaves (fruit), incubated at an RH exceeding 94%, occurred whether the wet period was 1 h or 18 h. Factors such as these that promote initial infection sometimes occur long before the appearance of visual symptoms of grey mould (Jarvis cited in Coley-Smith et al., 1980). This quiescent or latent period, as defined by Van der Plank (1963), is the time needed for one generation of a pathogen, i.e., the period from the arrival of infective propagules at the host surface until the new fungus colony is able to produce spores. The time needed for symptoms to appear is the incubation period.

Because conditions were often optimum, initial infection of seedlings via conidia in the MAIN greenhouse could have occurred during July and August. This may have been followed by a period of pathogen latency prior to new spore-producing colonies in September. Quite likely, grey mould did not occur in the HOOP house because conditions were not ideal for sufficient periods of time for conidia germination.

Host Shoot Growth and Water Content

Seedling shoot growth was continuous until age classes 17 weeks in the MAIN house and 16 weeks in the HOOP house. Throughout the growing season, the greater height of the MAIN house seedlings (Figure 11) resulted in a more dense canopy there. A taller and more dense canopy

accounts for less shortwave radiation penetration and reduced light intensities at the base of the MAIN house canopy (Table 1).

No differences between MAIN and HOOP house canopy temperature were detected during July; however, the canopy RH in the MAIN house was higher in July. For the next three months, after seedling shoot growth had stopped and the canopy had closed, no differences in canopy RH's were apparent between the greenhouses. The combination RH \geq 98% and temperature of 15-20°C which are optimum for B. cinerea conidia germination was most often present in the canopy in the MAIN house during July and August. The predominance of these conditions in the MAIN house likely resulted from the taller seedlings there creating a denser canopy.

Throughout the growing season, MAIN house seedlings contained about 30% more water (grams per mm² of root collar area) than those of the HOOP house. MAIN house seedlings had this higher water content during July and August (age class 14-22 weeks). High tissue water content can facilitate germ tube or mycelial penetration of host tissue (Herling cited in Jarvis, 1977).

The taller seedlings in the MAIN house resulted in a more dense and fully closed canopy by July 1. Because less of the total available solar radiation penetrated the canopy, daytime canopy temperatures were lower in the MAIN house. Most importantly, the more dense canopy likely restricted air movement. Reduced air circulation, combined with the slightly lower temperatures, produced higher canopy RH's. The higher water content in the MAIN house, plus the ideal conditions for

grey mould infection almost certainly accounted for the grey mould outbreak there. From September 1 onwards, changes in the airborne spore load in the MAIN greenhouse suggest that a quiescent or latent stage of the grey mould may have been present during July and August.

Airborne Spore Concentration

The airborne spore concentrations in the two greenhouses did not differ during August ($P = 0.05$), suggesting a common outside source of spores. In the early morning, during periods of dew evaporation and accompanying humidity changes, conidia are probably released from B. cinerea sporulating on dead material around the nursery. This is consistent with the pattern of B. cinerea spore release in a raspberry plantation observed by Jarvis (1962). Following increased concentrations of outside spores, daytime heating and convective forces, coinciding with operation of the ventilation fans, result in peak spore densities at about 12:00 in the greenhouses (Figure 13). The greenhouses apparently function as large traps, concentrating spores drawn in by the ventilating fans.

During September and October, diurnal spore densities were highest in each greenhouse between 04:00 and 20:00 (Figure 13). A large increase in the number of spores per m^3 occurred in the MAIN house during these two months. The diurnal pattern of spore density in the HOOP house during August, September and October likely resulted from spores being drawn in from outside. However, the increase in spore density in the MAIN house during September and October resulted from

sporulating grey mould colonies within the greenhouse releasing B. cinerea conidia.

In the MAIN house, but not the HOOP house, spore numbers increased dramatically during the application of overhead irrigation water. An irregular irrigation schedule based upon seedling demand for water resulted in high variations of spore density from day to day during September and October. Thus, in addition to high spore density values in the MAIN house, there were associated increases in standard deviation each month. Consequently, although it was intuitively obvious that airborne spore load in the MAIN house was often much greater than in the HOOP house, during September and October the between-greenhouse differences were not statistically significant at $P = 0.05$ (Appendices X and XI).

An association between irrigation times and increased numbers of spores in the MAIN house was clearly evident. Correlation and multiple regression analysis (MRA) also indicated associations between some microclimatic parameters and airborne spore load. The best correlations occurred between spore numbers and ambient rather than canopy parameters (Table 3). Changes to the canopy microclimate by the irrigating did not become apparent as quickly as those to the ambient conditions did. Thus, any irrigation-induced spore release was more closely associated with ambient microclimatic elements than with those in the canopy.

Observations of spore density occur in a time series with release usually building to a peak level and then declining over time (Royle

and Thomas, 1972). Thus, successive observations are not likely to be independent of one another; independence being a condition required in regression analysis. Grouping of spore data into 4-h time periods helped overcome this problem; however, total independence of each dependent variable observation was not a certainty.

