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EPIDEMIOLOGY OF MONILIOPHTHORA RORERI

A field study in the Atlantic Zone of Costa Rica

C.J. Tazelaar

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INVESTIGACION Y ENSEÑANZA - CATIE

AGRICULTURE UNIVERSITY
WAGENINGEN - AUW

MINISTERIO DE AGRICULTURA Y
GANADERIA DE COSTA RICA - MAG

PREFACE

The present study was carried out in Costa Rica in the period August - October 1990. During this time the support of Mr. J. J. Galindo (CATIE) and H. Waaijenberg (Atlantic Zone Program, Agricultural University Wageningen) was very valuable . I would like to thank them for their continuous help and interest.
I also would like to thank Mr. I. Staritsky (Department of Soil Science & Geology, Agricultural University Wageningen) for his help to enter in the unknown world of geostatistics. My special thanks go to my parents who supported me during my recovery of an accident and the writing of this report. This report could not be finished within reasonable time limits without them.

Many aspects of the spread of the disease are still poorly understood (LASS, 1986).

CONTENTS

1 INTRODUCTION

Cocoa (Theobroma cacao) is one of the twenty-two species belonging to the genus Theobroma, member of the Sterculiaceae family. Only T. cacao gives a product of commercial value. The cultivation of cocoa has been developed in several Central-American countries as an alternative for lowlands with a high precipitation and as a source of foreign currency. Actually cocoa is grown in the majority of tropical countries in a zone between 20° latitude South and 20° latitude North.

In the sixties and seventies the Centro Tropical de Investigacion y Enseñanza (CATIE) developed cocoa hybrids of the Trinitario type. Selection took place with an emphasis on yield and resistance to the diseases black pod rot (Phytophthora palmivora) and Ceratostomella wilt (Ceratocystis fimbriata). These hybrids were distributed in Costa Rica and other Central-American countries. Expectations were high but soon two mayor constraints were revealed: first, yields fell far behind those obtained at CATIE fields and secondly, hybrids did not have tolerance to a new threat: Monilia disease (WAAIJENBERG & WESSEL, 1989).

Monilia disease of cocoa, also called watery pod rot, is caused by the fungus Moniliophthora roreri. The fungus attacks the cocoa pods in each stage of its development. At the end of 1978 the fungus was detected in the Atlantic Zone of Costa Rica. This year annual production was about 10.300 tons. In a few years production declined with 80 - 95 %. In 1983 production was no more than 1850 tons (GALINDO & ENRIQUEZ, 1984). Monilia disease caused the abandonment of many plantations or a change in culture.

Little literature exists about the epidemiology of the fungus. It is generally accepted that spores are dispersed by the slightest breeze of wind but literature concerning the spread and progress of the disease in a parcel of cocoa is scarce. With a better understanding of these factors farmers could be advised about better methods to control the disease.

This report will serve as a first step in understanding the spread and progress of the fungus. A parcel of cocoa was infected with Monilia and weekly recordings of the spread were made. Several techniques were used to analyze the spread.

In the second chapter general information about the fungus will be given. The third chapter concerns a brief description of epidemiology and geostatistics. The fourth chapter describes the used materials and the followed methods. Chapter five deals with the results, chapter six with the conclusions, while chapter seven concerns the discussion of the results.

2 THE FUNGUS

2.1 Name of the disease:

The fungus was classified in 1933 by Ciferri and Parodi and was named Monilia roreri in honour of J.B. Rorer who was the first to describe the pathogen. Evans et al. (1978) redescribed the fungus because typical dolipores were discovered and thus indicating the fungus is the asexual stage of a hitherto unknown basidiomycete. In this report will be referred to Monilia because it is still the most widely used name.

An extensive amount of common spanish names exist. A few of them are: moniliasis del cacao, enfermedad de Quevedo, mancha ceniza, podredumbre acuosa, prinque, mal paludico (THURSTON, 1989). The most commonly used name in the english language is watery pod rot. The term frosty pod rot is less correct.

2.2 Causal organism:

The fungus is classified as:

Class	:	Deuteromycetes
Order	:	Moniliales
Genus	:	Moniliophthora
Species	:	roreri (Cif & Par) Evans et al.

2.3 Host range:

The only genera known to be susceptible are Theobroma and Herrenia. Theobroma cacao is the only species of commercial value.

2.4 Morphology:

Hyphae are hyaline, thin walled, septate, without clamp connections but with dolipores. Conidophores are branched, giving rise to a basipetal maturing chain of conidia (Fig. 1).
Conidia are easy separable, thick walled, pale yellow, brown en masse ,formed on basipetal strains, globose to sub globose, 8 - 15 µm in diameter or ellipsoid (8 - 20 x 5 - 14 µm) (EVANS ET AL., 1978; EVANS, 1981).

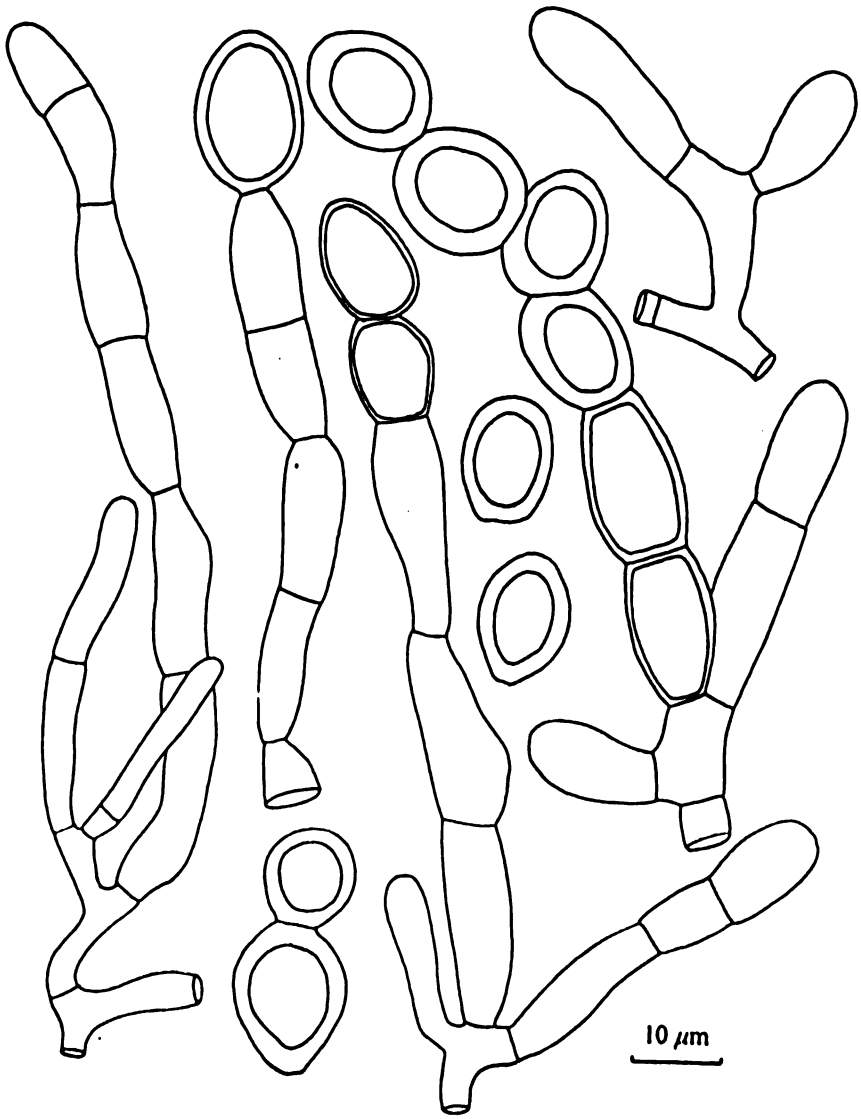


Figure 1. Conidial structures of M. roreri.

2.5 Symptoms:

The only known source of infection are the pods (DESROSIERS & SUAREZ, 1974). In pods younger then three months the symptoms are subsequently as follows: discoloured fatty spots, deformation, brown coloured lesions, formation of a stroma, sporulation and death.

In pods older then three months the first symptoms are little discolourations which turn in brown coloured lesions with a yellow halo. Later these lesions extend and are covered with a white stroma. The interior of the pod is usually watery and its content destructed (SANCHEZ, 1985).

SUAREZ (1971) describes the symptoms as follows:

1) Pods inoculated at 20, 40 and some at 60 days after podsetting
The entire infection process of penetration, intercellular and intracellular invasion before symptom expression takes 40 days. It results in necrosis of the fruit, which, especially in pods inoculated at 20 and 40 days, may easily be confused with cherelle wilt.

2) Pods inoculated at 80 days and some at 60 days after podsetting
The infective process last 60 days. These pods show symptoms of premature ripening such as the appearance of areas of mature coloration on pods which are immature, and deformation without externally visible necrosis. Some pods may appear healthy externally but may be totally destroyed internally.

3) Pods inoculated at 120, 140 and 160 days after podsetting.
Small localised lesions may develop and be visible on the pod surface after 60 days. In many cases, the endocarp is not penetrated and the seed from such pods may be salvaged.

2.6 Life cycle:

The only known source of inoculum are the conidia (EVANS ET AL, 1981). Conidia are principally dispersed by wind and rain. Other possibilities are animals, insects and man (ENRIQUEZ, 1985). Spores are transported by wind for distance between 30 and 375 m (GREEN, 1977, MERCHAN, 1981). For germination conidia need a film of water. Conditions favouring germination are a daily mean temperature of 21 - 22 °C and a high daily mean relative humidity of 91 - 93 % (PORRAS & GONZALEZ, 1984). The pathogen is capable to infect the pods in each stage of their development but pods with an age of less then three months are most susceptible. The fungus germinates and penetrates the pod through the epidermis. Eight hours after inoculation hyphae are growing intercellular between the cells of the parenchyme. Incubation period varies with age of the pod, the cultivar and climatological conditions BARROS, 1977). Conidia are capable to survive during a dry period on infected pods remaining on trees or at the ground. These pods are mummificated and the conidia conserve their infective capacity for over nine months (LASS, 1986).

3 EPIDEMIOLOGY

3.1 Introduction:

The cycle of Monilia roreri can be described as a polycyclic (compound interest) process with the epidemic systematically build up. Because infection cycles are identical the polycyclic process is said to be homogeneous (ZADOKS & SCHEIN, 1979). According to HEESTERBEEK AND ZADOKS (1987) the epidemic in the experiment can be called a zero-order epidemic. Expansion of the focus is limited to the boundaries of the field as is the establishment of daughter foci. Assumptions used in this model are:

- individuals are susceptible and homogeneous distributed
- all individuals are equally susceptible
- once an individual is infected it cannot be infected again
- the time kernel is measurable
- the contact distribution is measurable.

3.2 Botanical epidemiology:

Characterization of spread of diseases has been the subject of many studies. These studies vary from the study of disease dispersal gradients to complex computer simulations.

