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Response of black rot in cabbage to spatial distribution of inoculum

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Abstract

Disease progress of black rot in cabbage crop was studied over three years in field plots to compare the effects of uni-focal and multi-focal inoculum applied in equal amounts per plot. Disease progress (plant incidence and leaf incidence) was plotted over time, three dimensional maps were made, and disease aggregation was studied by means of geostatistics, black-black counts and Moran's *I* statistic. Black rot progress was primarily due to focus expansion. Secondary foci may appear at short distances from the initial focus but they usually merge with the expanding initial focus. Anisotropy occurred occasionally but was of minor importance. Disease proceeds faster in plots with multi-focal inoculation than in those with uni-focal inoculation. Probably, serious epidemics in Dutch cabbage fields originate from large numbers of foci.

Introduction

Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*X.c.* pv. *campestris*) is a serious disease of cabbage crops (Williams, 1980). *X.c.* pv. *campestris* is a seedborne bacterium occurring worldwide. Under warm, wet conditions black rot causes serious economic losses (Chupp and Sherf, 1960; Walker et al., 1958). *X.c.* pv. *campestris* colonises the vascular system resulting in V-shaped lesions with black veins, chlorosis and necrosis (Sutton and Williams, 1970).

The relationship between inoculum from various sources and disease development is a fundamental component of quantitative epidemiology. Once established, this relationship can be used to evaluate the effectiveness of biological, chemical, or cultural control practices (Mitchell and Kannwischer-Mitchell, 1983). Inoculum from various sources, such as soil (Alvarez and Cho, 1978; Ruissen et al., 1990; Schaad and White, 1974), seed (Schaad et al., 1980), weed (Schaad and Dianese, 1981), and cabbage refuse piles (Kocks and Zadoks, 1996) are recognized as factors in the epidemiology of black rot. If populations of *X.c.* pv. *campestris* increase rapidly by production and dispersal of secondary inoculum, low levels of initial inoculum at planting time would have the potential to cause severe black rot epidemics. Kocks and Ruissen (1996) and Kocks and Zadoks (1996) studied black rot epidemics initiated by artificial single source inoculations (uni-focal disease development). Under Dutch field conditions, the maximum spread of black rot in these studies was limited to six meters from the artificial inoculum source.

Since cabbage crops sometimes become completely diseased by black rot, we hypothesise that multiple sources were present in those completely infected cabbage crops (multi-focal disease development). An experiment was designed to compare uni-focal black rot development with multi-focal black rot development.

Materials and methods

Experimental plots

Black rot epidemics in the white cabbage (*Brassica oleraceae* L. convar. *capitata* (L.) Alef var. *alba* DC) cultivar Perfect Ball (susceptible to black rot) were studied in Wageningen (1992 and 1994) and Lienden (1993), The Netherlands. Cabbage plants were grown in the greenhouse until plants reached the six leaf stage, and were hardened outdoors two days before planting. Plots were planted on 16 May 1992, 10 May 1993 and 16 May 1994.

Each year at planting date, 150 hardened plants were randomly selected and returned to the greenhouse to be observed for black rot symptoms as a check on seed infection with *X.c.* pv. *campestris*.

The experimental design in 1992 had two treatments without replicates. In 1993 and 1994, a randomized block design with two treatments (four replicates) was used. Individual plots comprised 20 × 20 plants in a square grid with an interplant distance of 0.5 m. Plots were separated by 10 m borders of winter wheat. Distance between plot borders and wheat was 1 m. To detect possible interplot interference, as well as external sources of inoculum, 20 potted cabbage plants (15 cm diameter pots) were deployed in areas between and around the plots in 1992. Two untreated, non-inoculated control plots were used for detection of back-ground contamination and interplot interference in 1993 and 1994.

Preparation of inoculum

The isolate of the bacterium *X.c.* pv. *campestris*, isolate PD 714, Culture Collection Plant Protection Service, Wageningen, The Netherlands was used. Inoculum and focal plants were prepared as described by Kocks et al. (1998, in press).

Inoculum sources

Initial disease intensity per plot was 16 plants with 4 diseased leaves per plant. The spatial distributions of source plants were *i*) 16 source plants in a square at the centre of a plot (size 2 × 2 m) (uni-focal inoculation treatment), and *ii*) a regular spatial distribution of 16 source plants through a plot (multi-focal inoculation treatment). At introduction time, original plants were replaced by inoculated source plants, thus maintaining the spatial pattern of the plots. Source plants were introduced on 20 May 1992, 20 May 1993 and 23 May 1994, and were left in the plots.

