Appendix 1. Summary details of past investigations, published and grey literature (in chronological order), on the meteorological variables that affect a) infection (germination and penetration) and b) spore release by *Mycosphaerella nubilosa* and *M. cryptica* in eucalypts and c) other *Mycosphaerella* spp. on various hosts.

(a) **Studies investigating meteorological variables that affect infection by *Mycosphaerella* spp.**				
Author/Year	**Methodology**	***Mycosphaerella* species**	**Host**	**Results**
(Cheah, 1977)	**Field** -Visual assessment of infection in abscised and symptom free leaves.	*M. cryptica**	*E. delegatensis* (New Zealand)	-Peak infection occurred after several days of rainfall, and long periods of high relative humidity. -Infection was highest during late summer (1 month after peaks in ascospores as shown by water trapping), then slowed during autumn and reduced to none in winter. -New infection symptoms were evident in September (spring). An infection peak occurred in December, then again in late February. -Ascospores were always more frequently deposited on leaf surfaces than conidia. -Germination rate of ascospores was always higher than conidia. -Deposition of conidia was always observed along edges of leaves, around the midrib or where there were curves in the leaf. The deposition of the ascospores was more scattered over the entire leaf surface.
	Climate Chambers -Potted trees were exposed to infected trees during rain events in the field then incubated in chambers.	*M. cryptica**	*E. delegatensis* (New Zealand)	-The number of hours after exposure that relative humidity was maintained above 95% was positively correlated with appearance of infection symptoms.
	Laboratory conditions -Leaves with spores were collected from the field and stored under laboratory conditions for various lengths of time and then placed into petri dishes, soaked periodically, then examined for number of spores germinated.	*M. cryptica**	*E. delegatensis* (New Zealand)	-Ascospores germinated 48hrs after being soaked, then left to dry. -Percentage of spores that germinate was found to be proportional to the time the spores were stored under laboratory conditions. Germination dropped from 60-70% of total spore discharged after 8 days in the laboratory, to 20% at 14 days. All spores lost their ability to germinate after 14 days under laboratory conditions.

* refers to *Mycosphaerella cryptica* which has been misidentified and published as *M. nubilosa*. These studies observed the production of both conidia and ascospores indicating the detection of *M. cryptica.* The corrected species has been included in this table with an asterisk.

Appendix 2. This appendix includes two sections. The first explains the methodology of fitting mathematical functions to the ascospore and meteorological data and the second includes the methodology for calculating a prediction.

Section 1. Application of mathematical functions to daily and diurnal ascospore and meteorological data.

This section was completed in collaboration with Dr Scott Foster at the Tasmanian Institute of Agricultural Research.

Function application was split into three steps. The first ignored meteorological data, and investigated the probability of observing ascospores. The second incorporated the meteorological data and the third considered the concentration of a ascospore event. Initially, the meteorological data were ignored so that the effects on plantation age, diurnal and seasonal patterns could be understood. The second step integrated the effect of meteorological variables on atmospheric ascospore concentrations along with the diurnal, seasonal and plantation age effects. Finally the concentration component was considered.

The observations (atmospheric ascospore presence/absence) were assumed to arise from a binomial distribution with a probability of p, meaning there is a p probability of observing ascospores at any given tree age under any meteorological conditions. The probability p, of the j^{th} observation, is related to tree age and meteorological conditions by the linear logistic regression function:

$$\text{Logit}(p_j) = \log\left(\frac{p_j}{1-p_j}\right) = \sum_{i=1}^{q} x_{ij}\beta_i \qquad \text{Equation (1).}$$

where p_j is the probability of observing ascospores, X_i is the i^{th} explanatory variable for j^{th} observation and β_i is the coefficient for the i^{th} explanatory variable. The function for the probability of ascospore dispersal is a generalised linear model with binomial error and logit link (McCullagh and Nelder, 1989). This equation models a non-linear function of the probability using a linear combination of the explanatory variables. The function used ensures that the fitted probability remained within the range (0, 1). This function allows the probability of ascospore counts to change with tree age, diurnal patterns and meteorological data by inclusion of suitable explanatory variables (x_{ij}). The coefficients (β_i) were not known, and were estimated.

1.1 Model development for predicting an ascoascospore event ignoring meteorological data

This model had three components: a long-term or daily trend, a diurnal trend and the interaction between these, which allowed changes to occur as the trees aged. The long-term or global trend was modelled as a polynomial with the best fitting level (up to degree 3) used for the model. A biological model was suggested by the form of a smoothing spline curve that was applied to the data. The diurnal trend was incorporated using a periodic function (Fuller, 1996) of the hour within day measured in military time (t = 0 h, ... 2400 h). The function is defined to have a period of 1 day and can be expressed as a simple sum of trigonometric functions.

The function is,

$$h(t) = \beta_1 t_{sin} + \beta_2 t_{cos}$$

where

$$t_{sin} = \sin(2\pi \text{ hour} / 2400)$$

$$t_{cos} = \cos(2\pi \text{ hour} / 2400).$$

Since this periodic function is linear in t_{sin} and t_{cos}, it can be easily incorporated into the model explaining variation in the ascospore concentration data.

The diurnal trend was allowed to change smoothly with the global trend of the data by also including interactions between diurnal components and age. The variables and their interactions for the model were chosen using a stepwise model building procedure (e.g. Miller, 2002; Neter et al., 1996) using Akaike's information criteria. This method starts with a base model (age effects) and includes the model with the most important explanatory variable. This process is iterative and continues to add variables into the model until no additional explanatory variable is important enough to be included. The analysis was completed using R-software (Ihaka and Gentleman, 1994) using the stepAIC function in the MASS package (Venables and Ripley, 2002). This function respects marginality by adding main effects before taking into account the interactions.

1.2 Model development for predicting a ascospore event with meteorological data and the concentration of the ascospore event

The statistical methods used to develop the model to predict the concentration of a ascospore event were an extension of those presented for the previous model. Modelling consisted of two parts, firstly calculating the probability of observing a ascospore event; and secondly, extracting the distribution probability for the ascospore events and the way in which its parameters are modelled. These models are often referred to as 'hurdle' models (Ridout et al., 1998).

The probability distribution (P) that allows for excess zeros is,

$$P(Y_j - y_j) = \begin{cases} (1 - p_j) & y = 0 \\ \dfrac{p_j \exp(-\lambda_j)\lambda_j{}^{y}}{(1 - \exp(-\lambda_j))\,y!} & y > 0 \end{cases} \qquad \text{Equation (2).}$$

There are two parameters for observation j, one models the probability of an actual ascospore release (p_j), and the other models the concentration of the ascospore event (λ_j). The two parameters p_j and λ_j are considered to depend on the explanatory variables (meteorological variables). Firstly, p was modelled using a logit function (see Equation (1) and previous section for a description), and secondly, λ_j was modelled using a log-link function. The log-link function is:

$$\log(\lambda_j) = \sum_{i=1}^{r} \chi_{ij} \tau_i$$

where τ refers to the explanatory variable for the concentration component of the ascospore concentration.

The form of the function for p_j was assumed to be the same as found in section 1.2. Model development therefore remains for the parameter 'λ_j'. This was completed using a forward stepwise procedure (e.g. Miller, 2002; Neter et al., 1996). Computation was completed using the hurdle function from the 'pscl' library in the R computing environment (Ihaka and Gentleman, 1994).

Using the raw cubic polynomial for the global age term caused the estimation algorithm to fail. This appeared to be due to the high correlation between the polynomials explanatory variables. This was remedied by taking a linear transformation of the age variables i.e. Age^1 (see page 191 for transformation calculation). Tests of hypotheses were used (i.e. examining p-values) for the stepwise procedure and the process was terminated when no term not already in the model had a significant effect (at the $\alpha = 0.05$ significance level). Due to computational limitations only main effects of the observed and derived explanatory variables were considered.

Section 2. Methodologies for predicting an ascospore release. An example is given of how to calculate a prediction to forecast if a ascospore event will occur or not, both with and without meteorological variables, and the concentration of the event.

This section was completed in collaboration with Dr Scott Foster at the Tasmanian Institute of Agricultural Research.

2.1 Methods

Prediction for the presence/absence of a ascospore event ignoring meteorological data

To predict an ascoascospore event, two pieces of information are required; firstly, the coefficient estimates and secondly, the solution for the probability (p) in equation (1).

Represent the linear predictor for observation j in the right hand side of equation (1) as,

$$\hat{\mu}_j = \sum_{i=1}^{q} X_{ij}\beta_i. \qquad \text{Equation (1).}$$

The prediction of probability is then,

$$\hat{p}_j = \frac{\exp(\hat{\mu}_i)}{1+\exp(\hat{\mu}_i)}. \qquad \text{Equation (2).}$$

Example of calculating a prediction

The coefficient effects and their estimates required for the models are included in Table 1. These are used together with observed values for tree age (Table 2) to produce predictions. The series of calculations required to obtain a prediction for the probability of recording ascospores for a particular plantation age follow the Tables 1 & 2.

Table 1. Estimated model effects for the coefficients included in the predictive model ignoring meteorological variables.