VAN DER PLANK (1963) initiated the study of botanical epidemiology by studying the disease progress in time. GREGORY (1968) contributed with a study on disease progress in space.

Other studies compare different statistical methods for the study of spatial distribution in the field. Some common statistics used are: Fisher's variance to mean ratio, Lloyd's indices of mean crowding and patchiness, the Morisita index of dispersion or the Moran I Statistic. (CAMPBELL & NOE, 1985; MADDEN et al., 1982; NICOT et al. 1984; SCHUH et al., 1986; SHEW et al., 1986).

A more sophisticated approach was used by MINOQUE & FRY (1983 a,b). They studied the spread of disease in a one dimensional space, introducing the gradient parameter g (apparent infection rate (r) divided by the velocity of spread (v). Other methods to model development mathematically have been carried out by JEGER (1983) and VAN DEN BOSCH, METZ AND ZADOKS (1988 a,b).

ZADOKS & KAMPMEIJER (1977) provided a simulation model for the spread of disease in space and time.

3.3 Geostatistics:

A major limitation in most models is the difficulty of relating patterns of initial inoculum to disease incidence and the failure to recognize the degree of dependency among neighbouring observations. To compensate for these problems geostatistical can be used (CHELLIMI, 1988).

Geostatistics is based on the theory of regionalized variables, variables whose values are dependent on its position in space (JOURNEL & HUIJBREGTS, 1978). Therefor geostatistics detects spatial dependence by measuring the variation among samples separated by the same distance.

Spatial variability is measured by determining the average of the squared difference in values between pairs of samples separated by a given distance. This so called semi-variance is defined as:

$$\gamma(h) = \left(\frac{1}{2}N_h\right) \sum_{i=1}^{n} [F(x_i+h) - F(x_i)]^2 \quad (1)$$ Semi variance

here x denotes the position of a sample, x+h the position of another sample, F(x) the incidence at location x and n the total numbers of pairs separated by the same distance.
The factor 2 $\gamma(x,h)$ is the arithmetic mean of the squared difference between two experimental measures (DELHOMME, 1978).

When data are displayed as a graph of semi-variance versus distance a picture of the spatial variation within a field can be obtained. When semi-variances are calculated for different directions the spread of the disease could be tested for anisotropy.

Basically, geostatistical theory states that observations which are located close together are likely to have a higher probability of resembling each other then observations that are further apart. For that reason a better method is to give the observation points weights which are depending on the distance to the predicting point. When the weights are multiplied with a factor to make the sum of the weights equal to one, the sum of the products between weights and according values gives an unbiased prediction. To calculate the weights, different relations can be used. A function which can be used is:

$$W = C * 1/R$$

in which: W = Weight,
C = Constant to make the sum of the weights equals one
R = Distance to prediction point

It is also possible to analyze the spatial structure of the variable, which is measured in the separate observation points. For this analysis it is necessary to look at all the pairs of observation points and calculate the variance in relation to the distance. The resulting co-variance relation can be used to calculate the semi-variogram. An idealized semi-variogram shows that the variables are dependent up to a certain distance (RANGE). When the observation points are situated further apart than the RANGE they are independent and the semi-variance equals the variance (SILL). When prediction point and observation point are the same the semi-variance equals zero. In that case the two points are of course totally dependent. In practice the semi-variance does not equal zero when the distance reaches zero, but there is a small difference, which is called the NUGGET of the semi variogram. If a sill is reached at all, there is a close

resemblance between the and the covariance function. The covariance function is the inverse semi-variogram increased with the sill. The Kriging method uses the covariance function for calculating the weights of the different observation points to calculate the estimated value in the prediction point.
This method is called unbiased because it plots the mean and variance of the variable, restores the values measured at sample points and ensures the estimation variance is minimized.

A very useful possibility of this technique is to plot isovalue maps (JOURNEL & HUIJBREGTS, 1978; GASCUEL-ODOUX, 1987; LECOUSTRE et al.; 1986, 1989).

Geostatistics have not been used frequently as a tool to describe spatial characteristics in phytopathology but the obtained results indicate there is a big future for this technique applied in agronomical research (LECOUSTRE & DE REFFYE, 1986; CHELLEMI, 1988; LECOUSTRE et al., 1989, LANNOU & SAVARY, 1990).

4 MATERIAL AND METHODS

4.1 Location:

The field experiment described in this report was conducted at the experimental farm 'La Lola' of the Centro Agronomo Tropical de Investigacion y Enseñanza (CATIE), Costa Rica. This farm is situated in the district of Bataan, Canton of Matina, Province of Limon, 30 m above sealevel at 10°,05' N and 83°,25' W. Mean annual precipitation is 3573 mm, mean maximum and minimum temperature 30.1°C and 20.5°C and mean maximum and minimum Relative Humidity at 18.00 hours is 92.8% and 60.8%.
Meteorological data at La Lola for the period August 1990 to October 1990 are presented in Table 1 and Fig. 2.

Table 1. Climatic data.

weeks after inoculation [t]	precipitation (mm) [L]	temperature (°C) [K]	sunshine (h) [t]
1	16.2	24.8	1.3
2	33.8	25.1	3.4
3	14.7	25.5	4.0
4	4.9	25.9	5.5
5	2.0	25.5	4.4
6	1.0	25.9	6.4
7	16.3	25.0	4.6
8	1.1	25.8	5.5

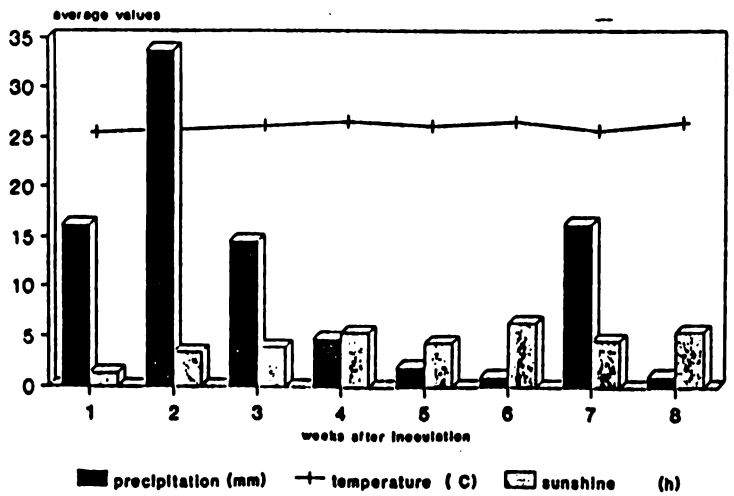


Figure 2. Climatic data.

4.2 Experimental design:

In this experiment the clone UF29 was used. This clone is reported to be highly susceptible to Monilia (ENGELS,1981; GALINDO 1990). Planting year was 1968. Plant distance was 2.5 x 4 m (average values). One side of the plot was surrounded by banana (Musa AAA), another by peach tree (Bactris gasipaes) whilst the remaining two were surrounded by cacao. (Figure 3.) To diminish the risk of invading Monilia from these sides border rows with a width of 20 m were established. One week before inoculation all trees were labelled and mapped. (Fig. 4, APPENDIX 6).

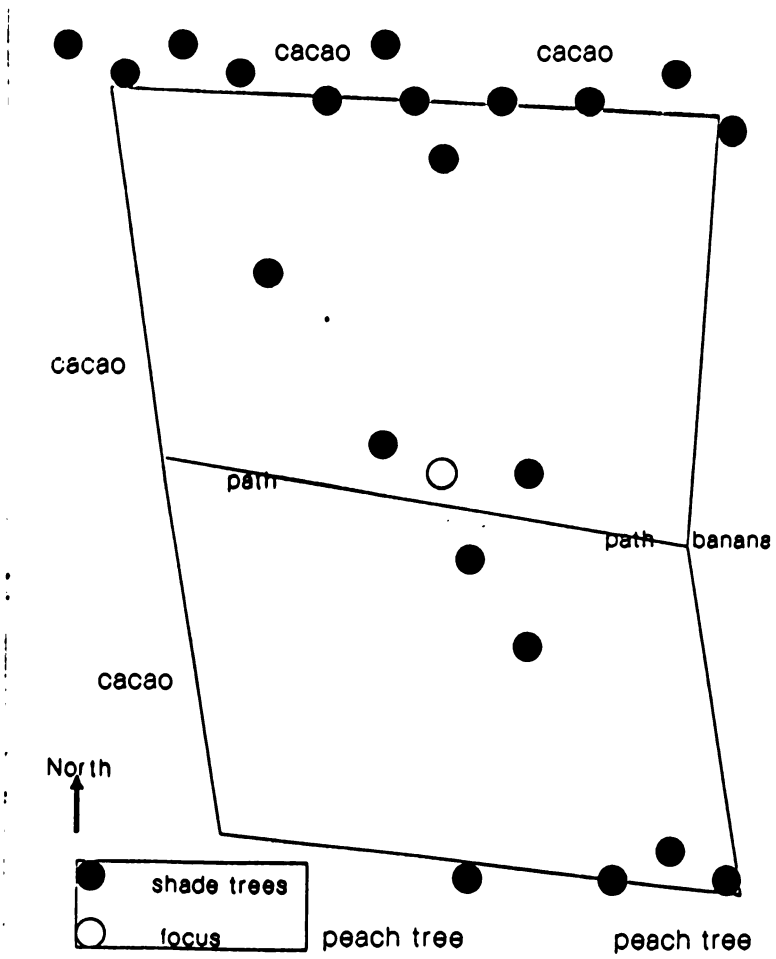


Figure 3. Surroundings of the cacao field.

