Black Rot of Cabbage in The Netherlands:

Studies on Spatial and Temporal Development

Promotor: Dr. J.C. Zadoks
Emeritus Hoogleraar in de Ecologische Fytopathologie

Co-promotor: Dr. Ir. M.A. Ruissen
Senior Scientist Norwegian Centre for Ecological Agriculture
te Tingvoll, Noorwegen

C.G. Kocks

Black Rot of Cabbage in The Netherlands: Studies on Spatial and Temporal Development

Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van de Landbouwuniversiteit Wageningen,
Dr. C.M. Karssen,
in het openbaar te verdedigen
op dinsdag 26 mei 1998
des namiddags te 13.30 uur in de Aula
van de Landbouwuniversiteit te Wageningen.

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Kocks, C.G.

Black rot of cabbage in The Netherlands: studies on spatial and temporal development

Thesis Landbouwuniversiteit Wageningen - With ref. - With summary in Dutch

ISBN 90-54858575

Subject headings: *Brassica oleracea* / *Xanthomonas campestris* pv. *campestris* / epidemiology

The research described in this thesis was conducted at the Wageningen Agricultural University (WAU), Department of Phytopathology, P.O. Box 8025, 6700 EE Wageningen, The Netherlands. This study was part of the C.T. de Wit Graduate School for Production Ecology.

Financial support was received from the Wageningen Agricultural University (WAU), The Netherlands.

Printed by Ponsen & Looyen BV

STELLINGEN

1. Ziekte-ontwikkeling en ziekte-verspreiding zijn vrijwel even effectief als parameters om koolcultivars te toetsen op veldresistentie tegen zwartnervigheid. (*Dit proefschrift*)

2. Zwartnervigheid is een polycyclische ziekte waarbij het aantal cyclussen bepaald wordt door guttatie in combinatie met regen. (*Dit proefschrift*)

3. Zware volveldse aantastingen kunnen alleen ontstaan vanuit een groot aantal bronnen per veld en zijn indirect terug te voeren op geïnfecteerd zaad of op besmetting van zaailingen tijdens het opkweken. (*Dit proefschrift*)

4. Analyse van de ruimtelijke verdeling van ziekten of plagen is alleen zinvol indien het locatie-effect en de ruimtelijke afhankelijkheid in acht genomen worden. (*Lecoustre et al., 1989; Nicot et al., 1984*)

   Lecoustre R, Fargette D, Fauguet C and Reffye P. 1989. Analysis and mapping of the spatial spread of African cassava mosaic virus using geostatistics and the kriging technique. Phytopathology 79:913-920.

   Nicot PC, Rouse DI and Yandell BS. 1984. Comparison of statistical methods for studying spatial patterns of soilborne plant pathogens in the field. Phytopathology 74:1399-1402.

5. Geostatistiek is een aanwinst in de plantenziektenkunde omdat daarmee het aantal monsters, het aantal bemonsteringsdagen en de bemonsteringstijd gereduceerd, de mate van aantasting op niet gemeten locaties op basis van de ruimtelijke afhankelijkheid geïnterpoleerd en een afbeelding van de mate van aantasting gegeven kan worden. (*Stein et al., 1994*)

   Stein A, Kocks CG, Zadoks JC, Frinking HD, Ruissen MA and Myers DE. 1994. A geostatistical analysis of the spatio-temporal development of downy mildew epidemics in cabbage. Phytopathology 84:1227-1239.

6. De in 1991 binnen het MJPG gestelde normen voor het reduceren van het gebruik van chemische bestrijdingsmiddelen worden waarschijnlijk niet gehaald. Daarom is het vreemd dat het CTB de EU-richtlijn 91/414/EEC (procedure voor toelating gewasbeschermingsmiddelen) anders interpreteert dan commissies in het buitenland. Hierdoor wordt de introductie van plantaardige extracten sterk vertraagd en zoeken leveranciers van deze extracten steeds meer hun toevlucht tot het buitenland om het product daar op de markt te brengen.

7. a) Teelt van agrificatiegewassen vraagt om veel kennis en kunde bij akkerbouwers. (*Verheul en Struik, 1995*)
   b) Agrificatiegewassen zijn gewassen die vooral in de veenkoloniën geteeld moeten worden om de akkerbouwers daar te helpen een beter inkomen te verwerven. (Stelling van klas 8P van de Christelijke Agrarische School, 1997)
   c) Conclusie: de betere akkerbouwers bevinden zich in de veenkoloniën.

Verheul MJ en Struik PC. 1995. Teeltkundige aspecten van agrificatie. 94 pp.

8. Volgens de prioriteitsregel is het correcter om bij '*lijnen van gelijke graad van infectie*' te spreken van '*isoblabs*' (*Thung, 1947*) in plaats van '*isopaths*'.

Thung TH. 1947. De verspreidingswijze van plantenziekten. Landbouwkundig Tijdschrift. No 711/712.

9. De artikels 7 en 25 van het Auteursrecht spreken elkaar tegen. Een wetswijziging om het auteursrecht op een juiste en eenduidige wijze te beschermen is dan ook noodzakelijk.

10. Het zelf boer zijn is uiterst effectief om praktijkgerichte onderzoeksvoorstellen te schrijven en verhoogt de doelmatigheid van praktijkgericht onderzoek.

11. De Russische landbouw drijft op de inzet van vrouwen.

12. Boer: iemand die snel rijk wordt, tenminste als hij zijn land als bouwgrond verkoopt.

Stellingen behorende bij het proefschrift van Corné G. Kocks: '*Black Rot of Cabbage in The Netherlands: Studies on Spatial and Temporal Development*'.

Wageningen, 26 mei 1998.

Iets is sterker dan de dood,
namelijk de aanwezigheid
van de afwezigen
in het geheugen van de levenden.

d'Ormesson

Aan mijn ouders en Ellen

Author's abstract

Kocks, C.G. 1998. Black rot of cabbage in The Netherlands: studies on spatial and temporal development. PhD Thesis, Wageningen Agricultural University, The Netherlands, 200 pp., 46 tables, 40 figures, English and Dutch summary.

Black rot in cabbage is caused by the bacterium *Xanthomonas campestris* pv. *campestris*. An exploratory survey at farm level suggested that major aspects contributing to black rot development are cultivar, initial inoculum, refuse management, origin of transplants, and seed quality. Black rot development was far more intensive with fresh than with old refuse piles, infected with *X.c.* pv. *campestris*. Increased levels of field resistance reduced the development of black rot in time and space. Field resistance to black rot is thought to be composed of several mechanisms. Black rot is hypothesised to be a potentially polycyclic disease. Fast disease development was related to the number of rain days. Secondary foci may appear at short distances from the initial focus but they usually merge with the expanding initial focus. Fast spatio-temporal development was related to high initial inoculum levels. Disease proceeds faster in plots with multi-focal inoculation than in those with uni-focal inoculation. Serious epidemics in Dutch cabbage fields originate from large numbers of foci. Temperatures $\geq$ 5°C stimulated decomposition of plant debris and subsequently hastened the decline of *X.c.* pv. *campestris*. Infestation foci of *X.c.* pv. *campestris* in soil dwindled away during winters and became extinct in spring of the successive year. Polyetic development of black rot was not found. Geostatistics was successfully applied to reduce field sampling needed to obtain insight in disease patterns.

Additional keywords: comparative epidemiology, cultivars, disease gradients, disease progress, dwindling foci, field resistance, geostatistics, initial inoculum level, refuse piles, source distribution, spatio-temporal analysis, survival, symptomatology, *Xanthomonas campestris* pv. *campestris*

Voorwoord

Het uitvoeren van een promotieonderzoek is niet het werk van de promovendus alleen, maar bestaat uit een wisselwerking tussen promovendus en een aantal personen. Zonder anderen tekort te doen, wil ik een aantal van deze personen met name noemen.

Professor Zadoks bedank ik voor het snel en grondig doornemen van mijn manuscripten, voorzien van gerichte maar vooral ook scherpe en opbouwende kritiek. Veel heb ik geleerd van deze kritiek en de daarbij behorende discussies. Vooral tijdens mijn laatste AIO-jaar, toen we beiden "boven" zaten, was ons contact intensief en was er veel steun van uw kant.

Met mijn directe begeleider, Theo Ruissen, heb ik vooral gedurende de eerste helft van mijn AIO-schap vele discussies gevoerd over de ecologie en epidemiologie van *X.c.* pv. *campestris* en over geostatistiek. Theo, bedankt voor deze discussies en het enthousiasme waarmee je ze voerde.

Professor Alfred Stein dank ik voor het beschikbaar stellen van de programma's betreffende de geostatistiek. Alfred, bedankt voor de uitleg betreffende het fenomeen geostatistiek. De discussies en het gezamenlijk schrijven van een publicatie (beloond met de PE-prijs) hebben mij veel geleerd over de (on)mogelijkheden van geostatistiek.

Verschillende personen hebben bijgedragen aan mijn onderzoek door middel van ondersteuning in kassen, op het laboratorium en op het proefveld of door middel van een afstudeervak. Hiervoor gaat mijn dank uit naar de kassendienst voor hun zorg betreffende mijn kasexperimenten en het opkweken van gezond plantmateriaal. De medewerkers van de proefaccommodatie (toentertijd nog optredend voor de Binnenhaven-vakgroepen en het IPO-DLO) dank ik voor de aanleg en verzorging van de veldproeven. Speciale dank verdienen Marchienus van de Scheur, Wim van Noordt en Marius van den Bogert die het leeuwendeel van dit werk voor hun rekening hebben genomen.