Disease assessment

Black rot symptom expression was recorded on each individual plant by visual assessment of the incidence of plants (expressed as proportion diseased plants per plot and referred to as disease incidence) and incidence of leaves (expressed as proportion diseased leaves per plot and referred to as diseased leaf incidence). In 1992, 1993, and 1994, black rot was assessed 10, 7, and 8 times, respectively) per summer until senescence of the crop or symptoms of other diseases interfered with the black rot assessments. Plots were visited only when leaves where dry to avoid mechanical spread of *X.c.* pv. *campestris* by the assessor.

Once per two assessment dates, one leaf with black rot symptoms was randomly selected from each plot to confirm that symptoms on initially healthy plants were indeed caused by *X.c.* pv. *campestris*. The selected leaves were surface-disinfected with ethanol 96%, crushed and incubated for 10 min in sterile water. After incubation, the resulting suspension was injected into petioles of leaves of cabbage plants (cv. Perfect Ball) in the six leaf stage. Injected cabbage plants were incubated for three weeks in the greenhouse (20-30°C) to develop black rot symptoms whereafter Koch's postulates were applied to leaves with symptoms.

Temporal analysis

Area under the disease progress curve (AUDPC) was determined per plot to characterize disease progress. The AUDPC in time was calculated as:

$$AUDPC = \sum_{i=2}^{n} [(y_i + y_{i-1})/2](t_i - t_{i-1})$$

where i is the time dimension (in assessment days), y is the disease intensity, t is time (in days), and n is the number of observed times. Units of AUDPC are expressed in proportion × days. Treatment effects within years were examined by analysis of variance and means were separated using the Bonferroni test at $P \leq 0.05$.

All experiments comprised uni-focal and multi-focal source inoculations, for which we calculated interaction effects of time × treatment. Gill (1978), Madden (1986), and Campbell and Madden (1990)

stated that repeated measures ANOVA is an appropriate technique to analyze interaction effects between time and treatments. The repeated measures analysis was performed in SPSS/PC+. Log transformation of y (where y is disease intensity) was applied to stabilize the variance. Since the transformation is undefined when $y = 0$, y was redefined as

$$y = \frac{I + 0.5}{N + 1}$$

in which I is the number of diseased plants or leaves (disease incidence or diseased leaf incidence data, respectively), and N is the total number of plants or leaves per plot (Campbell and Madden, 1990).

Spatial analysis

Geographic trends of disease were examined by 3D-response surface maps of diseased leaf incidence data. Maps were generated for each plot, treatment, and observation date, and were examined to determine formation of secondary foci and directionality of disease development.

Anisotropy is often encountered in ecological field data. The semivariogram (Matheron, 1962) was used to describe autocorrelation as a function of direction. The spatial structure of a disease pattern is defined as isotropic when the semivariograms are the same regardless of the directions of measurement. Semivariograms were fitted per plot in radial arms downwind and crosswind, with 163°, 268°, and 178° (1992, 1993, and 1994, respectively) as the prevailing wind direction (radial arms downwind) (programs SPATANAL and WLSFIT). Comparison of these semivariograms gave information on presence or absence of anisotropy. Semivariograms were calculated for diseased leaf incidence. Disease incidence per plant is a 2-class system (0 and 1), whereas diseased leaf incidence per plant (as number of diseased leaves per plant) is a multi-class system (0 to 14). Theoretical maximum semivariance in a 2-class system is 1^2, in a 14-class system (0–14) is 14^2. The discriminative capacity of a multi-class system increases with the square of the number of classes. Thus, diseased leaf incidence provides better discrimination of patterns.

Nicot et al. (1984) stated that frequency distribution does not adequately discriminate among random, aggregated, or regular dispersion of disease over the crop, but that methods accounting for the location of the samples allow to distinguish such patterns. Therefore, 'black-black' counts and spatial autocorrelation were used to analyze and characterize disease aggregation.

The spatial patterns of disease incidence were characterized using the black-black counts (Sokal and Oden, 1978a). Black-black refers to the number of times that two 'black'-units are neighbours in a plot with 'white' and 'black' units. In our study 'black' and 'white' mean a disease incidence of 1 and 0, respectively. The black-black counts (further referred as black-black statistic) were calculated as described by Sokal and Oden (1978a,b). High values of the black-black statistic indicate high degrees of aggregation in spatial patterns, while values approaching zero indicate randomness or absence of aggregation in spatial patterns.

Moran's I statistic of autocorrelation was used to assess disease aggregation and to describe the spatial patterns of diseased leaf incidence (Sokal and Oden, 1978a,b; Moran, 1950). With Moran's I, the diseased leaf incidence at each sampling location i is compared to the diseased leaf incidence at locations neighbouring i. We considered pairs as neighbours when distance between pairs was smaller than 2 m. Within this 2 m, spatial autocorrelation was determined according to the queen's moves (Sokal and Oden, 1978a,b). Moran's I varies from -1 to +1. Moran's I is positive if diseased leaf incidence tends to be high in some groups of neighbouring plants and low in other groups of neighbouring plants (positive correlation, aggregation or clustering). Moran's I is negative when high diseased leaf incidence values tend to be located near low diseased leaf incidence values and visa versa (regular pattern). Finally, Moran's I approaches 0 if no trend is present in the spatial pattern of disease (random spatial pattern or absence of autocorrelation).