Effect	**Estimate ($\hat{\beta}i$)**	**Std. Error**	**Pr(>|z|)**
Intercept	9.611	1.093	<0.001
Age^1	-0.0560	0.005451	<0.001
Age^2	0.00009299	0.000008347	<0.001
Age^3	-0.00000004335	0.000000004017	<0.001
HourCos	1.227	0.1716	<0.001
HourSin	0.1536	0.05261	0.004
$Age_1 \times$ HourCos	-0.0009042	0.0002313	<0.001

Table 2. Observed meteorological variables for 561 days after planting, at 2200hrs.

Variable	**Derivation**	**Value (xi)**
Age^1		561.9167 days
Age^2		315750.3777
Age^3		177425410.3
HourCos	$= \cos\left(2\pi \frac{2200}{2400}\right) =$	0.995
HourSin	$= \sin\left(2\pi \frac{2200}{2400}\right) =$	0.100

Note: Age is measured in days

Calculations required to complete a prediction:

The probability ($\hat{p}$) of observing a ascospore event on day 561 is

$$\hat{p} = \frac{\exp(\hat{\mu})}{1+\exp(\hat{\mu})}$$

where

$$\hat{\mu} = \sum_{i=1}^{q} x_i \beta_i.$$

given by equations 1 and 2. Now, for 561,

$\hat{\mu}$ = intercept + (Age^1 × $\hat{\beta}_1$) + (Age^2 × $\hat{\beta}_2$) + (Age^3 × $\hat{\beta}_3$) + (HourCos × $\hat{\beta}_4$) +

(HourSin × $\hat{\beta}_5$) + ((Age$_1$ × $\hat{\beta}_6$) × $\hat{\beta}_7$))

= 9.611 + (561.9167 × -0.0560) + (315750.3777 × 0.00009299) + (177425410.3 × -0.00000004335) + (0.995 × 1.227) + (0.100 × 0.1536) + ((561.9167 × 0.995) × -0.0009042))

=0.745

Therefore, the probability of observing a ascospore event is,

$$\hat{p} = \frac{\exp(\hat{\mu})}{1 + \exp(\hat{\mu})}$$

$$= \frac{\exp(0.745)}{1 + \exp(0.745)}$$

$$= \frac{2.106}{1 + 2.106}$$

$$= 0.678$$

Prediction incorporating meteorological data and the concentration of a ascospore event

To predict the concentration of a ascospore event the following components are required: 1) the coefficient estimates for p_j and λ_j and 2) the value of the explanatory variables (meteorological and age variables).

The respective linear predictors for 'p_j' and 'λ_j' are:

$$\hat{\mu}_j = \sum_{i=1}^{q} x_{ij}\hat{\beta}_i \qquad \text{and} \qquad \hat{e}_j = \sum_{i=1}^{r} x_{ij}\hat{\tau}_i.$$

The estimated parameters are given by:

$$\hat{p}_j = \frac{\exp(\hat{\mu}_j)}{1+\exp(\hat{\mu}_j)} \qquad \text{and} \qquad \hat{\lambda}_j = \exp(\hat{e}_j).$$

The predicted mean of the distribution given the explanatory variables (x_{ij}'s) is given by:

$$\mathrm{E}(y_j) = \frac{\hat{p}_j}{1-\exp(-\hat{\lambda}_j)}\hat{\lambda}_j.$$

Example of calculating a prediction

Table 3 includes the estimated coefficients for the probability of recording an ascoascospore event and the concentration of the event once it occurs. The coefficient estimates are used together with observed values for meteorological data and tree age (Table 4), to calculate predictions for 917 days at 1400 hours. The series of calculations required to obtain a prediction for the probability and concentration of

observing ascoascospores under particular meteorological conditions for a particular plantation age are given in the following example.

Note: The calculation required for the linear transformation of Age^1 variable to be used in the prediction example for day 917.583 is given as:

$TAge^1 = [2 (age – age\ min) / (age\ max – age\ min) – 1]$

Where: age = 917.583

age min = 308 (age of plantation at the start of experiment)

age max = 1097.958 (day experiment ended)

$\therefore \quad TAge^1 = [2 (917.583 – 308) / (1097.958 – 308) –1]$

$TAge^1 = 0.545$

Table 3. Estimated model effects for the two parts of the concentration model (Hurdle model). $TAge^1$, $TAge^2$ and $TAge^3$ are the first to third order orthogonal polynomials.

Effect	Estimate	Std. Error	Pr(>|z|)
Probability of a ascospore event	$(\hat{\beta}_i)$		
Intercept	-2.7644862	1.78E-01	<0.001
$TAge^1$	1.2798913	1.99E-01	<0.001
$TAge^2$	-0.1001044	1.77E-01	0.571
$TAge^3$	-2.5342073	2.98E-01	<0.001
HourSin	0.2091063	6.51E-02	0.001
HourCos	-0.413724	1.33E-01	0.002
$T_{HrsOutRange}$	0.1416989	3.00E-02	<0.001
VPD	0.4013152	4.32E-01	0.353
RH_{90Time}	2.1282315	3.37E-01	<0.001
$R_{TimeSince}$	-0.0883034	4.00E-02	0.0273
RH_{90}	0.1080417	1.47E-01	0.463
BOMSolRad	-0.0009216	8.61E-05	<0.001
Light	-0.3863652	1.81E-01	0.033
$TAge^1$ × HourCos	-0.2486563	1.05E-01	0.018
VPD × RH_{90Time}	-33.9047205	1.08E+01	0.002
VPD × BOMSolRad	-0.0002766	1.53E-04	0.071
RH_{90Time} × light	-1.5897356	5.88E-01	0.007
RH_{90Time} × BOMSolRad	0.000267	4.01E-04	0.505
RH_{90} × light	0.172168	1.87E-01	0.358
VPD × RH_{90Time} × BOMSolRad	0.0167958	5.45E-03	0.002
Concentration of a ascospore event	$(\hat{\tau}_i)$		
Intercept	1.19172	0.096142	<0.001
$TAge^1$	2.04595	0.092407	<0.001
$TAge^2$	-0.99306	0.074431	<0.001
$TAge^3$	-2.01195	0.141919	<0.001
HourSin	0.09044	0.02558	<0.001
HourCos	0.43531	0.049906	<0.001
RH_{90Time}	0.79318	0.070344	<0.001
Light	0.60371	0.064294	<0.001
T	0.03996	0.005765	<0.001
WindSpeed	0.05721	0.011316	<0.001
$T_{InRange}$	-0.20981	0.04382	<0.001
$R_{TimeSince}$	-0.09719	0.033955	0.004
$TAge^1$ × HourCos	-0.41522	0.050701	<0.001

HourCos and HourSin are the two effects for the diurnal pattern

Table 4. Observed meteorological variables for 917 days after planting, at 1400hrs. TAge = Linear transformed (see page 190 for how to calculate the transformation)

Variable	Derivation	Value (x_i)
$TAge^1$		0.545
$TAge^2$		0.297
$TAge^3$		0.088
HourCos	$= \cos\left(2\pi \frac{1400}{2400}\right) =$	-0.866
HourSin	$= \sin\left(2\pi \frac{1400}{2400}\right) =$	-0.500
T		13.5
$T_{InRange}$		1
$T_{HrsOutRange}$		0
WindSpeed		4.336
VPD		0.4806
RH_{90Time}		0
$R_{TimeSince}$		0
RH_{90}		0
BOMSolRad		980.682
Light		1
$TAge^1$ × HourCos	0.545 × -0.866 =	-0.472
VPD × RH_{90Time}	0.4806 × 0 =	0
VPD × BOMSolRad	0.4806 × 980.682 =	471.316
RH_{90Time} × light	0 × 1 =	1
RH_{90Time} × BOMSolRad	0 × 980.682 =	0
RH_{90} × light	0 × 1 =	0
VPD × RH_{90Time} × BOMSolRad	0.4806 × 0 × 980.682 =	0
HourCos × $TAge^1$	-0.866 × 0.545 =	-0.47197

Calculations required to complete a prediction:

If the probability (p) of observing ascospore dispersal and the density (λ) of the ascospore release is given by,

$$\hat{\mu} = \sum_{i=1}^{q} x_i \hat{\beta}_i \qquad \text{and} \qquad \hat{e} = \sum_{i=1}^{r} x_i \hat{\tau}_i.$$

Where $\hat{\mu}$ and $\hat{e}$ are calculated by,

$\hat{\mu}$ = intercept + ($TAge^1 \times \hat{\beta}_1$) + ($TAge^2 \times \hat{\beta}_2$) + ($TAge^3 \times \hat{\beta}_3$) + (HourSin $\times \hat{\beta}_4$) + (HourCos × $\hat{\beta}_5$) + ($T_{HrsOutRange} \times \hat{\beta}_5$) + (VPD × 0.4013152) + ($RH_{90Time} \times \hat{\beta}_7$) + ($R_{TimeSince} \times \hat{\beta}_8$) + ($RH_{90}$× 0.1080417) + (BOMSolRad ×

$\hat{\beta}_9$) + (Light × $\hat{\beta}_{10}$) + ((TAge^1 × HourCos) × $\hat{\beta}_{11}$) × ((VPD × RH_{90Time}) × $\hat{\beta}_{12}$) + ((VPD × BOMSolRad) × $\hat{\beta}_{13}$) + ((RH_{90Time} × Light) × $\hat{\beta}_{14}$) + ((RH_{90Time} × BOMSolRad) × $\hat{\beta}_{15}$) + ((RH_{90}× Light) × $\hat{\beta}_{16}$) + ((VPD × RH_{90Time} × BomSolRad) × $\hat{\beta}_{17}$).

