EPIDEMIOLOGY OF FUSARIUM FRUIT AND STEM ROT OF GREENHOUSE GROWN SWEET PEPPER

by

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Abstract

Spore monitoring, using Burkard Continuous Recording Air Samplers, in Lower Mainland commercial pepper crops during three different growing seasons strongly suggests that ascospores of *Nectria haematococca* are both the primary and secondary inoculum. *Fusarium solani* spores were seldom detected and apparently play a minor role, if any, in the spread of Fusarium fruit and stem rot. No ascospores could be detected in monitored greenhouses upon completion of the end-of-season cleanup procedures indicating that detectable spore inoculum was not being carried over from one growing season to the next in commercial greenhouses. In “clean” greenhouses, which obtained seedlings produced by one propagator, high ascospore levels were detected with the introduction of the new crop. No symptoms were observed on the new seedling plants but perithecia of *N. haematococca* were observed on the surface of the rockwool cubes in which the seedlings were growing. Spores trapped from these perithecia were pathogenic to pepper seedlings and caused typical fruit rot in pathogenicity tests. Thus, rockwool cubes contaminated during propagation are one way of introducing the fungus into “clean” commercial greenhouses.

Under controlled conditions of temperature and relative humidity (RH) ascospore discharge from moistened diseased fruit tissues was not inhibited by RH as low as 51% (the lowest tested). Ascospore discharge from air-dried diseased fruit tissue occurred only at RH ≥95%. Temperatures in the range of 15 to 30°C were not inhibitory for ascospore discharge, however, maximum discharge occurred at the lowest temperature tested (15°C). Ascospores discharged onto glass slides maintained at 55-60% RH (typical RH observed during the cleanup) survived from 6 to 18 days, depending upon temperature. Under “typical”
greenhouse conditions ascospores remained viable on glass slides for up to 12 days. Ascospore germination occurred at all temperatures tested (15-30°C), but germination was greater and occurred more quickly at higher temperatures. Substantial ascospore germination occurred only during prolonged periods (8-12 h) of very high (>98%) relative humidity. Therefore, although perithecia of *N. haematococca* maybe present on the cubes from the early stage of the growing cycle, losses due to Fusarium fruit and stem rot will depend on the greenhouse climate management. Ascospore discharge from the rockwool cubes, which are constantly saturated with nutrient solution, can occur independent of ambient RH. Therefore, aggressive climate management, which avoids extended periods of high RH, apparently reduces disease by preventing ascospore germination rather than preventing ascospore discharge.
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1. INTRODUCTION

Fusarium stem and fruit rot of greenhouse-grown sweet pepper is a fungal disease caused by *Fusarium solani* (Mart.) Sacc., which has *Nectria haematococca* Berk. & Broome as its perfect stage. It has generally been considered a minor disease associated with poor climate management. Therefore, details of the disease cycle have not been worked out and no special control measures have been recommended. However, since about 1990, for unknown reasons, the disease has been an increasing problem in greenhouse pepper production in both Europe and North America. In the Lower Mainland of British Columbia, Fusarium fruit and stem rot of greenhouse-grown pepper has increased in incidence and severity to the point where in one year it caused losses estimated between 1 and 2 kg/m² ($30,000 to 60,000/ha). The observed losses pointed out the need for control measures based on a thorough knowledge of the disease. Thus, the objective of this research was to determine how the pathogen is introduced into the new crops, how it spreads and specific conditions favoring its spread.
2. LITERATURE REVIEW

2.1. THE SPECIES *FUSARIUM SOLANI*

The species *Fusarium solani* is among the most common and widely distributed fungi on earth. It is mainly a soil inhabitant, but it can also be isolated from air, water and diverse organic substrates. It typically produces septate mycelium with macroconidia and microconidia. Under adverse conditions, chlamydospores (so-called survival spores) are also produced. The aerial mycelium is grayish-white, striate and sparse to dense. Potato Dextrose Agar typically develops a blue to bluish-brown discoloration. *Fusarium solani* is distinguished from other *Fusarium sp.* by the exact shape and size of its macroconidia and by the manner in which its macroconidia, microconidia, and chlamydospores are produced (Booth, 1971; Tousson and Nelson, 1976). All three spore types can be soil-borne and air-borne. The perfect stage of *Fusarium solani* is *Nectria haematococca*, which belongs to the class Pyrenomycetes - the flask fungi. The perithecia of *Nectria haematococca* are irregularly globose, pale orange to red and produce sexual spores, called ascospores, in groups of eight within an ascus (Booth, 1971). The ascospores are ellipsoid to obovate, two celled, 11-18 x 4-7 μm. There are hyaline but become light brown, slightly constricted at the single central septum, and develop longitudinal striations when mature. Because the ascus is a turgid cell that finally bursts violently liberating its spores *N. haematococca* ascospores are air-borne.

The imperfect stage, *Fusarium solani*, has been isolated from an extremely wide range of plants (Booth, 1971). Considerable physiologic specialization with respect to host plants has been demonstrated. Therefore, on the basis of host-specificity, pathogenic *F.*
solani are divided into formae speciales. Some of the more economically important are *F. solani* f.sp. *phaseoli* which causes root rot of beans, *F. solani* f. sp. *pisi* which causes rot of peas, *F. solani* f. sp. *eumartii* which causes dry rot of potatoes and *F. solani* f. sp. *cucurbitae* which causes root rot of cucurbits (Booth, 1971).

Although the imperfect stage of the fungus has long been known as a pathogen on numerous hosts (Snyder, 1934), its perfect stage *N. haematococca* has been described from very few hosts. The *Nectria* stage was first reported on trees (van der Goot, 1935). Only in 1961 was it found on a herbaceous plant (pumpkin) (Dingley, 1961). *N. haematococca* was also observed on passion fruit in Uganda (Emechebe and Mukiiibi, 1976) and Southern Florida (Ploets, 1991). More recently *Nectria haematococca* has been implicated in the sudden death syndrome of soybean (Abney et al., 1993). Finally, the *Nectria* stage has also been described from the greenhouse-grown sweet pepper (Fletcher, 1994; Jarvis, 1994).

### 2.2. ETIOLOGY AND EPIDEMIOLOGY OF FUSARIUM FRUIT AND STEM ROT OF GREENHOUSE-GROWN SWEET PEPPER

Fruit, stem and crown rot of greenhouse-grown pepper was first reported in Hungary (Lukács and Szarka, 1988). The same disease was also observed in greenhouse pepper production in Netherlands (Anonymous, 1989) and England (Fletcher, 1994). In 1990 the disease first appeared in Canada, in the greenhouse pepper crops in Ontario, where losses of up to 50% were noted (Jarvis, 1994). In British Columbia the disease was first observed in 1992 (R. Copeman, personal comm.). The causal agent was found to be the fungus *Fusarium solani* (Mart.) Sacc., which has *Nectria haematococca* Berk. & Broome as its perfect stage (Jarvis, 1994).
In all cases dark brown to black somewhat sunken lesions occurred at the junction of the rockwool blocks and the stem. These gradually spread until the plant was girdled. The root system of diseased plants was unaffected. The rot also occurred higher up the stem, usually at wounds resulting from careless leaf and axillary shoot removal or harvesting operations. The fruit rot generally started beneath the calyx and then spread to the shoulder of the fruit. The calyx lobes became brown and curled upwards. Diseased fruits contained fewer seeds, which were discolored brown.

In reports from Hungary (Lukács and Szarka, 1988), England (Fletcher, 1994) and Ontario (Jarvis, 1994), fruit, stem and crown lesions often have superficial whitish mycelium and sporodochia of *Fusarium solani* and reddish-brown perithecia of *Nectria haematococca*. In contrast to these reports, in the Lower Mainland of British Columbia, sporulation, both asexual and sexual, was not commonly observed on the stem lesions and was observed on the crown lesions only late in the season (R. Copeman, personal comm.). However, ascospores, macroconidia and microconidia were present on the fruit lesions. All three spore types were equally able to cause typical disease symptoms when freshly broken stubs of stems or picked fruit were inoculated (R. Copeman, personal comm.).

R. Copeman (personal comm.) has confirmed that species *F. solani* is responsible for crown, stem and fruit rot phase of the disease locally. Local pepper isolates of *F. solani* did not cause root or crown symptoms in young tomato, lettuce, cucumber and bean plants in replicated greenhouse trials. Therefore, the pathogen responsible for crown, stem and fruit rot of the greenhouse-grown peppers could be a new *forma speciales* of *Fusarium solani*, which has a host range limited to *Capsicum annuum*. 
A survey conducted by R. Copeman (personal comm.), as well as the earlier report from England (Fletcher, 1994), suggests that all of the commercial greenhouse-grown pepper cultivars are susceptible to *Fusarium solani*. Whether varying degrees of susceptibility to the disease exist among the cultivars is not known. Although it is mainly a problem in greenhouses, Fusarium fruit and stem rot has been found in field-grown peppers as well (Lukács and Szarka, 1988; Moens, 1990; Micosa and Ilag, 1977; Steekelenburg, 1976). Interestingly, the *Nectria* stage was not observed on the field-grown peppers.

Because Fusarium fruit and stem rot disease until recently has been considered of negligible economic importance, very little is known about its epidemiology. None of the published papers provides experimental evidence concerning survival of the fungus and its spread in the crop. Also, no attempts have been made to determine the conditions required for disease initiation and no control measures have been worked out.

Identification of sources of inoculum of *Fusarium solani* for greenhouse-grown peppers is an important first step in disease management. Control strategies based on sanitation and the eradication of the primary inoculum could be developed. Specific information on environmental conditions for spore release and germination is essential for a complete understanding of the initiation of epidemics and for any success in identifying conditions that constitute the infection period. With this knowledge, the greenhouse growers may be able to reduce disease spread by modifying the climate to avoid conditions favorable for spore germination and/or dispersal.

A puzzling aspect of this disease is that it appears first in the spring on maturing fruit at varying heights in the canopy. Plants having single, diseased fruit are randomly scattered in
the greenhouse and do not have stem or crown lesions (R. Copeman, personal comm.). The pattern of disease incidence in the greenhouse suggests that primary inoculum is airborne but which spore type(s) is (are) being spread and where the inoculum it is coming from is not known.

In recent years, production of greenhouse peppers in the Canada has shifted from ground-bed culture to rockwool or sawdust hydroponic systems. In this culture system pepper transplants grown in small rockwool cubes are placed on bags of sawdust or slabs of rockwool substrate enclosed in white plastic sleeves which are positioned on a plastic-covered greenhouse floor. During the standard end-of-season cleanup procedure all crop debris is removed from the greenhouse and ideally (but not always) removed from the site. Depending on the greenhouse, the rockwool slabs are either reused or replaced with the new ones. The plastic used to cover the soil surface is changed every year or every second year. Irrigation lines and tanks are flushed with buffered bleach. Finally, the whole inside structure of the greenhouse is power washed with a detergent followed by the application of disinfectant such as buffered bleach or Virkon®.

Covering all the soil in the greenhouse with intact plastic sheeting eliminates the soil as the primary source of inoculum within the greenhouse. The rockwool slabs are a possible concern because some of the growers use them for more than one growing season without steaming.

*Fusarium solani* was not recovered from commercial seed but was recovered from apparently healthy root systems of a low percentage of 8-week old plants indexed from the local 1995 crop (R. Copeman, personal comm.). This finding suggests that the fungus could
be introduced in a latent form with the new plants. However, sporulation that could be responsible for occurrence of the early spring lesions on fruit a meter or more above the greenhouse floor was not observed on the young plants. R. Copeman was also sometimes able to recover *F. solani* from debris remaining in the greenhouse from the previous crop. However, the fungus was not sporulating on the debris and the rate of recovery was too low to make it the major source of inoculum. Sawdust samples taken at random from broken bags in a commercial greenhouse containing diseased plants were found to contain *F. solani* (R. Copeman, personal comm.). Disposal sites for contaminated growing media and diseased plants at the end of the season might be a potential inoculum source because spores formed on decomposing material could be carried back into the greenhouse by wind.

During the growing season the causal agent of Fusarium fruit and stem rot does not appear to be spread by recirculation of the nutrient solution (R. Copeman, personal comm.). The fact that diseased plants do not occur in groups throughout the growing season but are randomly scattered within the greenhouses suggests that the secondary inoculum is also spread by air currents. However, which spore type is being disseminated and the factors that govern its dissemination are not known.

According to the very limited number of studies on dispersal of plant pathogens in greenhouses, airborne epidemics in greenhouses often start with the entry of fungal spores from the outside environment. Rowe et al. (1977) in Ohio proved that initiation of epidemics of Fusarium crown and root rot in greenhouse-grown tomatoes could be attributed partly to airborne microconidia of *Fusarium oxysporum* from outside dump piles. Nelson et al. (1974) implicated wind-blown spores of *Fusarium roseum* ‘Graminearum’ or its perfect stage sp.
*Giberella* from an adjacent cornfield as the possible primary inoculum for Fusarium stub dieback of greenhouse-grown carnations. Peterson et al. (1988) found that spores of *Botrytis cinerea* infecting Douglas-fir seedlings in greenhouses originated from neighboring fields and greenhouses. Similar results were reported by Kerssies (1993) who worked with *B. cinerea* on greenhouse-grown crops of gerbera. Schepers (1984) suggested that overwintering inoculum of cucumber powdery mildew (caused by *Sphaerotheca fuliginea*) is possible, because cucumber plants are grown in greenhouses all year round. According to Schepers, dispersal of spores takes place by wind. Spores are introduced into disinfested and newly planted greenhouses through open doors and windows. These spores originate from greenhouses in which the cucumber crop has not yet been harvested. The spores could also be introduced into the clean commercial greenhouses with the new plants (Hausbeck, 1991, Schepers, 1984). In the greenhouses which had not been cleaned properly at the end of the growing season the epidemics could also start from the spores originating in debris of the previous crop (Rowe at al., 1977).

So far very little is known about the factors influencing dissemination of airborne spores within the greenhouses. Frinking and Scholte (1983) indicated that the complex dispersal process involves aspects of pathogen, host, environment and human activity. Hausbeck and Pennypacker (1991), working with greenhouse-grown potted geraniums, showed that grower activity (e.g. pruning, harvesting and spraying) in a greenhouse resulted in peak concentrations of conidia of *B. cinerea* in the greenhouse air. Water-splashing, mechanical disturbance and increased air movement are all well recognized agents in the release and dispersal of spores of many imperfect fungi (Meredith, 1973). Jarvis reported dispersal of conidia of *B. cinerea* associated with rapidly changing relative humidity (1980).
The same author suggested that as the air near the conidial apparatus dries out from a near-saturated state, conidiophores undergo twisting movements with the varying degrees of violence, which dislodges mature conidia from the conidiophores. Peterson et al. (1988) observed massive conidial release in *B. cinerea* brought about by overhead greenhouse irrigation.

To my knowledge none of the published studies has addressed the factors which influence dispersal of ascospores of pyrenomycetes within greenhouses (not even of any of ascomycetes). Nevertheless, according to numerous field observations, the dissemination of ascospores in pyrenomycetes depends upon several interrelated factors including the amount and duration of rainfall or irrigation, temperature, light and relative humidity (Alderman, 1993; Dobrev, 1987; Froyd and French, 1967; Johnson and Kuntz, 1979; Lortie and Kuntz, 1963; MacHardy and Gadoury 1986; Paulitz, 1996; Perrin, 1978; Raynal, 1991; Rowe and Beute, 1974; Sanderson, 1970;).