Andere personen die ik dank verschuldigd ben, zijn Rob van der Vossen (laboratoriumondersteuning) en de studenten Thijs Banken, Marco van Etteger, Vera van Kesteren en Ard Lengkeek.

Tonnie Engels, Maarten Zwankhuizen, Gitte Schober, Herman Frinking en Corrie Geerds; jullie waren onmisbaar voor mij op Fyto. Bedankt voor jullie gezelligheid, steun, beoordeling van manuscripten en hulp bij computerproblemen.

Last but not least, bedank ik mijn ouders en Ellen. Zonder hun steun, vertrouwen en stimulans was het een moeilijke klus geworden. Jullie hebben me altijd weten te motiveren maar ook geleerd dat het af en toe "eruit breken" (lees: het

uitleven als "hobbyboer") nodig is. Ook ben ik jullie zeer erkentelijk voor het feit dat jullie mij betrokken hebben bij het "boerenleven", het telen van gewassen, het omgaan met problemen in de landbouw en dat jullie er op toezagen dat ik de boerenpraktijk niet uit het oog verloor tijdens mijn promotieonderzoek. Bovendien hebben jullie mij regelmatig geholpen bij aanleg en onderhoud van enkele proefvelden. Mijn verdere familie wil ik langs deze weg mijn verontschuldiging aanbieden voor het feit dat ik veel afwezig was, dan wel geestelijk afwezig scheen te zijn.

Corné Kocks

Contents

Chapter 1

General Introduction

1.1 Introduction

Cabbage (*Brassica oleracea* L.) is an old crop in Europe, portraited on several paintings of the Dutch painter Pieter Aertsen (1509-1575), as e.g. "Groentenverkoopster" ("Woman selling vegetables", *Berlin-West, Gemäldegalerie des Staatliche Museen Preußische Kulturbesitz*), "Groentestal" ("Vegetables stall", *Stockholm, Hallwyl Museum*), "Marktscene met Ecco Homo" ("Market scene with Ecco Homo", *München, Bayerisches Staatsgemäldesammlungen, Alte Pinakothek*), "Keukenmeid" ("Girl cook", 1569, residence unknown), and "The young fruitseller", *Berlin-Dahlem, Gemäldegalerie*). These paintings illustrate the importance of cabbage in people's diet. The first cauliflower quotations are from A. van der Venne, Middelburg, 1623, in "Tafreel van Sinnemal" ("Scene of Sinnemal"), and the first cabbage citation is from J. van Beverwijk (1660) in "Schat der Gesontheijt" ("Treasure of Health").

Cabbage's wild form, *Brassica sylvestris* (L.) Miller, grows on the maritime cliffs of the west coast of Britain, France, and the Mediterranean coastal area (Zeven and Zhukovsky, 1975). When cultivated, the wild form shows an enormous increase in morphological types (Zeven and Zhukovsky, 1975). Several classifications of the cultivated *B. oleracea*-types were published (e.g. Helm, 1963). The origin of these cultivated types is not yet fully understood and it is supposed that they developed gradually from several wild cabbage populations and were diversified by crossings with other cultivated types or wild species and by human selection pressure (Zeven and Zhukovsky, 1975). Helm (1963) presented a simplified pedigree but did not succeed to address the origin of white cabbage (*B. oleracea* L. convar. *oleracea* var. *capitata* DC), the cabbage type studied in this thesis.

In the 19th century, cabbage was grown intensively in Russia and northern Europe. During the second half of the 19th century, immigrant farmers from northern Europe introduced cabbage in the USA. The first record of black rot disease comes from Graham (1891), who described black rot in cabbage grown in Kentucky. In 1898 black rot was highly destructive in Wisconsin (Russell, 1898). In The Netherlands, black rot was first described by Van Hall (1900). At that time, black rot was not discussed in Russia, possibly because phytopathological studies there focused on club root caused by *Plasmodiophora brassicae* (Woronin, 1878), a major problem in cabbage around St. Petersburg. Nowadays, black rot of cabbage is a serious disease worldwide (Williams, 1980), which occurs on all the cultivated crucifers and on some wild species.

1.2 Black rot in The Netherlands

The first notes concerning black rot in The Netherlands were by Van Hall (1900). Several diseased cabbage plants coming from a farm at Broek op Langendijk were sent to the Laboratory of Phytopathology in Amsterdam. Broek op Langendijk is in the centre of the region where white en red cabbage were grown then and now. The symptoms were described as leaf dropping. Leaves became soft and leathery and were yellowing with brown margins and blackening of the veins. Van Hall noticed, when cutting the stem, that veins inside the stem were dark brown or black. These symptoms agreed with those described by Erwin Smith in 1897. Van Hall identified the cabbage disease as black rot, caused by *Pseudomonas campestris* (Pammel), later classified as *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939.

From the notes written by the sender (Mr. Porte) of the diseased plants it is clear that the disease existed already for some time in the area. Porte wrote: "already for a year of five we saw these symptoms in the cultivar Utrechtse Roode ("Utrecht Red"). At present almost all cultivars are attacked by the pathogen, even in cauliflower, but the problems are greatest with red cabbage where in some cases 90% of the harvest is lost". Porte noticed that in the crop, the symptoms appeared from inside the plant since the outer leaves did not show symptoms, in contrast to the inner leaves. Often plants seemed to be healthy but when stems were cut, black vessels could be seen. Porte also observed that infection already occurred in the seedbeds before transplanting. Plants from particular seedbeds resulted in diseased crops while other seedbeds gave healthy crops.

1.3 Black rot in cabbage

Causal organism and symptoms. Black rot is caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*X.c.* pv. *campestris*). *X.c.* pv. *campestris* is an aerobic, Gram-negative, non-spore-forming, small, rod-shaped bacterium 0.4-2.0 μm in length, with a single polar flagellum (Buchanan and Gibson, 1974). *X.c.* pv. *campestris* produces an extracellular polysaccharide, xanthan, which plugs and damages the xylem vessels (Sutton and Williams, 1970). The damaged xylem vessels cannot transport water and thus cause water stress and wilt.

Black rot infection of leaves may appear as *i*) infection of leaves through hydathodes, wounds or stomata (the latter when filled with water), or *ii*) infection

by the vascular system. Infected cabbage seedlings show chlorotic V-shaped lesions at the margins of the cotyledons (Cook *et al.*, 1952b; Drechsler, 1919) when infected through hydathodes. The cotyledons wilt and drop off prematurely. Symptoms reappear after a few weeks when *X.c.* pv. *campestris* invades the vascular system through the cotyledons' nodes.

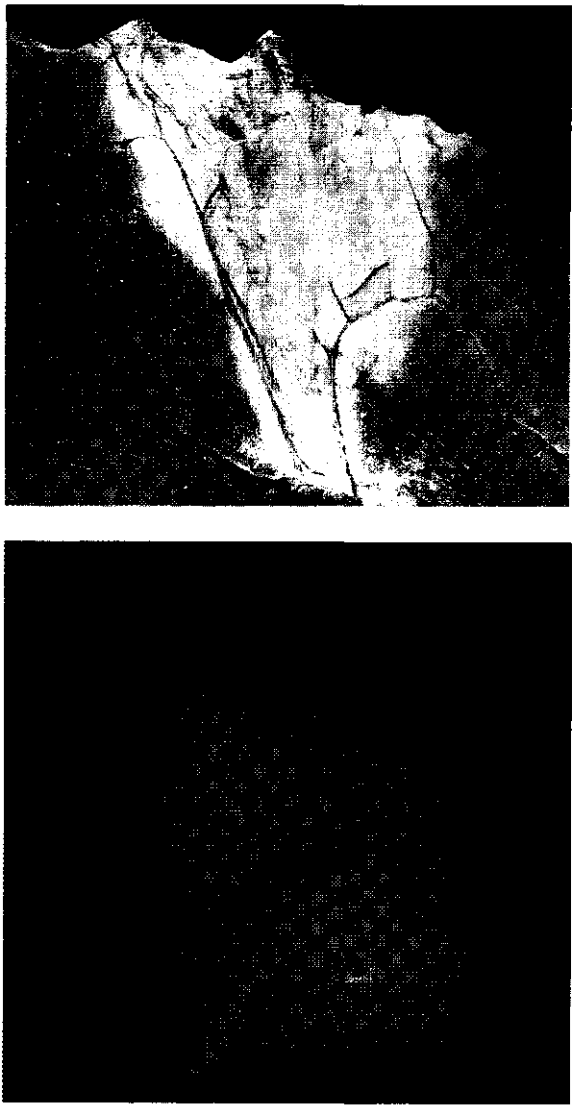


Figure 1.1. Black rot symptoms of cabbage. Upper: after invasion of hydathodes, Lower: after migration of *X.c.* pv. *campestris* downward through the xylem vessels.

Progress of leaf infection is usually seen as blackening of veins, soon followed by yellowing and subsequent death of the tissue, resulting in characteristic V-shaped lesions (Figure 1.1). After infection of the leaves, *X.c.* pv. *campestris* enters the stem, invading its vascular system in all directions. Chlorotic lesions from systemic invasion may appear anywhere on the leaves and defoliation may follow. Cabbage and cauliflower heads are invaded and discolour.