= -2.7644862 + (0.545 × 1.2798913) + (0.297 ×-0.1001044) + (0.088 × -2.5342073) + (-0.500 × 0.2091063) + (-0.866 × -0.4137240) + (0 × 0.1416989) + (0.4806 × 0.4013152) + (0 × 2.1282315) + (0 × -0.0883034) + (0 × 0.1080417) + (980.682 × -0.0009216) + (1 × -0.3863652) + (-0.472 × -0.2486563) × (0 × -33.9047205) + (471.316 × -0.0002766) + (1 × -0.5897356) + (0 × 0.000267) + (0 × 0.1721680) + (0 × 0.0167958).

= -3.323

$\hat{e}$ = intercept + (TAge^1 × $\hat{\tau}_1$) + (TAge^2 × $\hat{\tau}_2$) + (TAge^3 × $\hat{\tau}_3$) + (HourSin × $\hat{\tau}_4$) + (HourCos × $\hat{\tau}_5$) + (RH_{90Time} × $\hat{\tau}_6$) + (Light × $\hat{\tau}_7$) + (T × $\hat{\tau}_8$)+ (WindSpeed × $\hat{\tau}_9$) + ($T_{InRange}$ × $\hat{\tau}_{10}$) + ($R_{TimeSince}$ × $\hat{\tau}_{11}$) + ((HourCos × TAge^1) × $\hat{\tau}_{12}$).

= 1.19172 + (0.545 × 2.04595) + (0.297 × -0.99306) + (0.088 × -2.01195) + (-0.500 × 0.09044) + (-0.866× 0.43531) + (0 × 0.79318) + (1 × 0.60371) + (13.5 × 0.03996) + (4.336 × 0.05721) + (1 × -0.20981) + (0 × -0.09719) + (-0.47197 × -0.41522).

= 2.642

Therefore, now having $\hat{\mu}$ and $\hat{e}$, $\hat{p}$ and $\hat{\lambda}$ can be calculated,

$$\hat{p} = \frac{\exp(\hat{\mu})}{1+\exp(\hat{\mu})} \quad \text{and} \quad \hat{\lambda} = \exp(\hat{e})$$

$$= \frac{\exp(-3.323)}{1+\exp(-3.323)} \quad \text{and} \quad = \exp(2.642)$$

$$\therefore \hat{p} = 0.035 \quad \text{and} \quad \therefore \hat{\lambda} = 14.045$$

The expected concentration of atmospheric ascoascospores based on the observed meteorological data is,

$$E(y) = \frac{\hat{p}}{1-\exp(-\hat{\lambda})}\hat{\lambda}$$

$$E(y) = \frac{0.035}{1-\exp(-14.045)} \times 14.045$$

$$E(y) = 0.489 \mathrm{L}^{-1}\mathrm{h}^{-1}$$

(Beresford, 1978)	**Field trials** -Defoliated leaves were collected weekly and analysed for disease development. Meteorological variables were also monitored.	*M. cryptica* *	*E.delegatensis* (5-6 yr old plantation) (New Zealand)	-Leaves were infected within 3-4 weeks after emerging from the leaf bud. -Leaf fall of infected and senescent leaves was highest during summer and early autumn. -Young leaves that were infected consisted of 35% of the total leaf fall. These young leaves continued to fall during winter. Young leaves fell within 1-2 months of being infected. -Senescent leaves made up 32% of the leaf fall. -Extent of colonisation influenced the timing of leaf abscission. -Between 25-50% of leaf area of young expanding leaves was diseased at the time of abscission. -Weekly leaf fall and maximum temperature were positively correlated in spring and autumn. -Leaf fall and wind run time were negatively correlated in spring, winter and autumn.
	-Lesions on attached leaves were tagged and the progress of symptoms monitored weekly for 10 weeks. Meteorological data was also collected.			-A period for possible infection occurred approximately 2 months before infection symptoms were seen. These were: high relative humidity (95%+) for a constant 36 hours, mean temperatures of 14 °C, and 32 mm of rain in 24 hours. This was followed by another similar set of conditions. 2-3 weeks following the second set of suitable conditions infection symptoms were identified, which corresponds to the minimum incubation period. -Spore trapping from a nearby spore trap indicated spores were present during the possible infection period.
	-Disease development was monitored monthly at three tree height levels over two years.			-New disease symptoms first appeared during November then decreased in December and January. The disease symptom reduction corresponded with a decline in rainfall. -Disease in the lower levels of the canopy was greater due to the possible microclimate conditions being favourable to infection. -Infection increased again during April (autumn) following a rainfall event that occurred after a long dry period.
(Ganapathi, 1979)	**Cultures in climate chambers** -Effect of different media, pH, temperature and light on growth of colonies.	*M. cryptica**	*media*	-The colonies inoculated to agar media were first visible to the naked eye 4-6 days after inoculation. -The best growth was obtained in the pH range of 5 to 6 at 23 °C. -The optimal temperature for growth of colonies was 25 °C. -There was no effect of different light sources on growth of colonies.
	-Effect of different media on sporulation (production of conidia).			-Cultures grown on Emerson Yeast Phosphate soluble starch agar at 5 °C and 10 °C sporulated (conidia) the best. -Cultures grown on water agar germinated readily after 5-7 days producing numerous conidia.
	Seedlings in climate chambers -Seedlings were sprayed with conidia suspended in water. -Seedlings were placed in climate chambers and monitored for disease development under various treatments; a) temperature, b) light/dark, c) leaf age, d) inoculum concentration, e) leaf wetness (at 20°C/10°C day/night) f) nutrients.		*E. delegatensis* *E. regnans* (New Zealand)	-Disease symptoms appeared on *E. regnans* and *E. delegatensis* after four and seven weeks respectively at 18°C/12°C. -Under all light treatments symptoms of infection appeared four weeks after inoculation for both eucalypt species. Optimal light treatments for infection was 300 μE and 650 μE. First symptoms appeared on all trees under 300 μE and 200 μE within four weeks. Slowest symptom development was under the lowest light treatments (50 μE & 75μE). -Optimal temperature range for infection was day/night regime of 24 °C/18 °C. -Leaf age treatments indicated leaves of both species were most susceptible within 21days after expansion, becoming more resistant after this time. -Three weeks after inoculum was sprayed at $10^4 ml^{-1}$ symptoms were observed on both tree species. An increase in inoculum concentration resulted in earlier disease appearance and increased percentage of leaf area infected. Repetitive inoculation resulted in a reduction in tree height after 10 months. -1 day leaf wetness produced little infection compared to seedlings under 2, 4 and 7 days wetness treatments. After one day misting *E. regnans* had double the amount of infection symptoms exhibited by *E. delegatensis* although the level was low in both cases. -Leaves on branches of trees that had nutrients added daily and had been covered after one day of misting with polythene bags were the most highly infected.

	Glass house -Seedlings were artificially inoculated with a suspension of ascospores and conidia. They were exposed to leaf wetness duration treatments and then monitored for disease development.	*M. cryptica**	*E. delegatensis* *E. regnans* (New Zealand)	-Symptoms were first observed three weeks after inoculation. Stem cankers appeared after 6 weeks. This was followed by dieback of shoot tips. -Leaves that had severe infection surrounding the lamina abscised readily.
	Field trials **-**Leaves were collected weekly for 15 months and the level of infection development noted. Leaves were tested for ascospore discharge and inverted in petri dishes to monitor germination rate.	*M. cryptica* *	*E.delegatensis* (New Zealand)	-Under optimal laboratory conditions (23 °C) ascospores germinated after 5-6 hours. -Lesions were most abundant on leaves during September (Spring) to May (autumn) with most leaves with lesions being collected in November. The percentage of diseased leaves decreased until the following September.
	-Leaves were tagged on trees and disease development was monitored weekly for 9 months.		*E. delegatensis* *E. regnans*	-Leaf lesions took 8-10 weeks to develop from the initial to mature stages. -Stem cankers were observed in February (mid summer). -Leaves with several lesions tended to senesce early.
(Park, 1984; Park, 1988a; Park, 1988b; Park and Keane, 1982a)	**Field** **-**Disease development was monitored over 4 years by tagging leaves in the field. Daily rainfall records were also recorded.	*M. nubilosa*	*E. globulus* (Victoria, Australia)	-Small peaks of low infection symptoms were observed in late summer to early spring. A major peak in symptoms was observed later in the year between July and November. These symptoms were often large blighting events, which resulted in premature leaf abscission. -High levels of rainfall over consecutive days occurred during the four-week period prior to symptom appearance and is suggested to be when infection occurred. -*M. parva* was observed on older lesions caused by *M. nubilosa* (more than one species in a lesion).