Regression analysis also requires that each conditional distribution be normal with equal variance. This requirement for homogeneity of variance cannot always be met with arithmetic spore data. The variance of spore counts likely increases with increased spore concentration (Royle and Thomas, 1972). Accordingly, LOG_{10} values of spore counts were used (LSPS) to correct for heteroskedasticity. This procedure is common with MRA of microclimate and spore data (Royle and Thomas, 1972; Sutton, 1981; Sutton *et al.*, 1983).

Pearson product-moment correlation coefficients indicate the greatest association between LSPS and climatic variables occurred during September and October while the irrigation was on (Table 3). Also, the signs of the coefficients changed to expected values during these times. The correlations were also best, i.e., more statistically significant when the water was on. Correlations between LSPS and ambient conditions were higher than those between LSPS and canopy conditions in the MAIN house from August through to October. This is most apparent during the September and October while the irrigation was on.

Ambient microclimate was most closely associated with LSPS. Because ambient rather than canopy conditions respond more rapidly to the application of irrigation water and irrigating generates higher

spore loads, it appears that ambient conditions affect or are related to spore release. However, spores were trapped above the canopy, not within it. Thus, ambient conditions can only be associated with the release of spores from the canopy. These spores were most likely previously liberated through mechanisms such as hygroscopic twisting of conidiophores (Jarvis, 1962), triggered by changes in canopy microclimate.

The ambient climatic conditions present with the irrigation on, also occurred many times with the irrigation off. Therefore, the large increases in spore density associated with watering, were more likely due to mechanical effects upon the canopy rather than microclimate. Because noticeable increases in spore density coincided with watering, LSPS correlated relatively well with current ambient conditions. Prior liberation of conidia within the canopy explains lower correlation coefficients between LSPS and canopy climatic variables than between LSPS and ambient conditions.

Multiple regression analysis was used to regress LSPS on the climatic factors observed while the irrigation was on in each greenhouse. The best regression equation from the MAIN greenhouse data for September and October was: $LSPS = 2.48 + 0.02CH - 0.09AVD^*$. Limitations must be placed upon interpreting the MRA because the irrigation water was applied only 12 times during September and October. Thus,

* LSPS = LOG_{10} of spore density. CH = canopy humidity. AVD = ambient vapour pressure deficit.

rather than being a predictive or descriptive model, the MRA is best used to indicate trends between LSPS and climatic variables.

An R^2 value of 0.65 for the MRA indicates that 65% of the variation in LSPS can be accounted for by a combination of canopy relative humidity and ambient vapour pressure deficit in the MAIN house during September and October while the irrigation was on.

The variation in LSPS explained by the MRA, increased in the HOOP house during August through to October, in the MAIN house during the same months, and in the MAIN house during September and October (Table 4). The signs of the coefficients took on expected values during September and October in the MAIN house.

With the irrigation on, the intercept portion of the regression equation (2.48) can be seen to indicate background values of LSPS. Variations in LSPS are accounted for by the regression coefficients CH and AVD. The larger coefficient for AVD ($P = 0.004$) was included in the model with a greater degree of confidence than the smaller, less influential coefficient for CH ($P = 0.2$). The model indicates with the greatest confidence that a decrease in AVD will have more effect upon LSPS than would a corresponding increase in CH. Use of the regression equation shows the predicted values of LSPS for all 12 observations were within the 95% confidence limits of the actual values (Figure 14).

The indication that vapour pressure deficit is more important than relative humidity with regard to spore release is consistent with observations made by Unwin (1980). He states that with many dynamic

systems, significant biological events often follow small humidity changes. For a given temperature change, greater relative change in VPD occurs than for RH. Because of this, and its greater variability, i.e. it has a greater range than RH, VPD appears to be a more sensitive indicator of spore release. A more rapid response to the irrigation by ambient rather than canopy climate also helps account for the greater significance of AVD in the MRA. Ambient vapour pressure deficit appears to be the best indicator of increased spore densities associated with greenhouse irrigation. Despite the limitations of the MRA, the model is consistent with the correlations and helps explain observed trends. As with the correlation coefficients, the MRA indicates that humidity strongly affects spore concentrations. As with the correlations, a parameter of the ambient rather than canopy conditions appears to most readily be related to spore densities.

CHAPTER 6

CONCLUSIONS

Conclusions

Production of conifer seedlings in container nurseries has altered the nursery microclimate so that diseases such as grey mould have increased in importance. To investigate this problem, four research questions posed were: (i) is the greenhouse climate favourable for Botrytis cinerea disease outbreaks; (ii) what values of climatic elements are related to or are precursors of Botrytis outbreaks; (iii) does the disease cycle within the greenhouse or does it rely on outside inoculum; and (iv) are open-sided, polyethylene-covered or open-sided fibreglass-covered greenhouses more favourable for the disease?