Results

Checks

Seed infection was not detected in the greenhouse tests. None of the 150 randomly selected plants developed black rot symptoms in the greenhouse.

No disease was observed at any time on the cabbage plants placed around and between the plots to detect possible interplot interference (1992), and diseased leaf incidence in the control plots was 0.002 and 0.000 in 1993 and 1994, respectively. Therefore, we conclude that plot-to-plot movement of black rot did not influence the outcome of our experiments.

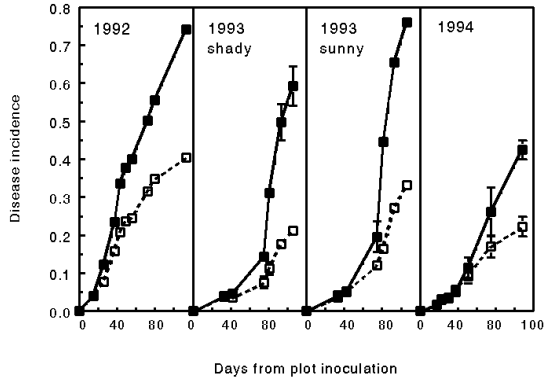

Figure 1. Black rot disease incidence for the uni-focal inoculation treatment (open squares and broken lines) and multi-focal inoculation treatment (closed squares and drawn lines) in 1992, 1993, and 1994. Markers represent non-replicated plots in 1992, means of two replicates in 1993, and means of four replicates in 1994. Error bars represent standard deviations of means. If markers have no error bars, the error bars are too small to plot.

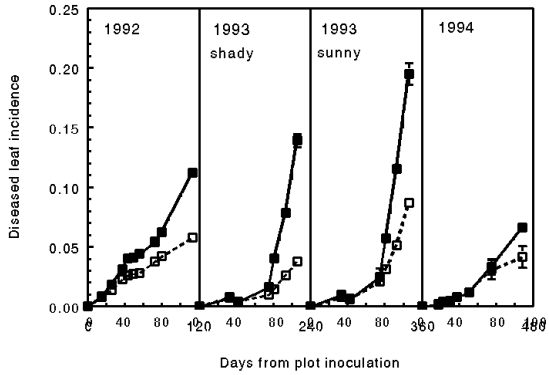

Figure 2. Black rot diseased leaf incidence for the uni-focal inoculation treatment (open squares and broken lines) and multi-focal inoculation treatment (closed squares and drawn lines) in 1992, 1993, and 1994. Markers represent non-replicated plots in 1992, means of two replicates in 1993, and means of four replicates in 1994. Error bars represent standard deviations of means. If markers have no error bars, the error bars are too small to plot.

Table 1. Minimum, maximum, and average temperature (Tmin, Tmax, and Tavg in °C respectively), average relative humidity (RHavg), and rain days during the experiments, 1992 to 1994

Year	Tmin	Tmax	Tavg	RHavg	Rain days
1992	3.6	34.4	17.6	72	60
1993	−0.6	31.4	15.3	77	43
1994	−1.7	36.3	17.1	75	37

Cabbage leaves with symptom expression obtained by random selection per plot once per two assessment dates were in 31 of 33 cases real black rot symptoms. In one case, no symptoms developed after injection in the petiole of a healthy cabbage plant. In the other case, the injected leaf of the cabbage plant died within three days.

Source plants

Source plants recovered well from inoculation and transplanting and became, apart from infection, indistinguishable from original plants. In 1994, four source plants over three replicates of the uni-focal treatment and four source plants over three replicates of the multi-focal treatment were damaged by pigeons which may have reduced the inoculum amount in the plots. Curiously, healthy plants around the source plants were not affected by the pigeons.

Temporal analysis

Characteristic black rot symptoms were observed on initially disease-free plants within 12–19 days after placement of the source plants. Weather conditions are summarized in Table 1.

In 1992, the highest disease incidence (0.74) and diseased leaf incidence (0.11) was found in the multi-focal treatment (Figures 1 and 2). AUDPC (Table 2) in the uni-focal treatment for disease incidence and diseased leaf incidence reached 5.70 and 1.02 proportion × days, respectively, which was lower than AUDPC's in the multi-focal treatment (12.01 and 2.33 proportion × days for disease incidence and diseased leaf incidence, respectively) ($P \leq 0.05$, using standard errors of 1994).