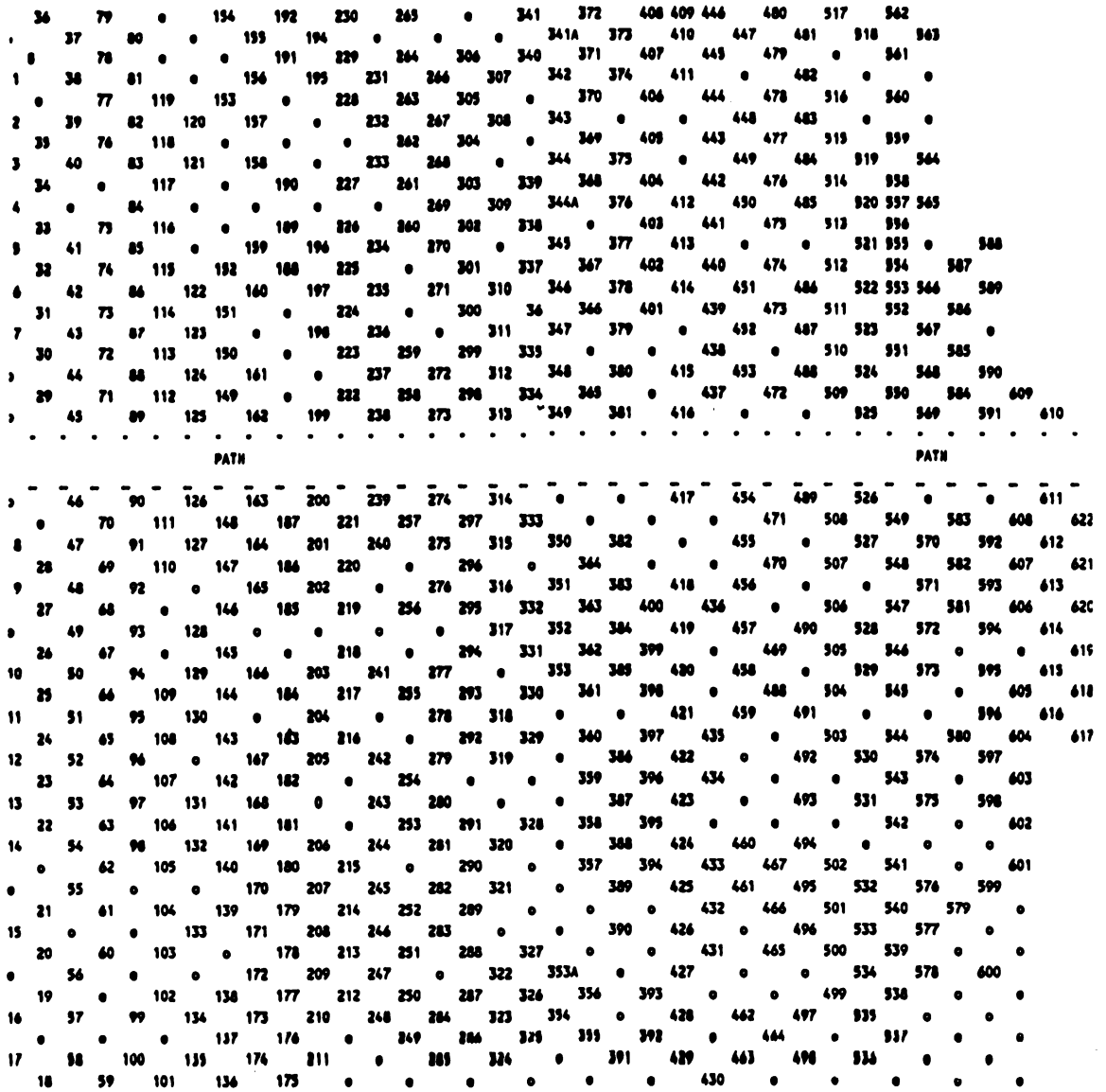


Figure 4. Lay out of the experiment.

4.3 Cultural practices:

Five weeks before inoculation weeds were controlled chemically by spraying Gramoxone at a rate of 2 kg commercial product to the ha. For a greater impact hand weeding was performed before spraying.
Four weeks before inoculation chupons were removed and border rows treated with a copper formula against Monilia. The fungicide used was Kocide at a rate of 2.25 kg commercial product to the ha.
Three weeks before inoculation all the pods with symptoms of Monilia were removed. This operation was repeated until two weeks after inoculation. As the incubation period of the fungus is about three weeks (GALINDO, 1990), all the expression of Monilia before three weeks after inoculation was not due to the artificial source of inoculum but to a natural source and thus disturbing the results.

4.4 Inoculation:

Inoculation took place at the fourth of August 1990. To create an adequate source of inoculum 30 freshly sporulating pods were attached with iron wire at different sites in a central tree. After two weeks these pods were replaced by others to provide a constant source of inoculum.

4.5 Spore trapping and counting:

To trap the Monilia spores several traps were placed in the plot. These traps consist of piles of wild sugarcane with a length of 2.35 m. A plastic tube with a length of 10 cm and a diameter of 3 cm covered the stake 5 cm below the top of the pile. At the top a plastic plate with a diameter of 8 cm was placed upside down to protect the plastic tube from rain. Tesa tape with a width of 1 cm and a length of 4 cm was attached to the plastic tube with

its adhesive side outwards to capture the spores.
The stakes were placed at a depth of 30 cm. in the soil. The tape is thus placed at a height of 2 m (Fig. 5). This is the height at which it is most likely to capture the majority of the spores (GALINDO, 1990).
Traps were situated in the eight main direction of the compass card. Distance between two traps was 5 m in the North, East, South and West directions and 10 m in the other directions (Fig. 6).
Trapping took place from 09.00 am to 15.00 pm, two times a week. During this period spore liberation is highest (GALINDO,1990).
Spore counting was done with a Bausch and Lomb Balplan microscope with a magnification of 10 x 10. To transform to a density to the m^2 data need to be multiplied but to keep them as simple as possible the original data were maintained. Data were stored in a LOTUS 123 file.

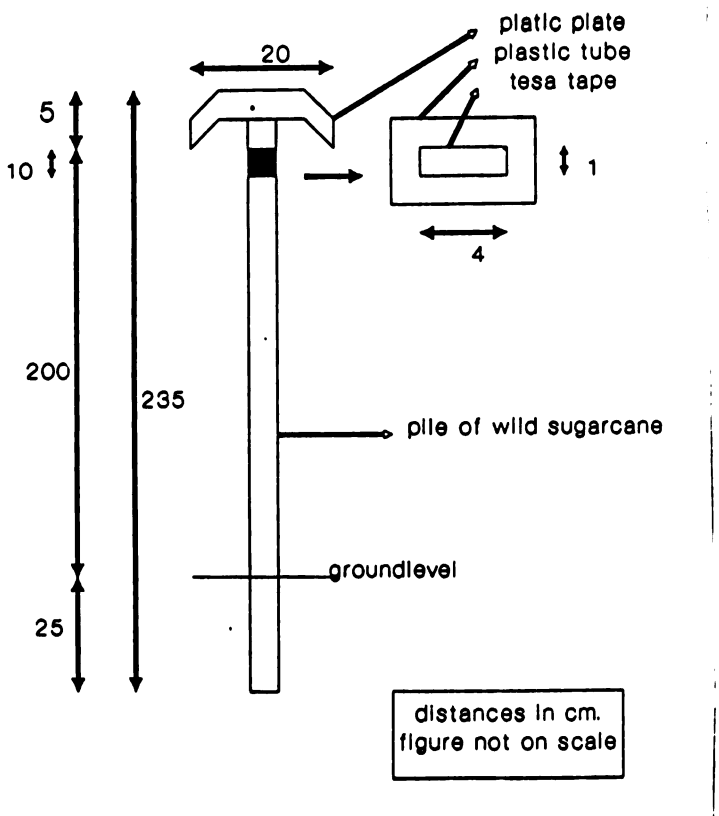


Figure 5. Trap to sample spores.

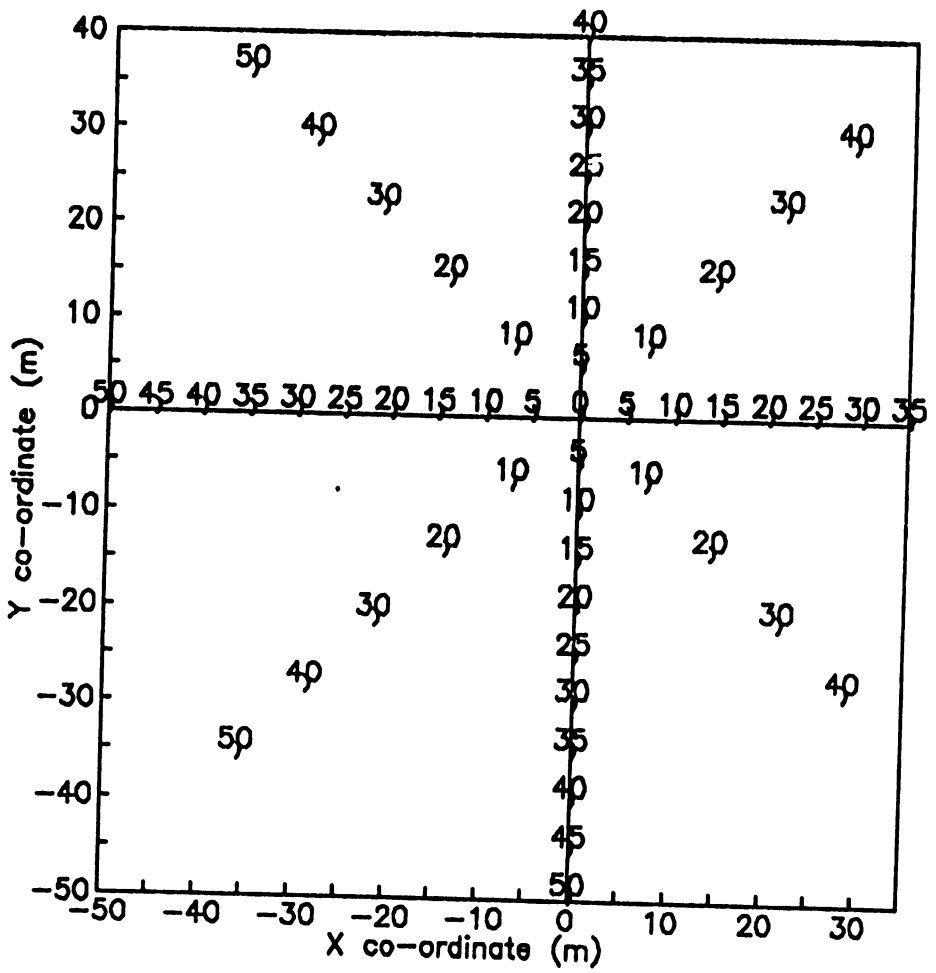


Figure 6. Position of the traps.

4.6 Pod counting:

In weekly intervals pods were counted. Counting occurred in three classes: not infected, pods with clear, visible symptoms of Monilia and pods with sporulating Monilia. Pods were left in place to allow for a polycyclic process of focus build up. Due to the difficult positions of the pods on the tree a more distinct classification was not possible. Data were stored in a LOTUS 123 file.

4.7 Data processing:

The LOTUS data were statistical treated with the program SPSS and geostatistical with SPATANAL and MAPIT. Graphs were made using HARVARD and SLIDEWRITE. The data were translated to SURFER for interpolation and plotting (Fig. 7).