Having addressed the four stated research objectives, it is possible to hypothesize about the occurrence of grey mould on container-grown Douglas-fir seedlings. Taller, succulent seedlings created a more dense canopy in the MAIN house than the HOOP house. Less total solar radiation penetrating the MAIN than the HOOP house roof, contributed to slightly lower ambient MAIN house temperatures. Also, more time with ideal conditions for spore germination was present more often in the MAIN house canopy. This results from lower canopy penetration of available sunlight, increased transpiration and restricted air movement in the denser canopy. Thus, during July and August, when spore density was the same in each greenhouse, spores from a common

outside source were better able to infect seedlings in the MAIN house than the HOOP house.

From September 1 onwards, grey mould was observed in the MAIN but not the HOOP greenhouse. Sporulation was evident despite low intensities of NUV light observed at the canopy base. Light apparently has little direct effect upon the grey mould fungus on container-grown seedlings. Sufficient NUV light is excluded at the canopy base (the site of B. cinerea sporulation) that the use of ultraviolet-absorbing film for grey mould control would be ineffective.

Increased spore densities in the MAIN greenhouse during September and October resulted from a grey mould outbreak. Although spores were not trapped within the canopy, this is likely where major secondary spread of the disease occurred via conidial and mycelial infection.

Large increases in the number of trapped spores coincided with irrigation time and the best correlations were obtained between trapped spores and ambient rather than canopy conditions. This indicates that ambient conditions likely affect the release of spores from the canopy where previously, canopy microclimate influenced their liberation from the fungus.

With the irrigation water on, multiple regression analysis indicated spore density to be a function of canopy humidity and ambient vapour pressure deficit. The limited number of observations used in the MRA makes prediction difficult. However, increased spore densities during watering are most likely due to mechanical effects of the water on the canopy. Coinciding with the irrigation was a decrease in

ambient vapour pressure deficit and an increase in canopy humidity. These two climatic variables, the ones most markedly influenced by the watering, were thus included in the MRA.

Following grey mould establishment in the MAIN house, the existence of favourable climatic conditions enabled it to cycle within the greenhouse. Secondary spread via conidia and mycelium was enhanced by the release of conidia from the canopy during watering.

In summary:

- Greenhouse microclimate can be favourable for grey mould outbreaks.
- Differences in greenhouse covering had a minimal direct effect upon grey mould of Douglas-fir seedlings.
- Most important to disease incidence was a dense canopy that restricted air circulation. The result was increased humidity and the occurrence of free water on the host as well as increased times of ideal conditions for spore germination.
- For Botrytis cinerea spores entering the greenhouse from outside, ideal conditions for germination were most often present on high-water-content seedlings comprising a dense canopy.
- Once established within a greenhouse, grey mould can cycle on its own. Progress of an internal epidemic is enhanced by the use of overhead irrigation.
- No single element could be identified as a predictor of disease occurrence. However, those microclimatic variables most readily

affected by the irrigation water were also most closely associated with increased spore densities.

Recommendations

Several recommendations can be directed to greenhouse growers and those interested in future research.

For greenhouse growers:

- Seedlings should be managed to minimize height and succulence. This will reduce canopy density and humidity and create conditions that are less favourable for infection by spore germination.
- Monitoring canopy temperature and humidity during mid-summer (July-August) would enable the use of profilactic fungicides to prevent grey mould establishment.
- Less fungicide may be applied into a less dense canopy early in the season than if used later to eradicate any established grey mould.
- Because one pass of the irrigation boom can significantly increase the airborne spore load, applying fungicides via the boom may increase the potential for secondary infection.
- The efficient filtering of NUV and B light by the seedling canopy precludes using UV-absorbing film as a control for grey mould on container-grown Douglas-fir seedlings.

For future research:

- Research should be aimed at examining ways of preventing optimum conditions for B. cinerea spore germination.
- Efficacy trials of seedling spacing may provide insight into reducing canopy humidity while maintaining the effectiveness of the canopy as an NUV filter.
- Detailed examination of canopy conditions associated with infection would provide better understanding of early latent infections.
- Investigation of secondary disease spread would enable more effective control of established grey mould.
- Trapping spores within the canopy while monitoring microclimate during irrigation times would better define mechanisms of spore release.

Grey mould occurring on container-grown Douglas-fir seedlings has resulted from environmental change due to technological innovation. This study has demonstrated the value of a research approach beyond the confines of one specific discipline. Future research into host/pathogen relationships may also benefit from the examination of environmental conditions conducive to such associations.