The relationship of ascospore discharge to wetting has been studied in a number of pyrenomycetes. Johnson and Kuntz (1979) showed that in *Eutypella parasitica*, which causes the canker of maple, a saturated environment (100% RH) alone will not induce discharge. Considerable amount of wetting (by rain or heavy dew) was necessary to initiate discharge from perithecia. High relative humidity, however, did influence the rate of drying of bark and prolonged discharge. Froyd and French (1967) observed a similar pattern in ejection of ascospores from Hypoxylon cankers and concluded that ascospores were formed and matured only when perithecia were moist. Rainfall, irrigation or heavy dew were also the most important factor initiating spore release in the perithecial pathogens *Claviceps purpurea*
Alderman, 1993), *Gnomonia erythrostoma* (Dobrev, 1987), *Nectria ditissima* (Perrin, 1978), *Gibberella zeae* (Reis, 1990) *Calonectria nivalis* (Sanderson, 1970). On rainless days, a sudden rise in relative humidity appeared to bring about ascospore discharge in *Gibberella zeae* (family Hypocreaceae) which is closely related to *Nectria sp.* (Paulitz, 1996). In the same fungus a decrease in water potential was also detrimental for the perithecial formation; it was maximum at -15 bars and almost completely inhibited below -50 bars (roughly corresponding to 98.9 and 96.5% RH, respectively) (Sung and Cook, 1981).

In spite of the fact that wetting seems necessary for discharge in so many pyrenomycetes, fruiting structures do not always continue to discharge at a high level once fully wetted. In some of them, it seems that a high level of discharge may occur only when the wetted fruiting bodies begin to dry, and therefore shrink. Lortie and Kuntzs (1963) claim that this occurs in *Nectria galligena*, but have been unable to demonstrate this phenomenon in the closely related species *Nectria cinabarina*. Similarly, in *Calonectria crotalariae* (the causal agent of stem and crown rot of peanuts), a rapid change in relative humidity (from 100% to 70 %) triggered massive discharge (Rowe and Beutte, 1974). In general ascospore release in most of the pyrenomycetes seems to be triggered by any sudden change in humidity whether it is a transition from long period of dry air to saturated air or vice versa.

Tschanz et al. (1975), working with perithecia of *Gibberella zeae*, reported that light has a profound influence but that it was second to wetness in triggering spore release. Appreciable discharge occurred only when wetted perithecia were exposed to a 12 h period of light. Very low spore discharge was noted after only 2 or 4 h of light. Similarly, Walker and Harvey (1966) found that a diurnal cycle of ascospore release in many Pyrenomycetes
could be induced by periods of dark and light. The tendency to release spores during the
dark or light periods varied from species to species. For example, ascospores of *Nectria
coccina* and *Xylaria longipis* were liberated in dark periods, whereas *Nectria cinnabarina*
liberated its spores in the light periods. Similarly, *Daldinia concentrica* has a nocturnal
rhythm of ascospore release that continued for a time after illumination ceased (Ingold,
1965). From the field studies, it seems that spore discharge in Pyrenomycetes tends to be
nocturnal, provided that water-supply is not limiting (Alderman, 1993; Paulitz, 1996; Raynal,
1991; Sanderson, 1970;).

In most examples where spore discharge in Pyrenomycetes has been examined
temperature was not the critical factor in determining whether or not spores were discharged,
although, it did influence the rate of discharge. For example, cool temperatures (11-20°C)
favored spore discharge from perithecia of *Gibberella zeae* (Tschanz et al., 1976), whereas
warmer temperatures (24-30°C) favored ascospore discharge in *Calonectria cotalariae*,
*Eutypella parasitica* (Johnson and Kuntz, 1979; Rowe and Beute, 1974).

In addition to knowing the factors which trigger spore release and influence the aerial
inoculum concentration, it is equally important to obtain estimates of the inoculum potential
which includes spore viability and the infection efficiency of viable spores under given
greenhouse conditions. As pointed out by Hirst and Stedman (1962), spore trapping usually
measures a “relative dose” of spores rather than “effective dose”. Given a susceptible host
and a virulent strain of the pathogen, the “effective dose” (the proportion of spores able to
cause infection) will vary with the climate regime and the condition of the host. Therefore,
even at times of abundant spore release, disease incidence could be low (Meredith, 1970;
Norse, 1971). For example, spores may be released during short periods of wetting or high relative humidity that may not be long enough to facilitate spore germination. Thus, deposited spores should be able to survive dry conditions until sufficiently long periods of free moisture or high relative humidity are available for germination.

The longevity of fungal spores, especially ascospores, has received considerable attention. Previous studies on ascospores of other fungi have shown that mortality of ascospores generally increased as the temperature increased (Caesar and Pearson, 1983; Johnson and Kuntz, 1979; Merc and Fergus, 1954; Patryka and Mai, 1962; Pearson et al., 1990). In addition, rapid change in temperature and humidity shortened the viability of ascospores of other species (Ceasar and Pearson, 1983; Merc, 1954). For example, the ascospores of *Eutypella parasitica* survived from 4 to 25 days depending on the temperature (Johnson and Kuntz, 1979). On the other hand, the viability of ascospores of *Calonectria crotalariae* dropped below 1% within 30 minutes after deposition (Rowe and Beute, 1974). Therefore, for this species sufficient moisture must be available almost immediately after spore deposition if appreciable germination is to occur. In *Guignardia bidwellii*, no germination was observed when leaves containing ascospores were dry for 48 h or more, prior to rewetting (Ferrin and Ramsdell, 1977). On the other hand, Semeniuk (1984) provided data indicating that ascospores of *Pseudopeziza medicaginis* on foliage in a hot dry greenhouse remained fully infective for at least 10 days.

Survival of conidia of *F. solani* and/or ascospores of *N. haematococca* is an important factor in the epidemiology of Fusarium fruit and stem rot because most commercial greenhouses are empty for 3-6 weeks, during the end-of-season cleanup. Spores of *F. solani*
and/or *N. haematococca* would have to remain viable for at least 21 days in order to span the gap between two growing seasons.

Although fungal spores generally require a moisture film on the plant surface for germination, some of them can germinate at high relative humidities. The germination process is also affected by temperature so that the interplay between temperature and periods of high relative humidity must be considered. For example, germination and germ tube growth of macroconidia of *Fusarium graminearum* and ascospores of its perfect stage *Gibberella zeae* took place at relative humidities above 95% (Sung and Cook, 1981). The spore germination of this fungus was greater and faster at higher temperatures (optimum 25-30°C). Ascospores of the pyrenomycetes *Gnomonia erythrostoma*, needed free water, or air humidity of >85%, for germination. However, at relative humidities below 95% the ascospores germinated only after 24 h and mass germination occurred after 36 h (Dobrev, 1987). When a requirement for plant surface wetness exists its duration also may be vital. A film of water or water droplets must persist for 3-6 h, depending on temperature (optimum 21-27°C), if substantial germination of ascospores of *Nectria galligena* is to occur (Lortie, 1964). By contrast germination of ascospores of *Venturia inaequalis* requires much longer periods of wetness, varying from 26 h at 5°C to 17 h at 25°C (Sys and Soenon, 1970). Therefore, it will be important to establish whether the spores of *N. haematococca* and/or *F. solani* require free water or periods of high relative humidity for germination and to determine the duration of these periods at different temperatures.

In the Lower Mainland of British Columbia the severity of Fusarium fruit and stem rot of the greenhouse grown peppers is sporadic. This disease is considered to be a problem in
some greenhouses while not in the others. Similarly, in a report from England, a crop near to the affected one of the same cultivar from the same propagation house was unaffected (Fletcher, 1994). The author speculated that the unaffected crop was generally grown in a more controlled environment and, in particular the relative humidity did not fluctuate as much. Such differences in disease severity between “problem” and “non problem” greenhouses could be extremely important because they suggest that high disease incidence and severity is avoidable. If we learn that certain growing environments or practices favor disease development, we may be able to use this information to develop a “disease avoidance program”. Ideally, Fusarium fruit and stem rot management would be achieved by altering current climate management and/or cultural practices so that the greenhouse environment would either prevent or deter disease initiation and subsequent spread.

The effect of the greenhouse climate management on a disease incidence has been addressed by several authors. For example, the probability of infection with *Botrytis cinerea*, which attacks both vegetable and flower crops has been shown to diminish as the period of the high humidity in the greenhouse decreases (Peterson et al., 1988; Yunis et al., 1990; Elad et al., 1992). According to Morgan (1984) and Winspear et al. (1970), nighttime ventilation, which lowered relative humidity, gave significant reductions in the incidence of *Botrytis cinerea* and *Cladosporium fulvum* in experimental greenhouse tomato crops, respectively. In addition to climate management, cultural practices such as watering regime could affect the incidence of disease. Overwatering of the growing medium can lower the defense system of the plants, through oxygen depletion (Agrios, 1988), and predispose them to pathogen attack. Root rot of *Phaseolus vulgaris* and *Pisum sativum* caused by *Fusarium solani* develops under conditions that reduce root growth such as anaerobic soil or drought (Burke
and Miller, 1983; Miller and Burke, 1985). Likewise, SDS (sudden death syndrome) of soybean also caused by *F. solani* has been associated with wet, cool weather which favors saturated, anaerobic soil conditions (Rupe, 1989).

The observation that the Fusarium fruit and stem rot disease appears in spring and again in the fall during the transition period when greenhouse climate is hard to control accurately is consistent with high moisture dependency. If poor climate (high relative humidity) is disease promoting as the pattern of disease incidence suggests, it is important to understand how relative humidity favors disease spread. Does a constant high relative humidity create sufficient periods of moisture to favor spore germination? Does a high relative humidity or fluctuating relative humidity facilitate the spore release, which enables spread by airborne spores?

Therefore, the objectives of this study were to:

1. Establish whether airborne spores dispersed from a source inside the commercial greenhouse and/or wind blown spores from an outside source serve as primary inoculum.

2. Determine whether the fungus is being introduced in a latent form with the new crop.

3. Establish which spore type is mainly responsible for spread during the growing season.

4. Establish the time of day or night when the spore dissemination occurs and whether this spread is correlated with the cultural practices or greenhouse climate change.

5. Determine and compare the environmental parameters affecting Fusarium fruit and stem rot epidemiology on greenhouse-grown peppers in “problem” and in “non-problem” greenhouses.
6. Determine the influence of environmental conditions on ascospore and/or conidia germination and viability, and correlate it with the greenhouse climate regime.

7. Determine the influence of environmental conditions on the release of spores of \textit{N. haematozoca} and/or \textit{F. solani} and correlate it with the greenhouse climate regime.
3. MATERIALS AND METHODS

3.1. SPORE SAMPLING AND MEASUREMENT OF ENVIRONMENTAL CONDITIONS

Burkard 24-hour Recording Air Samplers (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England) were used to monitor airborne dispersal of *Nectria haematococca* and/or *Fusarium solani* spores in commercial pepper greenhouses. To maintain anonymity, cooperating commercial greenhouses and their growers were assigned letter designations that will be used throughout the text. From July until September 1996, at 5-day intervals, the air samplers were operated in six Lower Mainland greenhouses experiencing varying levels of yield losses due to Fusarium fruit and stem rot disease. Commercial greenhouse GB, which had the greatest disease problem, was monitored for the remaining part of the 1996 growing season (from mid August until mid November).

Throughout the 1997 growing season, the numbers of airborne spores of *F. solani* and/or *N. haematococca* were measured in greenhouse GA, which historically did not have a disease problem and in greenhouse GB, which historically had a disease problem. From April 17th to 27th and from October 21st to 30th, 1997, the air of a newly built commercial pepper greenhouse GC was sampled as well. All three greenhouses grew peppers under regular cultural practice employed in greenhouse pepper production in Canada (Anonymous, 1996). No special treatment was given and no fungicides were applied.

Air samplers were attached to the greenhouse wires and positioned within the canopy (depending on the crop height) near the center of each greenhouse. The samplers operated continuously for 24 hours with a suction rate of 10 L min⁻¹ and slide speed of 2 mm hour⁻¹.
Glass slides (72 x 25 mm) coated with thin layer of gelvatol-glycerol mixture served as the trapping surface (for the detailed procedure for the glass slide coating refer to Appendix B). Slides were replaced daily by the growers. After the 24-hour exposure, slides were stained with Cotton Blue in lactic acid (preparation of the mounting medium is described in Appendix B), covered with 22 x 50 mm coverslips and examined under the light microscope. *F. solani* and *N. haematococca* spores were identified on the basis of size and shape (Booth, 1971; Tousson and Nelson, 1976). A “standard” spore trap slide, containing both spore types, was used for reference. Spores were counted under a 25-power objective of Zeiss Universal Microscope (Carl Zeiss, Germany) by making three traverses, 0.56 mm wide, along the middle portion of the slide at 2-mm (1 h) intervals (MacHardy and Gadoury, 1986). Hourly concentrations were obtained by correcting hourly counts for the portion of the slide examined and the volume of air sampled, and were recorded as the number of spores per cubic meter of the air (Aylor, 1993; Gadoury and MacHardy, 1983). Ascospore concentrations were reported as derived, rather than being rounded to the nearest hundred, so that spore numbers close to the limits of detection would not be reported as zeros.

At the beginning of the spore monitoring experiment, a Portable Air Sampler for Agar Plates (Burkard Manufacturing Co Ltd, Rickmansworth, England), containing a semi-selective medium for *F. solani* (Nash & Snyder, 1970), was used to confirm the spore identity and to determine spore viability and pathogenicity to peppers. Exposed plates were sealed with parafilm, returned to the laboratory and examined for the presence of *N. haematococca* and/or *F. solani* spores. After 7 days of incubation at 23°C, small portions of
those actively growing colonies visually identified as *F. solani* were subcultured on plates containing Difco potato dextrose agar (PDA) and stored for pathogenicity tests (section 3.3).

Records of temperature and relative humidity were collected from August 15 until November 13, 1996, in greenhouse GB and throughout the 1997 growing season in greenhouses GA and GB using a Campbell CR-10 datalogger. Sensors connected to the datalogger (Vaisala HMP 35C temperature and relative humidity probe, Campbell Scientific, Edmonton, Alberta) were attached to the greenhouse canopy wires and positioned close to the spore trap. Data were recorded every 15 minutes and averaged over 1-hour periods. Records of the time of sunrise and sunset, and the time of beginning and end of civil twilight were obtained from the Vancouver Planetarium (Dodge, Personal comm.).

Regression analyses were conducted to establish the relationship between spore release and each of the environmental variables, such as average temperature (T), maximum T, minimum T, average relative humidity, maximum RH and hours of RH>90% on a daily, weekly and monthly basis. Spore release was also tested against the above-mentioned independent variables recorded 24 hours, 1, 2 and 3 weeks preceding spore catches. To reveal the daily spore dispersal pattern, the relationship between spore release and various environmental factors (e.g., RH, temperature and light) was tested on an hourly basis. The significance of differences in environmental conditions and numbers of airborne spores of *N. haematococca* and/or *F. solani* trapped between greenhouses was determined by paired-comparison t-test (Zar, 1984). All statistical analyses were conducted using the Statistical Analysis System (SAS version 6.08).
3.2. IDENTIFICATION OF SOURCES OF PRIMARY INOCULUM

Burkard 24-hour recording air samplers were used to evaluate whether the standard commercial greenhouse end-of-season cleanup procedures were effective in preventing the carry-over of airborne inoculum of *N. haematococca* and/or *F. solani* from one growing season to the next.

The air samplers were operated continuously throughout 1996 end-of-season cleanup in commercial greenhouses GA and GB, and throughout 1997 end-of-season cleanup in greenhouses GA, GB and GC. The spore traps were removed from the greenhouses only when buffered bleach or Virkon® was applied. After the 1997 end-of-season cleanup, for 4 days before and after the introduction of the new crop, three other Lower Mainland commercial pepper greenhouses were monitored as well. The date, time of day, and specific grower activity, such as removal of the old crop debris, application of the Virkon® or the buffered bleach, and planting of the new crop was recorded by the greenhouse growers. The air samplers were operated and the glass slides prepared and examined as explained previously.

To determine whether the spores detected by the air samplers were viable and pathogenic to pepper, petri plates containing a Nash and Snyder’s semi-selective medium for *F. solani*, were placed on the floor of selected commercial greenhouses and exposed to the air for 10-12 h. Exposed plates were handled as described in section 3.1 and pathogenicity tests done according to procedures explained in section 3.3.