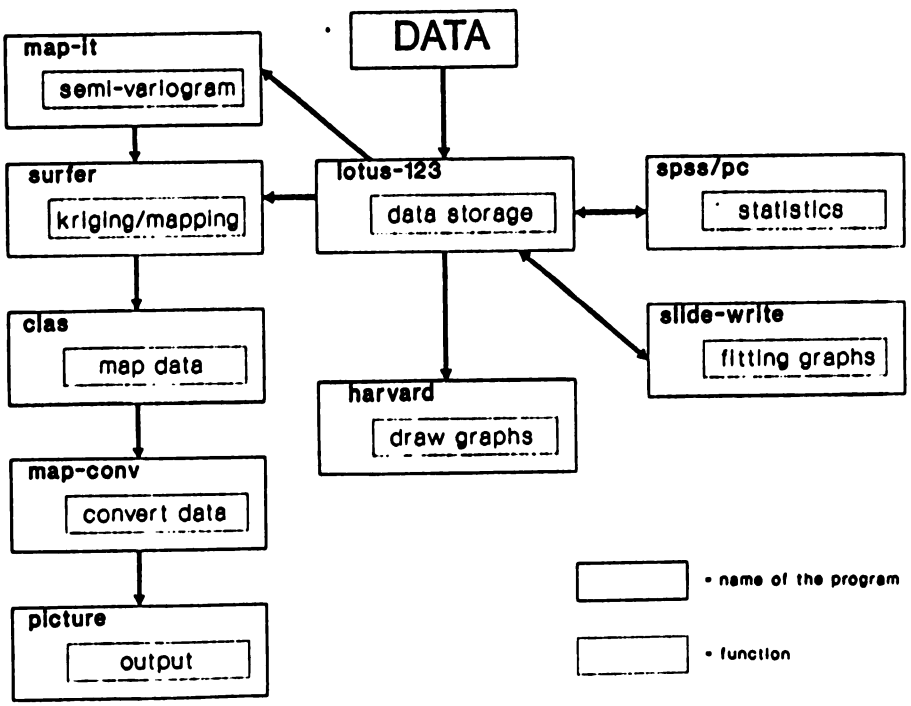


Figure 7. The relation between programs used in this report.

4.8 Data analyses:

The distribution of spore was visualized using SURFER, and studied by computing the semi variance and correlating the density of spores with the distance to focus.

To describe the disease progress and spread the percentages of symptom bearing and sporulating pods were first divided by 100, to express the incidence as a fraction on a 0 - 1 scale.

As it takes about three weeks from infection to the appearance of symptoms and another week to infection, the second generation will produce spores after seven to eight weeks. These first seven weeks the fungus is in a simple interest phase during which the amount of inoculum will be same as the original amount. After this period the compound interest phase starts with inoculum also coming from daughter foci.

To obtain a clear description of the Monilia disease progression data of the incidence were fitted to four different models. The first model assumes a monocyclic process, the second a polycyclic, the third a combination of the former two and the fourth uses the Gompertz model. The third model was used to compensate for the relatively long period of the simple interest phase. All four models are nonlinear but were transformed to a linear model in the following way:

$$f(x) = \ln\frac{1}{1-x} \qquad (2) \qquad \text{Simple interest Model}$$

$$f(x) = \ln\frac{x}{1-x} \qquad (3) \qquad \text{Compound Interest Model}$$

$$f(x) = \ln\left(\ln\left(\frac{1}{x}\right)\right) \qquad (4) \qquad \text{Gompertz Model}$$

The graphs were fitted using ordinary least square regression. The obtained straight lines are called logit or gompit lines. The tangent of the slope of this logit (or gompit) line is the logistic apparent infection rate r_l (ZADOKS & SCHEIN, 1979). Epidemics were described using r_l and y_0 (initial level of disease). Because different models were used, the parameters could not be compared directly but were transformed to the Weighted Mean Rate (ρ), where $\rho = r/n$. N is respectively 2, 6, 3.33 and 4 (MADDEN *et al.*, 1987). Different statistics were used to determine the appropriateness of each model.

As there exists a difference between predicted values and observations models were transformed into statistical forms, introducing μ_i; the unexplained variability at time i. When it is assumed that μ_i's are dependent (which seems reasonable with data collected over time) μ_i can be expressed as:

$$\mu_i = \rho * v_{i-1} + v_i \qquad (5)$$ unexplained variability

ρ is the autocorrelation parameter and v_i a normally, independently distributed error term with mean 0 and constant variance σ^2. The autocorrelation parameter ρ can be calculated as:

$$\beta = \frac{\sum_{i=2}^{n} (\beta_{i-1} * \beta_i)}{\sum_{i=2}^{n} (\beta_{i-1})} \qquad (6)$$ autocorrelation parameter

Disease gradients were studied using four different transformations:

1) log-log (GREGORY, 1968)
2) log-linear (KIYOSAWA & SHIYOMI, 1972)
3) logit-log (BERGER & LUKE, 1979)
4) logit-linear (MINOQUE & FRY, 1983a)

These transformations were fitted and analyzed using some common statistics.

To study the influence of the direction of the wind the plot was divided in eight parts. For each part the correlation with incidence was calculated. To obtain pseudo-replicates in the same plot eight transects were taken. Each replicate was surrounded by two transects and two arcs concentric around the focus (LEONARD & FRY, 1986).

To study the usefulness of geostatistics to determine spatial variability semi-variances wer calculated for each observation in four directions to test for anisotropy. A predicted value at each point was obtained using the Kriging technique.

5 RESULTS

5.1 Spore trapping:

Due to certain problems it was not possible the measure the spore density (data in APPENDIX 1) in the first two weeks. However, the first three measurements indicate a highly significant negative correlation with distance (Table 2). These three measurements took place in the period when there were no sporulating pods. After the first sporulation correlation declined rapidly and became small positive values instead of large negative values.

The semi-variances indicated a nugget effect in all observations (Fig. 8). The first two weeks the nugget effect could be transformed to a linear model but in the last weeks the semi-variogram immediately takes its maximum value, an indication of a pure nugget effect.

Maps (Fig 9.) show the wavelike spread in the first weeks but a decrease of this wavelike spread after the first weeks is clearly visible.

Table 2. Correlation spore density with distance to focus.

Count no. [1]	Correlation [1]
1	-0.44 **
2	-0.55 **
3	-0.53 **
4	-0.07
5	0.11
6	0.13
7	0.03
8	0.04
9	0.10
**: 1- tailed significance .001	