		M. cryptica	*E. obliqua* & *E. globulus* (Victoria, Australia)	-*M cryptica* infection levels on both eucalypt species were low. -Infection symptom appearance was observed during summer months. -Only young expanding leaves during the hosts growth flush were susceptible to infection. -High levels of rainfall over consecutive days occurred during the four-week period prior to symptom appearance and is suggested to be when infection occurred.
	Shade house/climate control chambers -*E. globulus* leaves were collected for sources of inoculum to inoculate seedlings (*M. cryptica* from Woolnorth in Tasmania and *M. nubilosa* from Nowa Nowa, Vic). Seedlings were investigated in respect to the influence of: a) inoculum concentrations (*M. nubilosa* for ascospores, *M. cryptica* for ascospores and conidia), b) leaf surface (abaxial & adaxial), c) leaf age, d) leaf wetness duration ('dew treatment'), e) temperature, on germination. Seedlings were placed in either shade houses or climate controlled chambers.	*M. cryptica* *M. nubilosa*	*E. globulus* *E. obliqua* (Victoria, Australia)	-*M. nubilosa & M. cryptica*: Blighting lesions on *E. globulus* caused premature leaf loss and were the result of the highest inoculation concentration (10^5 spores.ml^{-1}); Other concentrations resulted in spotting lesions of 1 cm diameter but no leaf loss. Seedlings with lower concentrations (10^2, 10^3 spores.ml^{-1}) exhibited symptoms of infection after 56 days, compared to higher concentrations (10^4, 10^5 spores.ml^{-1}), which was within 49-56 days. Similar effects of concentration treatments were observed on *E. obliqua* with *M. cryptica* (*E. obliqua* was not inoculated with *M. nubilosa* for this treatment). -*M. nubilosa* only infected via the abaxial surface of *E. globulus* leaves. *M. cryptica* could infect both surfaces of *E. obliqua*. *M. cryptica* was not applied to *E. globulus* leaves for this treatment. -Infection with *M. cryptica* and *M. nubilosa* was most severe in newly expanded leaves. Leaves became more resistant with age. -The leaves most susceptible to *E. obliqua* infection were between 15-30 days old. Leaves older than 39 days were resistant to infection. -Increasing duration of dew treatment increased the disease severity of *M. nubilosa*. Maximal disease severity was associated with a period of 5-7 days leaf wetness after inoculation. With this period of leaf wetness new lesions were first observed 28 days after inoculation and were still appearing 91 days after inoculation. -Infection levels by *M. cryptica* ascospores and conidia were similar for all dew treatments, increasing infection severity being observed by both hosts with increasing hours of dew. -*M. nubilosa* ascospores germinated and infected at all temperatures except 30 °C. The optimal range for ascospore germination and symptom development is 15 °C to 20 °C. -*M. cryptica* ascospores and conidia germinated at temperatures between 10 °C and 25 °C, with the optimal temperate range for symptom appearance and development being between 15 °C and 25 °C. -Ascopores infected more readily at 10°C than conidia. More lesions resulted from inoculation by ascospores, appearing earlier than those inoculated by conidia. -No symptoms appeared on seedlings at or below 10 °C and at 30°C. When trees that had been kept at non-lesion producing temps were transferred into 15 °C (if inoculated with *M. nubilosa*) and 20 °C (if inoculated with *M. cryptica*) they took between 7-24 days for lesions to develop. -*M. cryptica* epidemic cycle was typically polycyclic, and *M. nubilosa* was either mono or bicyclic.
(Hood et al., 2002a)	**Field** -Mature and juvenile branches were tagged on infected trees. Branches were assessed monthly for foliage and disease development. An automatic meteorological station on site recorded meteorological variables.	*M. cryptica* and *Phaeophleospora eucalypti* (synonyms *Kirramyces eucalypti*, *Septoria pulcherrima*)	*E. nitens* (2 yr old trees) (New Zealand)	-Growth rates were greatest during summer but new foliage was produced throughout the year. -Leaf spotting from *P. eucalypti* and *M. cryptica* appeared 1-3 months after new growth emergence and peaked during December-January. During these months mean monthly temperatures were above 17 °C for December and 19 °C for January, which were the highest for the year; leaf wetness hours were from 134 hrs and 200 hrs and relative humidity was above 75% and 77% for each of these months respectively. -*P. eucalypti* infection was seen on older foliage but was not as severe. Virtually no spotting from *M. cryptica* was seen on adult foliage.

	-*E. nitens* bait trees (nursery raised seedlings placed in pots into the field) were placed in the field for one month before returning to the glasshouse to monitor infection. This process was repeated for each month over an 18-month period. An automatic meteorological station on site recorded meteorological variables.			-*P. eucalypti* infected all seedlings after being exposed to infected trees in the field. -Infection incidence was low during April (Autumn) and September (Spring). -No leaf spots caused by *M. cryptica* were observed. The author suggests this occurred due to low inoculum density levels.

(b) Studies investigating spore production and release of *Mycosphaerella* spp.				
Author/Year	**Methodology**	***Mycosphaerella* species**	**Host**	**Results**
(Cheah, 1977)	**Field** -Spore trapping was completed using a Burkard automatic volumetric spore trap. Ascospores were stained and counted using the long transect method.	*M. cryptica* *	*E. delegatensis* & *E. regnans* (3-5 yr old) (New Zealand)	-Ascospore numbers peaked during late summer. -Ascospore numbers were positively correlated with monthly rainfall in summer and autumn and negatively related to monthly rainfall in winter and spring. However in winter low minimum temperatures reduced ascospore number even in the presence of rainfall. -No spores were trapped during dry periods. -The meteorological variables most frequently associated with ascospore dispersal were hours that relative humidity was above 95 %, total hours of rainfall and total daily rainfall. -Ascospore concentration was not always in proportion with increases in rainfall intensity. -There was a negative relationship between wind run (speed & time) and ascospore concentration. -Hourly spore trapping investigations indicated there was no diurnal pattern in spore release. -Hourly investigations during summer indicated ascospore discharge occurred immediately after rainfall, and increased in the 2nd and 3rd hour after rainfall, then ceased. During winter spore discharge was slower, commencing 2-3 hours after rainfall, and ceased before the rain stopped. -Stepwise regression results indicated the meteorological variables most important to seasonal ascospore concentration, in order of importance were: relative humidity above 95%, total rainfall, minimum temperature, maximum relative humidity, total rainfall hours, and total wind run.
	-Water collection traps (clustered test tubes attached to a pole located at different heights above ground; 60 cm, 160 cm, 260 cm, 340 cm). These traps favour conidia collection over ascospores.			-Few conidia were collected in early January, and only at 160-180 cm. -During February, ascospores were recorded at 60 and 160 cm, none were collected from 340 cm (above the canopy). -In March conidia and ascospores were collected at all levels apart from ascospores at 340cm. -During April when trees reached heights above the highest traps both ascospores and conidia were trapped. -Concentrations of ascospores and conidia were not related to height of trap, with the exception of the highest trap at 340 cm where both ascospores and conidia were rare. -The number of ascospores and conidia trapped peaked in late March and early Autumn. This result was not related to rainfall duration or intensity. -A large volume of conidia was trapped during December when there was a peak in infection symptoms.
	-Leaves as spore traps (symptom free leaves were collected at heights similar to the locations of the water traps).			-Ascospores were present on all leaves at all heights. Conidia were rarely present, and only observed at 160 cm and 260 cm.
	Laboratory -Climate chambers set up at various climatic settings with a Kramer-Collins 24 hr spore sampler.	*M. cryptica**	*-E. regnans* (New Zealand)	-Moisture was the most important variable in the release of ascospores followed by temperature. -No ascospore discharge occurred during dry periods. -Discharge began after lesions were misted with water, and then ceased when they were dry. Discharge did not occur even if relative humidity was above 95%, free moisture was always required for discharge. -There was no effect of light or dark on ascospore discharge. -Maximum discharge occurred at 22 °C, it reduced at 15 °C, then dropped markedly to near zero at 8 °C. -No ascospores were discharged when temperatures were above 30 °C.