FOOTNOTES

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APPENDIX I

AMBIENT RELATIVE HUMIDITY CORRECTION

MAIN Greenhouse

Sample size (N)	10
Curve fit	Logarithmic
Coefficient of Determination (r^2)	0.80
Standard Error of Estimate (Se)	8.93
Regression Equation	RH (%) (corrected) = -83.99 + 35.92 log RH (%) (observed)

HOOP Greenhouse

Sample Size (N)	11
Curve Fit	Logarithmic
Coefficient of Determination (r^2)	0.78
Standard Error of Estimate (Se)	7.91
Regression Equation	RH (%) (corrected) = -143.54 + 47.08 Log RH (%) (observed)

APPENDIX II

CANOPY PSYCHROMETER CALIBRATION

MAIN Greenhouse

<u>Wet Bulb</u>		<u>Dry Bulb</u>	
N	16	N	16
Curve fit	Linear	Curve fit	Linear
r^2	0.9	r^2	0.99
Intercept (a)	1.73	a	0.38
Slope (b)	0.85	b	0.97

Two-tailed t-test

$$H_0: \text{slope } (b_1) = 1 \quad (b_{11})$$

$$H_a: b_1 \neq b_{11}$$

$$\alpha = 0.01$$

$$t^* = -1.984$$

$$t_{\gamma}(0.995; 14) = \pm 2.977$$

accept H_0

$$H_0: b_1 = b_{11}$$

$$H_a: b_1 \neq b_{11}$$

$$\alpha = 0.01$$

$$t^* = -0.7407$$

$$t_{\gamma}(0.995; 14) = \pm 2.977$$

accept H_0

HOOP Greenhouse

<u>Wet Bulb</u>		<u>Dry Bulb</u>	
N	16	N	16
Curve fit	Linear	Curve fit	Linear
r^2	0.98	r^2	0.95
a	1.07	a	1.66
b	0.9	b	0.89

Two-tailed t-test

$$H_0: b_1 = b_{11}$$

$$H_a: b_1 \neq b_{11}$$

$$\alpha = 0.01$$

$$t^* = -2.94$$

$$t_{\gamma}(0.995; 14) = \pm 2.977$$

\therefore accept H_0

$$H_0: b_1 = b_{11}$$

$$H_a: b_1 \neq b_{11}$$

$$\alpha = 0.01$$

$$t^* = -2.144$$

$$t_{\gamma}(0.995; 14) = \pm 2.977$$

\therefore accept H_0

APPENDIX III

SEEDLING MORPHOLOGY

MAIN Greenhouse

<u>Age Class Week</u>	<u>Height cm</u>	<u>Root Collar Area mm²</u>	<u>Water Content gm</u>	<u>Water/Root Collar Area gm/mm²</u>
8	9.0	1.72	1.27	0.738
10	13.6	2.78	2.23	0.802
12	18.8	4.01	3.46	0.863
14	22.7	5.27	5.16	0.980
16	26.7	6.20	5.96	0.961
18	34.2	8.76	8.0	0.913
20	33.2	8.76	6.99	0.798
22	33.3	10.01	7.97	0.796
24	35.2	8.19	5.55	0.678
26	34.9	11.34	7.13	0.629
28	35.5	12.95	7.72	0.596

Means of 20 samples each observation

HOOP Greenhouse

<u>Age Class Week</u>	<u>Height cm</u>	<u>Root Collar Area mm²</u>	<u>Water Content gm</u>	<u>Water/Root Collar Area gm/mm²</u>
8	7.1	1.61	0.82	0.509
10	8.4	1.99	1.12	0.563
12	8.9	2.43	1.75	0.720
14	12.8	3.66	2.79	0.762
16	19.4	4.52	3.49	0.772
18	17.7	4.41	3.03	0.687
20	18.5	5.56	3.47	0.624
22	20.3	5.81	3.20	0.551
24	18.1	6.51	3.29	0.505
26	19.7	6.56	3.14	0.479

Means of 20 samples each observation

APPENDIX IV

AIRBORNE SPORE DENSITY CALCULATION

Area of tape exposed in 4 h 722 mm²

Area of 4 h tape segment microscopically scanned 10.26 mm²

Area scanned = 0.014 total area exposed

Spores counted = 0.014 total spores trapped

Total spores trapped = $\frac{\text{Spores counted}}{0.014}$

Trap is 80% efficient

Spores trapped = 0.8 spores drawn in

Spores drawn in = $\frac{\text{Spores trapped}}{0.8}$

Trap draws in 10 L/min.

= 600 L/h

= 2400 L/4 h

= 2.4 m³/4 h

#Spores/m³ 4 h = $[(\text{Spores counted}/0.014)/0.8]/2.4 \text{ m}^3$

APPENDIX V

DAILY MEAN AMBIENT TEMPERATURE

Paired comparisons t-test between the HOOP and MAIN greenhouses.

July

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 3.13$$

$$t_{\gamma, 0.05(2)}(30) = \pm 2.042$$

$$t^*(3.13) > t_{\gamma, 0.05} (\pm 2.042)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean ambient temperature between the HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 3.13$$

$$t_{\gamma, 0.05(1)}(30) = 1.697$$

$$t^*(3.13) > t_{\gamma, 0.05} (1.697)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean ambient temperature in the MAIN house is greater than in the HOOP house.