To determine if the growing medium and/or the seedlings were being contaminated during propagation, the air of two Lower Mainland propagation houses was monitored using
the previously described spore trapping procedures (18-21 December, 14-17 January and 20-
23 January 1997).

In January 1997, two-month-old apparently healthy plants (cultivar Spirit and Mazurka), provided by two commercial pepper greenhouse growers, were indexed to determine if the fungus was being introduced in a latent form with the new plants. Forty plants (twenty from each grower) were uprooted, and the root systems carefully removed from rockwool blocks. The root systems were washed under running tap water, rinsed in sterile distilled water and dried on sterile filter paper in a laminar air flow bench. Roots and crown were then cut aseptically into 5-mm pieces and plated onto the Nash and Snyder's medium. After 7 days of incubation at room temperature, any colonies resembling *F. solani* were subcultured onto the PDA and tested for pathogenicity.

3.3. **PATHOGENICITY TESTS**

**Pepper seedling bioassay.** Pepper seeds (California Wonder 300 TMR, Ferry-Morse Seed, Fulton) were surface disinfested in 10% sodium hydrochlorite for 7 minutes, rinsed in sterile distilled water, then soaked in 50% ethanol for 2 minutes followed by three sterile distilled water rinses. The seeds were dried on sterile filter paper disks in a laminar air flow bench. Ten seeds were placed around the edge of petri dishes containing 1% water agar and incubated at 23°C for 3 days. Agar discs (3mm diameter) cut from the edge of 7-day old cultures of the fungi to be tested, were placed in the center of the plates. The inoculated plates were incubated a further week in the dark at 23°C to facilitate seed germination. The plates were than placed under a bank of two fluorescent tubes (40W cool white) and incubated at room temperature for 3-4 weeks. The seedlings were examined weekly for the
presence of dark brown crown or lower stem lesions and the perithecia of *N. haematococca*. Pepper seeds inoculated with a known pathogenic isolate of the fungus isolated from a greenhouse pepper stem naturally infected with *F. solani* was included as a positive control throughout these studies; non-inoculated pepper seeds served as a negative control.

**Inoculum preparation.** 7-day old cultures of isolates to be tested were flooded with 3 ml of sterile distilled water. The surface of the colonies was gently rubbed with a sterile glass rod bent in the shape of a hockey stick to dislodge the spores. The spore suspension, containing mostly microconidia, was filtered through three layers of sterile cheesecloth. The spore concentration was estimated using a haemacytometer and adjusted to $1 \times 10^6$ spores per ml$^{-1}$.

**Fruit inoculation.** Peppers were soaked in Sparkleen detergent (Fisher Scientific) for 45 min then rinsed with running water. Individual peppers were moved to the laminar air flow bench, surface disinfested in 10% sodium hydrochlorite for 30 sec followed by three sterile distilled water rinses and sprayed with 70% ethanol. Peppers were dried on petri plates lined with sterile filter paper discs. Sterile paper towel was placed in the bottom of sterile beakers and moistened with sterile distilled water. One pepper was added to each beaker. Small injuries were made with the tip of a scalpel in the depression at the edge of the calyx and 10 µl of spore suspension (prepared as noted above) applied as a droplet to each wound site. The beakers were sealed with parafilm and incubated at room temperature on the laboratory bench for 3-4 weeks. At the weekly intervals, peppers were examined for the presence of lesions and perithecia of *N. haematococca*. Positive and negative controls were included as well.
3.4. **IN VITRO DISCHARGE STUDIES**

The influence of temperature and relative humidity on discharge of ascospores of *N. haematococca* was studied using the procedure described by Semeniuk (1993). Pepper fruit with mature perithecia, collected from Lower Mainland commercial pepper greenhouses during September and October 1997, were used in this experiment.

In the first study, pepper tissue bearing numerous perithecia was examined with a dissecting microscope and cut into smaller portions containing approximately 100 perithecia. Each portion, with the side bearing perithecia facing upward, was then placed on 25 × 75 mm glass microscope slide. In order to catch discharged spores, another glass slide, coated with a thin layer of a gelvatol-glycerol mixture, was supported above the tissue-bearing slide in a plastic frame with 5 mm spaced grooves. Plastic frames, each supporting two slides, were then placed in transparent plastic boxes (87 × 54 × 28 mm) containing 40 ml of aqueous salt solutions (indicated in Appendix C) or sterile distilled water used to produce humidities of 51, 76, 88, 95 and 100% (Robinson and Stokes, 1958). A space of 10 mm was left between the lower slide and the solution. The boxes were sealed with parafilm and placed in controlled temperature incubators at 15, 20, 25 and 30°C with a 12/12 h light/dark photoperiod (one fluorescent tube, 40W cool white). Slides containing the trapping surface were replaced every 24 hours. Exposed slides were then stained with Cotton Blue in lactic acid and covered with 22 × 50 mm coverslips. The number of ascospores present on the slides was recorded by microscopic observation.

Because of the moisture originating from the fruit tissue during cutting of the diseased peppers, it was not possible to avoid wetting the perithecia. Therefore, in the second study,
collected peppers with mature perithecia were cut into smaller pieces and air-dried on the laboratory bench at room temperature for 48 h before use in the experiment. The effect of temperature and relative humidity on discharge of ascospores of *N. haematococca* from air-dried tissue was studied using the above-explained procedure. The number of ascospores present after 24 h was determined.

Because of the limited number of available incubators, each set of treatments was replicated four times on different days. The experiment was repeated once. The experiment was conducted as a split-plot design, with temperature as a whole plot and relative humidity as a subplot. A separate analysis of variance was performed for each assessment time using the SAS PROC GLM (Anonymous, 1990). Linear, quadratic and cubic effects of temperature and relative humidity, and their interaction on ascospore discharge was also studied using SAS PROC GLM. The chosen regression equations were selected based on normality of residuals, $R^2$, adjusted $R^2$ and significance of estimated parameters (Schuh, 1991). Although being significant at $P<0.05$, terms which did not improve the $R^2$ significantly were not included in the regression equations.

3.5. **IN VITRO GERMINATION STUDIES**

The fruit inoculation procedure described previously was modified to provide a continuous supply of mature perithecia of *N. haematococca* used in the ascospore germination studies. Instead of placing whole, surface-disinfected peppers into the beakers, peppers were cut into smaller pieces, inoculated and then placed in petri plates (195 × 35 mm) lined with moistened sterile filter paper discs. Plates were then sealed with parafilm and incubated under constant light at room temperature. After 17-21, days numerous perithecia
formed on the surface of the pepper tissue. The isolate used to inoculate pepper tissue for perithecia production was obtained in October 1996 from naturally infected pepper and was maintained on PDA in test tube slants at 6°C until used in the study.

The effect of temperature and relative humidity on in vitro germination of ascospores of *N. haematococca* was studied using the isopiestic equilibration technique, described by Haris et al (1970) and modified by Arauz and Sutton (1989). Petri dishes (9 cm) containing 44 ml of 2% water agar amended with 0, 0.3, 0.6, 1.5, 2.2, and 3.1 M NaCl were used to maintain relative humidities of 100, 99, 98, 95, 92, and 88 %, respectively (Arauz and Sutton, 1989). The relative humidity in this chamber is related to the NaCl molality according to the values given by Lang (1967). A free water treatment was used as well, in which the spores were covered with a 10μl-droplet of sterile distilled water.

The ascospores used in germination studies were collected using a technique similar to that described by Semeniuk (1993). Small pieces of pepper tissue bearing mature perithecia, produced as described earlier, were soaked in sterile distilled water for 15-20 min and then blotted dry with sterile "delicate task" wipers (Kimwipes EX-L, Kimberly Clark Inc., Mississauga, Ontario). Each piece, with the perithecia facing upward, was then placed onto a piece of wet filter paper within the well (18 mm wide × 5 mm deep) of a depression slide. Each well was covered with an ethanol-sterilized microscope cover glass (18 × 18 mm) to catch ascospores. After 20-25 min incubation at a room temperature, approximately 150-250 ascospores had adhered to the cover glass. Cover glasses with collected ascospores were then placed in previously prepared relative humidity chambers preconditioned at the desired temperature for 24 hours. The chambers were sealed with parafilm and moved to...
incubators at 15, 20, 25 and 30°C. Ascospore germination was evaluated 4, 8, 12, 24, 48, and 72 hours after treatments were imposed. After each incubation period, cover glasses from each humidity chamber were inverted on a drop of Cotton Blue in lactophenol on a glass slide to stop further germination and to preserve the spores for future observations. Percent spore germination was calculated by observing 100 spores selected randomly on each cover glass. Ascospores were considered to have germinated when the length of the germ tube was greater than the width of the ascospore cells (>4μm). Germ tube length was determined at 8, 12, and 24 h by measuring 10 germ tubes randomly selected on each cover glass.

Each set of treatments was replicated four times on different days. The experiment was repeated once. The experiment was conducted in a split-plot design with temperature as the whole plot and relative humidity as subplot. Analysis of variance and regression analysis explained in the section 3.4 were used to analyze data. Data expressed as percentages were transformed using the arcsine $\sqrt{X}$ transformation to stabilize variances (Arauz and Sutton, 1989; Steel and Torrie, 1960; Sutton and Arauz, 1991).

3.6. **IN VITRO ASCOSPORE LONGEVITY STUDIES**

Hardiness and longevity of ascospores of *N. haematococca* were studied using a method similar to that described by Pearson et al (1991). In the first study, ascospores discharged onto cover glasses (as explained in the germination study) were placed in series of incubators adjusted to 15, 20, 25, and 30°C with relative humidity values ranging from 50-60% throughout the experiment. To assess the ascospore viability, cover glasses were removed from the incubators at various intervals (up to 20 days) and placed in 100 %-relative humidity chambers at 20°C for 24 h. After incubation, cover glasses were inverted on a drop of
Cotton Blue in lactophenol on a glass slide and examined under the microscope. Spore germination of 100 ascospores on each of four cover glasses per treatment was recorded as an indication of ascospore viability and was reported as percent survival. The experiment was repeated once.

In the second study, cover glasses with ascospores were placed in incubators and exposed to alternate “day/night” temperature, relative humidity and light regime. The regime used was 12 hours of 27°C under 50-60 % relative humidity (RH of incubators) and constant light followed by 12 hours of 18°C under 88 % relative humidity and constant darkness. The 88% RH chamber was created using the isopiestic equilibration technique explained in the section 3.1. Tests for viability, using the spore germination test described above, were conducted periodically for up to 16 days. The experiment was repeated once.

The experimental design used in the first study was a split-plot with temperature as the whole plot and assessment time (day) as the subplot. The experimental design for the second study was completely randomized. Analysis of variance and regression analysis described in the section 3.4 were used to analyze data. As in the germination studies, data expressed as percentages were transformed using the arcsine-√X transformation to stabilize variances.
4. RESULTS

4.1. SOURCES AND TYPE OF THE PRIMARY INOCULUM

The ascospore stage (*Nectria haematococca*) was detected in the air of all Lower Mainland commercial pepper greenhouses monitored during the 19-month spore trapping study. By contrast, only a few macroconidia of *Fusarium sp.* were trapped throughout the whole study. In all commercial pepper greenhouses monitored during 1996 and 1997 end-of-season cleanup, no spores of *Nectria haematococca* were detected in the air once the old crop was removed from the houses and disinfestant chemicals applied (Figure 1). *Fusarium sp.* macroconidia were also not trapped during this crop-free period.

At the beginning of the 1997 growing season, in commercial greenhouse GA (non-problem house) that obtained young plants produced by the propagation house X, ascospores were detectable as soon as the new plants were introduced (Figure 1). A similar pattern was observed at the beginning the 1998 growing season in all four commercial pepper greenhouses monitored, GA, GC (problem house), GD and GE, that obtained young plants produced by the propagation house X (Figure 1 and Figure 2). Daily spore levels as high as 5000-7000 ascospores m\(^{-3}\) air were trapped. No symptoms were observed on the new seedling plants but perithecia of *Nectria haematococca* were detected on the surface of the rockwool cubes in which the seedlings were growing (Figure 3). In four randomly selected rows, from two commercial greenhouses (two rows per house), nearly 10% of the rockwool blocks had perithecia. The spores trapped from these perithecia, using the agar plate technique, were viable and pathogenic when employed in the pepper seedling bioassay or fruit inoculation pathogenicity tests (Figure 5 and Figure 6). When roots and crowns of 8-week
old symptomless seedlings grown in rockwool blocks containing perithecia were indexed, *F. solani* was recovered from 10% (n = 40) of young pepper plants obtained from two commercial greenhouses.

At the beginning of the 1997 and 1998 growing season, in greenhouse GB, which obtained pepper seedlings produced by propagation houses Y and Z respectively, no ascospores could be detected upon the introduction of the new crop (Figure 7). Furthermore, no perithecia could be observed on the rockwool blocks in which the seedlings were growing. However, during both, the 1997 and 1998, growing seasons, a low level of spores of *N. haematococca* (daily average concentrations of 14 to 72 ascospores m$^{-3}$ air) was trapped approximately one month after the plants were placed in the greenhouse.

Similarly, at the beginning of the 1998 growing season in two other Lower Mainland greenhouses which obtained seedlings produced by the propagation house Z, no ascospores could be detected upon the introduction of the new crop. At the beginning of the same growing season, in another commercial greenhouse which obtained plants produced by the propagation house Y, low ascospore concentrations (daily average of 42-56 spores m$^{-3}$ air) were trapped upon the introduction of the new crop.

When propagation houses X and Z were monitored, the ascospores of *N. haematococca* were detected only in the air of the propagation house X. During the four days of monitoring in December 1996 and January 1997, daily average concentrations of 347 and 1316 ascospores m$^{-3}$ air, respectively, were recorded. Furthermore, perithecia of *N. haematococca* were observed growing on the rockwool blocks.
Figure 1. Levels of ascospores of *N. haematococca* trapped from commercial sweet pepper greenhouses during the end-of-season cleanup and the introduction of the new crop.
Figure 2. Levels of ascospores of *N. haematococca* trapped from commercial sweet pepper greenhouses after the end of season cleanup and introduction of the new crop.
Figure 3. Perithecia of *N. haematococca* observed on the surface of the rockwool blocks in which the symptomless pepper seedlings were growing.

A) Perithecia of *Nectria haematococca* (arrows) on the surface of a rockwool cube (scale bar 3 mm).

B) Close-up of perithecia showing the ostiole (arrows) through which spores are forcibly ejected at night (scale bar 300 μm).
Figure 4. Ruptured perithecium of *Nectria haematococca* with protruding asci (A) and numerous asci containing two celled ascospores (eight spores each ascus) (B).

A) scale bar 100 µm
B) scale bar 50 µm.
Figure 5. Typical *F. solani* colonies developed on Nash & Snyder's medium from ascospores of *N. haematococca* trapped in commercial pepper greenhouses.

Figure 6. Pepper seedling bioassay employed to test the pathogenicity of putative *F. solani* isolates trapped in commercial pepper greenhouses.

A) Control
B) Inoculated
Figure 7. Levels of ascospores of *N. haematococca* trapped from commercial sweet pepper greenhouses during the end-of-season cleanup and the introduction of the new crop.
4.2. GREENHOUSE ENVIRONMENT AND EPIDEMIOLOGY OF FUSARIUM FRUIT AND STEM ROT OF GREENHOUSE GROWN PEPPER

4.2.1. Concentrations of airborne ascospores of *Nectria haematococca*

Although ascospores were present in all the greenhouses surveyed, their concentration in the air differed greatly among the houses. The average 24-hr concentration of ascospores m$^{-3}$ from mid July till end of August 1996, in six Lower Mainland commercial pepper greenhouses, ranged from 49 (greenhouse GA) to 2647 (greenhouse GB) with negligible to 2% yield loses reported by growers, respectively (Figure 8). In October 1996, daily average ascospore concentrations in greenhouse GB varied from 1899 to 3737 spores m$^{-3}$ air with yield losses estimated at 9% (Grower B, personal communication). During the last week of the 1996 growing season, daily average concentrations of 906 ascospores m$^{-3}$ air were detected in greenhouse GA, but the disease severity remained negligible (Grower A, personal communication) (Figure 8).