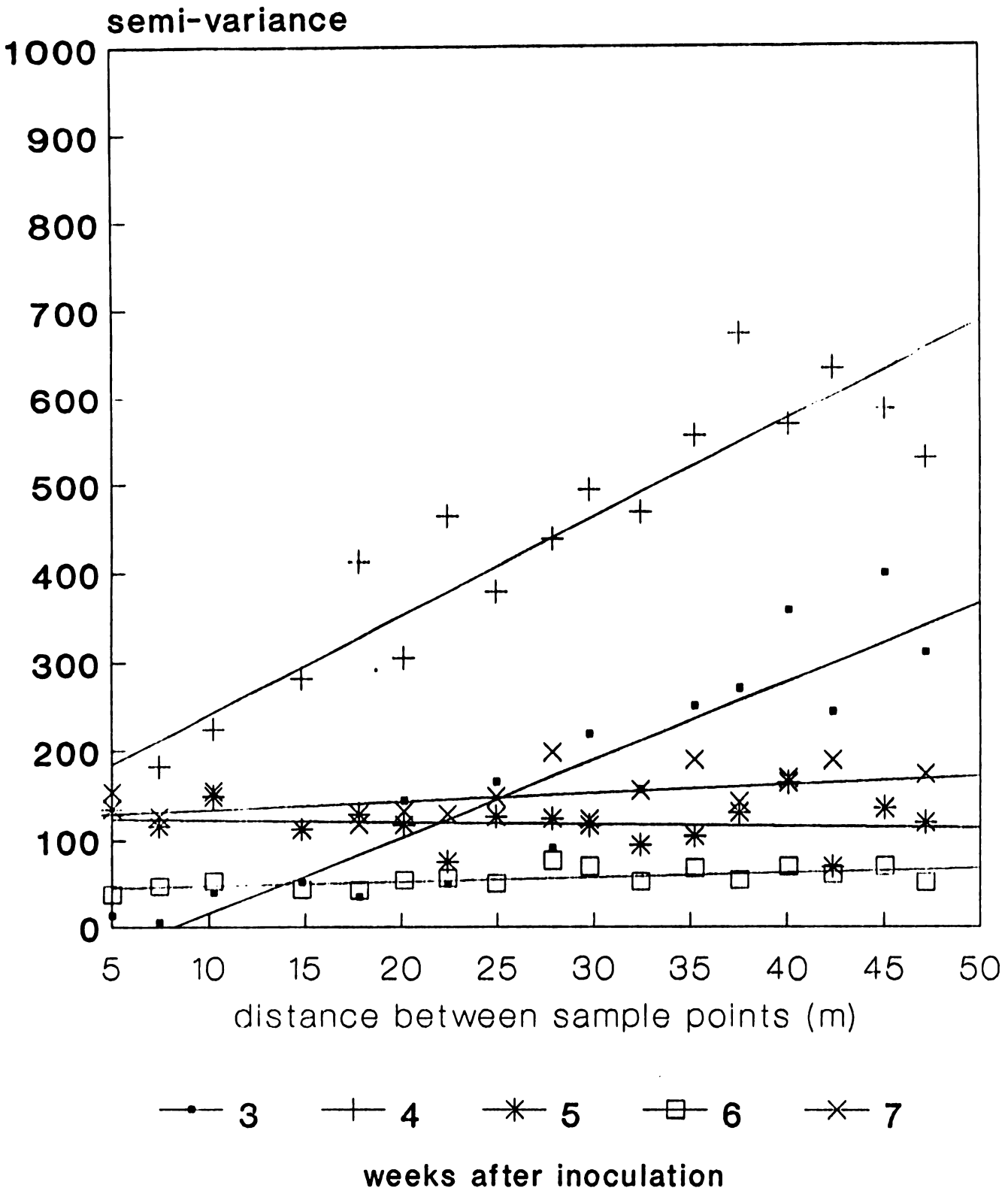


Figure 8. Distribution of spores

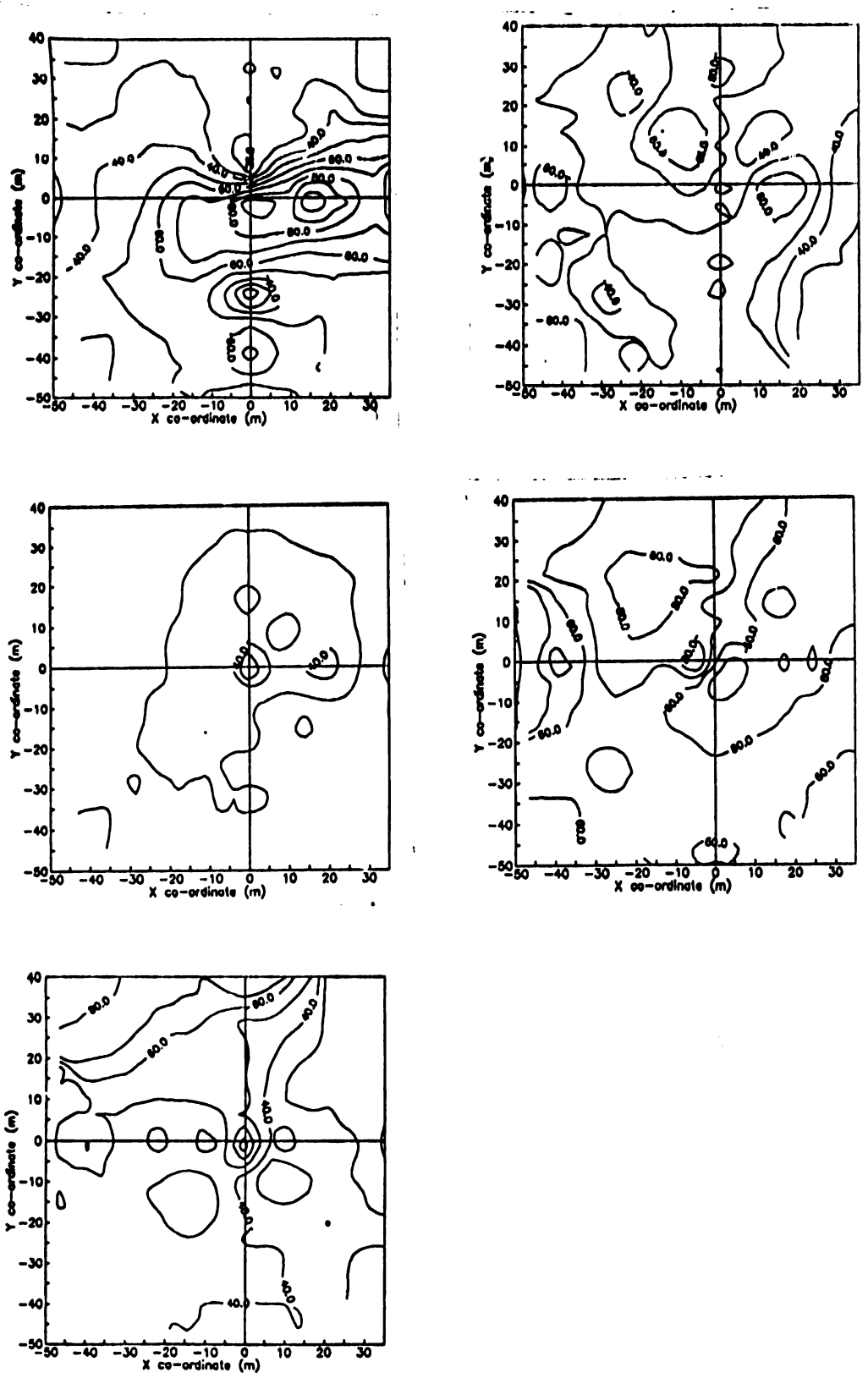


Figure 9. Distribution of spores at different days after inoculation.
Upper left: 21 Upper right: 42
Middle left: 38 Middle right: 49
Under left: 35

5.2 Disease progress in time (disease progress):

Observed data are presented in APPENDIX 2.
When average observations were plotted (Fig. 10), the appearing curve has a S shaped, sigmoid appearance, symmetrical around the point of inflexion. This point, reached when x (fraction of infected pods) becomes 0.5 is called the midtime ($t_{0.5}$).

After three weeks the incidence was already 0.17 but one week later the progress was even more quickly and incidence became 0.49. The following two weeks the increase of the incidence was equal (0.14) and declined the last two weeks.

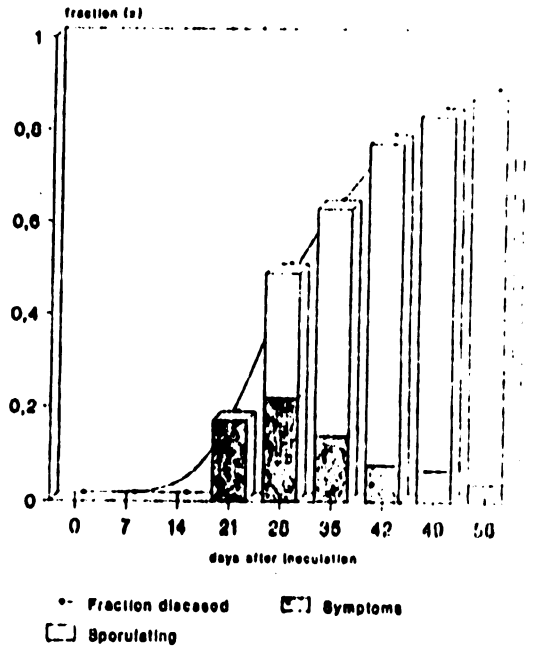


Figure 10. Disease progress.

Table 3. Results disease progress.

days after inoculation [T]	pods without symptoms [1]	pods with symptoms [1]	sporulating pods [1]	fraction diseased [1]
7	1	0	0	0
14	1	0	0	0
21	0.83	0.17	0	0.17
28	0.51	0.22	0.27	0.49
35	0.37	0.14	0.49	0.63
42	0.23	0.08	0.69	0.77
49	0.17	0.07	0.76	0.83
56	0.13	0.04	0.83	0.87

When the graph of the compound interest and the Gompertz model are studied there appears a bend in the curve after 28 days, indicating a changed susceptibility of the pods.

When models were de-transformed it is obvious the Simple Interest Model overestimates the disease although the R value is the highest of the four (Table 4, APPENDIX 3). The Compound Interest Model over-estimates the progress up to 25 days, under-estimates until 49 days and over-estimates the last week. The Combined Model overestimates until 35 days and the predicts almost correctly. The Gompertz Model over-estimates until 35 days and then under-estimates the progress (Fig 11.). Only the Combined Model was able to predict the midpoint value almost correctly. After 28 days the incidence was 0.49 and the Combined Model predicted 0.51. A t-test showed that all models were significant different from the expected values. Value $t_{0.90}$, 10 d.f. is 1.81 and observed values were 0.44, 0.12, 0.16 and 0.18.

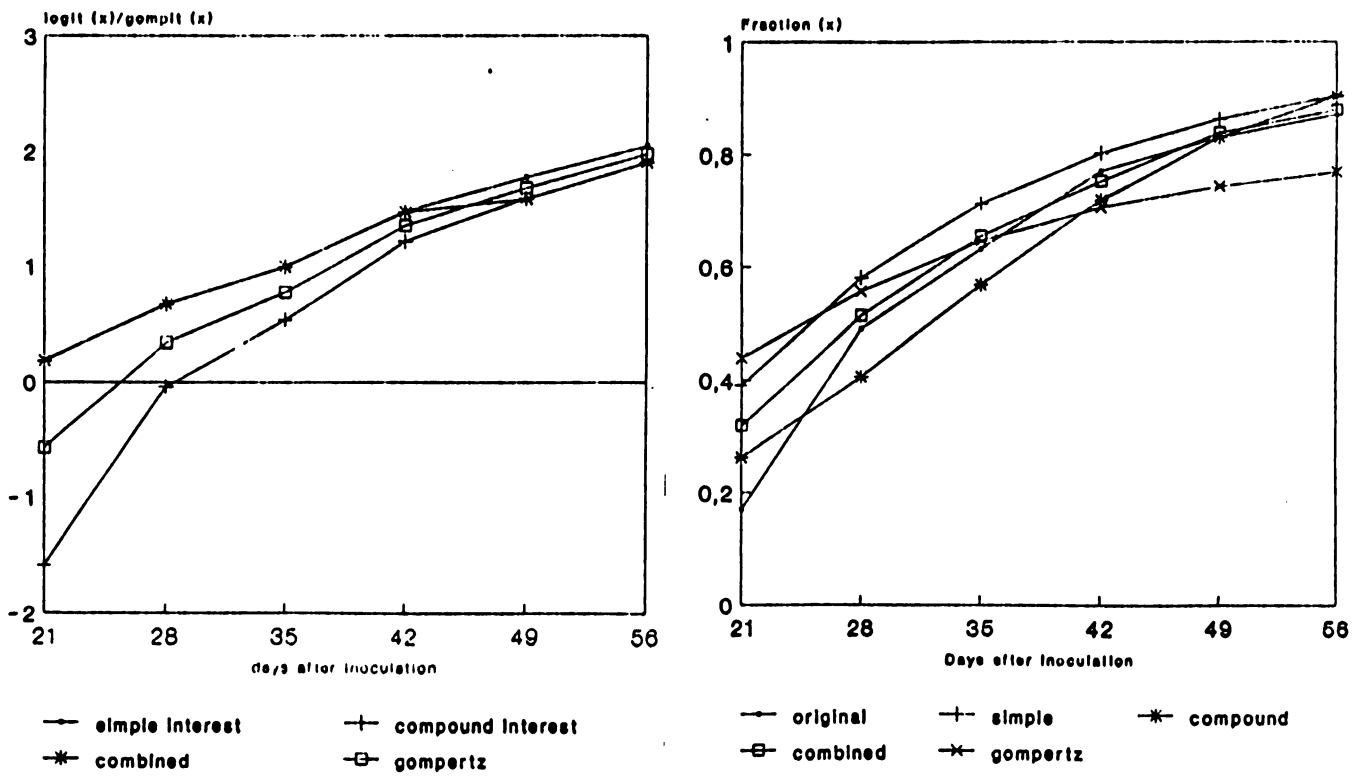


Figure 11. Transformed (left) and Detransformed models (right).

To study the independence of the y_i's the auto-correlation parameter ρ was calculated. Parameters were respectively -0.12, -0.04, 0.37 and -0.06. The parameters in the Simple Interest, Compound Interest and the Gompertz model are close to zero. The Combined Model has an parameter close to one and autocorrelation should be used in the analysis. To compare the results of the parameters all the models were treated in the same way as described before. The gives a quite remarkable result (Table 5, Fig. 12): when de-transformed the Simple Interest Model predicts the progress the best, followed by the Combined Model. The Simple Interest Model first underestimates the progress but predicts almost correctly in the last four weeks. The Compound Interest Model highly overestimates the progress in the first three weeks and less in the last three weeks. The Combined Model overestimates largely in the first three weeks but predicts almost correct in the last weeks. The Gompertz Model also overestimates the values in the first weeks but is again almost correct in the last weeks. Compared with the not corrected data the Simple Interest Model was improved by correction, the Compound Interest Model got worse, the Combined Model worse in the first two weeks but almost the same in the other four weeks and The Gompertz Model got worse in the first three weeks but improved in the last three weeks. After correction for autocorrelation no model was able to predict the midpoint value correctly. A t-test shoed that all models were significant different from the expected values. the t-expected value is 1.81 and observed values were 0.21, 0.65, 0.45 and 0.48.