In 1993, black rot development was influenced by shading from trees standing close to the experimental plots (Figures 1 and 2). Two replicates of each treatment were shaded till 8.00–8.30 h (defined as sunny plots), while the two other replicates of these treatments were shaded till 9.30–10.00 h (defined as shady plots). On sunny days, temperature could be up to 5 °C higher and RH up to 10% lower in the sunny plots than in the shady plots. The highest disease incidence (0.77) and diseased leaf incidence (0.195) were found in the sunny plots with multi-focal treatments (Figures 1 and 2). Treatments differed significantly in AUDPC's for both disease incidence and diseased leaf incidence (Table 2, $P \leq 0.05$). AUDPC was significantly lowest for uni-focal inoculation. Significant shade effects were found in both treatments, as disease

Table 2. AUDPC for disease incidence and diseased leaf incidence from experiments over 1992 to 1994 for uni-focal inoculation treatment (A) and multi-focal inoculation treatment (B). Units are give in proportion × days

Year	Disease incidence[1]						Diseased leaf incidence[1]					
	A[2]			B			A			B		
1992	5.70	a		12.01	b		1.02	a		2.33	b	
1993-sha[3]	4.00 ± 0.27	a	Y	9.07 ± 0.30	b	Y	0.75 ± 0.15	a	Y	2.02 ± 0.06	b	Z
1993-sun[3]	5.91 ± 0.01	a	Z	11.64 ± 1.16	b	Z	1.41 ± 0.02	a	Z	2.85 ± 0.31	b	Z
1994	4.69 ± 0.61	a		10.27 ± 0.33	b		0.94 ± 0.20	a		1.71 ± 0.06	b	

[1] Values within a disease assessment method and within a year (rows) followed by the same letter (lower case letters a and b) are not significantly different ($P \leq 0.05$). Values within a disease assessment method for 1993 and within an inoculation treatment (columns) followed by the same letter (capital letters Y and Z) are not significantly different ($P \leq 0.05$).
[2] Mean ± standard deviation from the mean, with two replicates for 1993-sha and 1993-sun, and with four replicates for 1994.
[3] Sha: plots shaded till 09.30-10.00 h. Sun: plots shaded till 08.00-8.30 h.

Table 3. Repeated measures ANOVA for disease incidence and diseased leaf incidence of black rot in cabbage with uni-focal and multi-focal inoculation treatments and shade effects, 1993

Source of variation	Treatment and shade effects				Time effects		
	df	MS	*P*		df	MS	*P*
Disease incidence							
Replicate plots	1	<0.01	0.495	Time	6	0.22	0.001
Treatment	1	2.93	<0.001	Time × Treatment	6	0.05	0.002
Residual	1	0.00		Time × Shade	6	<0.01	1.000
Shade	1	0.03	0.017	Time × Treatment × Shade	6	<0.01	0.982
Treatment × Shade	1	<0.01	0.058	Residual	31		
Residual	50						
Diseased leaf incidence							
Replicate plots	1	0.01	0.786	Time	6	3.01	0.029
Treatment	1	19.55	<0.002	Time × Treatment	6	0.73	0.001
Residual	1	0.04		Time × Shade	6	<0.01	1.000
Shade	1	1.01	0.001	Time × Treatment × Shade	6	0.22	0.976
Treatment × Shade	1	0.05	0.042	Residual	31		
Residual	50						

incidence revealed higher AUDPC's in the sunny plots than in the shady ones ($P \leq 0.05$). For diseased leaf incidence, shade had a significant effect on AUDPC for the uni-focal treatment but not for the multi-focal treatment ($P \leq 0.05$).

In 1994, disease incidence and diseased leaf incidence reached significantly ($P \leq 0.05$) higher levels in the multi-focal treatment (0.43 and 0.066 for disease incidence and diseased leaf incidence, respectively) than in the uni-focal treatment (0.22 and 0.042 for disease incidence and diseased leaf incidence, respectively) (Figure 1). AUDPC's for both disease intensity measures over four replicates were significantly affected by inoculation treatments (Table 2, $P \leq 0.05$), clearly showing that the multi-focal inoculation resulted in a faster disease development than the uni-focal inoculation.

Repeated measures analysis of disease development

The test for treatment effects on both disease incidence and diseased leaf incidence showed significant treatment, time, and time × treatment effects in 1992 (using standard errors of 1994) ($P \leq 0.05$). In addition, significant treatment, time, and time × treatment effects were found in 1994 ($P \leq 0.05$). In 1993, sig-

nificant treatment and shade effects were found for disease incidence and diseased leaf incidence. In 1993, treatment × shade effects were significant for diseased leaf incidence ($P \leq 0.05$) but not for disease incidence. Time and time × treatment effects were significant for disease incidence and diseased leaf incidence. Significant time × shade and time × treatment × shade effects were not found. Obviously (Figures 1 and 2), time (comprising effects of weather) was important in the development of disease intensity. Inoculation treatment and shade were the second and third determinant, respectively. Besides, effect of time was dependent on the spatial distribution of inoculum as indicated by the significant time × treatment interaction.

