European Journal of Plant Pathology **104:** 911–923, 1998.
© 1998 *Kluwer Academic Publishers. Printed in the Netherlands.*

Survival and extinction of *Xanthomonas campestris* pv. *campestris* in soil

C.G. Kocks[1,2], M.A. Ruissen[1,3], J.C. Zadoks[1] and M.G. Duijkers[4]
[1] *Dept. of Phytopathology, Wageningen Agricultural University, P.O. Box 8025, 6700 EE Wageningen, The Netherlands;* [2] *Present address and address of correspondence: Agrotransfer, Dept. Research and Consultancy, De Drieslag 30, 8251 JZ Dronten, The Netherlands;* [3] *Present address: Norwegian Centre for Ecological Agriculture, Tingvoll Gard, N-6630 Tingvoll, Norway;* [4] *Christian Agricultural College, De Drieslag 1, 8251 JZ Dronten, The Netherlands*

Accepted 8 October 1998

Abstract

Carry-over of inoculum of *X.c.* pv. *campestris* in the soil from one cropping season to the next was studied in field experiments over three years. These studies were supported by laboratory and greenhouse experiments on quantitative assessment of bacteria by bioassay using the Most Probable Number technique, and on recovery rates of bacteria from the soil. The mean recovery rate from artificially infested soil was 58%. Extinction of *X.c.* pv. *campestris* in soil infested with infected plant debris proceeded exponentially and extinction rates depended on temperature, as did the decomposition of plant debris. In replicated field plots, over three years, infection foci of black rot disease were established. At harvest time, all plants were chopped and resulting plant debris was rotovated into the soil. The resulting soil infestation was sampled and showed clear infestation foci reflecting the original infection foci of the crop. These infestation foci decreased with time and disappeared after the winter. Follow-up crops remained virtually uninfected. The results show that in The Netherlands good crop and soil management impedes survival of inoculum from one year to the next, so that cabbage can be grown continuously. Polyetic carry-over of inoculum by debris in the soil can be avoided in The Netherlands.

Introduction

A focus is a patch of crop with disease limited in space and time (Anonymous, 1953). An infection focus originating from a single diseased individual usually expands in a circular wavelike pattern. Disease foci and their expansion are characteristic features of many epidemics. Individual foci tend to be more or less regular in form, with well-defined gradients, enlarging at a constant velocity (Zadoks and Van den Bosch, 1994). These studies described focus build-up, but the opposite, disappearance of foci, has not been found in the literature. Where focus expansion is the result of favourable environmental conditions, focus contraction might occur under unfavourable conditions.

The present study reports on an experiment with black rot in cabbage. Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*X.c.* pv. *campestris*), is an important disease of cabbage (Williams, 1980). The disease can be managed by the use of resistant cultivars (Kocks and Ruissen, 1996), hot water treatment of seed (Clayton, 1924; Walker, 1923), crop rotation (Linn, 1958; Shropshire and Kadow, 1936) and cultural practices (Kocks and Zadoks, 1996; Walker et al., 1958). However, these management strategies do not provide consistent control of black rot. Schaad and White (1974) discussed crop rotation in relation to the survival of *X.c.* pv. *campestris* in soil. Likewise, Alvarez and Cho (1978) found that disease incidence in cabbage was influenced by rotation frequency. Since black rot is a polycyclic disease with a limited number of cycles per year (Kocks et al., 1998a,b), inoculum levels of *X.c.* pv. *campestris* in the soil at planting time may play a critical role in polyetic disease development.

Zadoks and Schein (1979) drew attention to matters of scale among which the time scale of monocyclic, polycyclic and polyetic processes. Polyetic epidemics build up over years, polyetic (a Greek neologism) meaning ‘over many years’. A polyetic epidemic implies carry-over of inoculum from one vegetation period to the next. An exploratory survey on farmers’

practices during 1991–1993 (Kocks, 1998) indicated that diseased cabbage plants with leaves and stems infected with *X.c.* pv. *campestris* are not uncommon at harvest time in The Netherlands. Since cabbage plants are ploughed under when the heads have been harvested, the diseased leaves and stems could serve as an important source of inoculum. To determine whether infested cabbage debris indeed carries inoculum over from season to season, survival of infected debris, and therewith survival of *X.c.* pv. *campestris*, was studied under Dutch conditions. The information on the survival of soilborne inoculum of *X.c.* pv. *campestris* is necessary in the planning of crop rotation as a control strategy. The present paper documents (i) the recovery of *X.c.* pv. *campestris* from soil by bioassay using the most probable number method, (ii) the survival of *X.c.* pv. *campestris* in soil at various temperatures, (iii) the extinction of the soil's *X.c.* pv. *campestris* infestation during the winter, and (iv) the polyetic development of black rot in cabbage.

Materials and methods

Recovery experiment (Experiment I)

An experiment was performed to test the recovery of *X.c.* pv. *campestris* from artificially infested soil, focused on the compound effect of blending and quantification by MPNs. A 600 g sample was taken from a field clay soil (35% clay, pH 7.2, and 2.8% organic compound) where no cruciferous crops had been grown and divided into samples A, B, and C of 200 g each. Distilled water (200 ml) was added to sample A. Sample B was treated by adding 200 ml suspension of a low inoculum density of *X.c.* pv. *campestris* (isolate PD 714, Culture Collection, Plant Protection Service, Wageningen, The Netherlands). Sample C was treated by adding 200 ml suspension with a high inoculum density of *X.c.* pv. *campestris*. Inoculum was prepared by suspending 48-h-old *X.c.* pv. *campestris* colonies (grown on Yeast Peptone Glucose Agar at 27 °C) in distilled water. Estimates of the actual densities of inoculum were obtained from plate counts with five replicates per density, and grown on YPG at 27°C (Table 1).

All treated samples were blended for 45 sec (Braun blender, low speed). After standing for 15 min to sediment soil and plant debris, the suspension was subsampled by collecting the upper ml of supernatant with a pipette. This sub-sample, served as stock suspension for the bioassay. The sub-sample was diluted in 10-fold steps from 10^0 to 10^8 and stored 5–10 min at 20–25 °C in plastic Erlenmeyer flasks (2.5 ml, SIGMA). Quantification of *X.c.* pv. *campestris* was done by bioassay (two replicates of four plants per dilution) and MPNs, and by plate counts (five replicates per density and grown on YPG at 27 °C). Recovery, in respect of the compound effect of blending and quantification by MPNs, was calculated as bacterial density obtained from plate counts before blending divided by the bacteria density obtained from MPN's after blending.

Table 1. Experiment I, recovery (%) of *X.c.* pv. *campestris* from three soil samples (A, B and C) by bioassay. The soil samples B and C were artificially infested by adding 200 ml of a suspension with known densities of *X.c.* pv. *campestris*

Test	Sample A[v]	Sample B[w]		Sample C[x]	
	Recovery%	Density[y]	Recovery%	Density[y]	Recovery%
1	0	1.1×10^3	52	7.2×10^8	51
2	0	3.4×10^4	63	6.5×10^6	57
3	0	3.9×10^3	43	8.0×10^8	71
4	0	5.5×10^3	41	1.9×10^9	70
5	0	7.4×10^2	61	8.5×10^7	67
Avg[z]	0		52		63

[v] Distilled water added to soil samples.
[w] Soil samples infested with low levels of *X.c.* pv. *campestris*.
[x] Soil samples infested with high levels of *X.c.* pv. *campestris*.
[y] Density of original inoculum obtained by plate counts averaged over five replicates, in cfu/ml supernatant/g soil.
[z] Avg = mean over five tests.