	-Effect of chemical solutions at different relative humidities on ascospore discharge (lesions were suspended above treatments in a petri dish).		*-E. delegatensis*	-Ascopores were only discharged at 100% relative humidity with the water treatment.
	-Lesions from leaves collected in the field were marked and placed in petri dishes. Lesions were soaked periodically and checked for spore discharge.			-After a single wetting, lesions could be actively discharging after a 38 hr period. -Ascospores were discharged from perithecia in lesions on leaves collected in the field 26 weeks after collection.
(Beresford, 1978)	**Laboratory** -Highly diseased leaves were collected from seedlings and tested for the effects of 7 wetting periods on ascospore discharge. After lesions were soaked for each test time period and blotted dry, a glass slide was placed on top of the lesion at various times after blotting. A glass slide was placed on top of a lesion and changed at various times during the drying process. Spore numbers were counted on the slides. The conditions in the laboratory were 22 °C, and 70% relative humidity.	*M. cryptica**	*E.delegatensis* (5-6 yr old plantation) (New Zealand)	-No spores were discharged from the controls (non-wetted lesions). -The 15min-wetting period gave the longest spore discharge period. Spores under this treatment began to discharge 15 min after wetting, and continued for 4.5 hours.
	Field -Leaf washings of leaves sampled over a two-year period from traps to investigate ascospore discharge over time.	*M. cryptica**	*E.delegatensis* (5-6 yr old plantation) (New Zealand)	-The number of conidia washed from young leaves was higher during the first year compared to the second. Peak concentrations occurred during February, March and April. -Large numbers of ascospores were washed from leaves in June to October, but only from mature severely infected leaves. No ascospores were collected from young leaves.
	-Spore trapping using a Burkard 7-day recording volumetric spore trap over 14 months. Counts were corrected for volume of air over a week. Additionally meteorological variables were monitored. *A comparison of daily spore concentrations at 3 sampling heights for 35 days.			-Ascospore concentrations increased sharply after a rainfall event that followed a dry period. -No conidia were found in spore trap samples. -Airborne ascospore concentrations were at a maximum between March (Autumn) and July (Winter), which is when number of mature lesions present was also high. Additionally temperatures at this time were warm (e.g. maximum temperature ~15 °C) and rainfall was high (e.g. monthly total rainfall ~ 15-20mm). *The highest total number of ascospores trapped was at 1.5 m above the tree crown. *High ascospore numbers occurred on rainy days when relative humidity was above 95%. *There was no clear effect of wind on ascospore concentration between the three levels. However results suggest on windy days when air turbulence was increased the trap recorded higher concentrations of spores. On days with light winds vertical movements of spores down through the canopy caused higher spore levels to be trapped in mid-height traps.

(Park, 1984; Park and Keane, 1982; Park and Keane, 1987)	**Field** -Ascopore discharge was investigated at monthly intervals for 12 months in 1) Attached leaves 2) Prematurely defoliated diseased leaves, 3) Senescent diseased leaves. Attached leaves with lesions had wet cotton wool clipped to their adaxial surfaces under a lesion. A cover slip was placed on the abaxial surface of the leaf covering a lesion. Slides were checked after 2 hours for the presence of ascospores. The defoliated leaves were left in cages in the field until sampling. Lesions from defoliated leaves were cut out and attached to the lid of a petri dish containing water agar supplemented with chloramphenicol, then kept in the dark at 20 °C for 5 days. The number of ascospores shed on the agar was counted as well as percentage that had germinated.	*M. nubilosa* (from Nowa Nowa, Victoria)	*E. globulus* (Victoria, Australia)	-The highest number of ascospores was produced between January and April irrespective of lesion age. -Ascospores were discharged for up to 17 months after lesion appearance. -In general, senescent leaves produced few ascospores. -Pseudothecia of *M. nubilosa* on leaves that had fallen between August (Winter) and October (Spring) took longer to produce ascospores than those that fell from November through to February. -Pseudothecia in leaf litter leaves were still producing ascospores 6-8 months after abscission.
		M. cryptica (from Narbethong)	*E. obliqua*	-The same numbers of spores were discharged from senescent leaves collected during summer months as in winter months. -Pseudothecia of leaf litter leaves produced the same amount of ascospores as attached leaves. Discharge continued for 3-4 months. Note: The viability of spores was high for both species
	Laboratory -Diseased leaves were washed in DWT20 (distilled water:tween) to remove surface spores. -Lesions were set up to test the effect of 6 different light and moisture treatments on ascospore production	*M. nubilosa*	*E. globulus* (Victoria, Australia)	-Few spores were discharged after 1 hour of wetting, irrelevant of the light and dark treatments. -Results indicate all spores were discharged after 2-3 days after 1 hour of wetting in all treatments and there was no further maturation of the pseudothecia. -Lesions that were under continuous high relative humidity or leaf wetness, or 12 hr wet/dry regimes produced ascospores for a long period of time. A slight decrease in ascospore production was observed between 1-4 days, before increasing again due to the production of new ascospores.
		M. cryptica	*E. obliqua* (Victoria, Australia)	-Similar results were observed for *M. cryptica*. However, more ascospores were observed under 12 hr light/dark conditions compared to constant light. -Abundant conidia were present when leaves were collected. -No conidia were produced as a result of the treatments. Note: The viability of spores was high for both species of *Mycosphaerella*

(Cheah and Hartill, 1987)	**Field** **(New Zealand)** -Daily spore trapping of ascospores with a Burkard automatic volumetric spore trap was completed. Weather variables were also monitored.	*M. cryptica*	*E.delegatensis* & *E. regnans* (Auckland)	-Spores were present throughout the sampling period. -Spores were predominantly trapped during summer and autumn, with a decline occurring with the onset of winter. -No ascospores were trapped on days with no rain. -Spores were mostly trapped on days when there were lengthy periods of rain and relative humidity was high (>95%). -Meteorological variables that were most closely correlated with ascospore discharge were daily total rainfall, rainfall duration and duration of relative humidity >95%. -Number of ascospores was not always proportional to rain intensity. -Wind run was negatively correlated with ascospore release. -During summer ascospore discharge began immediately with the onset of rainfall and increased in rate for the first 3 hours. The discharge rate then declined but also continued for up to 2 hours after rain ceased. -There was no diurnal discharge pattern observed. -During winter there was a 3-hour delay after the onset of rain before spores were recorded. Discharge usually ceased before the rain period ended.
	Laboratory **(New Zealand)** -Diseased seedlings were placed in a controlled climate chambers with a Kramer-Collins 24 hour spore sampler. The chambers were set with a 12h light/ dark photoperiod. Temperature and wetness effects on ascospore discharge were tested.	*M. cryptica*	3-5 yr old *E. regnans* (Auckland)	-No spores were released when foliage was dry. -Moisture initiated ascospore discharge. Discharge continued until leaf surfaces had dried. -There was no effect of light or dark periods on spore discharge. -At 90-98% relative humidity peak ascospore discharge occurred at 22 °C. Very few ascospores were discharged at 8 °C and increasing the temperature from 22 °C to 30 °C resulted in a decline in ascospore discharge.
(Hood et al., 2002a)	**Field** -Branches with juvenile foliage were tagged in order to follow seasonal production of spores. Leaf litter samples were collected and examined microscopically for spore production before and after damp incubation at room temperature.	*M. cryptica* &*Phaeophleospora eucalypti* (synonyms: *Kirramyces eucalypti; Septoria pulcherrima*) Teleomorph is unknown (Crous, 1996)	*E. nitens* (2 yrs old) (New Zealand)	-*P. eucalypti* conidia were first observed in lesions on the abaxial surface of juvenile leaves approximately 8 weeks after leaf emergence. *M. cryptica* ascospores were observed at the same time or slightly later. *M. cryptica* conidia were observed. -A decline in *P. eucalypti* conidia and *M. cryptica* ascospores occurred over the winter months on attached leaves. An increase in conidia and ascospores was recorded into the early summer on residual leaves remaining from the previous year. -No *P. eucalypti* conidia or pycnidia were found in the leaf litter samples. Ascospores of *M cryptica* were found in leaf litter before and after damp incubation.

(C) Comparative studies for spore release patterns				
Author/Year	**Methodology**	**Foliar pathogens**	**Host**	**Results**
(Sutton and Jones, 1979)	**Field** -Spore trapping of airborne conidia was conducted using a Burkard recording volumetric spore trap, or a Kramer-Collins spore sampler. Weather variables were also monitored.	*Podosphaera leucotricha*	Jonathan apple trees (25 and 21 year old orchard trees in Michigan and Grand Rapids).	-Dispersal was diurnal with a peak concentration observed in the early afternoon and a subsidiary peak after dark. -Atmospheric conidial concentration was positively correlated with wind velocity, temperature, and solar radiation and negatively correlated with relative humidity and leaf wetness. -The onset of rain resulted in a large peak in conidial concentration that declined with continued rain time.
(Shaw, 1993)	**Field** -Wheat plants were artificially inoculated in the field. Visual estimates were made weekly of the % area covered in lesions. An automated weather station recorded hourly temperature, rainfall, relative humidity and wind speed data. A splash meter was also used in the field.	*Mycosphaerella graminicola*	Winter wheat (cv. Longbow)	-Infection was related to periods of rainy weather. -Infection was determined by when rain splash occurred in relation to emergence of a new leaf layer. -No significant relationship was found between infection and temperature, relative humidity or wind. -Severe infection occurs as a result of the second generation of spores, which arise from existing lesions. -Rain splash moves spores between leaves. -Pattern of disease progress can be largely explained by patterns of rainfall and how they coincide with appearance of new leaves.
(Paulitz, 1996)	**Field** -Spore trapping was conducted, using a Burkard 7 day spore sampler in an artificially inoculated wheat plot. Meteorological data was collected.	*Gibberella zeae* (anamorph=*Fusarium graminearum*)	Wheat (*Triticium aestivum* L.cv.Max) (Canada)	-Ascospore release was highly correlated with the time at which relative humidity increased late in the afternoon. -Ascospore release occurred between 11 °C and 30 °C, relative humidity from 60-95%. -Ascospore numbers between 0800 hrs and 1600 hrs were low. -Spore release began at approximately 1800 hrs, and reached a peak at 2300 hrs. -Ascospore release continued throughout the night until approximately 0800 hrs. -Ascospore release began before leaf wetness was detected. -Peaks in ascospore release occurred 2 to 4 days after large rainfall events. -Ascospore release was reduced during the night on days when rainfall was less than 5 mm during the day and relative humidity was >80%.
(Hidalgo et al., 1997)	**Field** -Spore trapping was conducted during the growing season in two orchards aged 6 & 7 yrs old, from May to December and April to October respectively. Daily and hourly spore counts were completed. An automated meteorological station recorded temperature, leaf wetness, rainfall and relative humidity at the sites.	*Mycosphaerella citri*	Valencia orange orchard (Costa Rica)	-Very few conidia were trapped throughout the trial. -Ascospore dispersal began in late April or early May. Dispersal peaks occurred in late May and early June. Few ascospores were trapped after July. -Three ascospore discharge patterns were observed: *Firstly, ascospore discharge was associated with the onset of rain, as spores were observed an hour after rainfall occurred, and continued after rainfall ceased. The magnitude of spores trapped generally increased as rainfall intensity decreased. *Secondly, ascospore discharge was closely associated with dew, as ascospore counts were highest during early morning (0200 hrs – 0700 hrs) when dew was at its highest level. *The third pattern is a combination of the two. Frequently occurring afternoon rain initiated spore discharge. This was followed by another discharge in the morning from dew formation.