In 1997, the highest 24-h ascospore concentration of 13715 spores m$^{-3}$ air was detected in greenhouse GC in October, with yield losses estimated at 40 % (Grower C, personal communication). By that time approximately 50 % of the plants had either crown or stem lesions. From April 17-27, 1997, the same greenhouse had daily ascospore numbers ranging from 646-1101 spores m$^{-3}$ air. During this period fruit losses were estimated at 10%. At the same time, greenhouse GA, which obtained pepper seedlings from the same propagation house, had significantly lower (P<0.05, paired t-test) daily spore numbers ranging from 43 to 221 spores m$^{-3}$ (April, 1997) and from 284 to 1056 spores m$^{-3}$ (October, 1997) (Figure 9).
Ascospore levels in greenhouse GA rose during the first month after planting-out (January) with an average 24 h concentration of 2044 spores m$^{-3}$ air and then dropped to an average daily concentration of 113 spores m$^{-3}$ air during the second month. Ascospore numbers increased slightly during March (daily average 532 spores m$^{-3}$ air) and then remained below 250 spores m$^{-3}$ air until the end of August. During October, daily average ascospore levels ranged from 347 to 1033 spores m$^{-3}$ air and then rose to an average of 1290 spores m$^{-3}$ air in November (the end of 1997 crop) when the first stem and crown infections were observed (Figure 10). Throughout the growing season a negligible amount of fruit infection was reported by grower A.

As mentioned earlier, greenhouse GB started the 1997 growing season with undetectable ascospore levels. The first spores of *N. haematococca* were observed one month after planting-out, but, daily average ascospore concentrations stayed below 70 spores m$^{-3}$ air until November when their level slightly increased to an average of 181 spores m$^{-3}$ air (Figure 10). Throughout the year, virtually no diseased fruit and plants were observed by grower B.
Figure 8. Representative daily average airborne *N. haematococca* ascospore concentrations in non-problem (greenhouse GA) and problem (greenhouse GB) commercial sweet pepper greenhouses during August and October 1996.
Figure 9. Representative daily average airborne *N. haematococca* ascospore concentrations in non-problem (greenhouse GA) and problem (greenhouse GC) commercial sweet pepper greenhouses during April and October 1997.
Figure 10. Mean daily average concentrations of ascospores of *Nectria haematococca* in commercial sweet pepper greenhouses with: A, high ascospore levels present at the time of planting out (greenhouse GA) and B, undetectable ascospore levels present at the time of planting out (greenhouse GB) during the 1997 growing season.

In greenhouse GA the mean daily average ascospore concentration for November was based on 14 days because the crop was removed. In greenhouse GB the new crop was planted on January 27; thus, the average shown for January in greenhouse GB is based on three days only.
4.2.2. Greenhouse relative humidity and air temperature

In greenhouses GA and GB, throughout the 1997 growing season, the ambient relative humidity (RH) was usually at its highest early in the morning and at its lowest from noon to late afternoon. The minimum and maximum relative humidities measured were 37% and 98.8% respectively. On a monthly basis, the first period of relative humidity above 90% was observed in March. The highest relative humidity was measured in August and the highest daily mean relative humidity was measured in September and October (Table 1). Although the maximum relative humidity and the daily mean relative humidity showed a similar seasonal pattern in both greenhouses, greenhouse GB had significantly higher (P<0.05, paired t-test) RH levels throughout the observation period than those recorded in greenhouse GA. Furthermore, the periods of RH above 90% were significantly longer (P<0.05, paired t-test) in greenhouse GB than those noted in greenhouse GA (Table 1 and Figure 11).

In both greenhouses, for the duration of this study, the air temperature was usually at its highest early in the afternoon and at its lowest between 2400 and 0500 hours. The maximum and minimum monthly temperatures ranged from 28 to 36 and from 14 to 18°C, respectively. The average monthly air temperatures varied less than 3°C throughout the year (20.1 to 22.8). Minimum, maximum and mean daily temperatures were not significantly different (P<0.05) between the greenhouse GA and GB during the 1997-growing season (Table 2). However, different daily temperature patterns were observed in two greenhouses. In greenhouse GA, the rise in temperature began prior to sunrise, whereas in greenhouse GB it typically occurred after sunrise. Furthermore, early in the morning, short intervals with rapid temperature rise, which greatly increased the risk of condensation, were often observed.
in greenhouse GB. By contrast, the temperature rise in greenhouse GA was typically gradual (1°C per hour) (Figure 13 and Figure 14).

In both greenhouses, throughout the study, the calculated dew point temperature (Td) was never equal to or higher than the measured air temperature. However, periods during the night when the difference between Td and actual greenhouse temperature (condensation safety margin, CSM) (Seginer and Zlochin, 1997) was less than 1°C were often encountered in greenhouse GB, but were never observed in greenhouse GA (Table 1, Figure 12, Figure 13 and Figure 14).

The temperature and relative humidity observed during 1996 in greenhouse GB ranged from 11 to 34°C and 56 to 99% respectively. The relative humidity data, collected from mid August until mid November 1996 in greenhouse GB, were similar to data collected for the same months during 1997 at the same location. A significant difference (P<0.05) in year-to-year temperature data from greenhouse GB was observed only for October 1996 and October 1997.

Monitoring the environmental parameters in greenhouse GC was beyond the scope of this study, but, it was obvious that the grower had a problem with climate management. Drainage problems, caused by poor soil profiling, resulted in the accumulation of nutrient solution on the greenhouse floor. In addition, higher-than-needed volumes of the nutrient solution were applied per m² of the area, which resulted in waterlogged rockwool slabs and blocks. Based on the grower’s records, long periods of RH above 90% during the day were typical for the climate of greenhouse GC. Furthermore, fruit sweating and condensate drips
from the greenhouse structure were commonly observed in the early morning hours (Grower C, personal communication).

Table 1. Monthly relative humidities (RH) and hours with CSM (condensation safety margin) <1°C in commercial sweet pepper greenhouses GA and GB during 1996 and 1997 growing season

<table>
<thead>
<tr>
<th></th>
<th>Maximum monthly RH (%)</th>
<th>Mean monthly RH (%)</th>
<th>Hours of RH &gt; 90% per month</th>
<th>Hours of RH &gt; 95% per month</th>
<th>Hours of CSM&lt;1°C per month</th>
</tr>
</thead>
<tbody>
<tr>
<td>1996</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aug^3</td>
<td>-</td>
<td>99.2</td>
<td>-</td>
<td>95.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Sep</td>
<td>-</td>
<td>98.7</td>
<td>-</td>
<td>134.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Oct</td>
<td>-</td>
<td>97.7</td>
<td>-</td>
<td>88.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Nov^2</td>
<td>-</td>
<td>95.5</td>
<td>-</td>
<td>62.0</td>
<td>3.0</td>
</tr>
<tr>
<td>1997</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jan</td>
<td>77.8</td>
<td>-</td>
<td>54.9</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Feb</td>
<td>82.2</td>
<td>87.7</td>
<td>54.3</td>
<td>68.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Mar</td>
<td>91.8</td>
<td>95.6</td>
<td>69.2</td>
<td>75.3</td>
<td>7.0</td>
</tr>
<tr>
<td>April</td>
<td>87.6</td>
<td>94.6</td>
<td>66.5</td>
<td>73.7</td>
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</tr>
<tr>
<td>May</td>
<td>92.3</td>
<td>98.2</td>
<td>70.4</td>
<td>75.5</td>
<td>23.0</td>
</tr>
<tr>
<td>June</td>
<td>89.8</td>
<td>97.3</td>
<td>70.0</td>
<td>75.9</td>
<td>0.0</td>
</tr>
<tr>
<td>July</td>
<td>91.5</td>
<td>98.2</td>
<td>70.2</td>
<td>77.3</td>
<td>3.0</td>
</tr>
<tr>
<td>Aug</td>
<td>95.2</td>
<td>98.9</td>
<td>74.1</td>
<td>79.9</td>
<td>17.0</td>
</tr>
<tr>
<td>Sep</td>
<td>89.5</td>
<td>98.0</td>
<td>76.1</td>
<td>82.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Oct</td>
<td>85.4</td>
<td>96.0</td>
<td>74.8</td>
<td>84.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Nov^2</td>
<td>90.4</td>
<td>97.1</td>
<td>68.7</td>
<td>81.8</td>
<td>2.0</td>
</tr>
</tbody>
</table>

1 Hours with less than 1°C difference between calculated dew point temperature and actual greenhouse temperature (condensation safety margin, CSM).
2 For first two weeks of the month.
3 For last two weeks of the month.
Table 2. Monthly ambient air temperature in commercial sweet pepper greenhouses GA and GB during 1996 and 1997 growing season

<table>
<thead>
<tr>
<th></th>
<th>Mean monthly temperature (°C)</th>
<th>Maximum temperature (°C)</th>
<th>Minimum temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GA</td>
<td>GB</td>
<td>GA</td>
</tr>
<tr>
<td>1996</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August</td>
<td></td>
<td>21.9</td>
<td></td>
</tr>
<tr>
<td>September</td>
<td></td>
<td>21.5</td>
<td></td>
</tr>
<tr>
<td>October</td>
<td></td>
<td>18.5</td>
<td></td>
</tr>
<tr>
<td>November</td>
<td></td>
<td>19.9</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>20.4</td>
<td></td>
<td>28.5</td>
</tr>
<tr>
<td>February</td>
<td>20.6</td>
<td>20.5</td>
<td>31.6</td>
</tr>
<tr>
<td>March</td>
<td>20.1</td>
<td>20.8</td>
<td>28.9</td>
</tr>
<tr>
<td>April</td>
<td>21.1</td>
<td>21.5</td>
<td>30.2</td>
</tr>
<tr>
<td>May</td>
<td>21.4</td>
<td>22.1</td>
<td>35.5</td>
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<tr>
<td>June</td>
<td>21.9</td>
<td>22.1</td>
<td>34.4</td>
</tr>
<tr>
<td>July</td>
<td>23.2</td>
<td>22.8</td>
<td>35.4</td>
</tr>
<tr>
<td>August</td>
<td>22.7</td>
<td>23.0</td>
<td>32.3</td>
</tr>
<tr>
<td>September</td>
<td>22.8</td>
<td>22.7</td>
<td>31.8</td>
</tr>
<tr>
<td>October</td>
<td>22.8</td>
<td>21.7</td>
<td>29.2</td>
</tr>
<tr>
<td>November</td>
<td>22.4</td>
<td>20.8</td>
<td>30.8</td>
</tr>
</tbody>
</table>

1 For the last two weeks of the month.
2 For the first two weeks of the month.
Figure 11. Number of days per month with extended periods of uninterrupted relative humidity above 90 and 95% in commercial sweet pepper greenhouses GA and GB during 1997 growing season.

A) Days with more than five hours of uninterrupted RH above 90%.
B) Days with more than ten hours of uninterrupted RH above 90%.
C) Days with more than five hours of uninterrupted RH above 95%.
Figure 12. Typical air temperature (T), calculated dew-point temperature (Td) and air relative humidity (RH) profiles in commercial sweet pepper greenhouses GA and GB during May 1997.
Figure 13. Typical air temperature (T), calculated dew-point temperature (Td) and air relative humidity (RH) profiles in commercial sweet pepper greenhouses GA and GB during July 1997.
Figure 14. Typical air temperature (T), calculated dew-point temperature (Td) and air relative humidity (RH) profiles in commercial sweet pepper greenhouses GA and GB during August 1997.
4.2.3. Environmental parameters and concentrations of *Nectria haematococca* ascospores

Based on the observations from greenhouses GA and GB, the ascospore discharge was detected only during the night at relative humidities ranging from 50 to 99% and at temperatures between 11 to 23°C. In greenhouse GA during the 1997 growing season, on a daily, weekly or monthly basis none of the environmental variables (maximum RH, mean RH, hours of RH >90% and minimum and mean temperature) explained the variation in the ascospore numbers satisfactorily (R² from 0.08 to 0.22). In greenhouse GB during the 1997-growing season, the ascospore concentration was very low. In the same greenhouse during August, September and October 1996, daily, weekly and monthly ascospore concentration, RH and temperature data did not fluctuate much. Therefore, no analysis of data from the greenhouse GB was undertaken. However, significant differences in spore numbers and RH data were detected between greenhouse GA in 1997 and greenhouse GB in 1996, during August, September, and October (Figure 15). When temperature, relative humidity and spore trapping data from both greenhouses were pooled into one regression analysis, the ascospore concentration was best correlated (positively) with an average weekly relative humidity and number of hours of relative humidity above 90% (R²=0.58). The best linear regression model selected was the following: \[ y = -5426.04 + 84.77 \text{ARH} + 20.07 \text{HRH} \] where \( y \) = average ascospores m\(^{-3}\) air/week , \( \text{ARH} \) = average weekly relative humidity and \( \text{HRH} \) = hours of relative humidity above 90%.
Figure 15. Number of ascospores of *N. haematococca* trapped and canopy relative humidities recorded in problem (GB) and non-problem (GA) commercial sweet pepper greenhouses during August, September, October and November 1996 and 1997, respectively.
4.2.4. Patterns of ascospore discharge by *Nectria haematococca*

Although the ascospore concentration varied greatly from greenhouse to greenhouse, the ratio of spores trapped during daylight to spores trapped during darkness was consistent in all greenhouses surveyed. When major release events (≥ 4 spores per slide = 56 spores m⁻³ air/day) for the 1996 and 1997 growing seasons were considered, 90% of the ascospores were trapped during the night (from 1800 till 0800) with the highest percent of ascospores released around midnight (Figure 16). The selected day and night intervals (0800-1800 h and 1800-0800 h, respectively) were established from ascospore trapping records and provide convenient means of delimiting intervals when the airborne ascospore density was relatively low or high.

![Graph showing diurnal periodicity of release of ascospores of *Nectria haematococca* trapped in Lower Mainland commercial sweet pepper greenhouses from August 1996 until December 1997.](image)

Figure 16. Diurnal periodicity of release of ascospores of *Nectria haematococca* trapped in Lower Mainland commercial sweet pepper greenhouses from August 1996 until December 1997.

Each bar represents means (n=280) of the percentage of ascospores trapped at 1-hr intervals during major release events.
When all spore trapping data from greenhouse GA, during 1997 growing season were considered, 92% of the ascospores were trapped during the night, but, the length of the dissemination period changed significantly over the months ranging from 7 hours (July and August) to 20 hours (January) per day. The dissemination periods based on the average cumulative percentage of ascospores trapped per hour for each month are shown in Table 3. The beginning of an ascospore release event was counted from the time when the cumulative percent of ascospores trapped per hour exceeded 2% of the total spores trapped per day. The end of ascospore release was considered when cumulative percentage of ascospores trapped per hour reached 100% of the total spores trapped per day.

In greenhouse GB, the spore numbers detected during the 1997 growing season were too low to reveal hourly dissemination patterns for each month, therefore, only the spore trapping data collected at the end of 1996 growing season were considered. The length of the dissemination interval in August, September, October and November 1996 from greenhouse GB followed patterns similar to those observed in the greenhouse GA, however, the intervals were generally wider. (Table 3).