Life cycle and disease cycle. The life cycle of the causal agent *X.c.* pv. *campestris* is simple (Figure 1.2). Within a generation time of at least two hours, one single bacterium produces two bacteria by fission, each capable to infect a host. The generation time depends on temperature and is shortest at 27°C.

X.c. pv. *campestris* overwinters in infected plant debris, overwintering cabbage, seed, or weeds (Figure 1.2) (Alvarez and Cho, 1978; Cook *et al.*, 1952a, b). Seed is a major vehicle for transmission of *X.c.* pv. *campestris* from one area to another. *X.c.* pv. *campestris* invades the embryo through the xylem and subsequently infects the cotyledons. Heavily infected seedlings die. Seeds slightly infected with *X.c.* pv. *campestris* may produce seedlings which are potential sources of inoculum. Once *X.c.* pv. *campestris* has been introduced, disease can spread easily within the seedbed and field. By systemic invasion of seedstalks *X.c.* pv. *campestris* may reach the seed pods internally and infect the seeds. An epidemic can frequently be traced back to a given lot of infected seed or to seedbed which became infected early by inoculum from refuse or seed (Cook *et al.*, 1952a, b).

Spread of *X.c.* pv. *campestris* in fields occurs by overhead irrigation (splash dispersal) (Grimm and Vogelsanger, 1990), rain splash (Hunter *et al.*, 1975; Williams, 1980) and aerosols (Kuan *et al.*, 1986). Infection occurs through hydathodes, stomatal openings when filled with fluid, and wounds. Hydathodes are the most important port of entry (Cook *et al.*, 1952b; Ruissen and Gielink, 1994) whereas stomata are relatively unimportant for invasion. Hydathodes are often filled with guttation fluid whereas substomatal chambers are rarely congested under normal field conditions. Occasionally, lesions begin at wounds caused by insects. Infections can take place at any time during the life of the host plant. Black rot infection may render a large percentage of cabbage plants incapable of producing marketable heads whereas the harvested heads are unfit for storage or shipment (Williams, 1980).

Survival of *X.c.* pv. *campestris* in soil or in plant refuse and debris is affected by climatic factors, crop rotation, microbial activity in the soil, chemical soil properties, and protection by the host (Alvarez and Cho, 1978; Cook *et al.*, 1952b; Schaad and White, 1974b; Schuster and Coyne, 1975).

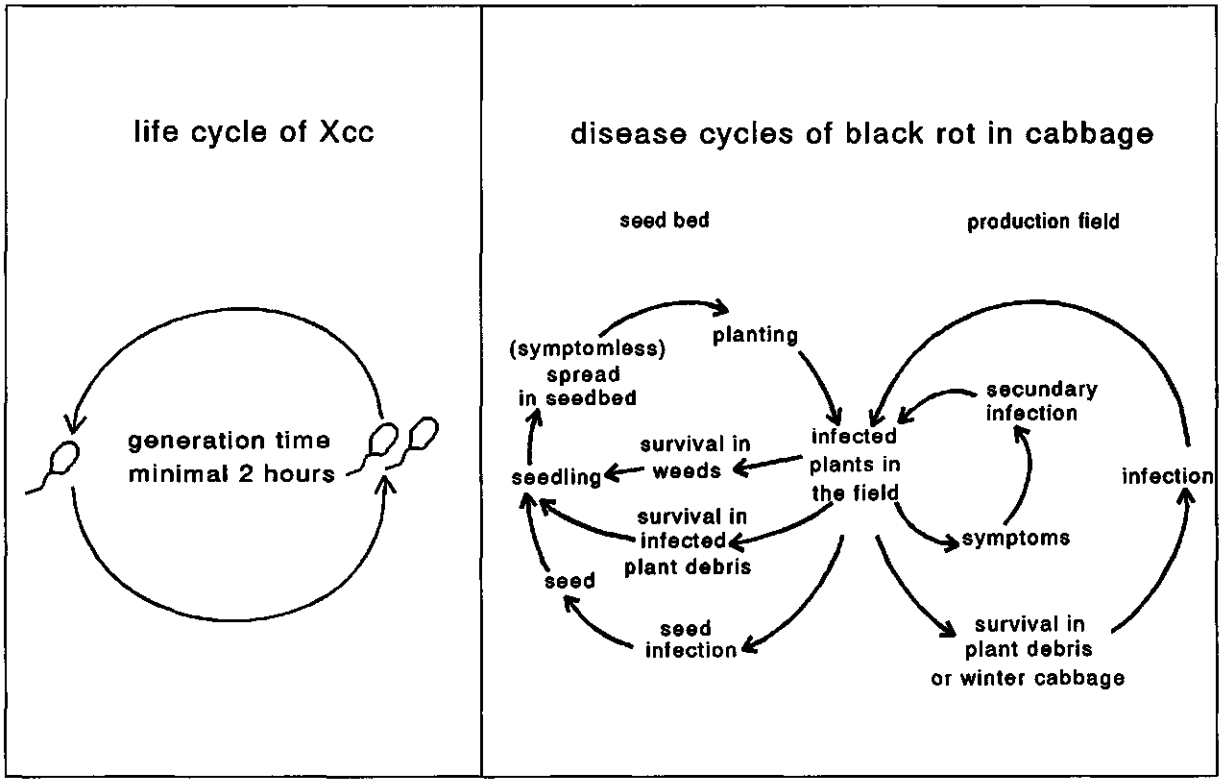


Figure 1.2. Life cycle of the causal agent of black rot, the bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*), and disease cycles of black rot in cabbage.

Host range. Black rot is found on cabbage, cauliflower, kale, turnip, radish, Chinese cabbage, Horse-radish, mustard, and on other crucifer crops and weeds. Several cruciferous weeds are hosts of *X.c.* pv. *campestris* (Walker, 1952; Schaad and Dianese, 1981). *Capsella bursapastoris* (L.) Medicus and *Raphanus raphanistrum* L. were found to be naturally infected by *X.c. pv. campestris* (Schaad,1976; Young, 1969). At least fifteen genera and more than 300 species have been reported as hosts of *X.c.* pv. *campestris* (Munz, 1963; Radford *et al.*, 1968).

Control. Since crucifers are susceptible to black rot during the whole cropping cycle, all aspects of crucifer production must be considered to control black rot. Williams (1980) gave guidelines to control black rot in cabbage. These guidelines emphasize seed treatment, sanitation, protection of seedlings during transplant, crop rotation, seedbed practices, use of production practices that reduce the disease, and resistance. Among his recommendations are:

- use hot-water treatment on seed before planting,
- use clean seedbeds since *X.c.* pv. *campestris* can survive in plant debris in the soil,
- if seedbeds are watered artificially, avoid sprinkling the foliage,
- practice rotation in the field so that at least two years elapse between cruciferous crops. Rotation is of no avail if infected plants debris is allowed to become a source of inoculum,
- use resistant varieties.

Observance of these recommendations may help to prevent the spread of black rot to a certain degree. Notwithstanding these recommendations, black rot appeared to be very destructive during several years in The Netherlands. In the 1990s, the disease is still an problem. As knowledge on the ecology of this pathogen was scantly, it was thought necessary to perform ecological and epidemiological studies on black rot in cabbage.

1.4 Arguments for ecological and epidemiological research

Various aspects of the ecology and epidemiology of black rot in cabbage have been analyzed but quantitative information on black rot development in time and space is

poor. In view of the limited possibilities to control black rot, more quantitative ecological knowledge is of scientific, economic and social importance since black rot causes problems at farm level in The Netherlands. Crop management practices may affect black rot development at farm level. Therefore, a black rot survey was performed to document cropping practices and black rot levels in The Netherlands.

Sanitation is an important component in the control of black rot (Williams, 1980). Sanitation is the action that reduces, excludes or eliminates the initial inoculum from which epidemics start (Van der Plank, 1963). Plant quarantine, crop rotation, seed disinfection, and eradication of alternate hosts are current sanitation practices (Zadoks and Schein, 1979). Considering sanitation of black rot in cabbage, the importance of seedborne inoculum (Schaad *et al.*, 1980), soilborne inoculum (Ruissen *et al.*, 1990; Schaad and White, 1974b), weedborne inoculum (Schaad and Dianese, 1981), and rootborne inoculum (Ruissen and Van Tol, 1988) has been described. No quantitative information was available on plant refuse piles as a factor in the origin of black rot epidemics, possibly a missing piece in the black rot puzzle.

Recently, Dzhalilov and Tiwari (1995) estimated the survival time of *X.c.* pv. *campestris* in soil and in host plant debris. This study and some other studies regarding soilborne inoculum (e.g. Schaad and White, 1974b) focus on survival of *X.c.* pv. *campestris*. The authors did not consider the appearance of foci and dwindling of focal soil infestation and the spatial distribution of soilborne inoculum. The spatial distribution of soilborne inoculum and the build-up of foci were studied to obtain information on polyetic development of black rot (Zadoks and Schein, 1979).

Classical black rot epidemiology was rather pre-occupied by identification of inoculum sources. A quantative assessment of the influence of initial inoculum on spatio-temporal black rot development was lacking. Likewise, the effect of spatial distribution of primary inoculum on black rot development was not known. Therefore, several experiments were performed to relate inoculum density, inoculum size, type of inoculum source, and spatial distribution of initial inoculum to epidemic development of black rot.