Experiment I was performed five times (five recovery tests) at intervals of four weeks.

Bioassay. Five week old (four and five leaf stage) cabbage plants (*Brassica oleraceae* L. convar. *capitata* (L.) Alef var. *alba* DC) of the black rot susceptible cultivar Perfect Ball were used for the bioassay. To facilitate inoculation, cabbage plants were not watered during the two days before inoculation.

Erlenmeyer flasks containing inoculum were thoroughly shaken for 30 sec to dislodge bacteria adhering to the inner surface of the flasks. Inoculation was performed by injecting 0.03 ml inoculum into the leaf close to the stalk of the second oldest leaf by means of a Hamilton microliter 1 ml syringe (1710RN) mounted with a sterile disposable needle (BD Plastipak 30G 1/2). A new sterile needle was used for each dilution and four plants were inoculated per dilution. Inoculated plants were placed in the greenhouse for five weeks at 20–25 °C and RH 75–85%. Absence or presence of black rot symptoms was scored at three or four day intervals during five weeks. Koch's postulates were applied to leaves with doubtful symptoms, and to ten randomly chosen leaves with black rot symptoms.

Data analysis. The bacterial density was assessed by the Most Probable Numbers (MPNs) method for the numerical interpretation of dilution data as applied by e.g. Ciafardini and Marotta (1989), Maloy and Alexander (1958), and Tuitert (1990). Tables of MPN's based on 10-fold dilutions with fixed numbers of replicates per dilution (Fisher and Yates, 1963) were used to estimate the bacterial densities in the sub-sample, and were converted to cfu/g fresh soil or log cfu/g fresh soil.

When no symptoms appear in the bioassay, MPN is -0.707 (Fisher and Yates, 1963). Taking into account that 18 bacterial cells initiate black rot symptoms within 20 days when inoculated in 5 week old cabbage plants (Lengkeek and Etteger, 1992), 600 cfu/g fresh soil (or log 2.78 cfu/g fresh soil) was our detection level.

Student's *t*-test was used to test for differences in recovery at low and high artificial soil infestations ($P \leq 0.05$).

Supernatant experiment (Experiment II)

Experiment II tested the effect of the sample volume of supernatant after blending. The experiment was performed with one sample of suspension B (5.5×10^3 cfu/ml; Test 4; Table 1) and one of suspension C (1.9×10^9 cfu/ml; Test 4; Table 1) of the fourth recovery test of experiment I (Figure 1). First, 1 ml was taken from the top of the supernatant obtained after blending. An aliquot of 0.1 ml was used for a first dilution series, as described above and the remaining 0.9 ml was set aside. Second, another 1 ml was taken from the top of the supernatant and added to the 0.9 ml saved from the previous sub-sample, yielding 1.9 ml suspension in total. The 1.9 ml was shaken thoroughly and 0.1 ml was used to prepare a second dilution series. Third, 8 ml was taken from top of the supernatant and added to the remaining 1.8 ml suspension, yielding 9.8 ml of suspension. The latter 9.8 ml was shaken and 0.1 ml was used to prepare a third dilution series. The three dilution series were further analyzed by bioassay and MPN, as described in Experiment I.

The bacterial density in the sub-sample, assessed by MPNs, was converted to cfu/g soil. Student's *t*-test was used to test the effect of sub-sample volume on recovery at low and high densities of artificial soil infestations ($P \leq 0.05$).

Sediment experiment (Experiment III)

Experiment III was designed to quantify *X.c.* pv. *campestris* in the sediment after blending, and was performed with the remaining sediments of suspension B and C of Experiment II (Figure 1). Supernatant left in the beakers after taking sub-samples for Experiments I and II was removed by means of a pipette. A sub-sample of 10 g sediment was mixed for 30 sec in 10 ml distilled water. After 1 min, 0.1 ml was used to prepare a dilution series as described in Experiment I. Quantification of *X.c.* pv. *campestris* was done by bioassay as described in Experiment I. The bacterial density in the sub-sample was converted to cfu/g soil.

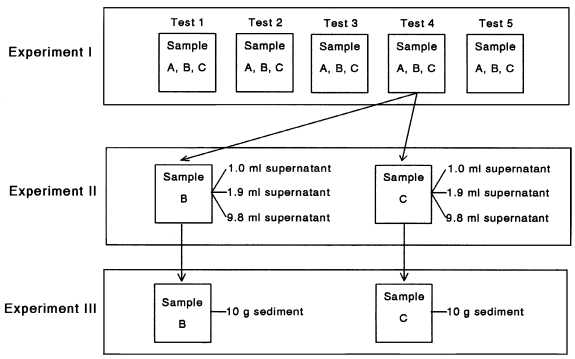

Figure 1. Flow chart for Experiment I–III.

Temperature experiment (Experiment IV)

An experiment was designed to test temperature effects on extinction of *X.c.* pv. *campestris* in plant debris exposed to soil. A bulk-sample (30 kg) of clay soil, taken from a grain field with no history of cruciferous crops for at least the last ten years, served as a soil without *X.c.* pv. *campestris*. A test-sample of 200 g was taken to check for presence of *X.c.* pv. *campestris* in the bulk-sample. Ten kilograms of heavily black rot infected cabbage plants (with 8–11 diseased leaves per plant) were chopped into pieces smaller than 15 cm^2. The chopped cabbage debris and the bulk-sample were thoroughly mixed by hand. The resulting mixture was divided over six plastic boxes ($30 \times 15 \times 12$ cm) with 4.5 kg each. Each box was incubated at a different constant temperature ($-12\,^{\circ}$C, $0\,^{\circ}$C, $5\,^{\circ}$C, $10\,^{\circ}$C, $15\,^{\circ}$C, or $20\,^{\circ}$C) for 20 weeks. Four-weekly samples of 600 g, taken from each box, were divided into three sub-samples of 200 g. Distilled water (200 ml) was added to each sub-sample. Suspension, dilution, and bioassay were prepared as described in Experiment I. The sediments were disregarded.

At the successive sampling dates, the degree of decomposition of plant debris in the soil was assessed visually.

Data analysis. Bacterial densities were expressed as log cfu/g soil to stabilize variance. At each temperature, log bacterial density was linearly regressed to time to obtain extinction curves (Zadoks and Schein, 1979). Differences in slope e due to temperature effects were examined by analysis of variance ($P \leq 0.05$).

Field experiment (Experiment V)

A field experiment was designed to study the decline in populations of *X.c.* pv. *campestris* in soil and the decline of infestation foci under natural conditions.