August

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 1.90$$

$$t_{\gamma,0.05(2)}(30) = \pm 2.042$$

$$t_{\gamma,0.05}(-2.042) < t^*(1.90) < t_{\gamma,0.05}(+2.042)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean ambient temperature between the HOOP and MAIN greenhouses.

September

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 15$$

$$df = 14$$

$$t^* = 1.71$$

$$t_{\gamma,0.05(2)}(14) = \pm 2.145$$

$$t_{\gamma,0.05}(-2.145) < t^*(1.71) < t_{\gamma,0.05}(+2.145)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean ambient temperature between the HOOP and MAIN greenhouses.

October

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 2.25$$

$$t_{\gamma, 0.05(2)}(30) = \pm 2.042$$

$$t^*(2.25) > t_{\gamma, 0.05}(\pm 2.042)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean ambient temperature between the HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 2.25$$

$$t_{\gamma, 0.05(1)}(30) = 1.697$$

$$t^*(2.25) > t_{\gamma, 0.05}(1.697)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: daily mean ambient temperature in the MAIN house is greater than in the HOOP house.

APPENDIX VI

DAILY MEAN CANOPY TEMPERATURE

Paired comparisons t-test between the HOOP and MAIN greenhouses.

July

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = -1.162$$

$$t_{\gamma,0.05(2)(30)} = \pm 2.042$$

$$t_{\gamma,0.05}(-2.042) < t^*(-1.162) < t_{\gamma,0.05}(+2.042)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean ambient temperature between the HOOP and MAIN greenhouses.

August

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 1.52$$

$$t_{\gamma,0.05(2)(30)} = \pm 2.042$$

$$t_{\gamma,0.05}(-2.042) < t^*(1.52) < t_{\gamma,0.05}(+2.042)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean ambient temperature between the HOOP and MAIN greenhouses.

September

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 15$$

$$df = 14$$

$$t^* = 2.94$$

$$t_{\gamma,0.05(2)}(14) = \pm 2.145$$

$$t^*(2.94) > t_{\gamma,0.05}(\pm 2.145)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean ambient temperature between the HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu \text{ MAIN} \leq \mu \text{ HOOP}$$

$$H_a: \mu \text{ MAIN} > \mu \text{ HOOP}$$

$$\alpha = 0.05$$

$$n = 15$$

$$df = 14$$

$$t^* = 2.94$$

$$t_{\gamma,0.05(1)}(14) = 1.761$$

$$t^*(2.94) > t_{\gamma,0.05}(1.761)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean canopy temperature in the MAIN house is greater than in the HOOP house.

October

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 4.72$$

$$t_{\gamma, 0.05(2)(30)} = \pm 2.042$$

$$t_{\gamma}(4.72) > t_{\gamma, 0.05}(\pm 2.042)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean canopy temperature between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu \text{ MAIN} \leq \mu \text{ HOOP}$$

$$H_a: \mu \text{ MAIN} > \mu \text{ HOOP}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 4.72$$

$$t_{\gamma, 0.05(1)(30)} = 1.697$$

$$t^*(4.72) > t_{\gamma, 0.05} (1.697)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean canopy temperature in the MAIN house is greater than in the HOOP house.

APPENDIX VII

DAILY MEAN AMBIENT RELATIVE HUMIDITY

Paired comparisons t-test between the HOOP and MAIN greenhouses.

July

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 5.28$$

$$t_{\gamma,0.05(2)}(30) = \pm 2.042$$

$$t^*(5.28) > t_{\gamma,0.05}(\pm 2.042)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean ambient RH between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu \text{ MAIN} \leq \mu \text{ HOOP}$$

$$H_a: \mu \text{ MAIN} > \mu \text{ HOOP}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 5.28$$

$$t_{\gamma,0.05(1)}(30) = 1.697$$

$$t^*(5.28) > t_{\gamma,0.05}(1.697)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean ambient RH in the MAIN house is greater than in the HOOP house.

August

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 2.85$$

$$t_{\gamma, 0.05(2)}(30) = \pm 2.042$$

$$t^*(2.85) > t_{\gamma, 0.05}(\pm 2.042)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean ambient RH between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 2.85$$

$$t_{\gamma, 0.05(1)}(30) = 1.697$$

$$t^*(2.85) > t_{\gamma, 0.05}(1.697)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean ambient RH in the MAIN house is greater than in the HOOP house.

September

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 15$$

$$df = 14$$

$$t^* = 3.71$$

$$t_{\gamma, 0.05(2)}(14) = \pm 2.145$$

$$t^*(3.71) > t_{\gamma, 0.05}(\pm 2.145)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean ambient RH between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 15$$

$$df = 14$$

$$t^* = 3.71$$

$$t_{\gamma, 0.05(1)}(14) = 1.761$$

$$t^*(3.71) > t_{\gamma, 0.05}(1.761)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean ambient RH in the MAIN house is greater than in the HOOP house.