Strandberg (1973) recognized the relevance of assessing the pattern of black rot to design experiments, sampling plans, and disease management studies. His spatial analysis was based on the use of the negative binomial distribution. Use of this distribution implies the assumption of independence of sampling points, an assumption which is often ignored. The introduction of geostatistics in phytopathology allowed to perform spatial analysis in dependence of sampling points

for small-scale (Lannou and Savary, 1991; Lecoustre *et al.*, 1988) and large-scale (Nelson *et al.*, 1994) experiments. Geostatistics appeared to be useful to design optimal sampling strategies and to perform spatial interpolation. Therefore, geostatistics was applied to optimize sampling plans and evaluate spatial interpolations for small scale experiments with black rot in cabbage.

Studies on resistance to black rot emphasized breeding (e.g. Williams *et al.*, 1982), genetics of resistance and molecular analysis of resistance mechanisms (e.g. Kamoun *et al.*, 1992). Some information on varietal resistance to black rot is available (Pillard *et al.*, 1991; Staub and Williams, 1972). A question is the effect of varietal field resistance on temporal and spatial dynamics of black rot. Remarkably, this question was not yet addressed. Increased levels of resistance might help to control the black rot by reducing the development of the disease in time and space. Therefore, resistance effects on temporal and spatial black rot development were quantified.

1.5 Outline of the thesis

The distribution and importance of black rot in The Netherlands is discussed in Chapter 2. Quantitative experiments were reported on the plant refuse pile as a factor in the origin of black rot epidemics (Chapter 3). The field performance of black rot on cabbage cultivars of different resistance levels was studied in time and in space (Chapter 4). Chapter 5 discusses the spatio-temporal response of black rot in cabbage to initial inoculum levels. A study of the spatio-temporal response to the distribution of initial inoculum is described in Chapter 6. Chapter 7 deals with dwindling foci, decrease of soilborne inoculum during the winter, and polyetic development during the subsequent growing season(s). Pattern analysis and sampling of cabbage plants naturally infected by black rot is studied by means of geostatistics (Chapter 8). The results are integrated and discussed in Chapter 9.

Chapter 2

Black Rot in The Netherlands: an Exploratory Survey on Farmers' Practices and Management

2.1 Introduction

Red and white cabbage and cauliflower, important vegetables in The Netherlands, can be damaged by black rot. Damage by black rot in cabbage in The Netherlands was first described in 1900 (Van Hall), and it still is a threat to cabbage production.

Black rot research, as carried out abroad, indicated that refuse piles (Dzhalilov and Tiwari, 1995), infected plant debris, winter cabbage, or seed (Alvarez and Cho, 1978; Cook *et al.*, 1952a, b), cultivar choice (Pillard *et al.*, 1991; Staub and Williams, 1982), and cruciferous weeds (Walker, 1952; Schaad and Dianese, 1981) may play a role in black rot epidemics. This information can be used to control black rot in cabbage. However, control methods in The Netherlands need to be evaluated within the context of management strategies relevant to the present status of cabbage growing in The Netherlands. Therefore, various aspects of the ecology of black rot in conventional cabbage production systems in The Netherlands had to be analyzed to obtain information on black rot epidemics at farm level and their relation to farming practices.

This chapter reports an exploratory survey of conventional cabbage production systems, with emphasis on incidence of black rot. An exploratory survey is a process by which researchers traverse target regions and informally interview farmers in order to arrive at a tentative understanding of farmers' existing technology for the target crop and to identify constraints limiting farmers' production and income (Beyerlee and Collinson, 1980; Vos, 1994). The goal of this survey was to describe and analyze farming systems to set a research agenda.

2.2 Cabbage production in The Netherlands

Red and white cabbage production. Cabbage growing as a specialized industry began around 1900. Cabbage is a cool-season crucifer of Mediterranean origin. Cabbage has been known in The Netherlands at least since the 16th century (Chapter 1). Red cabbage (*Brassica oleraceae* L. convar. *capitata* (L.) Alef var. *rubra* DC) and white cabbage (*B. oleraceae* L. convar. *capitata* (L.) Alef var. *alba* DC) are known for its heads which are generally eaten fresh in salads or cooked. White cabbage is also fermented (Sauerkraut).

Cropping of red and white cabbage is concentrated in the province of Noord-Holland, in the region of Heerhugowaard (Figure 2.1). Cabbage is planted in April and May and the heads are harvested from July until November. Fast growing,

early cultivars are used as summer cabbage for the fresh market. Mid-early, moderately fast growing cultivars can be used for both the fresh market and processing while late cropping, slow growing cultivars are only used for processing and long-term storage.

The total annual production in 1992 of red and white cabbage was 27,612 tonnes (1035 ha) and 74,052 tonnes (1687 ha), respectively (PAGV, 1995).

Cauliflower. Cauliflower (*B. oleraceae* L. var. *botrytis* DC) is of unknown origin. Although its history goes back to the second century when cauliflower was grown in Egypt and Turkey, the product was possibly known 1500 years earlier (PAGV, 1993). In The Netherlands, cauliflower is grown at least since 1550 (Chapter 1). Cauliflower production is concentrated in the province of Noord-Holland in the region De Streek (Figure 2.1). Other provinces for cauliflower production are Zuid-Holland, Noord-Brabant and Limburg. Almost all cauliflower is produced for fresh market. Cauliflower is grown as a summer, autumn and winter crop. The annual production of cauliflower in 1992 was 56,670,000 heads (2722 ha) (PAGV, 1995).

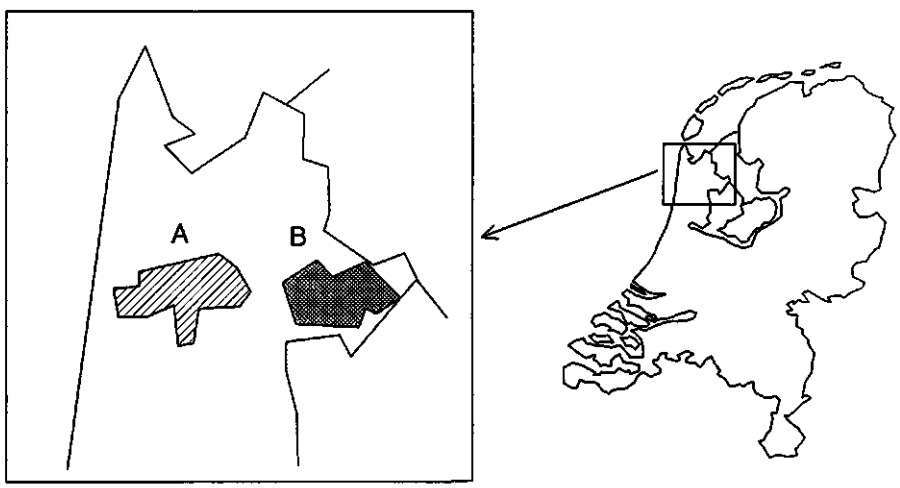


Figure 2.1. Survey regions Heerhugowaard (A) and De Streek (B) in The Netherlands during 1991 - 1993.

Table 2.1. Year of survey, date of survey (week number), sampled region, number of farms, number of fields, and number of surveyors.

Week	Region	Farms	Fields	Surveyors
1991				
28	De Streek	22	117	3
38	De Streek	22	117	3
45	De Streek	22	117	3
27	Heerhugowaard	28	131	3
37	Heerhugowaard	28	131	3
44	Heerhugowaard	28	131	3
1992				
37	De Streek	23 [w]	111	1
38	Heerhugowaard	28 [x]	130	1
1993				
35	De Streek	21 [y]	100	1
36	Heerhugowaard	28 [z]	130	1

[w] 13 farms were the same as selected for the survey of 1991
[x] 27 farms were the same as selected for the survey of 1991
[y] 10 farms were the same as selected for the survey of 1991 and 5 farms of 1992
[z] all farms were the same as for the survey of 1992

2.3 Materials and methods

Farm selection. Twenty-eight cabbage farmers were selected in the region of Heerhugowaard (red and white cabbage), and 22 farmers in the region of De Streek (cauliflower) (Figure 2.1). Farmers were selected randomly from a list, placed at our disposal by the Dienst Landbouw Voorlichting (agricultural extension service), to cover a range of farms, farming practices, cultivars, fields, and planting dates. Every farmer represents one individual farm.

Both regions were sampled three times in 1991 (Table 2.1). One sampling was performed in 1992 and 1993 (Table 2.1). Initially, the same farms were sampled each year as randomly selected for the survey of 1991. However, several farmers

Table 2.2. Exploratory survey form with questions on farm, cropping aspects, farmers' practices, and black rot level.

Question	Answer
Farm	
Cropping season	1991, 1992, 1993
Location	place name
Farm size	ha
Soil type	clay (%)
Nutrition	kg N/ha
Cropping aspects	
Crop	white or red cabbage/cauliflower
Cultivar	cultivar name
Hectareage	ha
Origin of transplants	seed company/nursing company/home grown
Planting date	week number
Farmers' practices	
Weed control	yes/no
Crop rotation	1:1/1:2/1:3/1:4/other
Plant refuse piles	destroyed/spread out over field/piled up on farmyard/other
Plant residue	ploughed under in autumn/ploughed under in spring/ chopped and ploughed under in autumn/chopped in autumn and ploughed under in spring/grazed by cattle
Black rot level	0/1/3/6/9

did not wish to participate in the survey for a second or third year. Therefore, new farms were added to the survey during 1992 and 1993 (Table 2.1).