History of the plots. Experiments on foliar black rot development, performed in Wageningen during 1992, 1993, and 1994, have been documented by Kocks et al. (1998a). Black rot epidemics were initiated by placing artificial inoculum sources in the centre of individual plots. During the growing season, foci developed naturally from the inoculum sources, one focus per plot. At harvest time, the black rot intensity was measured by disease incidence (proportion of diseased plants per plot) and diseased leaf incidence (proportion of diseased leaves per plot) (Table 2).

Description of the soils. The soils are described in Table 2. According the Dutch soil classification (Stichting Bodemkartering, 1973), the soil at Wageningen (1992 and 1994) was a 'kalkloze poldervaaggrond'. This soil type is a young, non calcareous clay soil in fluvial deposits with a heavy layer or a heavy sub-soil (map code Rn67 C). Ground water level in winter is 40–80 cm below surface, and in summer deeper than 120 cm below surface.

The soil at Lienden (1993), described according the Dutch soil classification is a 'kalkhoudende ooivaaggrond'. This is a young, calcareous clay soil in fluvial deposits (map code Rd90 A). The ground water level in winter is deeper than 80 cm below surface, and in summer deeper than 120 cm below surface.

Preparation of the plots. After the final disease assessment (Table 2), one (1992) or two plots (1993 and 1994) were prepared. Plants (including heads) were chopped and mixed with soil by a rotary cultivator (Sandri TCR-220) to a depth of at least 10 cm. All cultivator runs were made in the same direction to obtain a minimal and uniform displacement of soil and plant debris. Chopping was so intensive that plant pieces were, generally, smaller than 1 dm^2.

Soil sampling and bioassay. Soil samples were taken from the plots to determine bacterial density and distribution of *X.c.* pv. *campestris* by bioassay. The sample locations per plot are shown in Figure 2. A sample location corresponded to an area of 0.5×0.5 m (indicated by the open squares). To reduce within sample variation, composite soil samples were made for each sample location by mixing ten cores taken from the upper 10 cm of soil, each 60–75 ml per sample, using a 3 cm auger. Composite soil samples comprised soil and plant debris. Thirty-seven composite soil samples were taken per plot per sampling date (Table 2).

Two sub-samples of 200 g were taken from each composite soil sample. A sub-sample was added to a beaker containing 200 ml distilled water. Dilution series and bioassay were done as described in Experiment I. An analysis of a composite soil sample, including the bioassay, was fully completed before a next analysis was begun.

Table 2. History of the plots and summary of the soil experiment

Year	Plot size (m)	Soil type[x]	pH–KCl[x]	Organic compound%[x]	Plots	Plants per plot	DI[y]	DLI[y]	Date of chopping	Dates of soil sampling[z]
1992	7 × 7	clay loam	7.3	2.8	1	196	0.51	0.13	284	296,324,363,26,70,140
1993	7 × 7	sandy loam	6.8	2.6	2	196	0.56	0.14	269	298,323,363,17,48,143
1994	7 × 7	clay loam	7.6	2.4	2	196	0.47	0.16	286	286,314,356,28,88

[x] The soil samples were analysed on clay content, pH, and organic compound according to NEN-5753, and classified into texture classes (Roshing, 1995; Wagenaar and Wallenbrug, 1987).
[y] DI and DLI means, respectively, disease incidence and diseased leaf incidence at date of chopping. DI and DLI are roughly measures due to interference of maturity of the crop and presence of symptoms of other pathogens.
[z] Dates are given in Julian days.

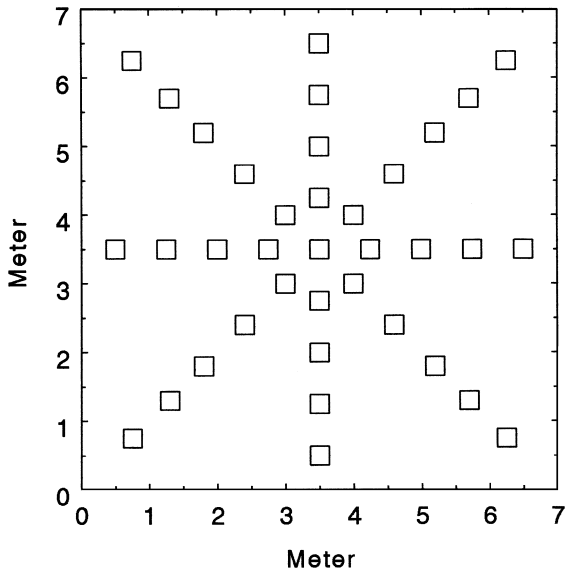

Figure 2. Experiment V, sample locations per plot with sample location corresponding to an area of 0.5 × 0.5 m (indicated by an open square).

Meteorological data. The mean daily soil temperature at 5 cm below surface was measured at a weather station of the Department of Meteorology, Wageningen Agricultural University. The distance between the weather station and the location of the plots was about 0.5 km (1992 and 1994) or about 6 km (1993).

Data analysis Bacterial densities in composite soil samples were assessed by MPN. The relative rate of extinction (e) per sampling time interval per plot was determined by

$$e = -\frac{(\log y_2 - \log y_1)}{t_2 - t_1},$$

where e is the relative rate of extinction, t is the time (days) and y is the bacterial density in soil (cfu/g soil). Relative rates of decline per interval were compared with soil temperatures during that interval. Differences in e between sampling dates were examined by analysis of variance. Means were separated using Fisher's Least Significant Difference (LSD) test ($P \leq 0.05$). Values of e per interval were correlated with soil temperatures during that interval.

Spatial distribution of disease as log cfu/g soil was examined by the use of 3D response surface maps (SURFER Access System Version 4.06, Golden Software Inc., 1989). Maps were generated for each plot and sampling date.

Gradients of bacterial density were averaged over sub-samples and plots within years and were calculated by use of the linearized form of the negative exponential model (Campbell and Madden, 1990) for the first four sampling dates. Larger values of b (slope parameter; m^{-1}) describe steeper gradients. The radial rate of focus expansion c (cm day^{-1}; Van den Bosch et al., 1988; Zadoks and Van den Bosch, 1994) between successive sampling dates was determined for the period from first to fourth sampling date.

Survival experiment (Experiment VI)

A field experiment was designed to test the extent to which disease-free seedlings become infected by soilborne inoculum. In the years following the field experiments, seedlings of the white cabbage cultivar Perfect Ball (five leaf stage) were planted as the susceptible crop about mid May. Individual plots (7 × 7 m) had 196 plants with an interplant distance of 0.5 m. Three-weekly observations were made of all individual plants and black rot incidence was recorded. Fifty seedlings

per plot were placed in greenhouses to check for seed infection.

Results

Cabbage leaves with symptom expression obtained by random selections were real black rot symptoms. Black rot symptom expression was usually seen as blackening of veins, sometimes followed by yellowing (chlorosis) and subsequent death of the leaf tissue. *X.c.* pv. *campestris* was not detected by Koch's postulates in leaves with doubtful symptoms. These latter leaves were scored as 'healthy leaves'.