October

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 5.4$$

$$t_{\gamma, 0.05(2)(30)} = \pm 2.042$$

$$t_{\gamma}(5.4) > t_{\gamma, 0.05}(\pm 2.042)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean ambient RH between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 5.4$$

$$t_{\gamma, 0.05(1)(30)} = 1.697$$

$$t^*(5.4) > t_{\gamma, 0.05}(1.697)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean ambient RH in the MAIN house is greater than in the HOOP house.

APPENDIX VIII

DAILY MEAN CANOPY RELATIVE HUMIDITY

Paired comparisons t-test between the HOOP and MAIN greenhouses.

July

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 6.55$$

$$t_{\gamma,0.05(2)}(30) = \pm 2.042$$

$$t^*(6.55) > t_{\gamma,0.05}(\pm 2.042)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean canopy RH between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 6.55$$

$$t_{\gamma,0.05(1)}(30) = 1.697$$

$$t^*(6.55) > t_{\gamma,0.05}(1.697)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean canopy RH in the MAIN house is greater than in the HOOP house.

August

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 0.06$$

$$t_{\gamma,0.05(2)}(30) = \pm 2.042$$

$$t_{\gamma,0.05}(-2.042) < t^*(0.06) < t_{\gamma,0.05}(+2.042)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean canopy RH between HOOP and MAIN greenhouses.

September

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 15$$

$$df = 14$$

$$t^* = 0.026$$

$$t_{\gamma,0.05(2)}(14) = \pm 2.145$$

$$t_{\gamma,0.05}(-2.145) < t^*(0.026) < t_{\gamma,0.05}(+2.145)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in the daily mean canopy RH between HOOP and MAIN greenhouses.

October

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = -1.35$$

$$t_{\gamma, 0.05(2)(30)} = \pm 2.042$$

$$t_{\gamma, 0.05}(-2.042) < t^*(-1.35) < t_{\gamma, 0.05}(+2.042)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean canopy RH between HOOP and MAIN greenhouses.

APPENDIX IX

MEAN AIRBORNE SPORE LOAD DURING
DAILY FOUR-HOUR PERIODS - AUGUST

Paired comparisons t-test between the HOOP and MAIN greenhouses.

00:00-04:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 26$$

$$df = 25$$

$$t^* = -0.21$$

$$t_{\gamma,0.05(2)}(25) = \pm 2.06$$

$$t_{\gamma,0.05}(-2.06) < t^*(-0.21) < t_{\gamma,0.05}(+2.06)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

04:00-08:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 26$$

$$df = 25$$

$$t^* = 1.53$$

$$t_{\gamma,0.05(2)}(25) = \pm 2.06$$

$$t_{\gamma,0.05}(-2.06) < t^*(1.53) < t_{\gamma,0.05}(+2.06)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

08:00-12:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 26$$

$$df = 25$$

$$t^* = 1.1$$

$$t_{\gamma,0.05(2)}(25) = \pm 2.06$$

$$t_{\gamma,0.05}(-2.06) < t^*(1.1) < t_{\gamma,0.05}(+2.06)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

12:00-16:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 26$$

$$df = 25$$

$$t^* = -0.96$$

$$t_{\gamma,0.05(2)}(25) = \pm 2.06$$

$$t_{\gamma,0.05}(-2.06) < t^*(-0.96) < t_{\gamma,0.05}(+2.06)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

16:00-20:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 26$$

$$df = 25$$

$$t^* = -0.77$$

$$t_{\gamma,0.05(2)}(25) = \pm 2.06$$

$$t_{\gamma,0.05}(-2.06) < t^*(-0.77) < t_{\gamma,0.05}(+2.06)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

20:00-24:00

Two tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 26$$

$$df = 25$$

$$t^* = -1.26$$

$$t_{\gamma,0.05(2)}(25) = \pm 2.06$$

$$t_{\gamma,0.05}(-2.06) < t^*(-1.26) < t_{\gamma,0.05}(+2.06)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

APPENDIX X

MEAN AIRBORNE SPORE LOAD DURING
DAILY FOUR-HOUR PERIODS - SEPTEMBER

Paired comparisons t-test between the HOOP and MAIN greenhouses.

00:00-04:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = -0.052$$

$$t_{\gamma,0.05(2)}(29) = \pm 2.045$$

$$t_{\gamma,0.05}(-2.045) < t^*(-0.052) < t_{\gamma,0.05}(+2.045)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