Table 4. Parameters of spherical semivariograms for diseased leaf incidence data in the downwind direction (DW) and crosswind direction (CW) to detect directionality of black rot development, with the downwind direction as the prevailing wind direction (1992, no replicates; 1993 and 1994, average over four replicates)

Julian day	Uni-focal treatment				Multi-focal treatment			
	DW		CW		DW		CW	
	sill	range	sill	range	sill	range	sill	range
1992								
196	1.8	5.1	1.9	4.7	1.3	1.0	2.1	0.9
212	2.5	5.5	2.6	5.0	1.4	1.2	1.6	0.9
220	2.9	5.5	3.0	5.0	1.0	1.4	1.1	1.3
253	4.1	6.1	4.1	5.2	1.4	1.9	1.4	1.9
1993								
215	1.1	4.9	1.1	4.9	0.4	0.8	0.4	0.8
221	2.2	5.0	2.2	4.7	0.5	1.9	0.5	2.0
229	4.8	5.4	4.9	4.9	2.0	2.3	2.0	2.3
246	9.7	5.8	9.6	5.0	2.5	4.0	5.0	2.2
1994								
181	1.1	4.5	1.1	4.4	1.0	1.2	1.0	1.4
194	1.9	4.8	2.0	4.4	0.8	1.1	0.9	1.2
218	2.6	5.8	2.6	4.9	1.3	1.2	1.2	1.1
251	3.8	6.5	3.9	5.2	1.7	1.1	1.6	1.2

Spatial analysis

The average treatment effects over replicates and years for final disease incidence and final diseased leaf incidence are shown in Figs. 3A and B, which show that multi-focal inoculation is more dangerous to the cabbage crop than a uni-focal inoculation with the same amount of initial inoculum. The spatial pattern for the uni-focal treatment was structured and strongly aggregated around the centre of the plot (4 × 4 source plants). For the multi-focal treatment, the observed symptoms became homogeneously dispersed throughout the field. The result points to the effectiveness of multi-focal development relative to uni-focal development for subsequent disease development.

Directional disease development was examined by semivariogram analysis for diseased leaf incidence. Some directionality of disease development was present, found by semivariogram analysis of the data from the last four assessment dates (Table 4). Two different kinds of anisotropy were observed in our experiments, geometric anisotropy and stratified anisotropy. Geometric anisotropy (same sill, different ranges) occurs when the longest and shortest ranges in two directions differ significantly. Stratified (or zonal) anisotropy (different sills, same range) refers to the fact that sills of the semivariograms are not the same in different directions. Generally, no differences in sill values were observed between plants in radial arms downwind and crosswind for the uni-focal treatment, but ranges were higher for downwind than for crosswind direction. Directional effects in the multi-focal treatment were found for day 196 (1992, different sill), day 212 (1992, different range), and day 246 (1993, different range and sill). Black rot symptoms were observed over a longer distance from the source in the downwind direction. Presence of many small foci developing around the individual sources probably prevented demonstration of anisotropy in plots with multi-focal inoculation. Wind was generally from 163°, 268°, and 178° at a mean speed of 3.6 m/s, 2.5 m/s, and 2.5 m/s (1992, 1993, and 1994, respectively). Thus, differences attributable to wind direction were observed on several days for the uni-focal treatment, but rarely for the multi-focal treatment. The directional effects were of minor importance.

For both directions, the sill and the range was higher for the uni-focal treatment than for the multi-focal treatment (Table 4). Besides, sill values increased with time in the uni-focal treatment. The low values for sill and range for the multi-focal treatment was due to the fact that the focal fronts arising from the 16 regularly distributed artificial introduced sources merged with time (Figure 3B).

Spatial patterns, as examined by black-black statistics on disease incidence varied over time (Figure 4). In most plots, black-black statistics were similar within replicates, as indicated by the absence of error bars but different between treatments. Depending on assessment date, either randomness (values approach-