Recovery

Experiment I. The bioassay showed that the indigenous bacterial density of *X.c.* pv. *campestris* in the soil was below the detection level (Table 1, sample A). Recovery of *X.c.* pv. *campestris* for soil with a low infestation level of *X.c.* pv. *campestris* (sample B) varied from 41% to 63%, with a mean of 52%. At high infestation level, recovery ranged from 51 to 71%, with a mean of 63%. The difference between these means was not significant (paired *t*-test, $P = 0.20$; independent *t*-test, $P = 0.10$). The overall mean for recovery was about 58%. Thus, the compound loss of *X.c.* pv. *campestris* in soil by blending and quantification by MPN was 42%.

Experiment II. We compared samples from the supernatant of 1, 1.9, and 9.8 ml. Bacterial density was 2.8×10^3, 2.2×10^3, and 9.7×10^2 for samples 1 ml, 1.9 ml, and 9.8 ml, respectively (sample B). No difference in bacterial densities was found between sampling 1 ml or 1.9 ml (both 1.3×10^9 cfu/g fresh soil). Bacterial density decreased to 3.1×10^8 cfu/g soil when sampling 9.8 ml water. Therefore, we used the top 1 ml of supernatant in subsequent bioassays.

Experiment III. Density of *X.c.* pv. *campestris* in the sediment was 9.2×10^3 cfu/g fresh soil at the high infestation level (sample C). At low infestation level (sample B), no *X.c.* pv. *campestris* could be found in the sediment. Probably, the bacterial density was too low to detect by bioassay and MPN's. The difference of about 10^5 between supernatant and sediment was so large that the supernatant (top 1 ml) was considered to justify to neglect the bacteria in the sediment.

Temperature experiment (Experiment IV)

The test-sample of 200 g soil did not contain detectable levels of *X.c.* pv. *campestris*.

After inoculation, the density of *X.c.* pv. *campestris* did not decline within 20 weeks at $-12\,^{\circ}$C or $0\,^{\circ}$C (Figure 3). Populations of *X.c.* pv. *campestris* declined with time when temperatures were $5\,^{\circ}$C or higher. Extinction rate was greatest at $20\,^{\circ}$C. After 12 weeks at $20\,^{\circ}$C, low bacterial densities were detected. Differences in *e* between temperatures were significant except between $-12\,^{\circ}$C and $0\,^{\circ}$C ($P \leq 0.05$, Table 3). The linear correlation coefficient between *e* and temperature was -0.93 ($P = 0.001$, $n = 18$) (linear regression equation for the range $0\,^{\circ}$C till $20\,^{\circ}$C was $-0.0018 - 0.0145 \times T$, with T as temperature).

Visual assessment of plant debris did not indicate any changes in the physical condition of the cabbage debris when exposed for 20 weeks to $-12\,^{\circ}$C or $0\,^{\circ}$C. The leaf tissue was intact and no signs of rotting were observed. After 20 weeks at 5 and $10\,^{\circ}$C, leaf tissue was partly decomposed. After 20 weeks at $15\,^{\circ}$C most leaf tissue was decomposed but petioles were still present. After 12 weeks of storage at $20\,^{\circ}$C, leaf tissue could hardly be distinguished from soil, and after 20 weeks

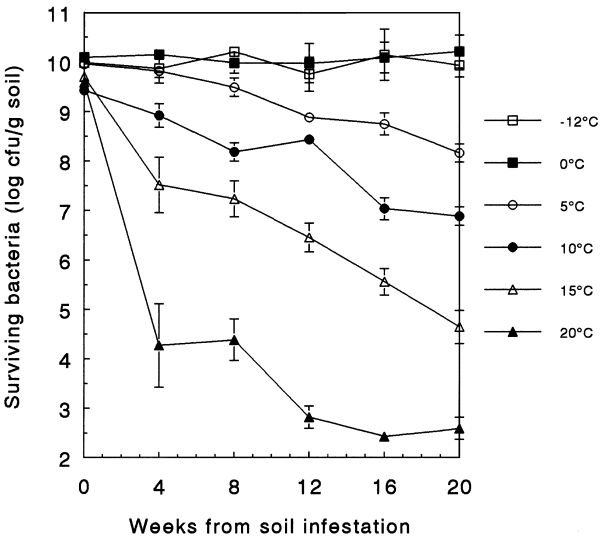

Figure 3. Experiment IV, extinction of *X.c.* pv. *campestris* in soil artificially infested with debris from black rot diseased cabbage and stored at six different temperatures. Bacterial densities (log cfu/g soil) were obtained by bioassay. Markers represent means of three sub-samples. Error bars represent standard deviations of each mean. Where error bars are absent, they were too small to plot.

Table 3. Experiment IV, extinction of *X.c.* pv. *campestris* (log cfu/g soil) in artificially infested soil stored at different constant temperatures for 20 weeks. Bioassay of three sub-samples per treatment, dilution ratio 10, and 4 sub-samples per dilution

Temperature	a^w	e^x	R^2
−12 °C	10.43 ± 0.29	− 0.001 ± 0.011 a[y]	0.26[z]
0 °C	10.52 ± 0.31	− 0.003 ± 0.010 a	0.30[z]
5 °C	10.55 ± 0.20	− 0.089 ± 0.007 b	0.91
10 °C	9.88 ± 0.36	− 0.123 ± 0.012 c	0.84
15 °C	9.55 ± 0.55	− 0.217 ± 0.019 d	0.89
20 °C	7.52 ± 1.51	− 0.301 ± 0.052 e	0.68

[w] Intercept and its standard error from linear regression of log cfu/g soil on time.
[x] Slope and its standard error from linear regression of log cfu/g soil on time (day^{-1}).
[y] Values *e* followed by the same letter are not significantly different ($P \leq 0.05$).
[z] Regressions are not significant.

petioles were partly decomposed but stem parts were still recognizable.

Soil experiment (Experiment V)

Densities of *X.c.* pv. *campestris* in soil declined during the winter (Figure 4) with time until log cfu/g soil reached 2.78 (i.e. no symptoms in the bioassay). Bacterial density in interval 296–363 (winter '92/'93) was higher than in the interval between days 298–363 ('93/'94) and interval 286–356 ('94/'95) ($P \leq 0.05$), although disease incidence and diseased leaf incidence were nearly the same at all chopping dates (Table 2). On Julian days 69 ('92/'93), 48 ('93/'94) and 87 ('94/'95) bacterial survival at low densities. *X.c.* pv. *campestris* was not detected at days 140 ('93/'94) and 142 ('94/'95) in the bioassay.

Relative rates of extinction (*e*) and daily mean soil temperature at 5 cm below ground are given in Figures 5 and 6, respectively. In '92/'93, values for *e* were highest for the intervals 296–324 and 324–363 (0.035 and 0.036, respectively) ($P \leq 0.05$). The mean soil temperatures over these intervals were 6.7 °C and 5.1 °C, respectively.

The *e* dropped to 0.012/day for interval 363–26, an interval with an mean soil temperature of 2.7 °C and 9 days of soil temperatures below 0 °C. The *e* for interval 26–70 did not differ significantly from the previous interval ($P \leq 0.05$).