Sampling and measurements. Samples were taken on three different dates in 1991, and in September of 1992 and 1993 (Table 2.1). Each cultivar on each farm was observed to obtain a general impression of black rot levels. The disease level was estimated by considering a number of rows over several tens of meters and classifying black rot in one of four classes 0, 1, 3, 6, and 9 (no disease, 1-10%, 11-

50%, 51-100% diseased plants, and visual damage to cabbage heads). Besides, a global impression was given on aggregation of diseased plants (randomly distribution or aggregation in some specific parts of the fields). Per farm, all fields were studied. A field is defined here as a coherent area with one single cabbage cultivar. Therefore, each field was assessed by completing by a form with a set of fifteen variables: cropping season, location, soil type, nutrition, farm size, crop, cultivar, hectareage, origin transplants, planting date, weed control, crop rotation, plant refuse piles, plant residues, and black rot level at observation date (Table 2.2).

On several farms, leaves with black rot symptoms were collected and checked in the lab for presence of *X.c.* pv. *campestris*. This test facilitated a comparative assessment of samples to limit surveyor errors. Continuity between seasons was maintained by the involvement of one surveyor in all three years. The survey was made in three seasons (1991-1993) to describe fluctuations between years in disease intensity and in farmers' practices.

The program STATGRAPHICS 7.1 was used to perform the Chi-Square Goodness-of-Fit Test (χ^2-test), comparison of two samples (Wilcoxon-test), Chi-Square Test with Yates correction (Yates-test), and the Kolmogorov-Smirnov test (KS-test). All test were performed with $\gamma = 0.05$.

2.4 Results and discussion

Results obtained on farms, cropping aspects and farmers' practices varied little between the years, since farmers' practices are related to tradition, soil, machinery, and climate. Therefore, only the results from 1991 are summarized and discussed. Black rot is described for all years.

Farm

Farm size. In 1991, 28 farms (131 fields) and 22 farms (117 fields) were visited in Heerhugowaard and De Streek, respectively. Farm size varied from 0.9 to 10.9 ha in Heerhugowaard and from 2.8 to 16.0 ha in De Streek (distributions were significantly different using KS-test and Wilcoxon). The median in farm size was significantly smaller in Heerhugowaard than in De Streek (9.3 and 5.3 ha, respectively) (Table 2.3) (χ^2-test, $P = 0.05$). The majority of farms was smaller than 7.0 ha in Heerhugowaard whereas many farms in De Streek measured over 9.0 ha. Thus, white and red cabbage was cropped on small farms while cauliflower was cropped on larger farms.

Field size. The majority of the fields were smaller than 2.0 ha (Figure 2.2). A field size of 1 ha was popular in Heerhugowaard, 62 out of 131 fields having this size. Obviously, farmers prefer to crop cabbage on small fields. The smallest field size was 0.15 ha. Large fields were found in De Streek, where several fields measured over 4 ha.

Clay content and nutrition. The soil types can be described as sandy clay to clay and as clay (Heerhugowaard en De Streek, respectively). Clay content in Heerhugowaard differed significantly from clay content in De Streek (χ^2-test, P = 0.05). In Heerhugowaard, clay content was quite heterogeneous (varied from $<15\%$ to 40% between fields) but it varied little between fields in De Streek (84 out of 117 fields had a clay content between 21 - 30 %) (Table 2.4).

The fertility status of the soil was characterized by a high organic matter content with medium tot high cation content (Ca was moderate, K, Mg and K were high) and neutral pH (Wagenaar and Wallenburg, 1987; Roshing, 1995). Generally, the N-value in the soil was about 280 kg N/ha in both Heerhugowaard and De Streek (Table 2.4).

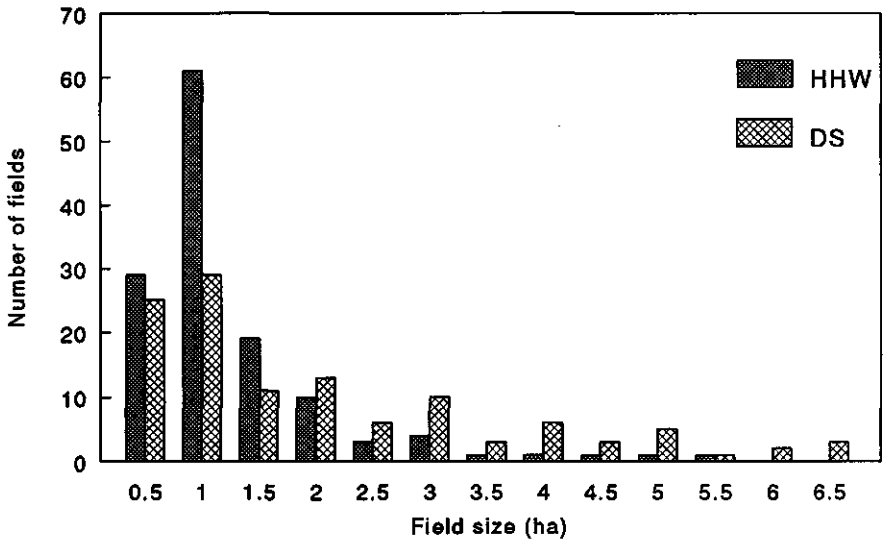


Figure 2.2. Field size (ha) for cabbage fields in Heerhugowaard (HHW) and De Streek (DS) in The Netherlands, 1991.

Table 2.3. Farm size for cabbage production in the regions Heerhugowaard and De Streek in The Netherlands, 1991.

Region	Farm size (ha)														
	<2.1	2.1-3.0	3.1-4.0	4.1-5.0	5.1-6.0	6.1-7.0	7.1-8.0	8.1-9.0	9.1-10.0	10.1-11.0	11.1-13.0	13.1-15.0	>15.0	Total	Median
Heerhugowaard	1	4	3	5	4	3	3	2	1	2	0	0	0	28	5.3
De Streek	0	1	3	1	1	2	1	0	3	3	3	1	3	22	9.3

Table 2.4. Clay content (% clay) and N in soil (kg/ha) for cabbage fields in the regions Heerhugowaard and De Streek in The Netherlands, 1991.

Region	% clay							N-value in soil (kg N/ha)		
	< 15	16 - 20	21 - 25	26 - 30	31 - 35	36 - 40	> 40	< 250	260 - 300	> 300
Heerhugowaard	16	39	19	28	20	9	0	11	106	14
De Streek	0	4	36	48	18	11	0	16	94	7

Cropping aspects

Cultivars. Figures 2.3 and 2.4 show that many different cultivars were used in 1991, 31 (Heerhugowaard) and 36 cultivars (De Streek) being grown. Cultivars 20, 22, 48, and 52 are present in both figures, since some farmers in De Streek cropped a few white or red cabbage cultivars. The difference between the two regions is remarkable. In Heerhugowaard, 10 cultivars are cropped more than 5 times (Galaxy [code 9], Roxy [20]; Bartolo [26]; Slawdena [27]; Bingo [33]) (Figure 2.3). In contrast, farmers in De Streek prefer three cultivars (Sernio [5]; Plana [10]; Frimond [14]) (Figure 2.4). Interpretation of frequency or hectareage are comparable (Figures 2.3 and 2.4).

The number of cultivars grown per farm is presented in Figure 2.5. Most farmers cropped 3 or more cultivars. Individual farmers in Heerhugowaard (white and red cabbage) cropped fewer cultivars than farmers in De Streek (cauliflower). The latter make use of many cultivars since they produce cauliflower for harvest year-round. Therefore it is necessary to crop many different cultivars suitable for different planting and harvest times. Two farms had only one or two cultivars (in Heerhugowaard) and three farms had more than nine cultivars (in De Streek). The highest number of cultivars on one single farm was found in Heerhugowaard (21 cultivars on 11 ha).

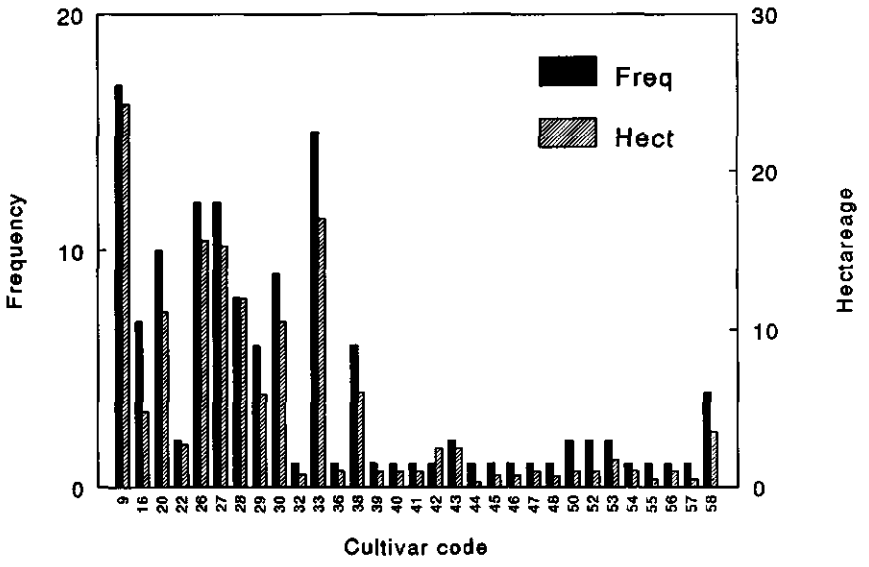


Figure 2.3. Frequency of cultivars (Freq) and hectareage per cultivar (Hect) in Heerhugowaard in The Netherlands, 1991.