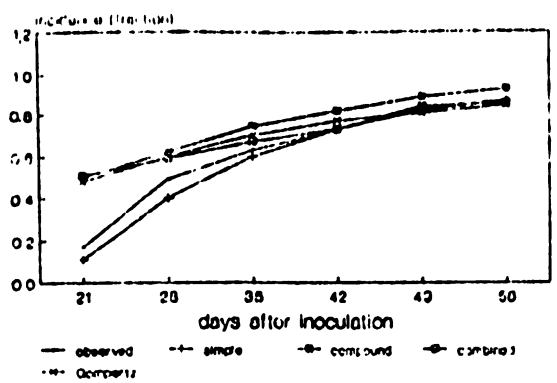


Figure 12. Detransformed models after correction for autocor relation

Table 4. Results from de-transforming the fitted data.

week [T]	Observed [1]	Simple [1]	Compound [1]	Combined [1]	Gompertz [1]
3	0.17	0.39	0.26	0.31	0.43
4	0.49	0.58	0.40	0.51	0.55
5	0.63	0.71	0.56	0.65	0.64
6	0.77	0.80	0.71	0.75	0.70
7	0.83	0.86	0.83	0.83	0.74
8	0.87	0.90	0.90	0.87	0.76

Table 5. Results from de-transforming the fitted data after correction for autocorrelaton.

week [T]	Observed [1]	Simple [1]	Compound [1]	Combined [1]	Gompertz [1]
3	0.17	0.11	0.50	0.51	0.48
4	0.49	0.40	0.62	0.59	0.60
5	0.63	0.60	0.74	0.67	0.70
6	0.77	0.73	0.82	0.73	0.77
7	0.83	0.82	0.89	0.84	0.81
8	0.87	0.87	0.93	0.86	0.85

5.3 Progress in space (disease spread):

The results of the disease spread are presented in Fig 13. (see APPENDIX 4 for data). Only in the first observation the influence of the focus seems obvious, the further the distance the lesser the incidence. After the second observation this pattern changes: observations near the focus have a lesser incidence then observations further away. The tangent of the line of the fifth and sixth week and the tangent of the seventh and eighth week are almost the same. The tangent becomes higher when time passes. Statistical analyze shows there is no correlation (r=0,05) between the distance from the focus and the incidence as was already shown by the low skewness of the logit lines.

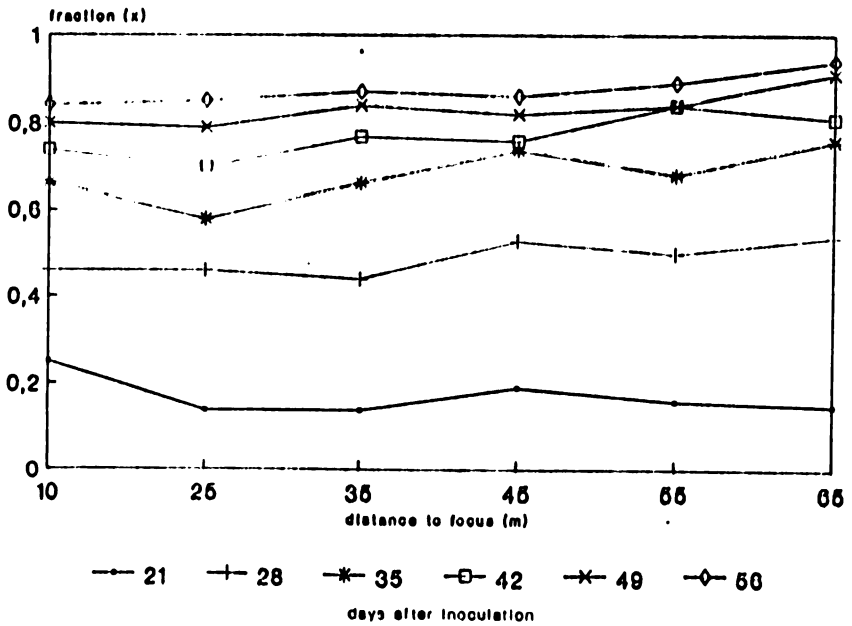


Figure 13. Disease spread.

To study the possible influence of the wind the correlation with each octant was calculated. As r = 0,077 a correlation with winddirection could not be found. When the plots was divided into pseudo-replicates correlation became 0.083. Correlation of distance with pseudo-replicates was 0.124 and octant with pseudo-replicates 0.9923 which indicates again the weak influence of distance to focus in relation to incidence.

5.5 Geo-statistics:

Spatial dependence was hard to recognize in this experiment. When semi-variances for all directions were plotted (Fig. 14) five observations show an almost pure nugget effect with only a very small slope. The first observation (21 days after infection) had a nugget variance of 0.005, the second and third 0.08 and the fourth 0.12. The slope of the weighted linear function could not be counted. Semi-variances differed greatly from one distance to another. This indicates a completely random distribution.

When observations were tested for anisotropy (Fig. 15) it appeared there was no anisotropy in the first and second observation. In the third observation there was a slight anisotropy in the 0° direction. The fourth observation showed anisotropy in all directions, especially in the 0° and 135° direction. In the last two observation no anisotropy could be found.