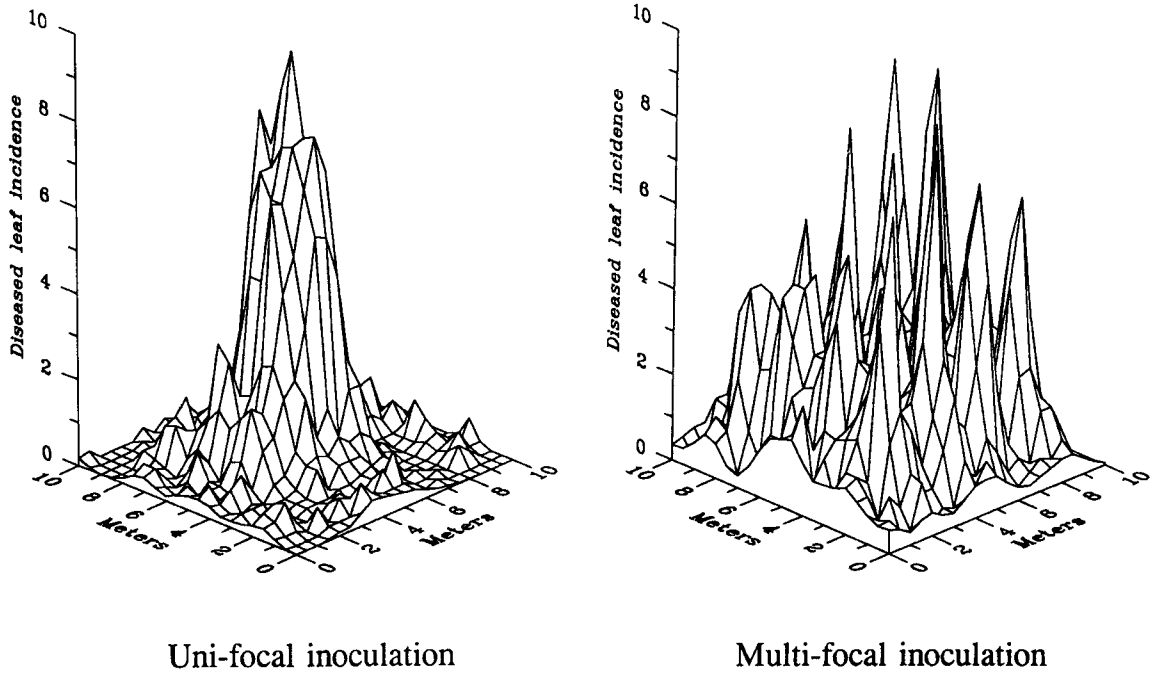

Figure 3. Three-dimensional response maps for diseased leaf incidence of black rot on cabbage. A: average final diseased leaf incidence over all replicates and years, uni-focal inoculation treatment (9 plots over 3 years). B: average final diseased leaf incidence over all replicates and years, multi-focal inoculation treatment (9 plots over 3 years).

ing 0), aggregation (values significantly different from 0), or an intermediate spatial pattern was indicated (Figure 4). Despite the variation, a general process can be described with only minor exceptions for all three years. Generally, spatial autocorrelations indicated aggregation in the uni-focal treatment. Randomness was found at the earliest assessment dates of the epidemics in 1992 and 1994 for the multi-focal treatment. Aggregation did not increase with time in the uni-focal treatment. In contrast, the spatial pattern of black rot changed from random over non-aggregated to aggregated in the multi-focal treatment. Aggregation reached a maximum at the last assessment date in the multi-focal treatment. In 1993, data for sunny and shady plots were averaged since differences between sunny and shady plots within treatments were small, except for day 106 (indicated by a large error bar). In 1994, treatment effects on aggregation of disease incidence were less clear than in 1992 or 1993, but the trend was still present since the aggregation level of disease incidence remained nearly constant in the uni-focal treatment but increased in the multi-focal treatment. The low value at the first assessment date (1994) was due to the damage of source plants by pigeons.

Moran's *I* statistic, calculated for diseased leaf incidence, was about 0.6 at the beginning of the season in 1992 and 1993 (the uni-focal treatment, Figure 5). The initial *I*-value in the uni-focal treatment indicated similar disease intensities on adjacent plants. During the development of black rot only minor changes in Moran's *I* were observed in the uni-focal treatment, pointing at low change in aggregation level with time. In the multi-focal treatment, Moran's *I* was about zero (randomness) at the beginning of the season, which means that healthy plants and plants with high disease intensities were not clustered. The increase in autocorrelation over time during the growing season reflects the change of a random spatial pattern to an increasing level of aggregation. Aggregation was significant during the later stages of the epidemics. In contrast to 1992 and 1993, diseased leaf incidence in the uni-focal treatment of 1994 was not aggregated, probably due to pigeons which damaged several diseased leaves on source plants.