Origin of transplants. Region had a significant effect on the origin of transplants (Yates-test, P = 0.05)). Most farmers in Heerhugowaard grow their own plants from seed (Table 2.5). Only 29 fields were planted with transplants obtained from commercial nurseries. Four fields were planted with transplants bought from fellow farmers. Farmers who grew their own transplants preferred to sow seed in nursing beds which were covered with different types of plastic sheets (54%). Other methods were growing transplants in glasshouses (20%) or nursery beds without plastic cover (24%).

In De Streek, 96 fields were planted with transplants obtained from commercial nurseries. Farmers who produced their own transplants grew transplants in glasshouses. None of the interviewed farmers applied direct sowing.

Differences in planting date (date of planting transplants in the fields) between the two regions are evident (Figure 2.6). White and red cabbage were planted within a short period (generally from week 19 to 23). Cauliflower had a long planting period (from week 11 to 31), related to the dispersion of harvest time.

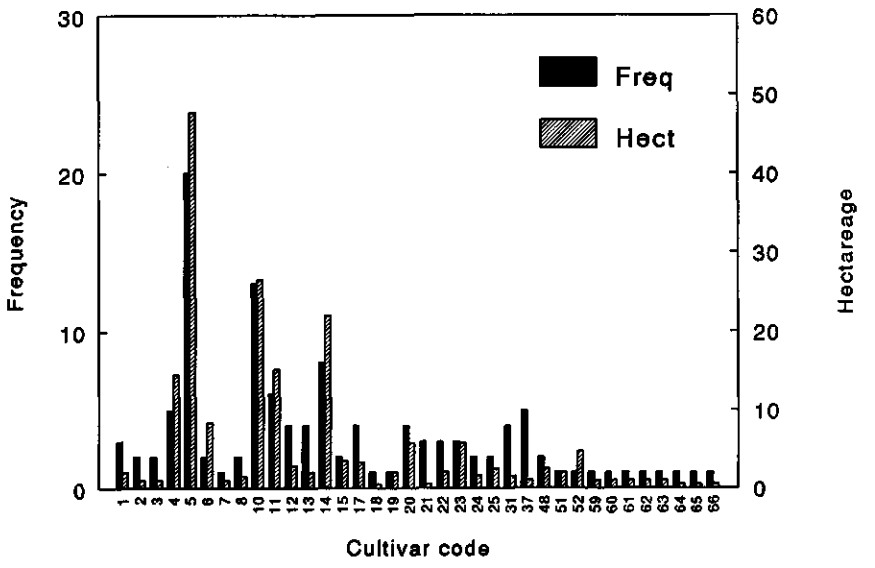


Figure 2.4. Frequency of cultivars (Freq) and hectareage per cultivar (Hect) in De Streek in The Netherlands, 1991.

Table 2.5. Origin of transplants of white and red cabbage (Heerhugowaard) and cauliflower (De Streek), and production method when home grown (The Netherlands, 1991).

	Heerhugowaard		De Streek	
	Origin of transplants	Method	Origin of transplants	Method
Home grown	98		21	
- Glasshouse		20		18
- Nursing bed		24		2
- Nursing bed covered with plastic		54		1
Commercial nursery	29		96	
Direct seeding	0		0	
Other	4		0	

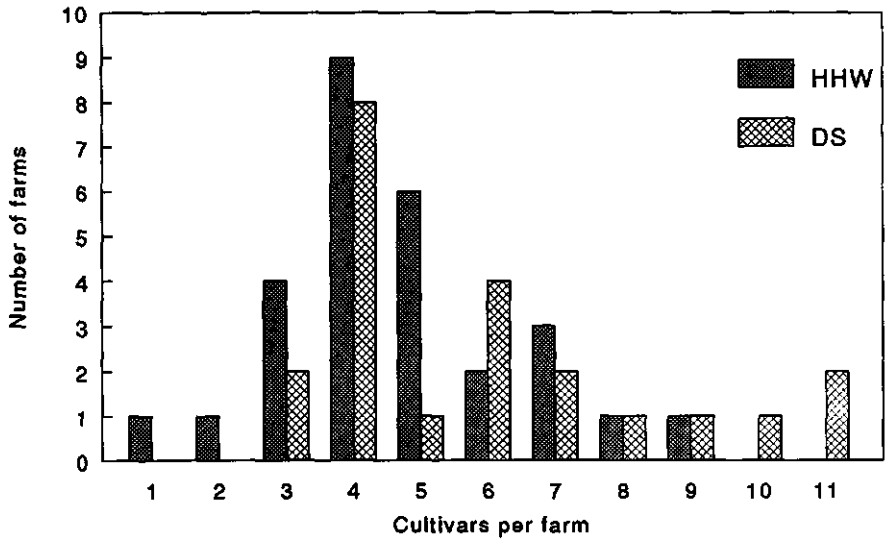


Figure 2.5. Cultivars per farm in Heerhugowaard (HHW) (white and red cabbage) and in De Streek (DS) (cauliflower) (The Netherlands, 1991).

Farmers' practices

Weed control. Farmers' practices on weed control were generally the same for Heerhugowaard and De Streek (Table 2.6). All farmers performed weed control in the fields by herbicide treatment or hand weeding. Very few farmers practised weed control in the ditches surrounding their fields because the regional waterway management provides for weed control in ditches, done during autumn and winter.

Crop rotation. Generally, farmers in Heerhugowaard cropped cabbage without crop rotation (50%) or with a rotation 2:3 (38%) (Table 2.6). The latter rotation was applied when farmers also grew flower bulbs. Fields were used for cabbage during two years and for bulbs for one year. Cauliflower (De Streek) is cropped practically continuously. A few farmers exchange fields with fields of life-stock farmers (rotations 3:4 and 2:3).

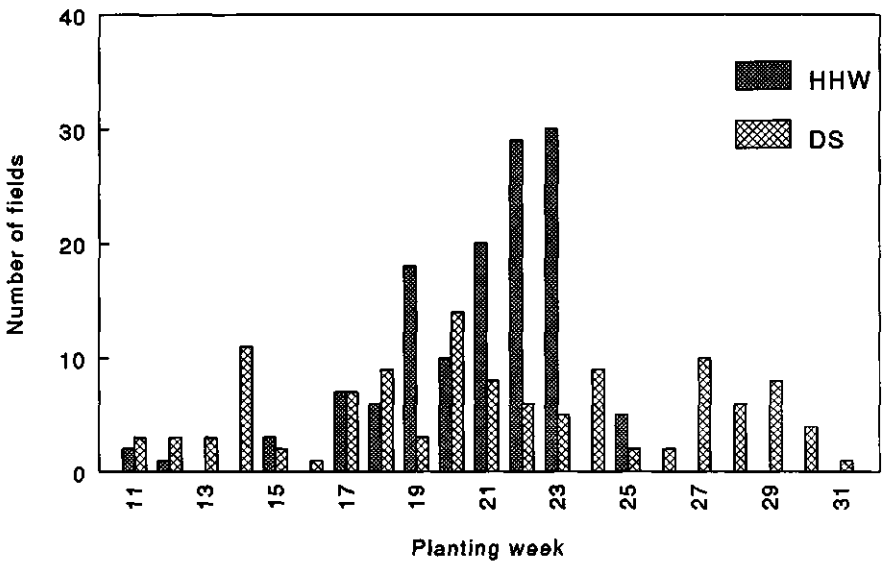


Figure 2.6. Number of fields per planting date (week number) in Heerhugowaard (HHW) (white and red cabbage) and in De Streek (DS) (cauliflower) (The Netherlands, 1991).

Table 2.6. Weed control and crop rotation in white and red cabbage (Heerhugowaard) and cauliflower production (De Streek) (The Netherlands, 1991).

	Weed control [y]				Crop rotation [z]				
	Field		Ditch						
	Yes	No	Yes	No	1:1	3:4	2:3	1:2	1:3
Heerhugowaard	28	0	3	25	66	3	50	11	1
De Streek	22	0	1	21	108	4	5	0	0

[y] Per farm
[z] Per field

Plant refuse piles. Results on plant refuse piles differed between the regions (Figure 2.7). Farmers in Heerhugowaard destroyed plant refuse by feeding it to cattle or by delivering it to commercial composters. On eleven farms, refuse piles were found on farmyards, behind barns, in ditches, at field entrances, and close to nursery beds. Refuse piles arose from preparing cabbage heads for commercial delivery. Farmers in De Streek only produced refuse piles when they grew white or red cabbage. In the case of cauliflower, farmers did not produce refuse piles since preparation of the cauliflower for commercial delivery is done on the field.

Plant residue management. Plant residue management was significantly different between the two regions (Yates-test, category "other" excluded, $P = 0.05$). Farmers in Heerhugowaard chopped the plant residue into small pieces before ploughing (Table 2.7). Plant residue was both chopped and ploughed under in autumn for the majority of the fields (50). Depending on clay content, weather and labour availability, several fields were chopped in autumn and ploughed under in spring. Within farms, both types of plant residue management could exist side by side. Remarkable are the results from De Streek, where plant residue was grazed by cattle for several fields. Besides, several life-stock farmers collected cabbage residue to feed to their cattle.