Low *e* for interval 323–363 in the winter '93/'94 coincided with an mean soil temperature of 2.4 °C and 12 days of soil temperatures below 0 °C. The *e* for

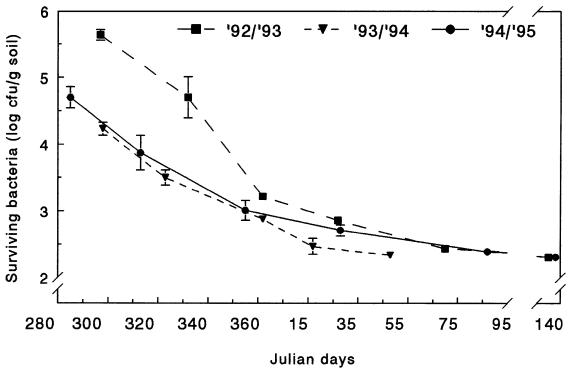

Figure 4. Experiment V, density of *X.c.* pv. *campestris* in soil (log cfu/g soil). Densities are obtained by bioassay. Markers represent means of two sub-samples from one plot ('92/'93) or means of two plots ('93/'94 and '94/'95). Error bars represent standard deviations of means. Where error bars are absent, they were too small to plot.

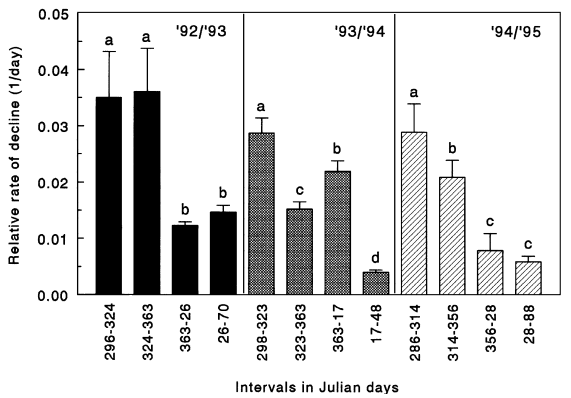

Figure 5. Experiment V, relative rate of extinction (*e* in 1/day) for *X.c.* pv. *campestris* in soil obtained by bioassays. Columns show means of two sub-samples from one plot ('92/'93) or means of two plots ('93/'94 and '94/'95). Error bars represent standard deviations of means. Columns within a year coded with the same letter are not significantly different ($P = 0.05$).

interval 17–48 was lowest ($P \leq 0.05$), since the bacterial density at day 17 had approached the detection level. The highest *e* (0.029) was found for interval 298–323 at an mean soil temperature of 6.2 °C.

In the winter of '94/'95, differences in *e*-values were not significant for the intervals 356–28 and 28–88 ($P \leq 0.05$). Obviously, *e* decreased with time. Mean temperatures were 8.7 °C, 7.2 °C, 3.4 °C, and 5.3 °C for the intervals 286–314, 314–356, 356–28, and 28–88, respectively. High *e*-values were found for the intervals corresponding with the high soil temperatures (Figures 5 and 6).

Values of e were correlated with mean soil temperature for 11 intervals (last interval of '94/'95 was excluded). The linear correlation with r was -0.49 which was indicative only ($P = 0.063$).

The 3D response surfaces of foliar black rot intensity at chopping date are given in Figure 7. The maps illustrate the spread of the disease from the infection source placed at the centre of each plot and the persisting dominance of that source. Peaks further from the centre of the plots indicate secondary foci.

The spatial distribution of the density of *X.c.* pv. *campestris* in soil at first sampling date (Figure 8, upper row) was compared visually with the spatial distribution of foliar disease in Figure 7. Evidently, the maps reproduced the locations of the sources and their dominance. Foliar disease decreased rapidly over the first two meters around the source, as reflected in the maps based on soil sampling. Maps of foliar disease intensity were based on 196 sampling sites (individual plants) while maps of bacterial densities were based on 37 sample sites only. As a result, the curves in Figure 8 were smoothed due to intrapolation and the irregularities in Figure 7 were levelled.

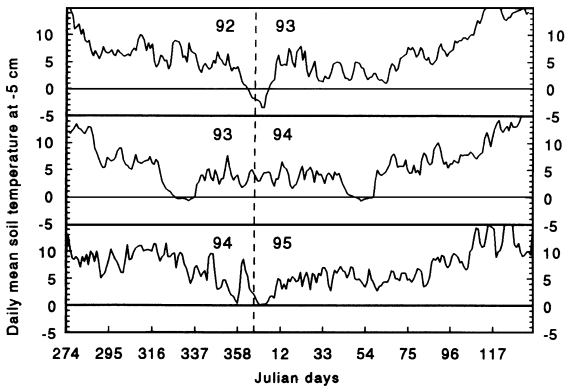

Figure 6. Experiment V, mean daily soil temperature at 5 cm below ground for the meteorological station during the winters '92/'93, '93/'94, and '94/'95.

The decline in infestation foci of *X.c.* pv. *campestris* in soil during winter is clearly evident in Figure 8. The bacterial density decreased both in and around the source during winter. The demonstrable size of the foci decreased with time. At the final observation dates (lower row), the foci were reduced to small sizes with low bacterial densities at their centres. The infestation foci became extinct before the next crop was planted.

Gradients of bacterial density in soil over distance from the plot centre were calculated by linear regression (Table 4). Residuals generally had a random scatter and R^2 values ranged from 0.75 to 0.99. The gradients were initially steep ($b < -4.2\,\mathrm{m}^{-1}$) and gradually flattened at the end of the winter ($b > -1.3\,\mathrm{m}^{-1}$).

Radial rates of focus expansion c ($\mathrm{cm\,d}^{-1}$) were negative and varied according to time interval. Since

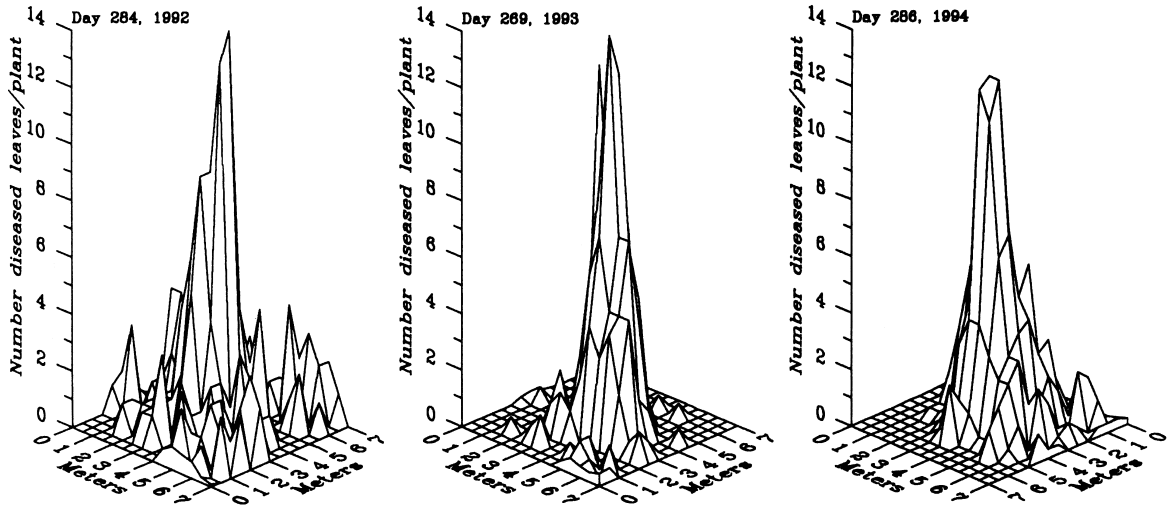

Figure 7. Experiment V, disease intensity of black rot in cabbage at date of chopping in 1992, 1993, and 1994. Intensity is expressed by number of diseased leaves per plant. Maps are derived from 196 plants with interplant distances of 0.5 m.