Discussion

The greenhouse checks, trap plant tests and control plots indicated that seed contamination of planting

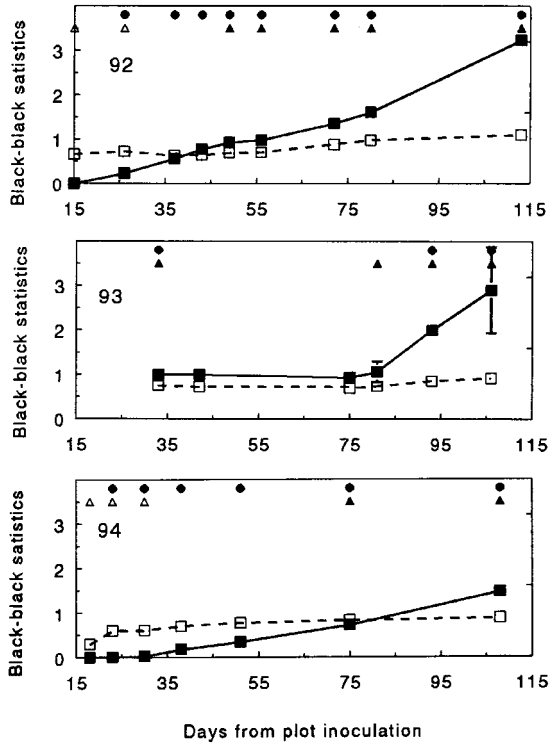

Figure 4. Black-black statistics plotted against time for cabbage plots inoculated with black rot. Black-black statistics refer to disease incidence in 1992, 1993, and 1994. Open squares and broken lines represent uni-focal inoculation treatment, closed squares and drawn lines represent multi-focal inoculation treatment. Markers represent non-replicated plots in 1992, means of two replicates in 1993, and means of four replicates in 1994. Error bars represent standard deviations of means. If markers have no error bars, the error bars are too small to plot. In top of the Figs.; open dots = significant randomness uni-focal treatment, closed dots = significant aggregation uni-focal treatment, open triangles = significant randomness multi-focal treatment, closed triangles = significant aggregation multi-focal treatment ($P \leq 0.05$).

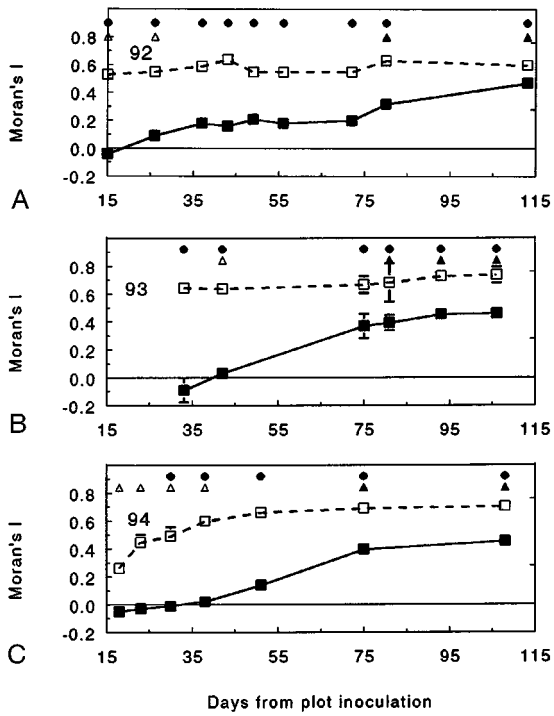

Figure 5. Moran's *I* statistics plotted against time for cabbage plots inoculated with black rot. Moran's *I* statistics refers to diseased leaf incidence in 1992, 1993, and 1994. Open squares and broken lines represent uni-focal inoculation treatment, closed squares and drawn lines represent multi-focal inoculation treatment. Markers represent non-replicated plots in 1992, means of two replicates in 1993, and means of four replicates in 1994. Error bars represent standard deviations of means. If markers have no error bars, the error bars are too small to plot. In top of the Figs; open dots = significant randomness uni-focal treatment, closed dots = significant aggregation uni-focal treatment, open triangles = significant randomness multi-focal treatment, closed triangles = significant aggregation multi-focal treatment ($P \leq 0.05$).

material and natural infection of transplanted material were absent or at least did not interfere with the results. Likewise, misinterpretation of results as a consequence of misidentification of black rot symptoms did not interfere since in at least 31 of 33 cases field identification was confirmed. We conclude therefore that the black rot epidemics in the plots were due to inoculum from the artificial inoculations and that observational data had only minor errors due to undesirable cross-contamination. We conclude that the bacterial isolate chosen was infectious and adequate for the purpose of the experiment.

The experiment of 1993 demonstrated that shade influenced disease development. Plots shaded till 09.30–10.00 h had a reduced disease progress in comparison to plots shaded till 08.00–08.30 h. Thermohygrographs showed that average daily temperature was higher in the sunny plots on sunny days. Since bacterial growth depends on temperature (Pinches and Pallent, 1986; Ruissen et al., 1993; Shu and Yang, 1990), *X.c.* pv. *campestris* had the opportunity to grow faster at higher temperatures. As a result, black rot symptoms may have appeared faster, and more bacteria may have been available for dispersal. We conclude that a higher temperature in the sunny plots was more important for black rot progress than a prolonged guttation period in the shady plots.