Table 2.7. Plant residue management for cabbage and cauliflower cropping in Heerhugowaard and De Streek (The Netherlands, 1991).

	Heerhugowaard	De Streek
Ploughed under in autumn	8	14
Ploughed under in spring	8	0
Chopped and ploughed under in autumn	50	24
Chopped in autumn and ploughed under in spring	39	11
Grazed by cattle	21	3
Other	5	60 [z]

[z] Other means no clear management

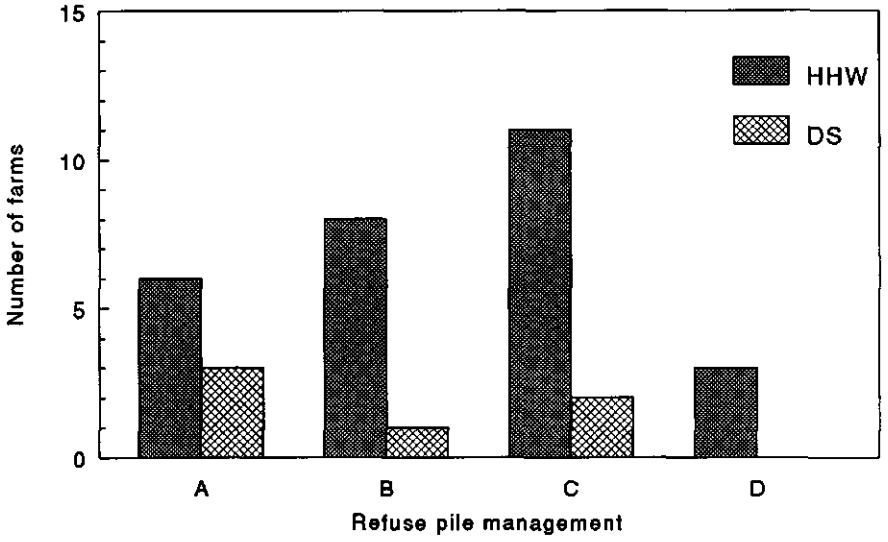


Figure 2.7. Plant refuse piles per farm in Heerhugowaard (white and red cabbage) and in De Streek (cauliflower) (The Netherlands, 1991). A = destruction, B = spread out over field, C = piles on farm, D = other method.

Table 2.8. Black rot levels in Heerhugowaard and De Streek in The Netherlands, 1991 to 1993. A, B and C: observation in week 27-28, 37-38, and 44-45, respectively.

Black rot level	Heerhugowaard					De Streek				
	91A	91B	91C	92	93	91A	91B	91C	92	93
0	131	78	23	91	74	72	65	37	82	50
1	0	33	19	28	24	22	23	26	24	31
3	0	6	12	10	5	0	15	4	5	15
6	0	0	8	1	0	0	0	1	0	4
9	0	0	0	0	0	0	0	0	0	0
Other [z]	0	14	69	0	0	23	14	49	0	0

[z] Crop was harvested or field was not visited

Black rot level

Black rot level and seasonal effects. Black rot levels were time dependent (Table 2.8). Black rot levels increased during the season 1991. Black rot was not found on the first observation date, possible because this date was close to the start of the growing season. Results of the third observation date are based on limited information since 59 fields (out of 131) had been harvested in week 44. A few fields could not be visited as the farmers' cooperation was limited.

Annual effects influenced black rot levels. In both regions, black rot level was highest in 1993, at least in De Streek.

At all observation dates, the surveyors gave a general impression on aggregation of black rot. In a few fields, surveyors found some aggregation at field entries and along the tractor tracks in the field made by the application of chemicals. Aside from this aggregation, supposedly due to dispersal by machinery, black rot was randomly distributed. Random distribution of black rot might mean that many different sources were present in the fields, that one or a few individual sources infected entire fields, or that the black rot infection came from soil.

Table 2.9. Frequency of black rot level in several selected fields cropped with the cultivars Bartolo (BA), Bingo (BI), Galaxy (GA), Roxy (RO), and Slawdena (SL) (Heerhugowaard) and Frimond (FR), Plana (PL) and Sernio (SE) (De Streek) in The Netherlands, week 37 and 38, 1991.

Black rot level	BA	BI	GA	RO	SL	FR	PL	SE
0	9	9	12	6	10	5	10	12
1	3	4	4	3	2	1	2	5
3	0	2	1	1	0	2	1	3
6	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0
Total	12	15	17	10	12	8	13	20

Black rot level and cultivar effects. The black rot level may depend on cultivar since cultivars differ in resistance against pathogens. To obtain more insight in cultivar effects, black rot levels of cultivars Bartolo, Bingo, Galaxy, Roxy, and Slawdena (Heerhugowaard) and Frimond, Plana, and Sernio (De Streek) were listed (Table 2.9).

A difference in cultivars may have existed. However, black rot levels within cultivars varied per field. Many fields were free from black rot symptoms while other fields with the same cultivar were infected up to 50% of the plants (level 3). Thus, the black rot level may be related to cultivars, but other factors as refuse piles and plant residues also may have played a (major) role in disease development.

Black rot level and plant refuse piles. Plant refuse piles can be a source of inoculum for black rot infections. Therefore, black rot level was compared with presence/absence of plant refuse piles per farm (Table 2.10). A significant relation between refuse pile management and black rot was found (Yates-test, $P = 0.05$), meaning that existence of refuse piles corresponds often with presence of high black rot intensity. Although destruction may prevent fields from black rot infection, 50% of the fields in De Streek, where destruction was the used method, were found infected. This result suggest that refuse piles were not the only infection source of black rot.

Table 2.10. Plant refuse piles and black rot in cabbage (week 37 and 38) and cauliflower in Heerhugowaard and De Streek (The Netherlands, 1991).

	Heerhugowaard		De Streek	
	No [x]	Yes [y]	No	Yes
Destruction	24	1	18	9
Spread over fields	27	11	3	8
Piles on farmyard	36	17	11	6
Other method	13	2	38	15
No data available [z]	14		9	

[x] No: black rot level was 0
[y] Yes: black rot level was 1, 3, 6, or 9
[z] No data available since fields were harvested

Black rot level and plant residue management. Black rot levels were compared with plant residue management (Table 2.11). Obviously, none of the practised plant residue management methods completely prevented black rot development. The result could mean that none of the methods is good enough to prevent cabbage for black rot infection. However, plants may be infected in the nursing beds, infection may have come from other inoculum sources, or seeds may have been infected. Thus the effect of plant residue management on black rot infection, based on this survey, is not clear.

2.5 Conclusions

The results of the exploratory survey illustrate that farmers' practices and crop management varied per region, per farm, and per field. A few practices and cropping aspects were uniform within one or both regions, such as N-nutrition and weed control.

Table 2.11. Plant residue management and black rot level (in weeks 37 and 38) in cabbage and cauliflower in Heerhugowaard and De Streek (The Netherlands, 1991).

	Heerhugowaard		De Streek	
	No [w]	Yes [x]	No	Yes
Ploughed in autumn	6	2	17	2
Ploughed in spring	3	1	- [y]	-
Chopped and ploughed under in autumn	30	4	16	8
Chopped in autumn and ploughed under in spring	28	16	6	4
Grazed by cattle	8	13	30	22
Other	3	2	3	-
No data available [z]	15		9	

[w] No: black rot level was 0
[x] Yes: black rot level was 1, 3, 6, or 9
[y] -: this type of plant residue management was not used
[z] No data available since fields were harvested

Major themes in black rot development are shown in Figure 2.8. In the present survey, no attention was given to seed infection, although it is know from literature that black rot can originate from infected seed (Williams, 1980). The effect of seed could not be analyzed because no seed was available. It was all used to obtain the transplants.

As black rot infection varied over the years, the climate may have influenced black rot development.

Survey data indicated that cropping aspects and farmers' practices may be important. Each individual factor, such as field size and plant refuse piles, has to be analyzed individually to point out if the factor is important to black rot development or not (monofactorial experiments). However, black rot development at farm level can be the outcome of a combination of different cropping aspects and farmers' practices. An interrelation between various aspects and practices may exist. The survey data cannot be analyzed completely for interrelations, since the number of fields with equal background for cropping aspects and farmers' practices is low (almost all combinations give frequencies of one or two).