	-Disease incidence and severity were recorded as % leaf area and crown with infection symptoms.			-Disease incidence remained low during the growth flush in May, and then dramatically increased from 41% incidence to 100% in August. Severity on each leaf remained low and increased from 1% leaf area affected to 5%. Increase in disease incidence occurred approximately three months after peaks in ascospore number were observed.
	-Leaf litter decomposition was monitored by placing a frame at the base of trees and recoding leaf fall and level of decomposition weekly.			-Total number of leaves in each stage of decomposition was a good predicator of ascospore catch but only for one site.
(Pinkerton et al., 1998)	**Field** -Spore trapping was conducted for 12 week periods (beginning at budbreak) over two seasons, using a Burkard 7 day spore sampler. Meteorological data was collected.	*Anisogramma anomala*	European hazelnut trees (*Corylus avellana*) (Oregon, U.S.A)	-Ascospores were released when branch surfaces were wet from rain, but not from dew. -Release ceased after surfaces dried. -The presence and duration of surface moisture from rain regulated the initiation and rate of ascospore release. No significant effects of temperature, relative humidity, wind or light on ascospore release were apparent. -Most ascospores (>90%) were trapped when rain exceeded 20 hrs in duration. -The hourly rate of spores trapped increased until 5 hours after the onset of rain and reached a plateau at 12 hours, at which time a decline occurred.
(Stensvand, 1998)	**Field** -Spore trapping was conducted over 4 years, using a Burkard 7 day spore sampler. Meteorological data was collected.	*Venturia inaequalis*	Apple trees (cultivar Gravinstein) (Norway)	-Ascopores were at their highest between 2200 hrs and 0900 hrs, with a peak occurring before 0400 hrs. -During 6 successive nights when dew was observed, 20% of the season's total ascospores were recorded within two of these nights. -Fewer ascospores were recorded during rain events preceding dew than during dew alone. -On days when ascospore release was triggered by dew, night time minimum temperatures ranged from 5 °C –10 °C, followed by daytime maximum temperatures between 20 °C and 26 °C, and relative humidity of 95%+ at night and 20-40% during the day.
(Burt et al., 1999)	**Laboratory** -Leaves with more than 16% necrosis were collected from banana trees. Numbers of perithecia were counted in lesions to determine the relationship between lesion size and perithecial density as perithecial density indicates ascospore release potential.	*Mycosphaerella fijiensis*	Bananna plantation (variety Grande) Costa Rica	-An increase in necrotic lesion area resulted in increased perithecial density.

	-Ascospores were counted from a known number of perithecia. Lesions with mature perithecia were cut out, attached to filter paper and glued to the lids of petri dishes. Lesions were suspended above nutrient free agar. Petri dishes were placed into a dew and growth chamber under two temperature and relative humidity treatments (28 °C/60 % RH, and 21 °C/100 % RH) and wetting and re-wetting regimes.			-Ascospore release was greater from lesions re-wetted every 12 hours than those re-wetted every 48 hours. -Most ascospores were released at 28 °C/60 % RH under a 12 hr rewetting regime. -Once ascospore release was initiated, a change in temperature/relative humidity treatment did not influence the amount of release. -Lesions kept at low temperatures released non-germinating ascospores.
(Villalta, 2001)	**Field** -Spore trapping was conducted, using a Burkard 7 day spore sampler from 1992-1999. Each year ascospores were trapped over a 3-month period beginning in September and ending in late November. Meteorological data was collected.	*Venturia pirina*	Pear trees (*Pyrus communis L.*) (Australia)	-Ascospore concentration was associated with rain and dew. 90-98% of the season's total number of ascospores were trapped during rain events and 2-10% were trapped during dew events. -82.5-99.9% of ascospores were trapped between 0600 and 1800 h. The remaining 0.1-17.5% ascospores trapped during the night (1900 h to 0500 h), were recorded within 1-3 hours of dawn or dusk.
(Prados-Ligero et al., 2003)	**Field** -Spore trapping of ascospores and conidia were completed around garlic plots in which infected garlic debris was fixed onto the soil surface.	*Stemphylium vesicarium* (teleomorph *Pleospora allii*)	*Allium sativum* (Spain)	-Ascospore release occurred mainly in February and March, coinciding with rainfall periods, temperatures 14 °C-21 °C, 14 h with vapour pressure deficit <5 mb and solar radiation <145 W m^{-2} on the current day of the capture. Daily ascospore concentrations were erratic with 30% being trapped between 0000 and 0600 hours. -Conidial concentration patterns were periodic with the highest concentrations being observed between 1200 and 1800 h, with a pronounced peak between 1400 and 1600 h. -Rainfall was highly correlated with aerial concentration of ascospores and conidia. -The greatest concentrations of conidia occurred in late April and continued into May, and were correlated with rainfall in days previous to the capture. During this time temperatures were high and solar radiation was 109-345 Wm^{-2}. - There was a relationship between atmospheric conidia concentration and number of hours with temperature in the range 12-21 °C. -When rainfall was absent, high relative humidity was essential for presence of ascospores and conidia.

(Zhang, 2005)	Field -Daily spore densities of ascospores and pycnidiospores were monitored using 16 Rotorod spore samplers placed at specific locations at various distances away from an artificially inoculated inoculum source. Additionally, a Burkard spore sampler was placed in the middle of the inoculum source to gather spore density data that was calculated for 4-hour periods during the day. Data was collected for 53 days beginning in June for two consecutive years. Hourly rainfall and temperature data were collected during the time of the trial.	*Mycosphaerella pinodes*	*Pisum sativum* (Canada)	-For both years the mean hourly density of ascospores trapped was 3.5 to 50.8/m^3, and 1.1 to 8.2/m^3 for mean hourly pycnidiospores. -Most ascospores and pycnidiospores were trapped between 1700 and 0400 h, with a large peak in ascospore density occurring at night between 2100 and 2400. The smallest numbers of spores were trapped between 0500 and 1600 h. -During rainfall, ascospore concentration remained similar to that of a dry day. Higher ascospore numbers were recorded 16 hours after a rainfall event, and these levels persisted for 3 days after rain, when they began to decline. -Pycnidiospore density was higher during rainfall events than 3 days after rainfall. Peaks in pycnidiospores occurred within 8 hours after rainfall began. -Release of pycnidiospores and ascospores was associated with rainfall events $\geq$2 mm during the first 27 days after infestation but was not associated with rainfall events after the 27 days.
	-Disease assessments were conducted twice a week from the first visual sign of infection symptoms. Assessments were carried out in a grid pattern to test the effect of distance from inoculum source. Hourly rainfall and temperature data were collected during the time of the trial.			-Disease symptoms were observed within 15-22 days after infestation and increased over time. -Rainfall was highly correlated with rate of disease severity development. Rain 10 days after disease infestation initiated disease development and symptoms were observed after five more days. -In the absence of rain disease severity increased but the rate of incidence did not. -Ascospore density was negatively correlated with distance from inoculum source: ascospore density decreased with increasing distance from inoculum source. -Disease severity was positively correlated with ascospore density: the observed severity was sourced from the inoculum source. -No results were given for pycnidiospores due to insufficient data.

Appendix 3. Mapping the spatial and temporal progression of Mycosphaerella Leaf Disease in a *E. globulus* plantation in north-west Tasmania.