Based on the Logistic model, cumulative percentage of ascospores trapped per hour for each month in both greenhouses was highly correlated with the time of day, with an $R^2$ ranging from 0.89 to 0.97 (Figure 17). Several functions including Gompertz, Weibull and Morgan-Mercer-Flodin, used to model sigmoidal or “S-shaped” curves, were tried (Prodan, 1968). The Logistic model was selected on the basis of goodness of fit, F-values, t-ratios and $R^2$. 
When spore trapping data collected from greenhouses GA and GB were combined, the time of initiation of ascospore release was significantly correlated (P<0.05) with the time of sunset (R² = 0.73) (Figure 19, A and Figure 18). The time of termination of ascospore release in both greenhouses was best correlated with the time of sunrise (R² of 0.57) (Figure 19, B and Figure 18). On days, which showed rapid change in relative humidity, the ascospore release commenced 2-3 hours after relative humidity started to rise (Figure 18, July and October). However, the same daily cycle of ascospore release was maintained during the days that had very little or no change in relative humidity (Figure 18, November).

In greenhouse GA, during the first month after planting out (January 1997) no connection was observed between the time of initiation or time of termination of ascospore release and change in any of above mentioned environmental variables. Therefore, spore trapping and environmental data collected for this month were excluded from the statistical analysis.
Table 3. Diurnal cycle of dissemination of ascospores of *Nectria haematococca* trapped in commercial sweet pepper greenhouses from August 1996 until December 1997

<table>
<thead>
<tr>
<th>House GA (1997)</th>
<th>Ascospore release</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Began (h)</td>
<td>Ended (h)</td>
<td>Sunset (h)</td>
<td>Sunrise (h)</td>
</tr>
<tr>
<td>January</td>
<td>14:00</td>
<td>10:00</td>
<td>16:25 – 17:08</td>
<td>7:30 – 8:00</td>
</tr>
<tr>
<td>March*</td>
<td>17:00</td>
<td>07:00</td>
<td>17:56 – 18:43</td>
<td>5:51 – 6:54</td>
</tr>
<tr>
<td>April</td>
<td>19:00</td>
<td>07:00</td>
<td>18:44 – 20:28</td>
<td>5:43 – 6:39</td>
</tr>
<tr>
<td>May</td>
<td>20:00</td>
<td>07:00</td>
<td>20:30 – 21:09</td>
<td>5:12 – 5:51</td>
</tr>
<tr>
<td>June</td>
<td>21:00</td>
<td>07:00</td>
<td>21:10 – 21:22</td>
<td>5:06 – 5:12</td>
</tr>
<tr>
<td>July</td>
<td>21:00</td>
<td>05:00</td>
<td>21:21 – 20:53</td>
<td>5:11 – 5:43</td>
</tr>
<tr>
<td>August</td>
<td>22:00</td>
<td>06:00</td>
<td>20:52 – 19:57</td>
<td>5:45 – 6:28</td>
</tr>
<tr>
<td>September</td>
<td>20:00</td>
<td>06:00</td>
<td>18:53 – 19:55</td>
<td>6:29 – 7:11</td>
</tr>
<tr>
<td>October</td>
<td>19:00</td>
<td>06:00</td>
<td>16:52 – 18:55</td>
<td>6:59 – 7:13</td>
</tr>
<tr>
<td>November¹</td>
<td>18:00</td>
<td>07:00</td>
<td>16:32 – 16:55</td>
<td>7:01 – 7:22</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>House GB (1996)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>August²</td>
<td>22:00</td>
<td>07:00</td>
<td>19:56 – 20:30</td>
<td>6:03 – 6:28</td>
</tr>
<tr>
<td>September</td>
<td>20:00</td>
<td>08:00</td>
<td>18:52 – 19:54</td>
<td>6:29 – 7:11</td>
</tr>
<tr>
<td>October</td>
<td>19:00</td>
<td>10:00</td>
<td>16:52 – 18:50</td>
<td>6:53 – 7:13</td>
</tr>
<tr>
<td>November¹</td>
<td>18:00</td>
<td>07:00</td>
<td>16:17 – 16:50</td>
<td>7:01 – 7:46</td>
</tr>
</tbody>
</table>

* February was omitted because only a few ascospores were trapped throughout the whole month.

¹ For the last two weeks of the month.

² For the first two weeks of the month.
Figure 17. The prediction models for cumulative percentage of airborne ascospores of *Nectria haematococca* trapped during 1997 growing season in the commercial sweet pepper greenhouse GA.

A) Curve fitted by the Logistic model to describe the relationship between the annual cumulative % of the ascospores trapped and the time of the day (n = 279). B) A fitted cumulative % spores trapped curve for January, August and October with $R^2$ of 0.95, 0.90 and 0.96, respectively (n = 22).

The form of the Logistic function used to fit the curves was $y = \frac{\alpha}{1 + \exp(\beta - \gamma x)}$

where $y = \text{cumulative} \% \text{ spores trapped}$, $x = \text{time of the day}$, $\alpha = 100\%$ (the asymptote), $\beta = \text{intercept}$ and $\gamma = \text{slope}$. Regression parameters with $R^2$ values for each month are shown in appendix a, table.REG 1.
Figure 18. Typical relationship between the time of initiation and termination of *N. haematococca* ascospore release and selected environmental variables in commercial pepper greenhouse GA during July 12, October 12 and November 12 1997.

Triangles denote time of sunset and sunrise for each date.
4.3. EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON IN VITRO DISCHARGE OF ASCOSPORES OF NECTRIA HAEMATOCOCCA

During the first 24 hours, from fresh diseased pepper tissue (where perithecia were moistened as explained in section 3.4), ascospore discharge occurred at all temperatures tested (15-30°C), and at relative humidities as low as 51% which was the lowest tested. In general ascospore release increased with increasing relative humidities and decreased with increasing temperatures. After 24 hours at the optimum temperature of 15°C, discharge values per 100 perithecia ranged from 162 ascospores at 51% RH to 1198 ascospores at 100% RH. After 24 hours at 30°C, discharge values per 100 perithecia ranged from 21 ascospores at 51% RH to 533 ascospores at 100% RH (Figure 20, A and C).
Compared to the number of spores discharged during the first day, a slightly lower number of ascospores was discharged during the second day under 100% RH at all temperatures tested. However, during the second day at relative humidities lower than 95% ascospore release was reduced and at a relative humidity of 51% it was completely inhibited (Figure 20, B and D).

Ascospore discharge from the air-dried perithecia occurred only at relative humidities above 95% at all temperatures tested (Figure 20, E and F). It followed a pattern similar to that observed for ascospore discharge from the fresh diseased tissue.

In both studies, highly significant (P < 0.001) effects of temperature, relative humidity and their interaction on discharge were observed. Interactions involving replication effect were not significant (see Appendix A, ANOVA 1, 2 and 3). Therefore, the data presented are combined means. The effect of temperature, relative humidity and their interaction for both studies was best described by the equations given in Table 4. Predicted values associated with these regressions can be observed in Figure 20.

Table 4. Regression equations for the effect of temperatures (T) and relative humidity (RH) on discharge of ascospores of Nectria haematococca.

<table>
<thead>
<tr>
<th>Equation</th>
<th>Regression equations</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study 1</strong></td>
<td>(1) Y = -7288.88 - 338.35T + 4.82T² + 451.74RH - 7.38RH² + 0.03RH³ + 5.95TRH - 0.06T²RH - 0.02TRH²</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>(2) Y = -8907.17 - 114.61T + 424.21RH - 6.63RH² + 0.03RH³ + 3.72TRH - 0.03TRH²</td>
<td>0.95</td>
</tr>
<tr>
<td><strong>Study 2</strong></td>
<td>(3) Y = 512511.94 - 16027.65T - 10641.28RH + 55.23RH² + 332.53TRH - 1.72TRH²</td>
<td>0.86</td>
</tr>
</tbody>
</table>
Figure 20. Effect of temperature and relative humidity on the discharge of ascospores of *Nectria haematococca* from fresh and air-dried diseased pepper tissue.

A) and B) Actual data for discharge from fresh pepper tissue for the first and the second day, respectively. C) and D) Predicted values from regression equations 1 and 2, respectively in Table 4. E) Actual data for discharge from air-dried pepper tissue. F) Predicted values from regression equation 3 in Table 4.
4.4. EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON IN VITRO GERMINATION OF ASCOSPORES OF NECTRIA HAEMATOCOCCA

Ascospore germination occurred over the complete range of temperatures tested (15-30°C). However, at 15°C germination was very low (8 to 36%) and required longer wetness duration (24 h). By that time, at temperatures greater than 15°C, at 100% RH, germination ranged from 80 to 94 % (Figure 21 and Figure 22). After 8 hours, 22, 38 and 69% germination were recorded at 100% RH for 20, 25 and 30°C, respectively (Figure 21).

Generally, percent germination increased and took a shorter time with the increasing relative humidities (RH). No ascospore germination was observed at 88% and 92% RH even after 72 hours. After 24 hours, at 95% RH, low germination (16%) was recorded only at 30°C. Greater than 50% germination occurred only under prolonged periods (8 -12 h) of RH above 98% at temperatures higher than 15°C. The average maximum (94%) germination of ascospores occurred under 100% RH at 30°C after 24 hours. Germination of ascospores was slightly lower in the free-water treatment than in the 100% RH treatment after 4, 8, 12 or 24 hours at all temperatures tested (Figure 21 and Figure 22).

Germinated ascospores typically developed one or rarely a second germ tube. The germ tube length generally increased with increasing temperatures and increasing RH. It followed a similar pattern to that observed for percent germination, except that, the germ tube elongation was greater in the free water treatment than in the 100% RH treatment (Figure 23 and Figure 24).

When analysis of variance was performed, highly significant (P < 0.05) effects of temperature, humidity and their interaction on both the germination and the germ tube length
were observed (see Appendix A, ANOVA 4, 5, 6, 7, 8 and 9). A significant positive cubic effect of temperature on percent germination in free water can be seen after 24 hours. After 24 hours, the germ tube length in free water was determined by a quadratic response on temperature. The combined effect of temperature and relative humidity on ascospore germination and germ tube length was described by the equations given in Table 5. The prediction models are given in Figure 21, Figure 22 and Figure 23.
Table 5. Regression equations for the effect of temperature (T) and relative humidity (RH) on germination of ascospores of *Nectria haematococca* 8, 12 and 24 hours after treatments were imposed.

For relative humidities from 95 to 100%.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Hours</th>
<th>Regression equations</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Germination</strong></td>
<td>8 h</td>
<td>$Y = 19435.15 - 64.92T - 390.34RH + 1.95RH^2 + 0.68TRH$</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>$Y = 29371.85 - 500.11T + 9.64T^2 - 549.30RH + 2.53RH^2 + 5.16TRH - 0.09T^3H$</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>$Y = 15598.06 - 435.62T + 9.04T^2 - 281.20RH + 1.24RH^2 + 4.48TRH - 0.09T^2RH$</td>
<td>0.92</td>
</tr>
<tr>
<td><strong>Germ tube length</strong></td>
<td>8 h</td>
<td>$Y = -8657.27 + 822.49T + 1.54T^2 + 189.45RH - 1.03RH^2 - 17.91TRH - 0.01T^3RH + 0.09TRH^2$</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>$Y = 29167.29 - 286.35T + 4.97T^2 - 567.35RH + 2.74RH^2 + 2.95TRH - 0.05T^2RH$</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>$Y = -124869.28 + 8995.41T + 2588.89RH - 13.41RH^2 - 186.69TRH + 0.96TRH^2$</td>
<td>0.97</td>
</tr>
</tbody>
</table>

For the free water treatment.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Hours</th>
<th>Regression equations</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Germination</strong></td>
<td>8 h</td>
<td>$Y = -394.29 + 51.73T - 2.15T^2 + 0.03T^3$</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>$Y = -183.00 + 16.74T - 0.29T^2$</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>$Y = -679.65 + 92.23T - 3.78T^2 + 0.05T^3$</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Germ tube length</strong></td>
<td>8 h</td>
<td>$Y = -55.19 + 3.84T$</td>
<td>0.93</td>
</tr>
<tr>
<td></td>
<td>12 h</td>
<td>$Y = -219.28 + 14.77T$</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>24 h</td>
<td>$Y = -1296.87 + 120.38T - 2.07T^2$</td>
<td>0.85</td>
</tr>
</tbody>
</table>

* where $Y = \arcsin \sqrt{X}$ (in radians).
Figure 21. Effect of temperature and relative humidity on germination of ascospores of *Nectria haematococca* 4, 8 and 12 hours after treatments were imposed. Left: Actual data. Right: Predicted values from regression equations given in Table 5. The nonlinearity of scale results from transforming back to percentage the arcsine $\sqrt{X}$ scale used in the analysis of data.
Figure 22. Effect of temperature and relative humidity on germination of ascospores of *Nectria haematococca* 24, 48 and 72 hours after treatments were imposed.

Left: Actual data.
Right: Predicted values from regression equations given in Table 5. The nonlinearity of scale results from transforming back to percentage the arcsine $\sqrt{X}$ scale used in the analysis of data.
Figure 23. Effect of temperature and relative humidity on germ tube length of ascospores of *Nectria haematococca* 8, 12 and 24 hours after treatments were imposed.

Left: Actual data.
Right: Predicted values from regression equations given in table 5.
Figure 24. Effect of relative humidity on germination of ascospores of *Nectria haematococca* at 25°C, 12 hours after treatments were imposed.
4.5. EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON *IN VITRO* SURVIVAL OF ASCOSPORES OF *NECTRIA HAEMATOCOCCA*

Ascospore survival after 8 days at 55-60% RH was the highest at 15°C (46%), followed by 20°C (41%). However, a great reduction in germination was recorded after 10 days at 15°C and viability was completely lost after 13 days at this temperature. At 20 and 25°C, the ascospores remained viable for 16 and 17 days, respectively. After only 2 days, a rapid decline in germination (54%) was observed at 30°C and complete mortality was reached sooner (after 6 days) at this temperature than at lower temperatures (Figure 25). Ascospores kept under alternating day/night temperature, relative humidity and light regimes remained viable for up to 12 days. However, ascospore viability was reduced to 25% after 4 days (Figure 25).

In the first ascospore survival study, significant (P<0.05) effects of temperature, days of air-drying and their interaction on ascospore viability were identified in the analysis of variance. A significant effect of number of days of air drying on ascospore viability was observed in the second study (see Appendix A, ANOVA 10 and 11). The regression models based on arcsine transformed percent viability, which were developed to predict ascospore viability in both survival studies, were the following:

**Study 1**  
\[ Y = -56.954 + 11.948T - 0.240T^2 + 20.798D - 3.006D^2 + 0.092D^3 - 1.491TD + 0.172TD^2 - 0.005TD^3 \]  
\[ R^2 = 0.92 \]

**Study 2**  
\[ Y = 71.846 - 13.992D + 1.160D^2 - 0.03D^3 \]  
\[ R^2 = 0.94 \]

where \( Y = \text{arcsin} \sqrt{X} \) (in radians), \( X \) = proportion of ascospore viability, \( T \) = temperature, and \( D \) = days of air drying.
Figure 25. Effects of various greenhouse temperature and relative humidity regimes on survival of ascospores of *Nectria haematococca*.