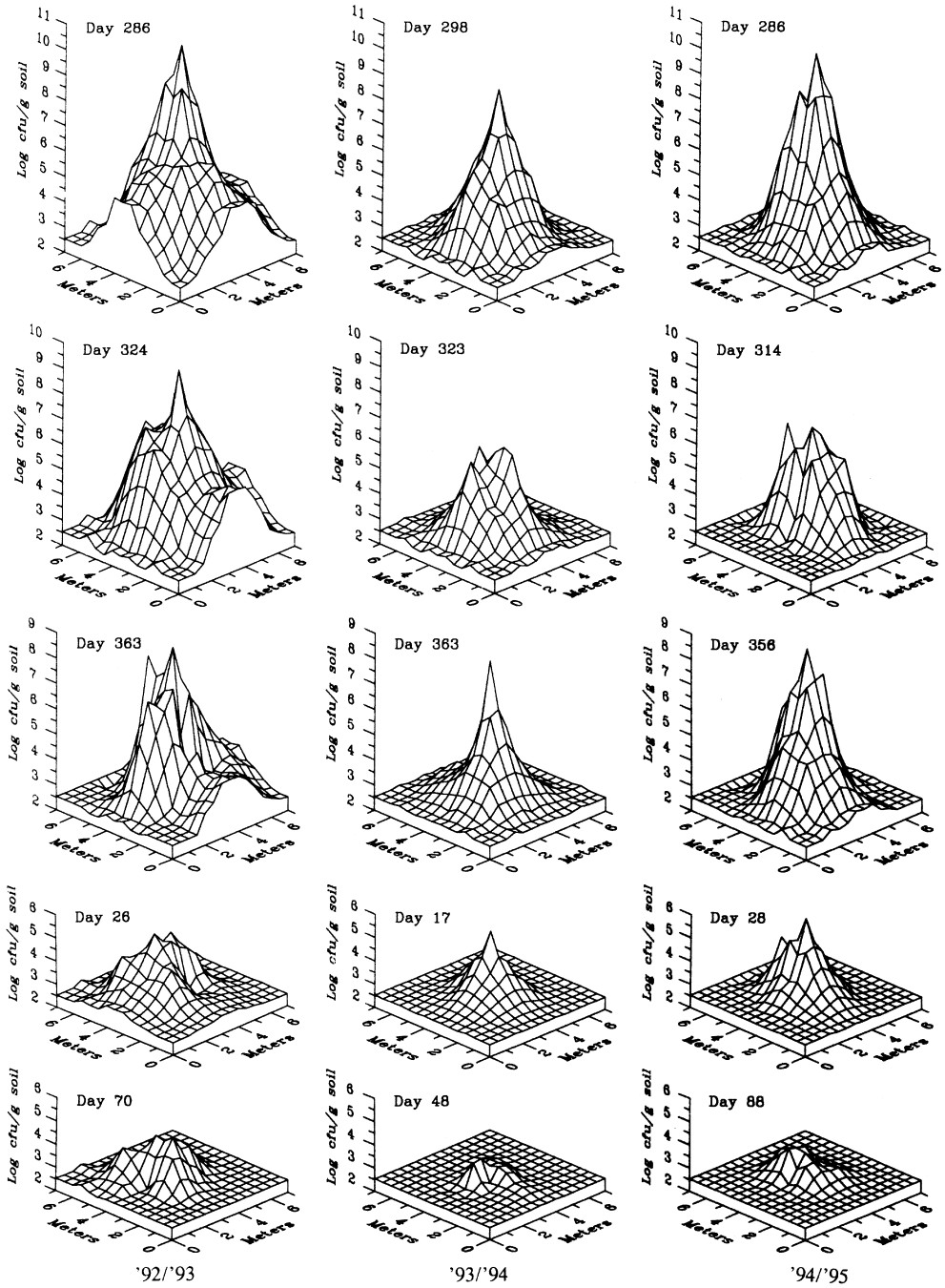

Figure 8. Experiment V, spatial distribution of the bacterial density of *X.c.* pv. *campestris* from plots previously cropped with diseased cabbage. Bacterial densities are obtained by bioassay and expressed as log cfu/g soil. Maps are derived from 37 composite soil samples. Theoretical detection threshold for bacterial density is 2.78 log cfu/g soil.

Table 4. Experiment V, estimated parameters for the linearized negative exponential model fitted to mean gradients per sub-samples and date of soil sampling. Time interval and rate of focus contraction for concerning time interval. (b = slope and standard deviation; R^2 = the coefficient of determination for agreement between observed and predicted disease density; c = radial rate of focus contraction)

Date of sampling	b	R^2	Time interval	c (cm day^{-1})^z
'92/'93				
296	-4.57 ± 0.45	0.97		
324	-3.85 ± 0.48	0.96	296–324	1.86 ± 0.14
363	-1.61 ± 0.07	0.99	324–363	3.97 ± 0.63
26	-1.28 ± 0.14	0.96	363–26	3.12 ± 0.22
'93/94				
298	-4.26 ± 0.47	0.97		
323	-3.18 ± 0.37	0.88	298–323	1.83 ± 0.36
363	-2.07 ± 0.43	0.89	323–363	2.31 ± 0.23
17	-0.88 ± 0.30	0.75	363–17	3.86 ± 0.38
'94/'95				
286	-4.52 ± 0.51	0.96		
314	-3.14 ± 0.40	0.96	286–314	2.09 ± 0.22
356	-2.12 ± 0.39	0.91	314–356	2.05 ± 0.40
28	-0.91 ± 0.15	0.83	356–28	2.73 ± 0.13

^{z} Here, c is the radial rate of focus contraction. To obtain the radial rate of focus expansion a minus sign should be added.

negative rates of focus expansion are in fact the radial rate of focus contraction, negative expansion rates correspond to positive contraction rates (Table 4). Generally, c (as contraction rate) increased with time, which means that the contraction rate was relatively high shortly before the infestation foci became extinct.

Survival experiment (Experiment VI)

In the greenhouse checks none of the seedlings was diseased. Disease-free seedlings planted as a follow-up crop in plots previously cropped with diseased cabbage did not develop black rot symptoms, except for one plant in 1993. At planting, hardly any plant debris was found by visual inspection of the seedbed. *X.c.* pv. *campestris* was not recovered from soil samples taken either at planting time or at maturation time of the follow-up crop.