04:00-08:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = 1.688$$

$$t_{\gamma,0.05(2)}(29) = \pm 2.045$$

$$t_{\gamma,0.05}(-2.045) < t^*(1.688) < t_{\gamma,0.05}(+2.045)$$

Accept H_0 with $\alpha = 0.05$

$$\alpha = 0.1$$

$$t_{\gamma,0.1(2)}(29) = \pm 1.699$$

$$t_{\gamma,0.1}(-1.699) < t^*(1.688) < t_{\gamma,0.1}(+1.699)$$

Accept H_0 with $\alpha = 0.1$

$$\alpha = 0.2$$

$$t_{\gamma,0.2(2)}(29) = \pm 1.311$$

$$t^*(1.688) > t_{\gamma,0.1}(\pm 1.311)$$

Reject H_0 . With $\alpha = 0.2$ conclusion: there is a significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = 1.688$$

$$t_{\gamma, 0.05(1)}(29) = 1.708$$

$$t^*(1.688) > t_{\gamma, 0.05}(1.708)$$

Accept H_0 with $\alpha = 0.05$

$$\alpha = 0.1$$

$$t_{\gamma, 0.1(1)}(29) = 1.316$$

$$t^*(1.688) > t_{\gamma, 0.1}(1.316)$$

Reject H_0 . With $\alpha = 0.1$ conclusion: daily mean airborne spore load is higher in the MAIN house than in the HOOP house.

08:00-12:00

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = 1.818$$

$$t_{\gamma, 0.05(2)}(29) = \pm 2.045$$

$$t_{\gamma, 0.05}(-2.045) < t^*(1.818) < t_{\gamma, 0.05}(+2.045)$$

Accept H_0 with $\alpha = 0.05$

$$\alpha = 0.1$$

$$t_{\gamma, 0.1(2)}(29) = \pm 1.699$$

$$t^*(1.818) > t_{\gamma, 0.1}(\pm 1.699)$$

Reject H_0 . With $\alpha = 0.1$ conclusion: there is a significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = 1.818$$

$$t_{\gamma, 0.05(1)}(29) = 1.699$$

$$t^*(1.818) > t_{\gamma, 0.05}(1.699)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean airborne spore load is higher in the MAIN house than in the HOOP house.

12:00-16:00

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = 1.386$$

$$t_{\gamma, 0.05(2)}(29) = \pm 2.045$$

$$t_{\gamma, 0.05}(-2.045) < t^*(1.386) < t_{\gamma, 0.05}(+2.045)$$

Accept H_0 with $\alpha = 0.05$

$$\alpha = 0.1$$

$$t_{\gamma, 0.1(2)}(29) = \pm 1.699$$

$$t_{\gamma, 0.1}(-1.699) < t^*(1.386) < t_{\gamma, 0.1}(+1.699)$$

Accept H_0 with $\alpha = 0.1$

$$\alpha = 0.2$$

$$t_{\gamma, 0.2(2)}(29) = \pm 1.311$$

$$t^*(1.386) > t_{\gamma, 0.2}(\pm 1.311)$$

Reject H_0 . With $\alpha = 0.2$ conclusion: there is a significant difference in the airborne spore load between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = 1.386$$

$$t_{\gamma, 0.05(1)(29)} = 1.699$$

$$t^*(1.386) < t_{\gamma, 0.05(1.699)}$$

Accept H_0 with $\alpha = 0.05$

$$\alpha = 0.1$$

$$t_{\gamma, 0.1(1)(29)} = 1.316$$

$$t^*(1.386) > t_{\gamma, 0.1(1.316)}$$

Reject H_0 . With $\alpha = 0.1$ conclusion: daily mean airborne spore load is higher in the MAIN house than in the HOOP house.

16:00-20:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = 0.804$$

$$t_{\gamma, 0.05(2)(29)} = \pm 2.045$$

$$t_{\gamma, 0.05(-2.045)} < t^*(0.804) < t_{\gamma, 0.05(+2.045)}$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

20:00-24:00

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 30$$

$$df = 29$$

$$t^* = -0.033$$

$$t_{\gamma, 0.05(2)}(29) = \pm 2.045$$

$$t_{\gamma, 0.05}(-2.045) < t^*(-0.033) < t_{\gamma, 0.05}(+2.045)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

APPENDIX XI

MEAN AIRBORNE SPORE LOAD DURING
DAILY FOUR-HOUR PERIODS - OCTOBER

Paired comparisons t-test between the HOOP and MAIN greenhouses.

00:00-04:00

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = -0.05$$

$$t_{\gamma,0.05(2)}(30) = \pm 2.042$$

$$t_{\gamma,0.05}(-2.045) < t^*(-0.05) < t_{\gamma,0.05}(+2.042)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

04:00-08:00

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 1.42$$

$$t_{\gamma,0.05(2)}(30) = \pm 2.042$$

$$t_{\gamma,0.05}(-2.042) < t^*(1.42) < t_{\gamma,0.05}(+2.042)$$

Accept H_0 with $\alpha = 0.05$

$$\alpha = 0.1$$

$$t_{\gamma,0.1(2)}(30) = \pm 1.697$$

$$t_{\gamma,0.1}(-1.697) < t^*(1.42) < t_{\gamma,0.1}(+1.697)$$

Accept H_0 with $\alpha = 0.1$

$$\alpha = 0.2$$

$$t_{\gamma,0.2(2)}(30) = \pm 1.31$$

$$t^*(1.42) > t_{\gamma,0.2}(\pm 1.31)$$

Reject H_0 . With $\alpha = 0.2$ conclusion: there is a significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