Repeated measures analysis (Table 3) demonstrated shade effects to be significant for both disease incidence and diseased leaf incidence. However, the AUDPCs for diseased leaf incidence did not differ sig-

nificantly between sunny and shady plots (Table 2), possibly because AUDPC analysis cannot discriminate between increase and decrease of epidemics. Since AUDPC-values may be the same for an increasing and a decreasing epidemic, misinterpretation cannot be excluded. Therefore, we recommend to use repeated measures analysis (or a comparable method) in stead of AUDPC.

Visual examination of the 3D-maps, black-black statistics (Sokal and Oden, 1978a,b) and Moran's *I* statistic of autocorrelation (Moran, 1950) were used to analyze and describe the spatial patterns of black rot. Aggregation of black rot increased with time after multi-focal inoculation, and was greatest at the end of the epidemics, whereas aggregation after uni-focal inoculation did not change much. Black rot symptoms were found close to the source plants (Figure 5), pointing at black rot dispersal over small distances. These observations confirm earlier work on spread of black rot of cabbage (Kocks and Ruissen, 1996; Kocks and Zadoks, 1996; Walker and Tisdale, 1920) and cauliflower (Clayton, 1929), and support the conclusions of Strandberg (1973) that infection of new plants is highly dependent upon their proximity to infected plants.

Black rot progress was significantly increased by multi-focal inoculation in comparison to uni-focal inoculation, notwithstanding the same amount of initial inoculum. Multi-focal inoculation resulted in higher disease levels with time and also in some progress of aggregation level during the experiments. If black rot disease progress in cabbage would be polycyclic (stated by Alvarez et al., 1987), the influence of initial aggregation of inoculum should have been overcome, at least partially, by multiple cycles of inoculum production and dispersal. Such a levelling off did not take place. Our results demonstrate clearly that aggregation of inoculum had a major influence on disease development and suggest that black rot had few multiplication cycles.

Although spatial development of black rot was limited to dispersal around the initial sources, several distinct secondary foci established during the seasons. Zadoks (1961) described for *Puccinia striiformis* that the disease early in the season was strongly aggregated due to short distance dispersal only. Later in the season, disease became generalized due to establishment of secondary and tertiary foci combined with increasing dispersal distances. The two processes, enlarging of new foci and initiation of foci, involve different methods of dispersal (dual dispersal (Zadoks and Van den Bosch, 1994; Zawolek and Zadoks, 1992)). This dual dispersal was e.g. found by Zadoks (1961) who noticed that *Puccinia striiformis* spreads both by rubbing diseased and healthy leaves together (increases a focus in size) and by spore dispersal through the air (initiation of secondary foci). The black rot disease of cabbage appeared to be strongly aggregated and sharply defined around the source of inoculum after uni-focal inoculation. The small foci, which developed around individual source plants after multi-focal inoculation, merged with time. The dispersal of black rot in cabbage under Dutch conditions is generally a short distance dispersal, resulting in increase of focus size. The development of secondary foci by long distance dispersal seems to be of minor importance due to the sharp decrease of inoculum density of *X.c.* pv. *campestris* with distance. Such a sharp decrease is associated with splash dispersal (Butterworth and McCartney, 1991 and 1992).

The possibilities for *X.c.* pv. *campestris* to cause serious black rot epidemics will depend largely upon initial inoculum levels (Kocks et al., 1998), level of host resistance (Kocks and Ruissen, 1996; Staub and Williams, 1972), degree of inoculum aggregation (the present study), and temperature (the present study). Dispersal of black rot in cabbage occurs especially during rainfall (Williams, 1980), and can be stimulated by overhead irrigation (Grimm and Vogelsanger, 1990). The experiments reported here were performed during relative dry seasons, and lack of rainfall may have reduced the rate of focal expansion and the establishment of secondary foci. Black rot may become an important disease in The Netherlands when many initial infection points are scattered over the field, the growing season is long, the temperature is relatively high, and rainfall is regular.

The dispersal of black rot from various inoculum sources is limited to a few meters only under the Dutch field conditions (Kocks and Ruissen, 1996; Kocks and Zadoks, 1996). Nevertheless, cabbage fields sometimes become completely diseased by black rot in The Netherlands. The experiments discussed here support the supposition that multiple sources were present in those completely infected cabbage fields (multi-focal disease development). Such multiple sources could come from infected seed, or infected seedlings, or from *X.c.* pv. *campestris* surviving in the soil (more or less homogeneous infestation of the soil). Continued research on these topics should provide the necessary information on effects of seed infection, seedling in-

fection and soil infestation on subsequent black rot development in cabbage.

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