Discussion

The present study consisted of six discrete steps. Experiment I showed that recovery of bacteria from infested soil was satisfactory to study the dynamics of *X.c.* pv. *campestris* in soil for the present purpose. Experiments II and III demonstrated that a bioassay using a dilution series based on the top 1 ml of a supernatant was adequate for our purpose. Experiment IV indicated that survival of *X.c.* pv. *campestris* was temperature and time dependent, thus permitting a dynamic interpretation of the field experiment. The field experiment (experiment V) showed the build-up of foci and the subsequent regress of the soil's infestation level with time. Finally, the survival experiment (VI) demonstrated that appropriate treatment of infected plant debris contributed to the decline in inoculum so that a follow-up cabbage crop remained virtually uninfected.

Bioassay

A bioassay was used to estimate densities of *X.c.* pv. *campestris* in soil. Mean recovery was about 58% which was high enough for studying survival and extinction of *X.c.* pv. *campestris* in soil and plant debris. Our extraction method and bioassay permitted the assessment of the densities of *X.c.* pv. *campestris* in the soil without confounding by other pathogens, which reasons not to use semi-selective media.

Quantitative assessment of plant pathogens in soil by means of a bioassay is sensitive to incubation

period (Maloy and Alexander, 1958; Pfender et al., 1981; Tuitert, 1990). Incubation period, determined by the population growth of *X.c.* pv. *campestris* after inoculation, depends on temperature (Pinches and Pallent, 1986; Shu and Yang, 1990; Ruissen et al., 1993), mature plant resistance (Bain, 1952, 1955; Hunter et al., 1987), and inoculum dosage (Ruissen, unpublished). These variables may be interrelated. Symptom expression may be delayed due to low inoculum dosage (the higher dilutions of the dilution series) or to temperature effects ($< 25\,^{\circ}$C or $> 28\,^{\circ}$C). During incubation plants mature so that mature plant resistance may develop and symptom expression may be further delayed by a combination of increased resistance with unfavourable temperatures and/or low inoculum dosages. Such interrelationships may explain part of the incomplete recovery of bacteria by the bioassay. Recovery would have been improved with greenhouse temperatures of 25–27 °C instead of 20–25 °C, and an observation over periods longer than five weeks. We expect that the recovery level has no effect on the interpretations of temperature effects and extinction of *X.c.* pv. *campestris* in infestation foci as presented here.

We demonstrated the presence of bacteria in the sediment, adhering to or hidden between soil particles. The density ratio of supernatant : sediment was about 10^5, a figure justifying neglect of the bacteria captured in the sediment (Experiment III), as also found for *Bacillus cereus* (Young et al., 1995).

In natural situations, bacterial cells are protected by plant tissue so that recovery from plant debris will be lower than in the laboratory situation of Experiment I. Thus, population densities of *X.c.* pv. *campestris* in soil will be underestimated notwithstanding the 58% recovery.

Temperature experiment

Alvarez and Cho (1978) suggested that survival of *X.c.* pv. *campestris* in soil may be affected by climatic conditions. Our study showed that temperature had a major effect on the survival of *X.c.* pv. *campestris*. Survival of *X.c.* pv. *campestris* decreased at temperatures $>5\,^{\circ}$C. Higher temperatures also stimulated decomposition of plant debris. Schaad and White (1974) found that survival of free *X.c.* pv. *campestris* in soil was limited to a relative short time, whereas survival in host debris lasted for a comparatively long time. Schultz and Gabrielson (1986) recovered *X.c.* pv. *campestris* from debris as long as debris remained undecayed. We tentatively conclude that higher temperatures lead to faster decomposition of plant debris so that *X.c.* pv. *campestris* becomes less protected by host debris and populations decrease at a faster rate.

Field experiment

The 3D response surfaces (Figure 7) illustrate the spread of black rot from the infection focus and the persisting dominance of that focus. Peaks further from the centre of the plots indicate secondary foci, resulting in a heterogeneity of the spatial pattern.

Notwithstanding the difference in sampling sites, we were able to reconstruct patterns of high and low soil infestation (Figure 7, upper row). Figure 7 (columns) shows the disappearance of the infestation foci of *X.c.* pv. *campestris* in soil during winter. The bacterial density decreased both in and around the source during winter and finally, in the next spring, no infestation could be demonstrated.

Where infection foci usually enlarge under favourable circumstances, infestation foci may shrink under unfavourable conditions. Rates of focus contraction c (cm d^{-1}) increased with time, generally. The increase is attributed to increasingly rapid decomposition of plant debris during winter, resulting in loss of protection for the bacterial cells. Free *X.c.* pv. *campestris* cells survive only for short periods (Schaad and White, 1974) (Experiment V). Therefore, densities of *X.c.* pv. *campestris* decreased during winter and infestation foci disappeared in the following spring.

The extinction rate of *X.c.* pv. *campestris* in soil correlated with soil temperature (Experiment IV). The correlation between relative rate of extinction and mean soil temperature at -5 cm was -0.49. A high e corresponded to an mean daily soil temperature of $>5\,^{\circ}$C whereas a low e was found for intervals having days with frost and with intervals with mean soil temperature $<3\,^{\circ}$C (Experiment V). Schultz and Gabrielson (1986) found that survival of *X.c.* pv. *campestris* was similar in residues buried in soil. Although rotavated soil (experiment V) may differ in temperature from untreated soil (as at the weather station), we expect that such a difference does not affect the essence of the relation between extinction rate and soil temperature.

Van den Bosch et al. (1988), Zadoks and Kampmeijer (1977) and Zadoks and Van den Bosch (1994) reasoned that isopaths of infection foci due to foliar fungal pathogens will be parallel and move outward at a constant velocity. Obviously, their theory

does not apply to bacterial infestation foci in the soil. Though curves of equal bacterial density in the soil were roughly parallel, they moved inward, and not at constant velocity. The lack of constancy may be due to temperature effects. Besides, plant decomposition and *X.c.* pv. *campestris* mortality is likely due to microbial activity that is impacted by factors as temperature, soil moisture, and soil type.

Survival experiment

Polyetic carry-over of inoculum from one vegetation period to the next obviously did not occur in the survival experiment covering three crop-free winters, one stray infection excepted. In contrast, Alvarez and Cho (1978) found 90% diseased plants after infesting a disease-free field with a small amount of infected plant debris. Seedlings were planted directly after a homogenous soil infestation and plots were sprinkler-irrigated to increase spread of black rot. In our study, plant debris was chopped after harvest. The essential difference between our survival experiment and the experiments by Alvarez and Cho (1978) and Kocks and Zadoks (1996) is in the condition of the plant debris. Whereas in the latter two reports the carry-over of inoculum from fresh plant debris to plants-to-be-infected was facilitated, directly by contact or indirectly by water splash, the plant debris in the soil experiment, neatly chopped and mixed with soil, had ample time to disintegrate. While others suggested a three-to-five-year-rotation (Alvarez and Cho, 1978; Richardson, 1945; Walker, 1952; Williams and Wade, 1973) or a two-year-rotation (Schaad and White, 1974) to control black rot, our data indicated that in The Netherlands cabbage can be grown without rotation, provided that the crop residue is treated carefully and has ample time to decompose. Thus, an effective rotation will be influenced by the time required for decomposition of host debris since the rate of decomposition varies with type of tissue, degree of tissue diminution, and environmental conditions (Williams, 1980).