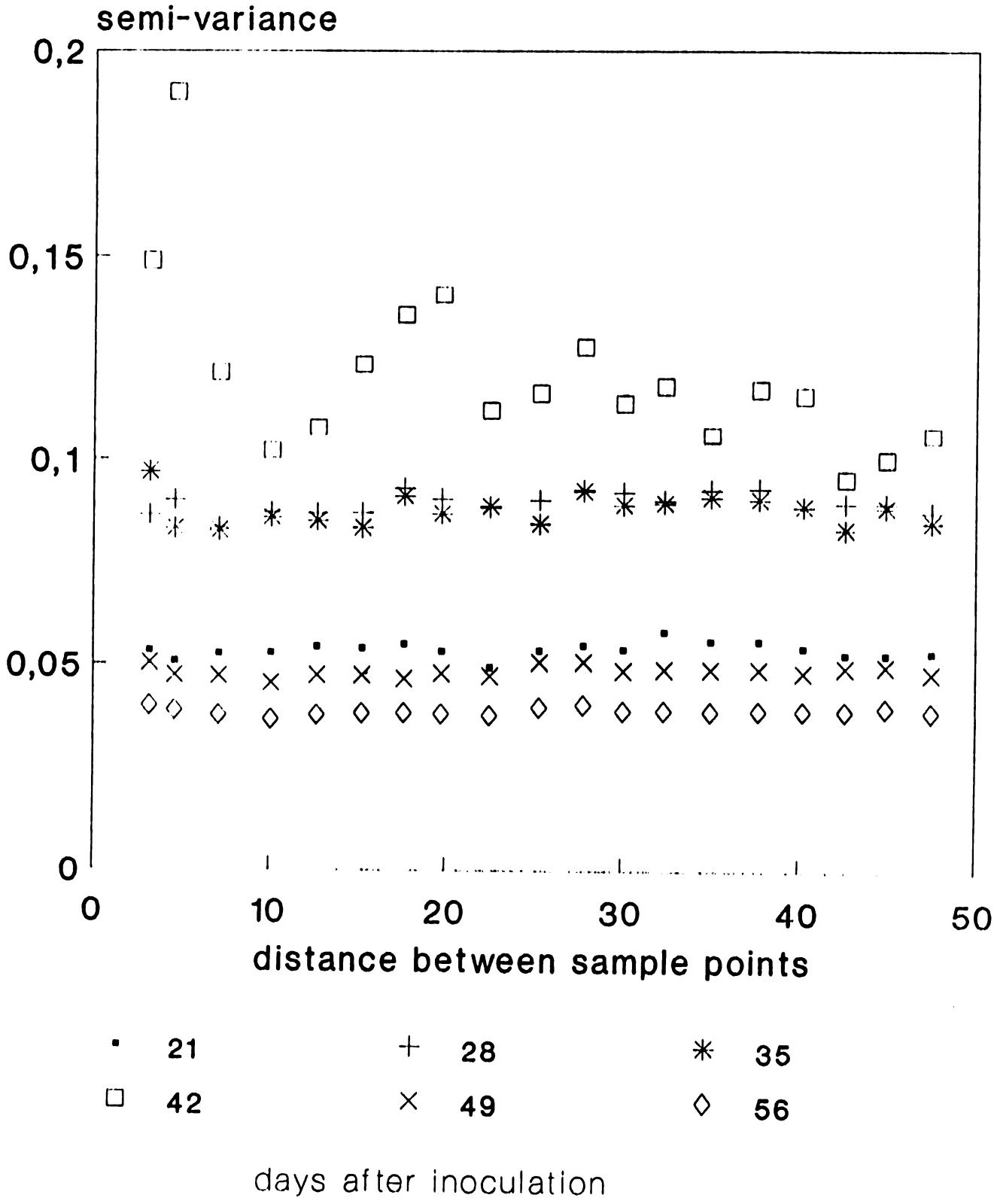


Figure 14. Semi-variogram

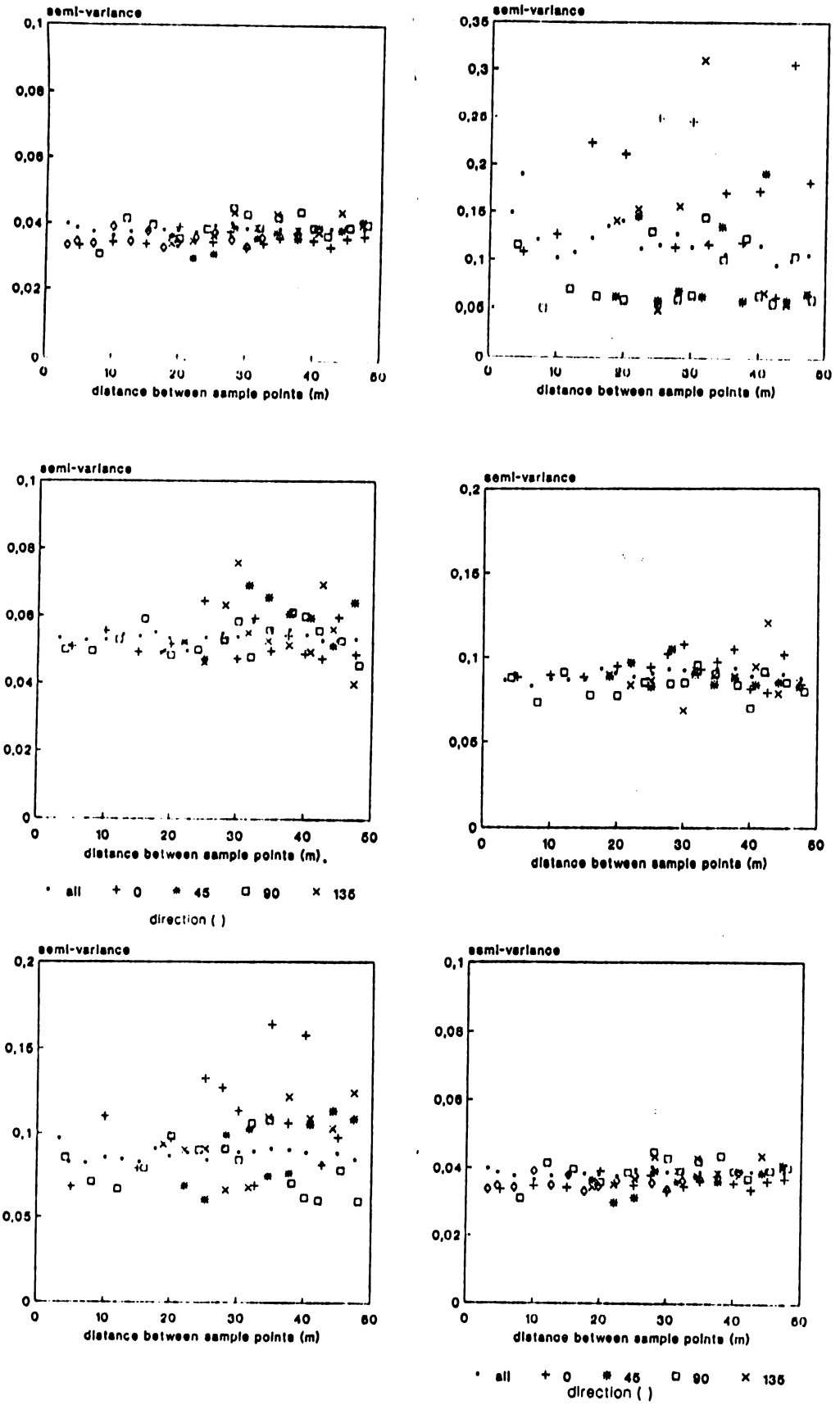
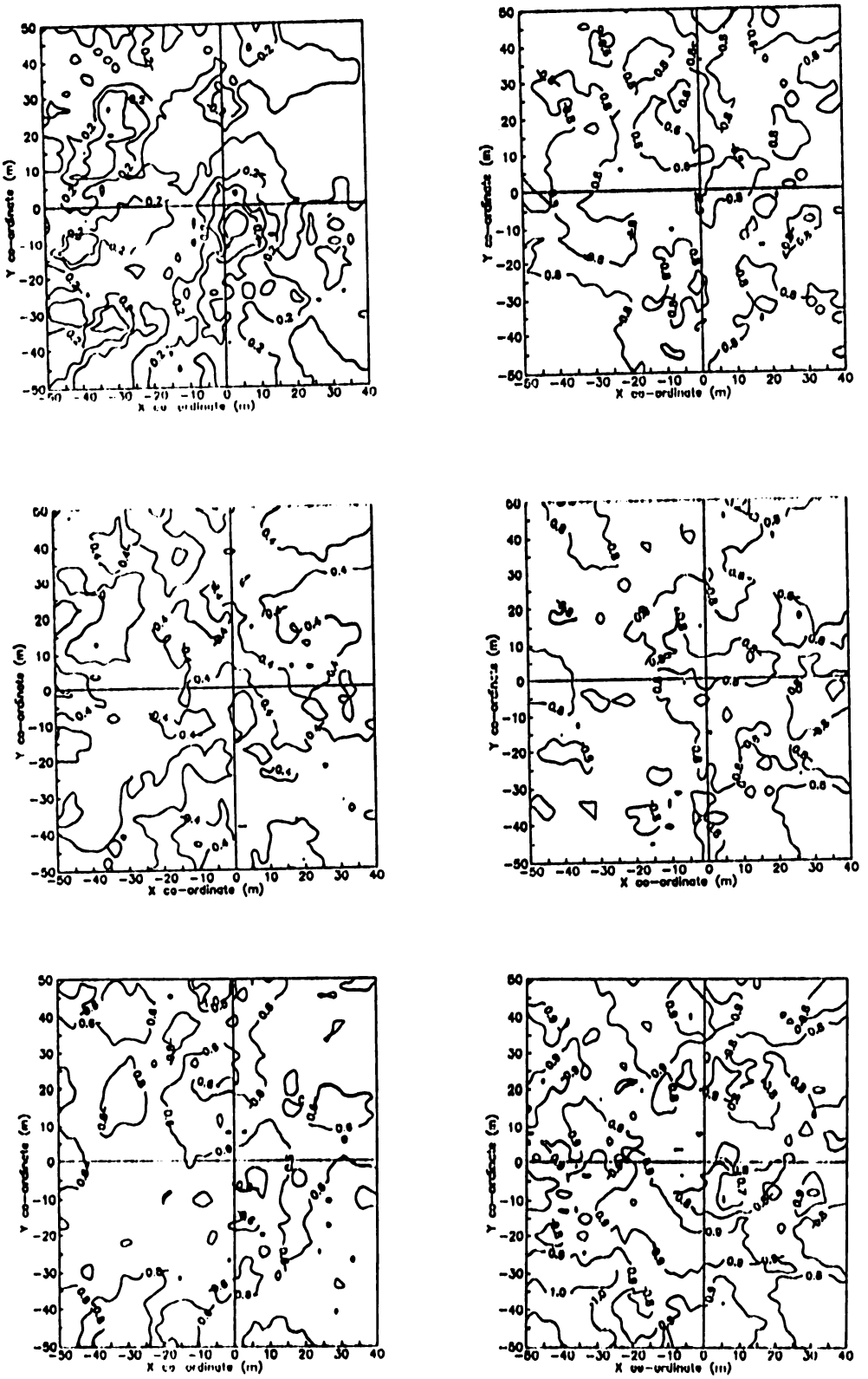


Figure 15. Semi-variograms for four directions.
(Upper left 21 Upper right 42
Middle left 28 Middle right 49
Under left 35 Under right 56)



Distribution different days after inoculation.

(Upper left 21 Upper right 42
Middle left 28 Middle right 49
Under left 35 Under right 56)

6 DISCUSSION

6.1 Spore trapping:

Because the first three observations are highly negative correlated with distance to focus it seems reasonable to suggest that in the preceding weeks the same results could be expected. When sporulating began, the significance dropped and no correlation with distance could be found anymore. Therefore it seems obvious that after sporulation the distribution of spores in the field became more equal as a result of dispersion of spores from secondary foci in all directions.

This assumption is supported by the study of the variogram. The first two observations (21 and 28 days) could be fitted to a linear model without a range but the other variograms indicate a pure nugget effect. The first observations has a nugget variance close to one, the others were clearly higher. This nugget effect is due to sampling errors, counting errors or sampling technique. Another possibility is the existence of a microstructure. This microstructure could not be revealed due to the large distances of samplers to each other.

6.2 Disease progress:

The progress in the third and fourth week was remarkable. It is possible the most susceptible (younger) pods were attacked first and the more resistant (older) pods later. When inoculum pressure becomes higher even these more resistant pods were infected. Another possibility is the undercorrection by the factor (1-x). This factor assumes equal susceptibility, constant infection pressure, constant weather conditions and so on (VAN DER PLANK, 1963). It was observed that pods of the same age were not equally susceptible as one pod was heavily infected whilst the neighbouring pod was uninfected, even when they touched each other.

Weather conditions were not very regular (Fig. 2) and the infection pressure changed because the pods in the focus lost their initial inoculum density.

When it is assumed there is an over-estimation in the assessment of the symptoms (because the first symptoms; deformations; were hard to recognize) but not in the assessment of sporulating pods the Combined model seems to be the most appropriate to predict the progress of the disease. This can indicate there is large source of inoculum already present in the plot (comparable to soil-borne fungal diseases). The use of the Compound Interest phase in the Combined describes the influence of the daughter foci.

The negative autocorrelation values were unexpected but probably due to the influence of other sources of inoculum. Applying the autocorrelation parameters changed performance of the models which is in contrast to the theory as described by MADDEN (1986) who stated that values of $\rho < 0.50$ have little impact on the progression results.

The fact that all the models had problems to describe the progression in the first weeks seemed to be caused by the increase of the incidence from 0 to 0.17. Possible explanations are that first symptoms were not observed, an weekly interval between first countings is too long or the symptoms in week three were overestimated. Supposing however correct procedures were followed it can be possible there is a large pool of inoculum in the trees and sanitation could not prevent infection of pods due to this source instead of the artificial source.

6.3 Disease spread:

It seems the spread of the disease is very quick between the third and fourth week, is constant between the fifth and sixth week and between the seventh and eighth week. The steeper angle between these two groups is probably caused by the influence of secondary foci. Because the incidence increased with distance the idea rises there is an influence of spores coming from other plots or from spores still in the plot after the first four weeks of sanitation. This idea is confirmed by the results of the analyze of disease progress (see 6.2).

When models are reviewed it is clear there is no need for a transformation. The spread was better predicted by fitting the original data and using these parameters then using the parameters of the fitted, transformed models.

6.4 Geostatistics:

Although the use of geostatistics is a powerful tool in detecting the spatial dependence between values (e.g. disease incidence) in this experiment spatial dependence could not be found. The appearance of a nugget variance is normally due to sampling errors or to a lag distance greater than the scale at which the processes of disease spread take place. The influence of sampling errors has already been discussed in chapter 6.2. The influence of a microstructure with a radius smaller than 2.5 m is possible but not likely. When a lag distance of 1 m was tested the distribution of number of pairs differed too much too be accepted for interpretation. A microstructure could be explained by the fact that pods of one tree are not located at 2.5 m of each other as is the intra row distance but this seems hard to justify.
The fact that in the first weeks the almost pure nugget effect appears strengthens the theory of an already existing source of inoculum in the parcel. The variation after 42 weeks is remarkable but possibly due to the emerging of secondary foci as has already been remarked in chapter 6.3 and 6.4.

6.5 Constraints:

As the study is a longitudinal study with no control of biotic and a-biotic factors some problems in observations have occurred. The problems with sampling techniques were already mentioned. Climatic data are very important as high rainfall and high disease incidence are highly correlated and windspeed and winddirection influence the liberation and spread of spores. These factors are known and therefor not considered in this study although correlating incidence to rainfall could explain the rapid spread in the first weeks. Wind direction was not included because correlations of incidence with octants could not be found.

Although Diaz (in LASS, 1986) suggested there is no difference between shaded and unshadowed cacao it is clear shade influence the relative humidity within the parcel and thus the incidence (WAAIJENBERG & TAZELAAR, 1990). Jorgensen (in LASS, 1986) could not find an effect of spacing on disease incidence but this factor also affects the relative humidity (TAZELAAR, 1990, 1991). One side of the parcel was heavily shaded and it seems worthwhile to incorporate this effect in the calculations. Potassium content seems to be related to production and disease incidence and could explain the high levels of incidence (WAAIJENBERG & TAZELAAR, 1990) but samples on a small scale were not available to study this effect.

7 CONCLUSIONS

This report was carried out to study the epidemiology of Monilia roreri in a parcel of cocoa. Several models were tested at their possibility to describe the progress of the disease incidence.

It seems the Simple Interest Model is the most accurate model to describe the progress. Due to the enormous amount of inoculum it was not possible to follow the spread of disease originating in an artificial source of inoculum. This Simple Interest Model is comparable to a soilborne fungus with a constant source of inoculum.

Observations show that many aspects of the infection process are still poorly understood and more research is needed to study the resistance mechanisms of the cocoatree.

For farmers results indicate they need to enter their parcel frequently to control the disease. The recommended interval of three weeks (ENRIQUEZ, 1985) is appropriate but is clearly the maximum. Waiting some days or, worse, forgetting a time gives rise to a rapid spread of the disease. The high incidence in farmers fields as observed in Costa Rica is probably due to an inadequate management and not only to non-resistant cultivars as is the farmers complaint.