Methods:

Study site

The study was conducted at a *E. globulus* plantation (CH033B) 15 km from Smithton at Christmas Hills, in North-West Tasmania, Australia (S40° 55' 21": E144° 59' 39") (Chapter 2, Figure 2.1). The plantation was 65.8 ha in size and was planted in 2001. Infection from species of *Mycosphaerella* was identified soon after planting in 2001. The plantation was surrounded by native wet sclerophyll forest and a number of other eucalypt plantations, including an *E. nitens* (CH033D) plantation established in 2002. All plantations showed signs of infection by MLD. Smith et al. (2004) identified a suite of *Mycosphaerella* species infecting leaves; however, the most abundant in the *E. globulus* was *M. nubilosa*.

Spatial monitoring

Trees in the plantation were two years old when they were assessed. Trees were assessed on a scale of 1-100% for defoliation, necrosis incidence and severity. These results were further calculated to give a Crown Damage Index (CDI) as described by Stone et al. (2003). Continuous CDI % scores were categorized for data presentation. Every 5^{th} tree in every 5^{th} row of the *E. globulus* plantation (CH033B) and *E. nitens* plantation was assessed (CH033D) (Figure 2.1, Chapter 2). In addition each tree was marked with a GPS point, which would be used in mapping the spatial distribution of CDI severity.

Note: The spore trap was running throughout the time of the spatial monitoring of MLD.

Results:

The distribution of CDI's for the *E. globulus* and *E. nitens* plantations for three separate assessments are given in Figure 1. Tables 1 & 2 give a summary of the proportion of the plantation for each severity score.

In 2003, tree crowns were already exhibiting symptoms of MLD infection. No spatial infection pattern was evident in the *E. globulus* plantation between the three assessments [Figure 1 (a)-(c)]. The summary results indicated 89.1% of the plantations crowns had infection when the first assessment was conducted (Table 1). Of these 89.1%, 24.2% had a CDI of 10 and 28.7% had a CDI of 20 (Table 2). The following two assessments indicate incidence of crown damage increased to 100% (Table 1). An increase in CDI severity was observed from one assessment period to the next [Figure 1 (a)-(c)] (Table 2). At the third assessment, results indicate 86% of crowns had a CDI score under 50 (Table 2).

In the *E. nitens* plantation (planted in 2002) only 2% of the crowns assessed exhibited any signs of damage symptoms at the first assessment (Table 1). The 2% damage included 1.4% with a CDI of 5 and 0.7% with a CDI of 10 (Table 2). During the next assessment the incidence of damaged crowns increased to 77% (Table 1). A spatial pattern in disease incidence was also observed from one assessment period to the other with more crowns exhibiting MLD infection symptoms towards the north-east of the plantation [Figure 1(a)-(c)]. The CDI severity remained low (Table 2) with 98.3% of crowns infected having a CDI less than 10 at the last assessment.

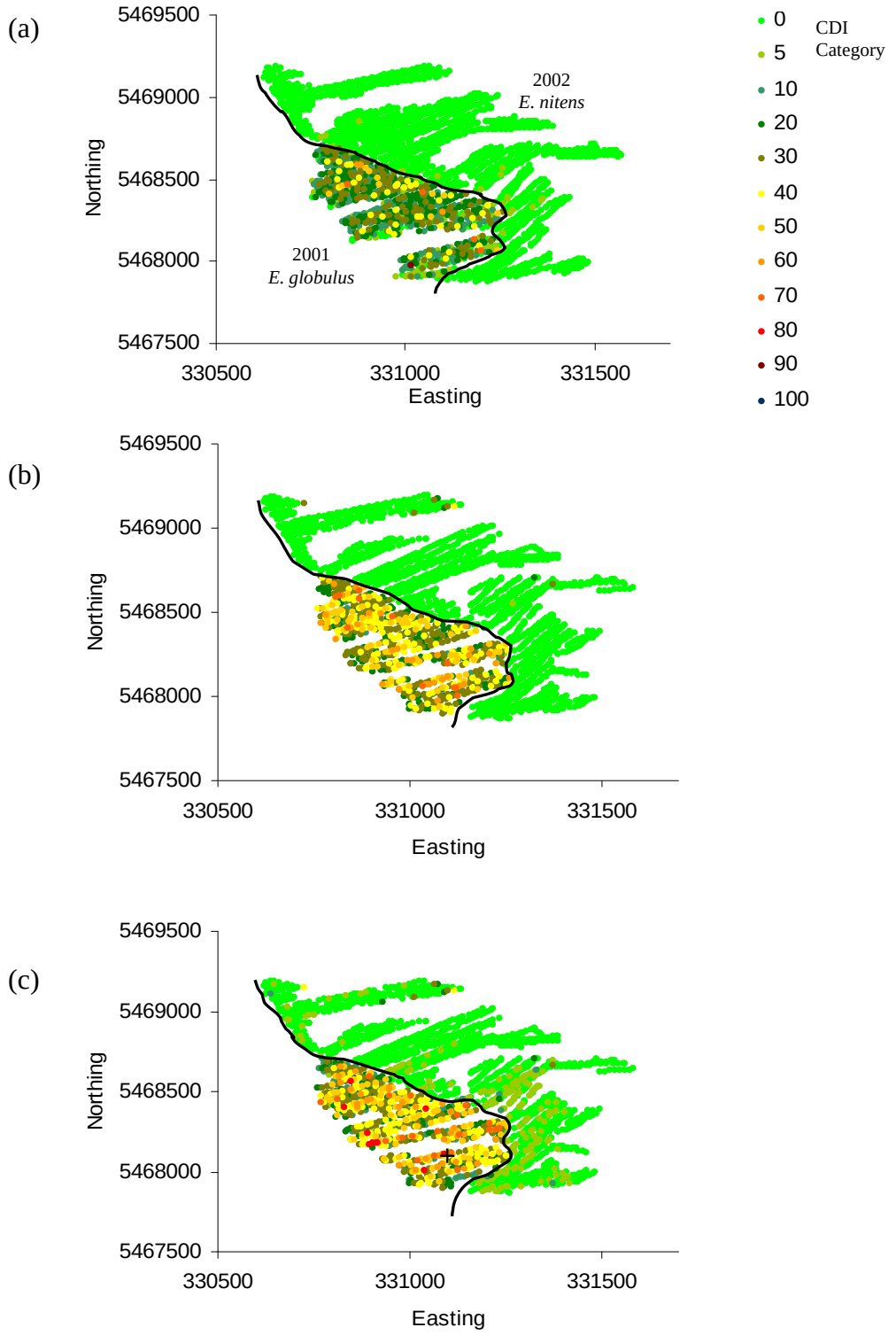

Figure 1. Spatial distribution of CDI severity at the study sites *E. globulus* (planted 2001) and *E. nitens* (planted 2002) plantation at Christmas Hills. Each point marks a tree crown assessment. The three figures refer to 3 assessments completed on (a) 7th July 2003 (b) 10 November 2003 and (c) 16th February 2004. The spore trap was present in the plantation throughout the assessment periods and the'+' indicates the location of the spore trap. The black line indicates the boundary between the *E. nitens* and *E. globulus* plantations.

Table 1. Summary of CDI severity spatial monitoring results of *E. globulus* and *E. nitens* crowns in plantations at Christmas Hills.

	Assessment Date					
	7th July 2003		10th November 2003		16th February 2004	
	E. globulus	*E. nitens*	*E. globulus*	*E. nitens*	*E. globulus*	*E. nitens*
Days since planting	615	189	741	315	839	413
Trees with NO damage (%)	10.9	98	0	99	0	23
Trees WITH damage (%)	89.1	2	100	1	100	77

Table 2. Proportion of *E. globulus* and *E. nitens* crowns in each CDI category in plantations at Christmas Hills. The CDI category represents the continuous CDI calculated from incidence and severity assessments (0-100%) completed in the field.

E. globulus		Assessment date		
		7th July 03	10th November 03	16 February 04
		Days since planting		
CDI (%)	**CDI Category**	**615**	**741**	**839**
0-2	0	10.9	0	0
3-7	5	9.1	0.3	1.4
8-15	10	24.2	4.3	9.7
16-25	20	28.7	19.1	15.3
26-35	30	19.1	33.7	26.9
36-45	40	5.4	21.3	21.0
46-55	50	1.5	10.4	11.7
56-65	60	0.4	4.3	5.0
66-75	70	0.6	1.5	2.8
76-85	80	0.0	0	1.4
86-92	90	0.1	0	0
93-97	95	0	0	0
98-100	100	0	0	0

E. nitens		Assessment date		
		7th July 03	10th November 03	16 February 04
		Days since planting		
CDI (%)	**CDI Category**	**189**	**315**	**413**
0-2	0	97.9	99.3	22.9
3-7	5	1.4	0.1	62.1
8-15	10	0.7	0.0	13.3
16-25	20	0.0	0.2	1.6
26-35	30	0.0	0.4	0.1
36-45	40	0.0	0.1	0.0
46-55	50	0.0	0.0	0.1
56-65	60	0.0	0.0	0.0
66-75	70	0.0	0.0	0.0
76-85	80	0.0	0.0	0.0
86-92	90	0.0	0.0	0.0
93-97	95	0.0	0.0	0.0
98-100	100	0.0	0.0	0.0

Discussion:

The results from this study illustrate how Mycosphaerella Leaf Disease increases in severity at the plantation scale over time. Infection severity in the *E. globulus* remained low, however incidence was 100% by the third assessment indicating the spread of the infection from crown to crown. *E. nitens* was planted 1 year later than the *E. globulus*. The pattern of disease spread in the *E. nitens*, out from the boundary with *E. globulus* and the fact that prevailing winds were west-east across this boundary suggest the inoculum source was the adjacent *E. globulus* plantation.