Acknowledgements

The authors are grateful to Dr. AHC van Bruggen of the Dept. of Plant Pathology at the University of California, Davis, and to Dr. g Tuitert of the Dept. of Phytopathology of the Wageningen Agricultural University for their assistance during the design of the experiments, and the anonymous referees for their valuable comments on the submitted manuscript. We thank M van den Bogert, W van Noort, MAJ van de Scheur, CE Winterswijk, FGHH van Kesteren, and RThM van der Vossen for technical assistance, field maintenance and data collection.

References

Alvarez AM and Cho JJ (1978) Black rot of cabbage in Hawaii: Inoculum source and disease incidence. Phytopathology 68: 1456–1459

Anonymous (1953) Some further definitions of terms used in plant pathology. Trans. British Mycological Society 4: 177–188

Bain DC (1952) Resistance of cabbage to black rot. Phytopathology 42: 35–37

Bain DC (1955) Disappearance of black rot symptoms in cabbage seedlings. Phytopathology 45: 55–56

Campbell CL and Madden LV (1990) Introduction to plant disease epidemiology. John Wiley & Sons, New York

Ciafardini G and Marotta B (1989) Use of the most-probable-number technique to detect *Polymyxa betae* (Plasmodiophoromycetes) in soil. Applied and Environmental Microbiology 55: 1273–1278

Clayton EE (1924) Investigation of cauliflower diseases on Long Island. New York State Agricultural Experimental Station. Bulletin 506

Fisher RA and Yates F (1963) Statistical tables for biological, agricultural and medical research. 6th edition, Longman, London

Hunter JE, Dickson MH and Ludwig JW (1987) Source of resistance to black rot of cabbage expressed in seedlings and adult plants. Plant Disease 71: 263–266

Kocks CG (1998) Black rot in The Netherlands: studies on spatial and temporal development. PhD-Thesis. Wageningen Agricultural University

Kocks CG and Ruissen MA (1996) Measuring field resistance of cabbage cultivars to black rot. Euphytica 91: 45–54

Kocks CG, Zadoks JC and Ruissen MA (1998a) Spatio-temporal response of black rot in cabbage to initial inoculum levels. In press (Plant Pathology)

Kocks CG, Zadoks JC and Ruissen MA (1998b) Response of black rot in relation to spatial distribution of inoculum. In press (European Journal of Plant Pathology)

Kocks CG and Zadoks JC (1996) Cabbage refuse piles as sources of inoculum for black rot epidemics. Plant Disease 80: 789–792

Lengkeek AG and Van Etteger M (1992) De afname van *Xanthomonas campestris* pathovar *campestris* in de bodem. Dept. of Phytopathology, Agricultural University Wageningen

Linn MB (1958) Vegetable diseases. University of Illinois, College of Agriculture and Extension service in Agriculture and Home Economics

Maloy OC and Alexander M (1958) The 'most probable number' method for estimating populations of plant pathogenic organisms in the soil. Phytopathology 48: 126–128

Pinches A and Pallent LJ (1986) Rate and yield relationships in the production of xanthan gum by batch fermentations using complex and chemically defined growth media. Biotechnology and Bioengineering 28: 1484–1496

Pfender WF, Rouse DI and Hagedorn DJ (1981) A 'most probable number' method for estimating inoculum density of *Aphanomyces euteiches* in naturally infested soil. Phytopathology 71: 1169–1172

Richardson JK (1945) Black rot of rutabagas. Sci. Agriculture 25: 415–425

Roshing H (1995) Bodemkaart van Nederland 1 : 50.000. Toelichting bij de kaartbladen Blad 9 West Texel (gedeeltelijk), Blad 14 West Medemblik, Blad 14 Oost Medemblik, Blad 15 West Stavoren (noordhollands gedeelte), Blad 19 West Alkmaar, Wageningen, DLO-Staring Centrum

Ruissen MA, van der Vossen RTM and Kocks CG (1993) Growth of *Xanthomonas campestris* pv. *campestris* populations at constant and variable temperatures. Netherlands Journal of Plant Pathology 99. Supplement 3: 173–179

Schaad NW and White WC (1974) Survival of *Xanthomonas campestris* in soil. Phytopathology 64: 1518–1520

Schultz T and Gabrielson RL (1986) *Xanthomonas campestris* pv. *campestris* in Western Washington crucifer seed fields: Occurrence and survival. Phytopathology 76: 1306–1309

Shropshire LH and Kadow KJ (1936) Diseases and insect pests of cabbage and related plants. Identification and control. University of Illinois, College of Agriculture and Extension service in Agriculture and Home Economics. Circular 454

Shu H-H and Yang S-T (1990) Effects of temperature on cell growth and xanthan production in batch cultures of *Xanthomonas campestris*. Biotechnology and Bioengineering 35: 454–468

Stichting voor Bodemkartering (1973). Toelichting bij de kaartbladen 39 West en 39 Oost Rhenen, Wageningen

Tuitert G (1990) Assessment of the inoculum potential of *Polymyxa betae* and beet necrotic yellow vein virus (BNYVV) in soil using the most probable number method. Netherlands Journal of Plant Pathology 96: 331–341

Van den Bosch F, Zadoks JC and Metz JAJ (1988) Focus expansion in plant disease I: The constant rate of focus expansion. Phytopathology 78: 54–58

Wagenaar K and Wallenbrug C (1987) Bodemkaart van Nederland 1: 50.000. Toelichting bij de kaartbladen Blad 19 Oost Alkmaar en 20 West Lelystad (noordhollands gedeelte), Wageningen, Stichting voor Bodemkartering

Walker JC (1923) The hot water treatment of cabbage seed. Phytopathology 13: 251–253

Walker JC. 1952. Disease of crucifers. In: Diseases of vegetable crops. JC Walker (ed) (pp 128–131) McGraw-Hill, New York

Walker JC, Larson RH and Taylor AL (1958) Diseases of cabbage and related plants. US Dept. Agric. Handb. No. 144

Williams PH (1980) Black rot: A continuing threat to world crucifers. Plant Disease 64: 736–742

Williams PH and Wade EK (1973) Recommendations for minimizing the threat of blackleg and black rot of cabbage. Control of Plant Diseases #78. Cooperative Extensive Programs. University of Wisconsin. Madison

Young CS, Letherbridge G, Shaw LJ and Burns RG (1995) Survival of inoculated *Bacillus cereus* spores and vegetative cells in non-planted and rhizosphere soil. Soil Biological Biochemistry 27: 1017–1026

Zadoks JC and Kampmeijer P (1977) The role of crop populations and their deployment, illustrated by means of a simulator, EPIMUL 6. Annals of the New York Academy of Sciences 287: 164–190

Zadoks JC and Schein RD, 1979. Epidemiology and plant disease management. Oxford University Press, New York

Zadoks JC and Van den Bosch F (1994) On spread of plant disease: A theory on foci. Annual Review of Phytopathology 32: 503–521