One-tailed t-test

$$\begin{aligned}
 H_0: \mu_{\text{MAIN}} &< \mu_{\text{HOOP}} \\
 H_a: \mu_{\text{MAIN}} &> \mu_{\text{HOOP}} \\
 \alpha &= 0.05 \\
 n &= 31 \\
 df &= 30 \\
 t^* &= 1.42 \\
 t_{\gamma, 0.05(1)}(30) &= 1.697 \\
 t^*(1.42) &> t_{\gamma, 0.05}(1.697)
 \end{aligned}$$

$$\begin{aligned}
 \text{Accept } H_0 &\text{ with } \alpha = 0.05 \\
 \alpha &= 0.1 \\
 t_{\gamma, 0.1(1)}(30) &= 1.31 \\
 t^*(1.42) &> t_{\gamma, 0.1}(1.31)
 \end{aligned}$$

Reject H_0 . With $\alpha = 0.1$ conclusion: daily mean airborne spore load is higher in the MAIN house than in the HOOP house.

08:00-12:00

Two-tailed t-test

$$\begin{aligned}
 H_0: \mu_d &= 0 \\
 H_a: \mu_d &\neq 0 \\
 \alpha &= 0.05 \\
 n &= 31 \\
 df &= 30 \\
 t^* &= 1.49 \\
 t_{\gamma, 0.05(2)}(30) &= \pm 2.042 \\
 t_{\gamma, 0.05}(-2.042) &< t^*(1.49) < t_{\gamma, 0.05}(+2.042)
 \end{aligned}$$

$$\begin{aligned}
 \text{Accept } H_0 &\text{ with } \alpha = 0.05 \\
 \alpha &= 0.1 \\
 t_{\gamma, 0.1(2)}(30) &= \pm 1.697 \\
 t_{\gamma, 0.1}(-1.697) &< t^*(1.49) < t_{\gamma, 0.1}(+1.697)
 \end{aligned}$$

$$\begin{aligned}
 \text{Accept } H_0 &\text{ with } \alpha = 0.1 \\
 \alpha &= 0.2 \\
 t_{\gamma, 0.2(2)}(30) &= \pm 1.31 \\
 t^*(1.49) &> t_{\gamma, 0.2}(\pm 1.31)
 \end{aligned}$$

Reject H_0 . With $\alpha = 0.2$ conclusion: there is a significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 1.49$$

$$t_{\gamma, 0.05(1)}(30) = 1.697$$

$$t^*(1.49) < t_{\gamma, 0.05}(1.697)$$

Accept H_0 with $\alpha = 0.05$

$$\alpha = 0.1$$

$$t_{\gamma, 0.1(1)}(30) = 1.31$$

$$t^*(1.49) > t_{\gamma, 0.1}(1.31)$$

Reject H_0 . With $\alpha = 0.1$ conclusion: daily mean airborne spore load is higher in the MAIN house than in the HOOP house.

12:00-16:00

Two-tailed t-test

$$H_0: \mu d = 0$$

$$H_a: \mu d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 2.73$$

$$t_{\gamma, 0.05(2)}(30) = \pm 2.042$$

$$t^*(2.73) > t_{\gamma, 0.05}(\pm 2.042)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in the airborne spore load between HOOP and MAIN greenhouses.

One-tailed t-test

$$H_0: \mu_{\text{MAIN}} \leq \mu_{\text{HOOP}}$$

$$H_a: \mu_{\text{MAIN}} > \mu_{\text{HOOP}}$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 2.73$$

$$t_{\gamma, 0.05(1)(30)} = 1.697$$

$$t^*(2.73) > t_{\gamma, 0.05}(1.697)$$

Reject H_0 . With $\alpha = 0.05$ conclusion: daily mean airborne spore load is higher in the MAIN house than in the HOOP house.

16:00-20:00

Two-tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 1.13$$

$$t_{\gamma, 0.05(2)(30)} = \pm 2.042$$

$$t_{\gamma, 0.05}(-2.042) < t^*(1.13) < t_{\gamma, 0.05}(+2.042)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is no significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

20:00-24:00

Two tailed t-test

$$H_0: \mu_d = 0$$

$$H_a: \mu_d \neq 0$$

$$\alpha = 0.05$$

$$n = 31$$

$$df = 30$$

$$t^* = 0.14$$

$$t_{\gamma, 0.05(2)}(30) = \pm 2.042$$

$$t_{\gamma, 0.05}(-2.042) < t^*(-0.14) < t_{\gamma, 0.05}(+2.042)$$

Accept H_0 . With $\alpha = 0.05$ conclusion: there is a significant difference in daily mean airborne spore load between HOOP and MAIN greenhouses.

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Forest Nursery Seedlings

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AUGUST 25, 1986
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