A) After exposure to 55-60% relative humidity (RH) at temperatures of 15, 20, 25 and 30°C. B) After exposure to alternating day/night temperature, RH and light regimes. Day, 12 h of exposure to 55-60% RH at 27°C under constant light. Night, 12h of exposure to 88% RH at 18°C in darkness. C) and D) Values predicted from equations given in text; for study 1 and 2, respectively. The nonlinearity of scale results from transforming back to percentage the arcsine $\sqrt{X}$ scale used in the analysis of data.
5. DISCUSSION

5.1. SOURCES OF PRIMARY INOCULUM

Spore monitoring in Lower Mainland commercial pepper greenhouses determined that the standard end-of-season cleanup procedures are effective in preventing the carry-over of airborne inoculum of \textit{N. haematococca} and \textit{F. solani} from one growing season to the next. The results also demonstrated that detectable levels of airborne spores from an outside source are not being introduced into the clean commercial greenhouses during the crop-free period. Even in the cases where the old plastic cover and sawdust slabs were retained, no spores of \textit{Nectria haematococca} and \textit{Fusarium solani} could be detected in the air of greenhouses awaiting the new crop. Therefore, if some sources were present in these greenhouses, they did not produce spores at detectable levels. By contrast, Kerssies (1993) trapped conidia of \textit{Botrytis cinerea} during 6 consecutive weeks after a gerbera crop had been removed from the greenhouse. Similar results were reported by Peterson (1988) with \textit{Botrytis cinerea} on the greenhouse-grown Douglas-fir seedlings. Both authors determined that most of the spores originated from the neighboring fields and greenhouses. In contrast to non-host specific \textit{Botrytis cinerea}, the strain of \textit{N. haematococca} responsible for crown, stem and fruit rot of the greenhouse grown peppers seems to have a host range restricted to \textit{Capsicum sativum} (R. Copeman, personal communication). Thus, inoculum can only originate from infected peppers or pepper tissue. In the Lower Mainland peppers are not grown commercially except in greenhouses. Also, the greenhouse vents were closed during the crop-free period. Therefore, during this period, contamination of the clean commercial greenhouses with the spores of \textit{N. haematococca} and/or \textit{F. solani} originating from the “neighboring” crops is unlikely to occur.
Spore trapping results from three different growing seasons provided the first clear evidence that the rockwool cubes infested during propagation are the main source of primary inoculum for Fusarium stem and fruit rot epidemics in Lower Mainland commercial pepper greenhouses. The perithecia of *Nectria haematococca* were detected on nearly 10% of the rockwool blocks containing pepper seedlings produced by one propagator. In commercial greenhouses, daily average ascospore levels as high as 5000 – 7000 spores m\(^{-3}\) air were trapped upon the introduction of pepper seedlings grown in contaminated blocks. This is the first report that provides clear evidence that this inert medium can act as a primary source of inoculum, indicating that the rockwool system is more vulnerable than previously thought.

At the time of planting in Lower Mainland commercial pepper greenhouses, ascospores of *N. haematococca* were trapped only upon the introduction of the pepper seedlings originating from one propagator. However, one month after planting out in 1997 and 1998 growing season, a low level of ascospores was detected in greenhouses that obtained plants produced by two other propagators. It is quite possible that at that time the ascospore production on the blocks contaminated during the propagation process reached levels detectable by spore trapping (daily average concentration of 14 ascospores m\(^{-3}\) air – 1 ascospore per slide). It is also possible that other, still unknown, sources were responsible for the low level of ascospores trapped. Therefore, while rockwool blocks contaminated during the propagation process may not be the only source of primary inoculum, they are the only detectable source at the time of planting.

Although the fungus is present on the blocks from the early stage of the growing cycle and is able to colonize the roots and crown of the young pepper plants, the first crown
symptoms are occasionally observed as much as 2-3 months later, and do not occur in quantity until the end of the season. This observation suggests that the crown symptom development may be triggered only when the plant is stressed by heavy fruit load, adverse environmental conditions or by its senescence. The above mentioned hypothesis is supported by other experiments where 8-week old pepper plants whose root systems were deliberately inoculated with pathogenic isolate of *F. solani* took 10 months to show symptoms (R. Copeman, personal comm.). Similarly in my current experiments, even though high concentrations of ascospores of *N. haematococca* were introduced in the rockwool system at seeding, pepper seedlings did not develop crown lesions during the two months that experiment was conducted. Experiments are currently under way to further test this hypothesis.

Contamination of the rockwool blocks during the propagation cycle could be attributed to the following circumstances. All three pepper propagators in the Lower Mainland area have either their own or someone else's commercial pepper crop in greenhouses nearby the propagation houses. Therefore, there is always a possibility of overlap between the end of commercial crop and the beginning of the propagation of the new crop. Consequently, during the warm autumn days (September and October), when the vents are open, the airborne ascospores of *N. haematococca* could be spread from the old crop to the early seeded peppers and could build up during the propagation cycle. Also, in the case of two propagators where the same grower shares the propagation and commercial greenhouse facilities, the pathogen could be introduced accidentally via tools and equipment carrying diseased debris from adjacent greenhouses. Unless specific precautions are taken, workers moving from one greenhouse to another could transfer the spores via their shoes and clothes.
If this hypothesis is correct, it is quite possible that the occurrence of contamination of the rockwool blocks and its level in the propagation houses may vary from year to year. This could be influenced by several interrelated factors including greenhouse climate, hygienic measures, disease pressure in the adjacent old crop, planting dates and location of the old and new crop in relation to the prevailing winds. Monitoring of the propagation facilities should be done to test this hypothesis, but this was beyond the scope of the present study.

5.2. IDENTIFICATION OF PRIMARY AND SECONDARY INOCULUM

The results from spore monitoring over three different growing seasons strongly suggest that the ascospore stage of *Nectria haematococca* is the primary inoculum for *Fusarium* stem and fruit rot epidemics in Lower Mainland commercial pepper greenhouses. This study clearly demonstrated that the ascospore stage is responsible for secondary disease spread within the Lower Mainland commercial pepper greenhouses.

In the greenhouses which started the growing season with rockwool blocks contaminated during the propagation, ascospore release occurred throughout the growing season. The availability of ascospores in the early spring supports our expectations that the initial inoculum is airborne and may explain the random occurrence of early season fruit infections on plants which did not have stem or crown lesions. Although observed on the fruit lesions, only a few conidia of *Fusarium solani* were trapped throughout the study. Likewise, in greenhouse experiments, Z. Punja (personal comm.) trapped very low numbers of airborne propagules of *Fusarium oxysporum f. sp. radicis-cucurbitacearum*, even though pronounced spore masses were visible at the crown of the cucumber plants. It is quite possible that the air movements, which hardly ever exceed 3.6 km per hour within the crop,
even when greenhouse doors and windows are open (McCartney and Lacey, 1990), are not great enough to liberate *F. solani* conidia from sporodochia. This theory is supported by the wind tunnel experiments performed by Stepanov (1935) who found that the minimum wind speed required to release spores varied from fungus to fungus. For instance, less than 1 km per hour released the conidia of *Botrytis cinerea*, whereas spores of *Fusarium culmorum* and *Phytophthora infestans* were not released by wind of 12 km per hour. The changing relative humidity, reported to induce release of conidia of some other fungi (Jarvis, 1980) appears to have no effect on the release of conidia of *F. solani*. Also activity (e.g., harvesting, pruning etc.) which resulted in peak concentrations of conidia of *B. cinerea* in geraniums (Hausbeck and Pennypacker, 1991), does not appear to bring about release of conidia of *F. solani*, all suggesting that *F. solani* spores play a minor role, if any, in spreading the disease. Therefore, in such “closed” agricultural systems the active nature of ascospore discharge, where the ascospores are violently “ejected” 0.5-2.0 cm from perithecia (Ingold, 1965), is of outmost importance for Fusarium fruit and stem rot spread.

The concentration of airborne *Nectria haematococca* spores showed a well-defined diurnal periodicity. The ascospore release occurred mainly during the night (1800-600) with a pronounced peak between 2300 and 0100. This pattern is similar to that of three other perithecial pathogens *Gibberella zeae* (Paulitz, 1996), *Calonectria nivalis* (Sanderson, 1970) and *Epichloe typhina* (Raynal, 1991). Nocturnal release may provide the pathogen with an adaptive advantage, since spores would be released during a period of high relative humidity or even dew, providing conditions enabling germination.
The time of initiation and termination of the ascospore release varied throughout the growing season and was best correlated with the time of sunrise and sunset, respectively ($R^2$ of 0.73 and 0.53). This observation agrees with Walker and Harvey's (1966) work who found that a diurnal cycle of ascospore release in many Pyrenomycetes could be induced by periods of dark and light. For example, ascospores of *Nectria coccina* and *Xylaria longipes* were liberated in the dark periods, whereas *Nectria cinnabarina* liberated its spores in the light periods.

According to Paulitz (1996), a rapid rise in relative humidity plays an important role in triggering the release of ascospores of *Gibberella zeae*, which is closely related to *Nectria* sp. (family Hypocreaceae). He hypothesized that an increase in relative humidity over a 1-2 h period during the early evening hours may increase the turgor pressure of the asci to the bursting point. The increasing relative humidity could also play an important role in the initiation of ascospore release in *N. haematococca* because on days which had rapid changes in relative humidity, the ascospore discharge commenced 2-3 hours after the relative humidity began to rise (Figure 18, July and October). However, the same diurnal cycle of the spore release was observed on days with very little or no change in relative humidity (Figure 18, November). It is quite possible that a daily rhythm may have developed resulting in an endogenous response independent of external stimuli. *Daldinia concentrica* has a nocturnal rhythm of ascospore release that continued for a time after the illumination stimulus ceased (Ingold, 1965). This would explain releases still occurring even on days with a lack of low-high relative humidity stimulus. It is also possible that light and relative humidity may cooperate to stimulate the ascospore discharge.
The development of an endogenous rhythm may also explain the observations during the first month after planting out in the greenhouse GA (January, 1997) when no correlation between the time of initiation or termination of ascospore release and any of environmental variables was observed. The fungus could have still been responding to the stimuli that it had been subjected to in the propagation house. It is also possible that the lights used in the greenhouse compartment nearby the trapping location at the beginning of the growing season affected ascospore release pattern during this month. Further studies under controlled environmental conditions are needed to determine conclusively what controls the diurnal periodicity of ascospore release in *N. haematococca*.

During the 1997 growing season, ascospore release was continuous throughout the sampling period in all three greenhouses, suggesting a constant perithecial production. This generates a heterogeneous population of perithecia with different levels of maturity. Once the perithecia have released all of their ascospores, they cannot form new ones (Ingold, 1966), but younger perithecia may mature a few days later ensuring continuity of ascospore supply.

On a relatively small scale, ascospore release and perithecial production and maturation persisted in the greenhouse GA throughout the growing season, despite the unfavorable climate (Table 1). This observation is contrary to numerous field reports for other perithecial pathogens that show an association between ascospore release and high relative humidity or rainfall (Alderman, 1993; Dobrev, 1987; Perrin, 1978; Raynal, 1991; Reis, 1990; Sanderson, 1970). In addition, high moisture conditions are reported to be required for the formation and maturation of perithecia of other fungi (French, 1967; Sung and Cook, 1981). However, in this study, as mentioned earlier, the perithecia of *N.*
haematococca were observed on the rockwool blocks in which the pepper plants were growing. Because the nutrient solution was provided at timed intervals through drip irrigation emitters placed at the base of each plant, the rockwool blocks remained wet at all times. Therefore, perithecia produced on the blocks were adequately hydrated throughout the release period, which could explain attenuated ascospore releases still occurring in greenhouse GA despite the unfavorable climate. The low background level of spores (0-200) observed in greenhouse GB throughout the growing season also could have originated from the rockwool blocks as virtually no diseased fruit or plants were reported by the grower. Perithecial formation and maturation, ascospore release and ascospore germination on blocks can occur independent of ambient relative humidity. By colonizing the blocks the fungus has a means of surviving periods of unfavorable climate throughout the growing season and producing spores in the absence of diseased fruit or crowns and is therefore available to cause disease whenever greenhouse climate permits.

Under controlled environment conditions, ascospore release from moistened perithecia was not inhibited by relative humidity as low as 51%, the lowest tested (Figure 20, A). This is consistent with the greenhouse observations and further supports the theory that a low ambient relative humidity is not a limiting factor for N. haematococca ascospore discharge if the perithecia are adequately hydrated. Nevertheless, spore release was greater and longer at higher relative humidity values (Figure 20, A and B). These results are in general agreement with field observations for other perithecial pathogens, which indicate that ascospore release continues through dry periods as long as the substrate bearing perithecia is moist (Alderman, 1993; Dobrev, 1987; Froyd and French, 1967; Johnson and Kuntz, 1979; Paulitz, 1996; Perrin, 1978; Reis, 1990; Sanderson, 1970). My observations also confirm
that spore discharge from moist perithecia is prolonged by high relative humidity. The very limited or no spore discharge observed during the second day at low relative humidities (51 and 75\%, respectively - Figure 20, B) was also noted previously by the above authors. The cessation of ascospore discharge in Ascomycetes associated with dryness of the fruiting body probably results from a loss in turgidity in the cells involved in discharge (Ingold, 1971). Therefore, if continued ascospore release in these fungi is to occur, there must be a sustained supply of water to maintain the turgidity of active cells.

Results from the second study, where ascospore release from air-dried perithecia was examined, suggest that high relative humidity (≥95\%) alone is sufficient for rehydration of perithecia of *N. haematococca* and therefore is able to induce the ascospore release. This is contrary to field reports for other perithecial pathogens, which indicate that a saturated atmosphere alone will not induce discharge. The perithecia must be wetted either by heavy dew or rain (Alderman, 1993; Dobrev, 1987; Froyd and French, 1967; Johnson and Kuntz, 1979; Perrin, 1978; Reis, 1990; Sanderson, 1970). However, in these field studies, if the perithecia were not fully hydrated at some point prior to wetting, the free moisture may have been required to stimulate formation and maturation of asci and ascospores, but not to stimulate the actual release event. In my study pepper fruit bearing mature perithecia were air dried and then used for this experiment. The environmental conditions necessary for perithecial and ascospore formation and maturation were not examined. Studies in an *in vitro* system similar to mine with two other ascomycetes also support the hypothesis that high relative humidity may be enough to actuate discharge. Mature apothecia of *Pseudopeziza medicaginis* and *Leptotrochila medicaginis* on dry, infected alfalfa leaves rehydrated and discharged ascospores at RH≥94\% and RH≥98\%, respectively (Semeniuk, 1984 and 1993).
Studies in controlled environment conditions also showed that the greenhouse temperature (15-30°C) is not a limiting factor for ascospore discharge in *N. haematococca*. This may explain why ascospore release in the greenhouse was not correlated with ambient air temperature. Nevertheless, higher numbers of ascospores were released at lower temperatures (15-20°C, optimum at 15°C) than that at 25 and 30°C. Similarly, cool to moderate temperatures (11-20°C, optimum 16°C) favored ascospore discharge in *Gibberella zeae* (family Hypocreaceae) (Tschanz and Horst, 1976).

Based on these results, longer periods of spore release in *N. haematococca* may be possible only under longer periods of saturated moisture conditions or high relative humidity at the plant tissue surface. Ascospore release from the rockwool blocks can occur independent of ambient relative humidity. Whether free water (e.g., heavy dew) or only high relative humidity is required for the formation and maturation of perithecia and ascospores of *N. haematococca* is a different matter and remains to be determined.

In the *in vitro* survival study, complete mortality of ascospores of *N. haematococca* was first observed at 30°C (after 6 days), which was the highest temperature tested (Figure 25, A). Previous studies on ascospores of other fungi have also shown that mortality of ascospores increased as temperature increased (Caesar and Pearson, 1983; Johnson and Kuntz, 1979; Merc and Fergus, 1954; Patryka and Mai, 1962; Pearson et al., 1990). High temperatures may decrease longevity by increasing metabolic activities, which could cause quicker depletion of reserve foods and result in spore death. However, in my study the ascospores did not survive the longest at 15°C (12 days) as expected, but at 20°C and 25°C (16 days) (Figure 25, A). This could simply be due to adaptation of *N. haematococca* to
these prevailing greenhouse temperatures (20-25°C). The experiment was repeated once and similar results were obtained. Similar to observations for ascospores of other species (Ceasar and Pearson, 1983; Merc, 1954), rapid change in temperature and humidity shortened the viability of ascospores of *N. haematococca* (Figure 25, B). However, even under such adverse conditions nearly 30% of the spores survived for up to 4 days.

The results from this study suggest that ascospores deposited onto plant surfaces can survive prolonged periods (several days) of unfavorable climate until free moisture or a nearly saturated environment is available for infection. Nevertheless, because the situation on the actual plant surface could be different from the situation on the glass slides used in this study the results should be interpreted with caution. Although the ascospores of *N. haematococca* are quite hardy, the results suggest that there are not able to survive the crop free period (3-6 weeks) between the two growing seasons. The results also indicate that hot days with large fluctuations in temperature and relative humidity are most detrimental for ascospore survival.