The use of geostatistics in this study indicate the random spread of the disease and seems to be a useful technique to evaluate the spatial spread of a disease. One possibility not included in this study and not found in the studies of Lecoustre et al. and Lannou and Savary is the use of the co-kriging method to calculate to calculate crossed covariances. This method is used when variables are correlated. In the case of phytopathology, in a longitidunal study observations are highly correlated and as the autocorrelation parameter was calculated to correct this effect the co-kriging technique could do the same.

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APPENDIX 1 Distribution of spores.

Average values	distance to focus (m)										
	0	5	10	15	20	25	30	35	40	45	50
1	100	85	75	70	58	41	47	57	44	45	31
2	99	87	75	90	69	77	60	71	53	65	45
3	71	35	30	41	31	28	32	24	23	27	27
4	66	35	36	36	38	35	36	38	32	24	35
5	87	45	37	43	41	40	42	49	44	44	52
6	25	34	38	38	38	37	36	41	47	39	37
7	30	50	51	58	52	57	46	45	43	61	54
8	54	57	56	59	53	49	61	51	45	61	61
9	61	64	61	63	63	64	71	62	62	55	54

APPENDIX 3 Disease progress

Transformed model

week [T]	data [1]	simple [1]	compound [1]	combined [1]	Gompertz [1]
3	0.17	0.19	-1.58	0.19	-0.57
4	0.49	0.67	-0.04	0.67	0.34
5	0.63	0.99	0.53	0.99	0.77
6	0.77	1.47	1.21	1.47	1.34
7	0.83	1.77	1.59	1.59	1.68
8	0.87	2.04	1.90	1.90	1.97

Transformed, fitted model

week [T]	data [1]	simple [1]	compound [1]	combined [1]	Gompertz [1]
3	0.17	0.25	0.90	0.29	0.30
4	0.49	0.62	0.30	0.63	0.16
5	0.63	0.99	0.30	0.97	0.64
6	0.77	1.36	0.94	1.31	1.12
7	0.83	1.73	1.58	1.65	1.60
8	0.87	2.10	2.22	1.99	2.

Statistical data

	simple	compound	combined	Gompertz
mean	1.19	0.60	1.13	0.87
maximum	2.04	1.20	1.90	1.90
minimum	0.80	-1.50	0.18	0.57
S.D.[1]	0.70	1.28	0.64	0.94
S.E.[2]	0.29	0.52	0.26	0.38
95% Conf.[3]	0.56	1.02	0.51	0.75
99% Conf.[4]	0.74	1.35	0.67	0.97
R^2	0.99	0.92	0.97	0.96
r_1	0.37	0.66	0.34	0.49
Y_0	-0.12	-1.69	-0.04	0.81
W.M.R.[5]	0.19	0.11	0.10	0.12

---

[1] Standard Deviation

[2] Standard Error

[3] 95% Confidence

[4] 99% Confidence

[5] Weighted Mean Ratio

AVERAGE VALUES observations

count	week	day	incidence
0	0	0	0
0	0-7	7	0
0	7-14	14	0
1	14-21	21	0.17
2	21-28	28	0.49
3	28-35	35	0.63
4	35-42	42	0.77
5	42-49	49	0.83
6	49-56	56	0.87

	logistic transformation				fitted model			
count	sim	com	tog	gom	sim	com	tog	gom
1	0.186	-1.58	0.18	-0.57	0.24	-1.0	0.32	-0.3
2	0.673	-0.04	0.67	0.34	0.62	-0.3	0.70	0.18
3	0.994	0.532	0.99	0.77	0.99	0.27	1.07	0.67
4	1.469	1.208	1.46	1.34	1.36	0.93	1.44	1.16
5	1.771	1.585	1.58	1.68	1.74	1.58	1.82	1.66
6	2.040	1.900	1.9	1.97	2.11	2.24	2.19	2.15

	de-transformed data				de-transformed data, fitted for autocorrelation			
count	sim	com	tog	gom	sim	com	tog	gom
1	0.390	0.261	0.317	0.438948	0.11	0.50	0.51	0.48
2	0.580	0.405	0.512	0.556819	0.40	0.62	0.59	0.60
3	0.710	0.568	0.652	0.643794	0.60	0.74	0.67	0.70
4	0.800	0.717	0.751	0.703409	0.73	0.82	0.73	0.77
5	0.862	0.830	0.837	0.742466	0.82	0.89	0.84	0.82
6	0.905	0.903	0.878	0.767355	0.87	0.93	0.86	0.85

sim = Simple Interest Model
com = Compond Interest Model
tog = Combined MOdel
gom = Gompertz model

APPENDIX 4 Disease spread.

lin-lin	days after inoculation					
	21	28	35	42	49	56
S.D.	0.04	0.04	0.06	0.05	0.04	0.04
S.E	0.02	0.02	0.03	0.02	0.02	0.01
95% C.	0.03	0.03	0.05	0.04	0.03	0.03
99% C.	0.04	0.04	0.08	0.05	0.06	0.04
R^2	0.24	0.62	0.53	0.67	0.72	0.81
log-log	**days after inoculation**					
	21	28	35	42	49	56
S.D	0.10	0.04	0.04	0.02	0.02	0.02
S.E	0.08	0.01	0.02	0.01	0.01	0.01
95% C.	0.08	0.03	0.03	0.02	0.01	0.01
99% C.	0.10	0.04	0.04	0.03	0.02	0.01
R^2	0.42	0.44	0.30	0.45	0.52	0.62
log-lin	**days after inoculation**					
	21	28	35	42	49	56
S.D.	0.09	0.04	0.04	0.03	0.02	0.02
S.E	0.04	0.02	0.02	0.01	-	-
95% C.	0.08	0.03	0.03	0.02	0.02	0.01
99% C.	0.10	0.04	0.04	0.03	0.02	0.02
R^2	0.31	0.59	0.50	0.55	0.64	0.69
logit-log	**days after inoculation**					
	21	28	35	42	49	56
S.D.	0.05	0.08	0.20	0.22	0.31	0.36
S.E	0.02	0.03	0.08	0.09	0.12	0.15
95% C.	0.04	0.06	0.16	0.18	0.24	0.29
99% C.	0.06	0.09	0.21	0.24	0.32	0.38
R^2	0.50	0.45	0.34	0.40	0.45	0.50
logit-lin	**days after inoculation**					
	21	28	35	42	49	56
S.D.	0.05	0.08	0.20	0.22	0.31	0.36
S.E	0.02	0.03	0.08	0.09	0.72	0.15
95% C	0.04	0.06	0.16	0.18	0.24	0.29
99% C.	0.06	0.09	0.21	0.24	0.32	0.40
R^2	0.31	0.59	0.50	0.55	0.64	0.69

APPENDIX 5 Computerprogrammes

1) Spore distribution

```
* getting the data file *.
     TRANSLATE FROM 'c:\kees\spread\spore.wk1'
     /FIELDNAMES.
     SAVE FILE= 'spore.sys'.
     GET  FILE= 'spore.sys'.

*compute the distance to focus *.
     COMPUTE distance = RND (SQRT((x**2)+(y**2))).
     SELECT IF (distance GT 0).
     LIST t,distance.

*recoding the distance to focus *.
     RECODE distance ( 5=1) (10=2) (15=3) (20=4) (25= 5)
                     (30=6) (35=7) (40=8) (45=9) (50=10).

*statistics *.
     CORRELATIONS distance WITH s1 TO s9.

     REGRESSION VARIABLES= distance TO s9
     /DEPENDENT=s1 TO s9
     /METHOD=STEPWISE.
```

2) Calculating the transects.

```
* getting the data file *.
     TRANSLATE FROM 'c:\kees\spread\transect.wk1'
     /FIELDNAMES.
     SAVE FILE ='c:\kees\stat\transect.sys'.
     GET FILE= 'c:\kees\stat\transect.sys'.
     MISSING VALUES i(0).

*statistics *
     CORRELATIONS t,o,a,v,i
     /STATISTICS 1,2.

     ANOVA i BY t(3,8),o(1,8),a(1,6)
     /OPTIONS 4,8.
```

3) Calculating the incidence.

```
* getting the data *.
     TRANSLATE FROM 'c:\kees\spread\aangetast.wk1'
     /FIELDNAMES.
     SAVE FILE = 'c:\kees\stat\aangetast.sys'.
     GET FILE  = 'c:\kees\stat\aangetast.sys'.

* compute the distance to focus *.
     COMPUTE        x1 = 60-x.
     COMPUTE        y1 = 50-y.
     COMPUTE distance = SQRT ((x1**2)+(y1**2)).

* compute the incidence (i) *.
     COMPUTE i3 = (z3)/p.
     COMPUTE i4 = (z4+s4)/p.
     COMPUTE i5 = (z5+s5)/p.
     COMPUTE i6 = (z6+s6)/p.
     COMPUTE i7 = (z7+s7)/p.
     COMPUTE i8 = (z8+s8)/p.

* statistics, time *.
     DESCRIPTIVES z3 TO z8.
     DESCRIPTIVES s4 TO s8.
     DESCRIPTIVES i3 TO i8.


* recoding the distance  *.
     RECODE distance (LO THRU 20 =1)  (20 THRU 30 =2)
                     (30 THRU 40 =3)  (40 THRU 50 =4)
                     (50 THRU 60 =5)  (60 THRU HI =6).

* statistics; space *.
     MEANS         i3 TO i8 BY   distance.
     MEANS         i3 TO i8 BY   oct.
     MEANS         i3 TO i8 BY   distance BY oct
                   /OPTIONS 5 7.

     CORRELATIONS i3 TO i8 WITH distance.
     CORRELATIONS i3 TO i8 WITH oct.
```

APPENDIX 6 Activities.

Day	Activity
16-Jul	La Lola/Turrialba
20-Jul	La Lola preparing spore samplers
23-Jul	La Lola placing spore samplers, labelling trees
24-Jul	La Lola placing spore samplers, labelling trees
07-Aug	La Lola infection
14-Aug	La Lola observation 1.1
16-Aug	La Lola observation 1.2
21-Aug	La Lola observation 2.1
23-Aug	Turrialba microscope, discussion Galindo
24-Aug	La Lola observation 2.2 replacing pods
28-Aug	La Lola observation 3.1 spore 2
30-Aug	La Lola observation 3.2, spore 2
04-Sep	La Lola observation 4.1, spore 3
06-Sep	La Lola observation 4.2, spore 4
11-Sep	La Lola observation 5.1, spore 5
13-Sep	La Lola observation 5.2, spore 6
18-Sep	La Lola observation 6.1, spore 7
20-Sep	La Lola observation 6.2, spore 8
25-Sep	La Lola observation 7.1, spore 9)
27-Sep	La Lola observation 7.2
04-Oct	La Lola observation 8
11-Oct	La Lola cleaning