Molecular identification tools identified *Mycosphaerella nubilosa* as the most dominant species causing infection in the *E. globulus* plantation (Smith, 2006) although *M. cryptica* was present. MLD observed in the *E. nitens* was caused predominantly by *M. cryptica* (Smith, 2006). *M. nubilosa* was the evident source of inoculum present at the adjacent *E. globulus* site but, since *E. nitens* is resistant to *M. nubilosa*, trees only became infected by *M. cryptica*. This suggests that planting *E. nitens* adjacent to a MLD infected *E. globulus* plantation may be a management option for industry, allowing the use of an otherwise high *Mycosphaerella* risk site.

Appendix 4. Investigating the occurrence of meteorological conditions conducive to MLD infection at Smithton two years prior and during the spore-trapping study described in Chapter 2.

Introduction:

Previous studies have demonstrated that climate conditions are critical in influencing severity of MLD disease outbreaks (e.g. Cheah, 1977; Park, 1984). This section provides some evidence that total monthly rainfall patterns have an impact on severity of disease outbreaks caused by *Mycosphaerella* spp. in *E. globulus*.

Aims:

To investigate:

1. The number of occasions meteorological conditions were conducive to MLD infection (minimum of three consecutive days with RH% above 80%; rainfall > 0 mm; minimum daily temperature > 10ºC; maximum daily temperature > 18º) during the study and the two years prior to the study.
2. Total monthly rainfall patterns during the growing seasons throughout the study and the two years prior to the study (a growing season in Tasmania, Australia is between October and April).

Methods:

Total daily rainfall, average daily temperature and relative humidity data were acquired for Smithton (S40º 50’ 24”: E145º 6’ 53”) from the Bureau of Meteorology (Bureau of Meteorology, Hobart Tasmania) for the growing seasons between year 2000 and 2003. Smithton is 10km from the Christmas Hills study site, which is

described in Chapter 2. The effect of geographical distance between Christmas Hills and Smithton is assumed to be negligible.

Aim 1: Daily data were filtered to confine the data set to days with meteorological variables conducive to infection. The number of occasions these conditions occurred for a minimum of three consecutive days two years prior to and during the study were recorded.

Aim 2: Monthly totals of rainfall, average temperature and relative humidity were examined for each growing season two years prior and during the study.

Results:

Three-day sequences of meteorological variables conducive to MLD only occurred twice during the study period and three times in the two years prior.

Figure 1 presents the monthly rainfall totals for four consecutive growing seasons. The figure also indicates the year that the *E. globulus* trees at the Christmas Hills field site experienced a blighting epidemic (Tim Wardlaw, Forestry Tasmania, pers. comm.). The plantation with the blighting epidemic was adjacent to the plantation used for studies in this thesis.

The results indicate rainfall was higher in October (Spring) and March (Autumn) during the first growing season (2000-2001), and was followed by high October rainfall at the beginning of the following growing season in (2001-2002). Rainfall was low at the end of the 2001-2002 growing season. High rainfall was recorded at the beginning (October/Spring) and end of the 2002-2003 growing season. Rainfall

was lower during October (Spring) of the fourth growing season (2003-2004) compared to the same month in the previous 3 growing seasons. Both temperature and relative humidity were conducive to infection by *Mycosphaerella* spp. during all growing seasons at the site (data not shown).

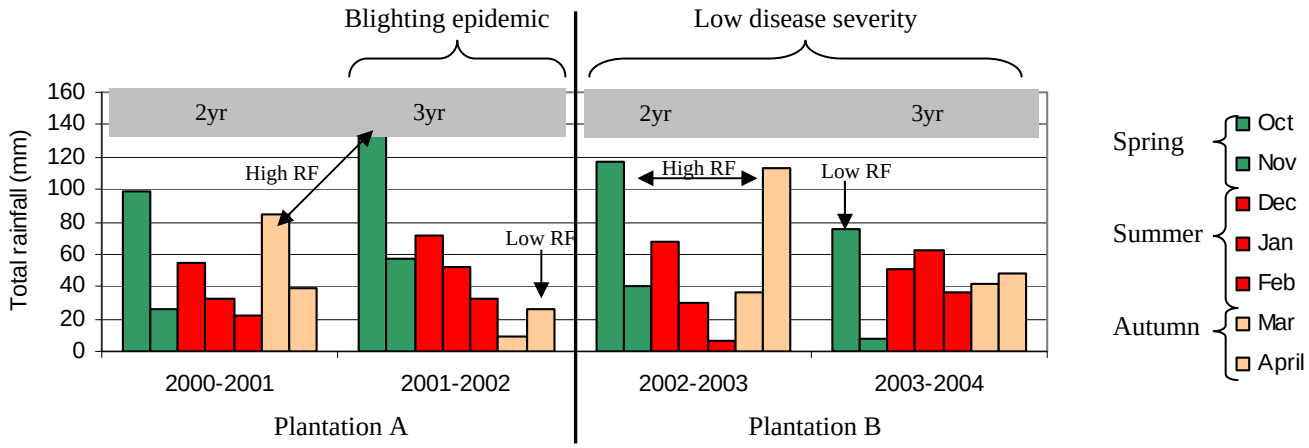

Figure 1. Total monthly rainfall for four consecutive growing seasons and observed disease severity for two plantations located at Christmas Hills. Plantation A was adjacent to the study site used in Chapter 2 (Plantation B). Years (yr) indicate the age of each plantation. Arrows point to months with high or low rainfall (RF) that have been discussed in the text.

Discussion:

While a number of meteorological variables were considered, the primary variable influencing *Mycosphaerella* spp. epidemic severity appeared to be total monthly rainfall pattern. The blighting epidemic which was observed in Plantation A was characterised by high autumn rainfall, followed by high spring rainfall. In contrast

the site used in Chapter 2, Plantation B, with low disease severity, had low rainfall in the previous autumn followed by high spring rainfall (Figure 1). These results suggest that a critical meteorological requirement for an occurrence of a blighting epidemic is the combination of high autumn rainfall followed by high spring rainfall - if one does not occur then there is no epidemic event.

Disease severity is positively correlated with spore concentration (Park, 1984). Both low disease severity and atmospheric spore concentration were recorded for plantation B. No such data was collected for plantation A, however the severity of the epidemic suggested that high atmospheric spore concentrations might have been present in this plantation. The combination of high atmospheric spore load (from an external source to the plantation in question) in addition to high autumn and spring rainfall may be essential for a blighting event to occur in the subsequent growing season OR the sequence of both high autumn and spring rainfall results in a higher atmospheric load and an epidemic event in the subsequent growing season.

It is recognised that this investigation of rainfall patterns presents only sufficient evidence to suggest a hypothesis that requires further testing.

Cheah L-H. 1977. Aerobiology and epidemiology of *Mycosphaerella nubilosa* (CKE) Hansf. on *Eucalyptus spp*. Thesis type. University of Auckland: Auckland.

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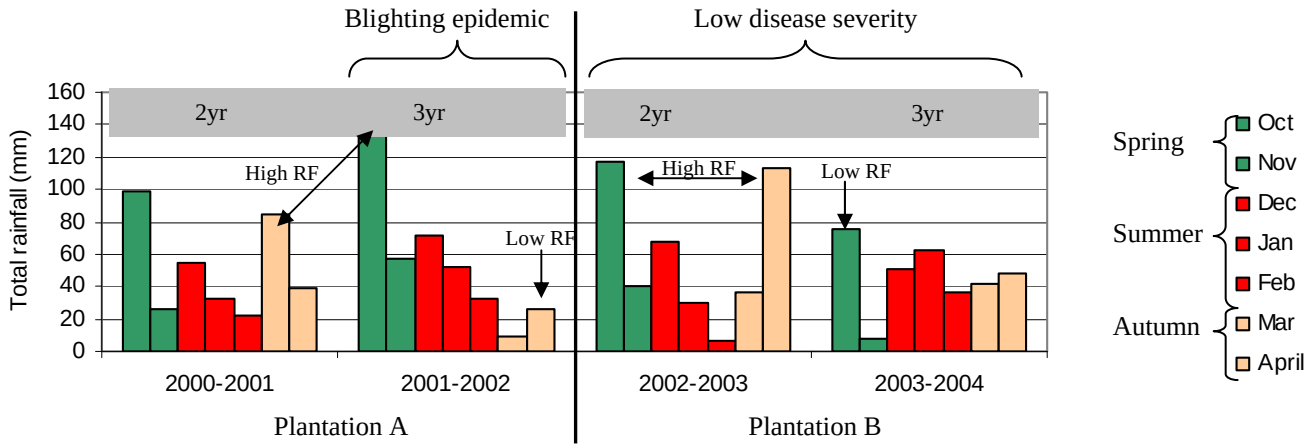

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