Under controlled environment conditions, spore germination and germ tube development of *N. haematococca* responded strongly to temperature and relative humidity. The optimum temperature for ascospore germination and germ tube elongation was 30°C, which was the highest temperature tested (Figure 21 and Figure 22). Nevertheless, very good germination was achieved at temperatures of 20 and 25°C (58 and 73%, respectively at 100% RH after 12 h). A temperature of 15°C delayed and reduced germination (<40% after 24 h at 100%) (Figure 22). Germination of ascospores of two other species from the family Hypocreaceae, *Nectria galligena* and *Gibberella zeae*, was also greater and faster at higher temperatures, with optima of 21-27°C and 25-30°C, respectively (Lortie, 1964; Ye, 1980).
Ascospores germinated only at relative humidities ≥95%, which is similar to observations for ascospores of *Gibberella zeae* (Sung and Cook, 1981). However, at an RH of 95%, low germination was recorded only at 30°C, even after 24 hours. Ascospores placed at relative humidities of 95-99% took a longer time to germinate than those exposed to a saturated environment (100% RH) or free water treatment (Figure 21 and Figure 22). The ability of spores to germinate in the absence of free water has been related to high osmotic pressure of the spore, which allows it to absorb water from the air (Sussman and Halvorson, 1966). Thus, the longer germination time observed in this study at 95-99% RH may be explained in terms of a longer absorption period. In many cases higher percent germination was observed in the 100% RH treatment compared to the free water treatment (Figure 21 and Figure 22). This may be due to a low oxygen supply in the water droplet used to wet the spores. Lilly and Barnett (1951) suggested that germination is greater in aerated than in nonaerated water because of the difference in oxygen supply.

Finally, it is important to note that relatively long periods (8-12 h) of free water or nearly saturated environment (RH>98%) accompanied by temperatures ≥20°C are required to induce a substantial rate of ascospore germination (Figure 21 and Figure 22). This requirement leaves the narrow range of climatic conditions that favor infection by *N. haematococca* and suggests that Fusarium fruit and stem rot disease in commercial pepper greenhouses could be diminished by reducing the periods of high relative humidity. The effects of duration of periods of high relative humidity in combination with specific temperatures on spore germination described in this study are useful elements in predicting the probable infection periods under greenhouse conditions. These parameters could be also used in the construction of disease forecasting models.
5.3. CONCENTRATIONS OF AIRBORNE ASCOSPORES OF *NECTRIA HAEMATOCOCCA*, GREENHOUSE ENVIRONMENT AND OCCURRENCE OF FUSARIUM FRUIT ROT OF THE GREENHOUSE-GROWN PEPPERS

The ascospore concentrations detected in the air showed good correlation with Fusarium fruit and stem rot disease incidence and severity, but this relationship was not entirely consistent. For example, daily average ascospore concentration of 13715 spores m\(^{-3}\) air resulted in yield losses estimated at 40% in the greenhouse GC during October, 1997 (Figure 9). In the same greenhouse during April, 1997, daily average ascospore levels of 646-1101 spores m\(^{-3}\) air were associated with yield losses estimated at 10% (Grower C, personal communication). Conversely, daily average ascospore numbers ranging from 347-1033 spores m\(^{-3}\) air were associated with negligible disease levels in greenhouse GA during October, 1997 (Figure 9). Norse (1971) also found that epidemic development of *Alternaria longipes* in glasshouse tobacco plants was not consistently related to the atmospheric spore content. Meredith (1970), who worked with *Mycosphaerella fijiensis*, the causal agent of black leaf streak disease of bananas, found that even at times of abundant ascospore release, disease incidence declined during winter months in Hawaii. Corresponding results were also reported by Hirst and Stedman (1962) who pointed out that spore trapping usually measures a “relative dose” of spores rather than an “effective dose”. Given a susceptible host and a virulent strain of the pathogen, the “effective dose”, which is the proportion of spores able to cause infection, will vary with the climate regime and the condition of the host.

In greenhouse GC, condensate drippings from the greenhouse structure along with “fruit sweating” associated with long periods of high relative humidity (Grower C, personal communication) created perfect conditions for ascospore germination, thus enabling
numerous fruit infections to develop in early spring (primary infections). The perithecia produced on the blocks along with the perithecia from fruit lesions provided further aerial inoculum, which, accompanied by the conditions favorable for spore germination, contributed to many secondary infections during the 1997 growing season. This resulted in a build up of inoculum (daily average concentration of 13715 ascospores m$^{-3}$ air) and yield losses estimated at 40% by the end of the growing season. In addition, the excessive moisture of the rockwool blocks, caused by overwatering, might have led to oxygen depletion and thus decreased the ability of the host to defend itself (Agrios, 1988) resulting in the high incidence of crown lesions observed in greenhouse GC in autumn. This observation agrees with reports for other diseases caused by *Fusarium solani*. Root rots of *Phaseolus vulgaris* and *Pisum sativum* caused by *F. solani* develop under conditions that reduce root growth such as anaerobic soil or drought (Burke and Miller, 1983; Miller and Burke, 1985). Likewise, SDS (sudden death syndrome) of soybean caused by *F. solani* has been associated with wet, cool weather which favors saturated anaerobic soil conditions (Rupe, 1989).

By contrast, in greenhouse GA, which had a drier climate (Table 1 and Figure 11), only a few spores of *N. haematococca* were able to germinate in the early spring and therefore to infect the plants and form new sources of inoculum. Thus, despite high inoculum levels present at planting out, daily average ascospore concentrations stayed relatively low (below 250 spores m$^{-3}$ air) for the main part of the 1997 growing season (Figure 10). From mid September until mid November (the end of cropping) daily average ascospore concentrations ranged from 347 – 1704, however, due to conditions unfavorable for the infection process (Table 1 and Figure 11) the incidence of disease remained negligible. In addition, the precisely adjusted drip irrigation, which maintained good aeration of the
rockwool blocks, could have kept the plants strong throughout the growing season. Therefore, despite the fact that the fungus was present on the blocks from the beginning of the growing season, relatively few crown lesions appeared only during the last two weeks of cropping. The observations from greenhouse GA suggest that even in the presence of adequate inoculum it is possible to avoid the disease through aggressive climate management.

In greenhouse GA, none of the environmental variables satisfactorily explained the variations in ascospore numbers. However, except for the especially high spore levels detected during the January, late October and early November 1997, the ascospore concentrations did not fluctuate much throughout the year (Figure 10). Because at least 2-3 weeks are required for the perithecial production (as shown in section 3.5), the high spore levels detected during the first month of cropping must have originated from the perithecia produced during the propagation cycle. Therefore, the spore levels trapped during the January in commercial greenhouse GA could be attributed to the factors from the propagation house. The significant drop in ascospore levels (thus in the perithecial formation and maturation) observed during the second month of cropping, suggests that conditions which influence the growth and sporulation of *N. haematococca* on the rockwool blocks in commercial greenhouse GA are not as favorable as those in the propagation house. The nutrient composition of the growing medium (Dehorter, 1982; Shipton, 1977) light intensity and its wavelength composition (Curtis, 1964; Leach, 1962; Tshanz et al., 1976) and temperature (Rowe and Beute, 1974; Silue and Notteghem, 1990; Ye, 1980) have been shown to be important for perithecial production and maturation of other fungi. Thus, the combination of the composition of the nutrient feed, temperature and supplemental light (sodium vapor) provided during the propagation could be responsible for those observations.
However, the cause of such big differences in perithecial production on the blocks between commercial greenhouse GA and the propagation house remains to be determined.

The rise in ascospore concentration observed at the end of the growing season in greenhouse GA could be ascribed to reduced crop hygiene. This resulted in accumulation of prunings and culled fruit on the greenhouse floor, which created an ideal substrate for perithecial production, ascospore formation and release. In addition, sporulation on crown lesions could have contributed to high ascospore numbers (daily average concentration of 1704 spores m$^{-3}$ air) observed in November (the end of cropping).

Ascospore levels monitored in a commercial greenhouse having low inoculum levels present at planting-out (greenhouse GB) remained low and variable (daily average concentration of 0-200 m$^{-3}$ air) until the end of 1997 growing season (Figure 10). In spite of periods of favorable climate being recorded (Table 1 and Figure 11), the disease did not develop probably because of insufficient primary inoculum being present. Conversely, in the same greenhouse during the October 1996, when sufficient inoculum was present (daily average concentrations of 1899 to 3737 ascospores m$^{-3}$ air, Figure 8), under a climate regime similar to that observed in October 1997 (Table 1 and Figure 15) yield losses were estimated at 9% (Grower B, personal comm.).

As determined by in vitro germination studies, a substantial rate of germination was observed only with prolonged periods of very high (>98%) relative humidity. According to environmental data collected during 1996 (from mid August until mid November) and 1997 growing season, the relative humidity values approaching these levels were never recorded in greenhouse GA and seldom in greenhouse GB (Table 1). The presence of numerous fruit and
stem lesions (yield losses estimated at 9%) in greenhouse GB at the end of 1996 growing season, indicates that the microclimate on the plant surface (where the spores are) in the canopy is more humid than that of ambient air recorded by the sensors.

Unfortunately, the instruments available for this study were not suitable for measuring the humidity of air adjacent to surface of the plant. However, wet periods can be roughly estimated from the duration of the RH≥90% on hygrothermographs charts (Sutton et al., 1984). The 90% RH threshold has been found to correspond closely to leaf wetting in onions (Vincelly, 1988; Vincelly and Lorbeer 1988). Furthermore, as a result of its ready availability in standard meteorological records, many successful disease prediction models use period of RH≥90% as a proxy for leaf wetness (Jensen and Boyle, 1966; Jones et al., 1980; Krause et al., 1975; Preece and Smith, 1961; Vincelli and Lorber, 1988; Wallin, 1962). Therefore, periods with relative humidities ≥90% were considered as an indication of the periods when the relative humidity around the plant surface could have reached the levels required for N. haematococca ascospore germination (RH≥98%). To further improve the estimate of near-surface humidity (as suggested by Seginer and Zlochin, 1997), the condensation safety margin (CSM), the difference between the actual air temperature and the calculated dew-point temperature of the greenhouse air, was used as a measure of the condensation risk (greater risk when CSM is small, e.g., <1°C).

Applying this ≥90% RH criterion to greenhouse GB, the number of hours per month when the combination of within-canopy conditions was ideal for germination of N. haematococca ascospores (calculated from greenhouse temperature and relative humidity data, Table 1 and Table 2), were 0, 5, 7, 62, 24, 84, 120, 147, 46, and 29 for February,
March, April, May, June, July, August, September, October, and November (until 15th), respectively. In contrast, in greenhouse GA there were 0, 0, 0, 8, 2, 16, 0, 0, 1 hours of ideal conditions for those months, respectively. Thus the length of time when conditions favored Fusarium fruit and stem rot was about 20 times greater in greenhouse GB than in greenhouse GA. As for the condensation safety margin, the 126 and 0 hours of CSM<1°C were recorded in greenhouse GB and GA, respectively, during the 1997 growing season.

During the period from August 15 to November 15 1996 in greenhouse GB, the number of hours per month with RH≥90% and temperature ≥20°C were 63, 128, 60, 25, respectively. 48 hours of CSM<1°C were recorded for this period.

The large differences in the humidity regimes between greenhouse GA and GB are most probably due to differences in ventilation management. The effectiveness of ventilation in reducing the incidence of Botrytis cinerea and Cladosporium fulvum in greenhouse-grown tomatoes has been demonstrated by Morgan (1984) and Winspear et al. (1970), respectively. In greenhouse GA, vents remained opened for most of the nights during 1997 growing season (Grower A, personal communication). Conversely, due to inadequate heating pipes and absence of growing pipes, nighttime ventilation was very limited in greenhouse GB (Grower B, personal communication). In addition, a drainage problem which resulted in the large quantity of puddled water on the greenhouse floor, could have contributed to the longer periods of high relative humidity observed in greenhouse GB.

The daily pattern of temperature increase in greenhouse GB (Figure 13 and Figure 14) also could have promoted the wetting of the plant surfaces. The thermal lag of the more massive parts of the plant which causes pepper fruit and stem temperature to increase slowly
in the morning is well known (Butler, 1980; Monteith, 1979; Huber, 1991 and Payen, 1983). Therefore, the rapid or late (after the sunrise) temperature rise in the morning could have resulted in dew formation because of the dew point temperature exceeding the fruit and stem temperature (Figure 13 and Figure 14). The deposition of dew on fruit during rapidly rising air temperatures in the morning was clearly demonstrated by Cotton (1969) in greenhouse-grown tomato crop. A slow temperature increase early in the morning (1°C per hour) ensures that tissue temperature reaches the daytime targets before the sunrise (the pattern typical for the greenhouse GA).

Epidemiologically the results of this study help in understanding why the fungus historically had been a problem in commercial pepper greenhouse GB while not in the greenhouse GA. In greenhouses with a wet climate *N. haematococca* exploits conditions allowing discharge and germination of ascospores, maintaining a high pressure of inoculum and increasing possibility of establishing itself on a large number of host plants.

The primary infections on the pepper fruit in commercial greenhouses are usually observed in April, which is about a month after the first periods of conditions favoring ascospore germination (based on the above mentioned ≥90% RH criterion) were recorded in commercial greenhouses (Table 1). This timing is consistent with the incubation period determined by lab experiments where pepper fruit inoculated with the spore suspensions showed typical rot symptoms within approximately 3 weeks (section 3.3). Although Fusarium fruit and stem rot usually becomes a serious problem in October, greenhouse data indicate that environmental conditions favoring spore germination and infection (Table 1 and Table 2) are most prevalent in July, August and September. The observations from
greenhouse GB during August and October 1996 (Figure 8 and Figure 15) indicate that low inoculum concentration is less likely to be responsible for the low disease incidence observed during the summer months. Other factors such as a combination of radiation, temperature, relative humidity, the structure and composition of plant cuticle, or the water relations (water and osmotic potentials) in the plant may contribute to the higher Fusarium stem and fruit rot disease incidence and severity in the autumn than in the summer. In summer, stem and fruit tissue may possess a thicker cuticle and more wax owing to high radiation, high temperatures and water stress (Skoss, 1955; Baker, 1974; Kolattukudy, 1985). Thus, germinated spores of *N. haematococca* may have greater difficulty penetrating the cuticle in summer than in autumn. Furthermore, the later period coincides with a maximum amount of senescent lateral tissue, which, as suggested by Fletcher (1994), may provide ideal entry points for the pathogen. Also, exposure to big fluctuations in temperature and relative humidity, which are typical for the greenhouse summer climate, can accelerate the decline in viability of *Nectria haematococca*. 
6. CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Spore monitoring in commercial pepper crops during three different growing seasons strongly suggests that ascospores of *Nectria haematococca* are both the primary and secondary inoculum. The conidia of *Fusarium solani* were seldom detected and are considered to play a minor role, if any, in the disease spread. Ascospores are discharged during the night and the duration of discharge appears to vary with photoperiod during the growing season. No ascospores could be detected in greenhouses upon completion of the end-of-season cleanup procedures, indicating that detectable spore inoculum was not being carried over from one season to the next in the commercial greenhouses.

This study demonstrated that the rockwool cubes infested during propagation could be the main source of primary inoculum for epidemics of Fusarium fruit and stem rot in Lower Mainland commercial pepper greenhouses (proposed disease cycle is shown in Figure 26). The presence of the perithecia of *N. haematococca* on the rockwool blocks is a great advantage for the fungus since ascospore formation, maturation, discharge and germination can occur independent of ambient relative humidity. By colonizing the blocs the fungus has a means of surviving periods of unfavorable climate throughout the growing season and producing spores in the absence of diseased fruit or crowns and is therefore available to cause disease whenever greenhouse climate permits.
Figure 26. Proposed disease cycle of Fusarium fruit and stem rot of greenhouse-grown sweet peppers caused by Nectria haematococa (anamorph Fusarium solani).
As determined by controlled environment studies, ascospore germination occurred only during prolonged periods of very high (>95%) relative humidity. This requirement narrows the range of climatic conditions that favor infection by *N. haematococca*. Therefore, although perithecia of *N. haematococca* are present on the blocks from the early stage of the growing cycle, losses due to Fusarium fruit and stem rot will depend on the greenhouse climate management. In a greenhouse where a restricted ventilation and poor drainage create a “wet” climate *N. haematococca* exploits conditions perfect for ascospore germination, thus enabling numerous fruit infections in the early spring (primary infections). The perithecia present on the blocks, along with the perithecia produced on the fruit lesions, provide further aerial inoculum, which when accompanied by a wet greenhouse climate in the autumn, results in numerous fruit and stem infections. Thus, the pathogen establishes itself on a large number of host plants, the inoculum builds up and high yield losses are observed. In addition, overwatering of the growing medium can lower the defense system of the plants, through oxygen depletion, resulting in a high incidence of crown lesions in autumn. Therefore, growers who restrict ventilation to save fuel, or have inadequate heating systems and poor soil profiles, may face extended period of high relative humidity, which in the presence of sufficient inoculum leads to an increased disease pressure.

By contrast, in the greenhouse where frequent ventilation and good drainage create a drier climate, only a few spores of *N. haematococca* are able to germinate and create new sources of inoculum in the early spring. By maintaining a dry climate, ascospore levels stay low for the main part of the growing season and disease incidence remains negligible despite high inoculum levels present at planting-out. In addition, good aeration of the rockwool blocks may allow the plants to remain strong and only a few crown lesions may occur at the
end of the growing season. This suggests that aggressive climate management, which avoids extended periods of high humidity and excessive wetness of the rockwool blocks, may reduce Fusarium fruit and stem rot disease to an acceptable level in Lower Mainland sweet pepper production.

In commercial greenhouse where low inoculum levels were present at planting-out time, ascospore levels remained low throughout the 1997 growing season and disease did not develop, in spite of periods of climate being recorded that should favor disease development. This points out the importance of the quantity of the primary inoculum.

Identification of the main source of inoculum for Fusarium fruit and stem rot epidemics in Lower Mainland commercial pepper greenhouses is an important first step in disease management. This work sets the stage for development of control strategies based on eradication or reduction of primary inoculum. Future work should be directed to determining how and where the pathogen is being introduced during the propagation cycle. In addition, attempts should be made to identify biocontrol agent(s) effective in preventing the contamination of the rockwool blocks and optimal use pattern should be established for their application in pepper propagation.
REFERENCES CITED


APPENDICES
## APPENDIX A

### STATISTICS

#### Table ANOVA 1. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore discharge from the fresh pepper tissue during the first day.

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
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<td>T</td>
<td>3</td>
<td>2043881.05</td>
<td>681293.68</td>
<td>161.66**</td>
</tr>
<tr>
<td>Error (a)</td>
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<td>50570.90</td>
<td>4214.24</td>
<td>0.60</td>
</tr>
<tr>
<td>RH</td>
<td>4</td>
<td>4253678.67</td>
<td>1063419.66</td>
<td>47.47**</td>
</tr>
<tr>
<td>T x RH</td>
<td>12</td>
<td>268807.82</td>
<td>22400.65</td>
<td>3.21**</td>
</tr>
<tr>
<td>Error (b)</td>
<td>48</td>
<td>335249.10</td>
<td>6984.35</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = significant at α=0.05; ** = significant at α=0.01

#### Table ANOVA 2. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore discharge from the fresh pepper tissue during the second day.

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
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<td>525133.83</td>
<td>175044.61</td>
<td>49.80**</td>
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<tr>
<td>Error (a)</td>
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<td>42180.85</td>
<td>3515.07</td>
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<tr>
<td>RH</td>
<td>4</td>
<td>4262443.05</td>
<td>1065610.76</td>
<td>26.71**</td>
</tr>
<tr>
<td>T x RH</td>
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<td>478799.85</td>
<td>39899.98</td>
<td>14.39**</td>
</tr>
<tr>
<td>Error (b)</td>
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<td>133067.90</td>
<td>2772.24</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>5441625.48</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Table ANOVA 3. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore discharge from the air-dried pepper tissue.

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>3</td>
<td>142265.67</td>
<td>47421.89</td>
<td>13.16**</td>
</tr>
<tr>
<td>Error (a)</td>
<td>12</td>
<td>43257.68</td>
<td>3604.80</td>
<td>2.22*</td>
</tr>
<tr>
<td>RH</td>
<td>3</td>
<td>457761.42</td>
<td>15287.14</td>
<td>11.38**</td>
</tr>
<tr>
<td>T x RH</td>
<td>9</td>
<td>120626.26</td>
<td>13402.91</td>
<td>8.25**</td>
</tr>
<tr>
<td>Error (b)</td>
<td>36</td>
<td>58487.56</td>
<td>1624.62</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>822398.60</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = significant at α=0.05; ** = significant at α=0.01
Table ANOVA 4. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore germination (%) 8 hrs after treatments were imposed.

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>3</td>
<td>11487.43</td>
<td>3829.14</td>
<td>333.98**</td>
</tr>
<tr>
<td>Error (a)</td>
<td>12</td>
<td>137.58</td>
<td>11.46</td>
<td>1.84</td>
</tr>
<tr>
<td>RH</td>
<td>4</td>
<td>11177.49</td>
<td>2794.37</td>
<td>7.18**</td>
</tr>
<tr>
<td>T x RH</td>
<td>12</td>
<td>4669.41</td>
<td>389.11</td>
<td>62.51**</td>
</tr>
<tr>
<td>Error (b)</td>
<td>48</td>
<td>298.80</td>
<td>6.22</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>27770.73</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = significant at $\alpha=0.05$; ** = significant at $\alpha=0.01$

Table ANOVA 5. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore germination (%) 12 hrs after treatments were imposed.

<table>
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<tr>
<th>Source of Variation</th>
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</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>3</td>
<td>20956.78</td>
<td>6985.59</td>
<td>8.25**</td>
</tr>
<tr>
<td>Error (a)</td>
<td>12</td>
<td>738.16</td>
<td>61.51</td>
<td>2.26*</td>
</tr>
<tr>
<td>RH</td>
<td>4</td>
<td>18633.18</td>
<td>4658.29</td>
<td>8.25**</td>
</tr>
<tr>
<td>T x RH</td>
<td>12</td>
<td>6778.36</td>
<td>564.86</td>
<td>20.80**</td>
</tr>
<tr>
<td>Error (b)</td>
<td>48</td>
<td>1303.65</td>
<td>27.15</td>
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<tr>
<td>Total</td>
<td>79</td>
<td>48410.15</td>
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<td></td>
</tr>
</tbody>
</table>

Table ANOVA 6. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore germination (%) 24 hrs after treatments were imposed.

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>3</td>
<td>14095.47</td>
<td>4698.49</td>
<td>41.52**</td>
</tr>
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<td>Error (a)</td>
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<td>1358.10</td>
<td>113.17</td>
<td>3.73**</td>
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<tr>
<td>RH</td>
<td>4</td>
<td>33584.25</td>
<td>8396.06</td>
<td>28.36**</td>
</tr>
<tr>
<td>T x RH</td>
<td>12</td>
<td>3552.46</td>
<td>296.03</td>
<td>9.75**</td>
</tr>
<tr>
<td>Error (b)</td>
<td>48</td>
<td>1457.20</td>
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<tr>
<td>Total</td>
<td>79</td>
<td>54047.50</td>
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* = significant at $\alpha=0.05$; ** = significant at $\alpha=0.01$
### Table ANOVA 7. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore germ-tube length 8hrs after treatments were imposed.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>3</td>
<td>49627.99</td>
<td>16542.66</td>
<td>137.73**</td>
</tr>
<tr>
<td>Error (a)</td>
<td>12</td>
<td>1441.30</td>
<td>120.10</td>
<td>1.54</td>
</tr>
<tr>
<td>RH</td>
<td>4</td>
<td>101983.65</td>
<td>25495.91</td>
<td>6.46**</td>
</tr>
<tr>
<td>T x RH</td>
<td>12</td>
<td>47386.96</td>
<td>3948.91</td>
<td>50.53**</td>
</tr>
<tr>
<td>Error (b)</td>
<td>48</td>
<td>3751.18</td>
<td>78.14</td>
<td>2.94**</td>
</tr>
<tr>
<td>Error (c)</td>
<td>720</td>
<td>19126.38</td>
<td>26.56</td>
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<tr>
<td>Total</td>
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<td>223317.47</td>
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<td></td>
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* = significant at α=0.05; ** = significant at α=0.01

### Table ANOVA 8. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore germ-tube length 12 hrs after treatments were imposed.

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</thead>
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<td>3</td>
<td>514556.84</td>
<td>171518.94</td>
<td>645.67**</td>
</tr>
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<td>Error (a)</td>
<td>12</td>
<td>3187.74</td>
<td>265.64</td>
<td>1.10</td>
</tr>
<tr>
<td>RH</td>
<td>4</td>
<td>1323118.90</td>
<td>330779.72</td>
<td>5.57**</td>
</tr>
<tr>
<td>T x RH</td>
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<tr>
<td>Error (b)</td>
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<tr>
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<td>720</td>
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* = significant at α=0.05; ** = significant at α=0.01

### Table ANOVA 9. Analysis of variance for data from effect of temperature (T) and relative humidity (RH) and their interaction on ascospore germ-tube length 24 hrs after treatments were imposed.

<table>
<thead>
<tr>
<th>Source of Variation</th>
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<td>2478693.33</td>
<td>826231.11</td>
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</tr>
<tr>
<td>Error (a)</td>
<td>12</td>
<td>154401.48</td>
<td>12866.79</td>
<td>1.18</td>
</tr>
<tr>
<td>RH</td>
<td>4</td>
<td>9104125.18</td>
<td>2276031.29</td>
<td>9.96**</td>
</tr>
<tr>
<td>T x RH</td>
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<td>2743151.91</td>
<td>228595.99</td>
<td>20.93**</td>
</tr>
<tr>
<td>Error (b)</td>
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<td>524351.59</td>
<td>10923.99</td>
<td>4.32**</td>
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</tr>
<tr>
<td>Total</td>
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Table ANOVA 10. Analysis of variance for data from effect of temperature (T) and days of air drying (D) and their interaction on ascospore viability.

<table>
<thead>
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<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>3</td>
<td>14002.99</td>
<td>4667.66</td>
<td>208.87**</td>
</tr>
<tr>
<td>Error (a)</td>
<td>12</td>
<td>268.16</td>
<td>22.34</td>
<td>0.91</td>
</tr>
<tr>
<td>D</td>
<td>7</td>
<td>40027.76</td>
<td>5718.25</td>
<td>22.91**</td>
</tr>
<tr>
<td>T x D</td>
<td>21</td>
<td>5240.89</td>
<td>249.56</td>
<td>10.13**</td>
</tr>
<tr>
<td>Error (b)</td>
<td>84</td>
<td>2069.16</td>
<td>24.63</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>127</td>
<td>61608.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = significant at α=0.05; ** = significant at α=0.01

Table ANOVA 11. Analysis of variance for data from effect of days of air drying on ascospore viability.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>6</td>
<td>6730.80</td>
<td>1121.80</td>
<td>72.48**</td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>325.03</td>
<td>15.47</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>27</td>
<td>7055.83</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* = significant at α=0.05; ** = significant at α=0.01

Table REG 1. Regression parameters for the relationship between the time (hours) and cumulative percentage of ascospores trapped per hour for each month in the greenhouse A.

<table>
<thead>
<tr>
<th>Month (1997)</th>
<th>( \beta )</th>
<th>( \gamma )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>4.4545</td>
<td>-0.52445</td>
<td>0.95</td>
</tr>
<tr>
<td>March</td>
<td>9.1118</td>
<td>0.92478</td>
<td>0.93</td>
</tr>
<tr>
<td>April</td>
<td>10.7650</td>
<td>0.90656</td>
<td>0.94</td>
</tr>
<tr>
<td>May</td>
<td>10.0030</td>
<td>-0.81415</td>
<td>0.95</td>
</tr>
<tr>
<td>June</td>
<td>11.3860</td>
<td>-0.83021</td>
<td>0.95</td>
</tr>
<tr>
<td>July</td>
<td>12.5370</td>
<td>-1.03430</td>
<td>0.93</td>
</tr>
<tr>
<td>August</td>
<td>13.3690</td>
<td>-1.01880</td>
<td>0.89</td>
</tr>
<tr>
<td>September</td>
<td>10.9490</td>
<td>-0.89121</td>
<td>0.95</td>
</tr>
<tr>
<td>October</td>
<td>10.8590</td>
<td>-0.88890</td>
<td>0.97</td>
</tr>
<tr>
<td>November</td>
<td>10.2750</td>
<td>-0.90364</td>
<td>0.96</td>
</tr>
</tbody>
</table>
**Table REG 2.** Regression parameters for the relationship between the time (hours) and cumulative percentage of ascospores trapped per hour for each month in the greenhouse B.

<table>
<thead>
<tr>
<th>Month (1996)</th>
<th>$\beta$</th>
<th>$\gamma$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>12.2680</td>
<td>-0.92982</td>
<td>0.95</td>
</tr>
<tr>
<td>September</td>
<td>11.7150</td>
<td>-0.93190</td>
<td>0.95</td>
</tr>
<tr>
<td>October</td>
<td>9.7937</td>
<td>-0.76530</td>
<td>0.96</td>
</tr>
<tr>
<td>November</td>
<td>10.0580</td>
<td>-0.87732</td>
<td>0.95</td>
</tr>
</tbody>
</table>
APPENDIX B

PROTOCOL FOR PREPARATION OF SLIDES FOR SPORE TRAP

**Slide base**

A base is prepared from the following mixture:

35 g Gelvatol (grade 40-20)  
100 ml Distilled water  
50 ml Glycerin  
2 g Phenol

The Gelvatol is added to the water and allowed to stand overnight. Glycerol is than added and the solution warmed slightly in a water bath (40- 50°C) and stirred vigorously until the Gelvatol is dissolved completely. Finally, the phenol is added.

**Slide mounting medium**

A mounting medium is prepared from the following mixture:

10 g Gelvatol (grade 40-20)  
25 ml Distilled water  
50 ml Glycerin  
25 ml Lactic acid (20%)  
6.25 g Phenol  
0.05 g Cotton blue

The Gelvatol is added to the water and allowed to stand overnight. Glycerol is than added and the solution warmed slightly in a water bath (50- 60°C) and stirred vigorously until the Gelvatol is dissolved completely. Finally, the lactic acid, the phenol and the cotton blue are added.
APPENDIX C

SOLUTIONS TO MAINTAIN CONSTANT HUMIDITY IN A CLOSED ATMOSPHERE

The salt solutions were prepared according to data given in the Electrolyte solutions (Robinson and Stokes, 1956) by dissolving salts in water on a magnetic stirrer overnight at room temperature. The following salts were chosen for the various humidities:

- **51%** = Mg(NO$_3$)$_2$ $\cdot$ 6H$_2$O (saturated) 586.0 g/l (use 805 ml water)
- **76%** = NaCl (saturated) 317.0 g/l (use 881 ml water)
- **88%** = NaCl (3.1M) 187.0 g/l
- **95%** = NaCl (1.5M) 87.6 g/l
- **98%** = NaCl (0.6M) 35.0 g/l
- **99%** = NaCl (0.3M) 17.5 g/l

Data are provided for making saturated solutions by volume (g of substance per 1 l of saturated solution and the ml of water required to make such a solution).