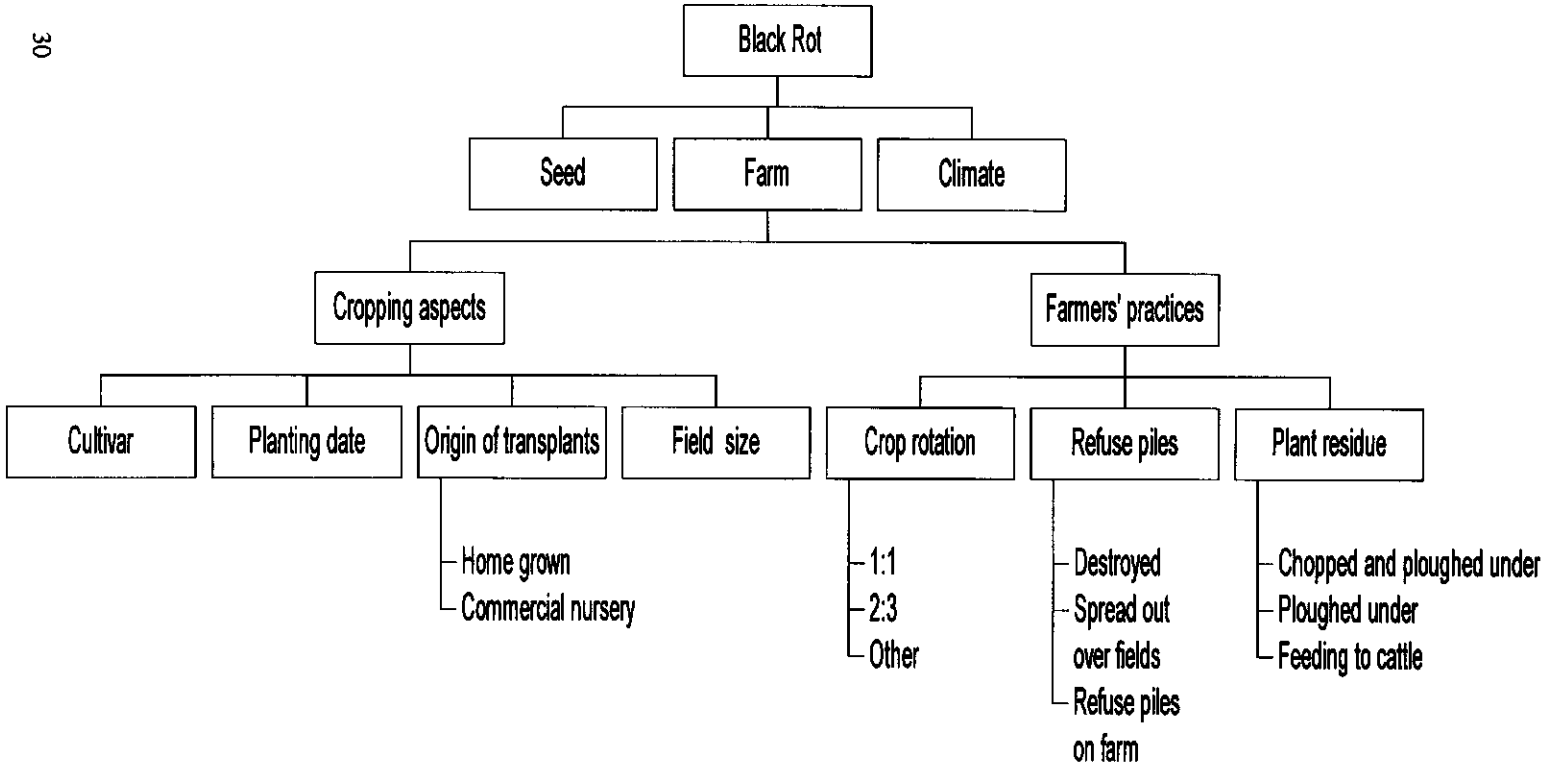


Figure 2.8. Major themes and aspects concerning black rot of cabbage.

Chapter 3

Cabbage Refuse Piles as Sources of Black Rot Epidemics

C.G. Kocks and J.C. Zadoks
Plant Disease 80: 789-792

Abstract: During three consecutive years, the effects of cabbage refuse piles, infected with the bacterium *Xanthomonas campestris* pv. *campestris*, on black rot epidemics in cabbage were investigated. Field plots of cabbage were infested by placing old (4-mo-old) or fresh (2-wk-old) refuse piles in the center. Infection of the plots from seed and from unknown sources in or around the plots could be excluded by appropriate experiments, farm history analysis and visual observation. Black rot development in the plots was far more intensive with fresh than with old refuse piles. During all three years, cabbage plots infested with old refuse piles had 1% diseased plants per plot and an average of 0.02 diseased leaves per plant. In contrast, fresh refuse piles resulted in 30-70% diseased plants and 1.0 to 3.5 diseased leaves per plant. Typical disease foci developed around the fresh refuse piles. Black rot development was positively correlated to the number of days with rainfall between 06.00-09.00 h during May and June. Refuse piles are common in Dutch growing areas and thus may be serious sources of inoculum for black rot epidemics.

3.1 Introduction

Black rot in cabbage crops, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*X.c.* pv. *campestris*), is an important disease of crucifers worldwide (Williams, 1980). *X.c.* pv. *campestris* colonizes the vascular system (Cook *et al.*, 1952a). Characteristic black rot symptoms are V-shaped lesions at leaf margins with black veins, chlorosis and necrosis (Sutton and Williams, 1970).

In spite of intensive research, black rot still is not controlled sufficiently. Sanitation is an important component in the control of black rot (Williams, 1980). Sanitation is the action that reduces, excludes or eliminates the initial inoculum from which epidemics start (Van der Plank, 1963). Plant quarantine, crop rotation, seed disinfection, elimination of refuse piles, and eradication of alternative hosts are general sanitation practices (Zadoks and Schein, 1979). The importance of sanitation has been demonstrated for e.g. *Phytophthora infestans* (Mont.) de Bary (Bonde and Schultz, 1943), *Puccinia graminis* Pers. (Lehmann, Kummer & Pannenmann) (Stakman and Fletcher, 1930), and *Puccinia striiformis* Westend. (Erikss. & Henn.) (Zadoks, 1961). For sanitation to be fully effective, the various primary inoculum sources must be identified.

The importance of seedborne inoculum (Schaad *et al.*, 1980), soilborne inoculum (Ruissen *et al.*, 1990; Schaad and White, 1974b) and weedborne inoculum (Schaad and Dianese, 1981) of *X.c.* pv. *campestris* has been demonstrated. Recently, Dzhalilov and Tiwari (1995) estimated the survival time of *X.c.* pv. *campestris* in soil and host plant debris, but they did not relate *X.c.* pv. *campestris* presence in host plant debris to black rot epidemics. In The Netherlands, where cabbage is grown on small fields by farmers using much manual labor, refuse piles occur frequently. Especially with storage of cabbage, farmers daily prepare cabbage heads by removing diseased parts of the heads so that the piles are plenished daily. The present study reports on infected host plant debris in old and fresh refuse piles as a source of bacteria for black rot epidemics.

3.2 Materials and methods

Plot establishment. Black rot epidemics in cabbage were studied in Emmen (coordinates 52.46N, 6.58E), The Netherlands, during 1992, 1993, and 1994. The white cabbage (*Brassica oleraceae* L. convar. *capitata* (L.) Alef var. *alba* DC) cultivar Perfect Ball was used as the host plant. Perfect Ball is very susceptible to black rot. Plants were grown in a greenhouse until the six leaf stage and acclimatized to outside conditions for two days before transplanting. To test for seed infection with *X.c.* pv. *campestris*, 80 randomly selected transplants were returned to the greenhouse to be observed for symptoms. Four plots were planted in May 1992, 1993, and 1994 (Table 3.1) (in general, farmers plant cabbage in April and May). Plots were planted in soil where no cabbage had been grown for at least 30 yr, according to the farm records. To prevent infections by soilborne inoculum from the plots of 1992 or 1993, plots of 1993 and 1994, respectively, were planted on other locations in the field than in 1992 or 1993.

Each year there were two treatments, "fresh" and "old" refuse piles in two replicates. Plots comprised 14 x 14 plants in a square grid with interplant distances of 0.5 m. Plots were separated by 10 m borders of oats to avoid interplot interference. Trap plants (25 each yr) were randomly placed in the vicinity of the plots (up to 70 m distance away from the plots) to check for *X.c.* pv. *campestris* infections outside the plots. Presence and absence of rain between 06.00 and 09.00 h were recorded daily.

Table 3.1. Dates of planting, plot infestation with old and fresh refuse piles, sampling pile infectivity determination, and black rot assessment during 1992, 1993, and 1994. Dates are given in Julian days.

		Plot infestation		Sampling pile infectivity		
Year	Planting	Old	Fresh	First	Second	Black rot assessment
1992	126	42	112	126	180	126, 171, 192, 206 [z], 213
1993	133	41	111	133	186	133, 176, 206, 229, 251
1994	129	47	117	129	188	129, 176, 193, 224

[z] Only one replicate observed due to heavy rainfall

Preparation of refuse piles. One isolate of *X.c.* pv. *campestris* (isolate PD 714, Culture Collection Plant Protection Service, Wageningen, The Netherlands) was bused in all years. A culture of *X.c.* pv. *campestris*-PD-714 was grown on Yeast-Peptone-Glucose agar (YPG). Inoculum was prepared by suspending 48-h-old *X.c.* pv. *campestris* colonies in distilled water (density approximately 10^8 cfu/ml).

Five leaves of eight-wk-old cabbage plants were injected at the base of the leaf close to the petiole with about 0.03 ml inoculum. Plants were placed in the greenhouse until symptoms developed. Plants with five symptomatic leaves were harvested (ten-wk-old plants). Leaves were cut off close to the stem and stems were cut off just above the ground. Stems and leaves were chopped and then mixed to form cabbage 'debris'.

Each plot was infested, within 4 h after debris was prepared, by building a refuse pile with 15 kg of debris on the soil surface in the plot center. At introduction, the piles measured 50 x 50 cm with a height of 30 cm. The four central plants of each plot were not planted since the refuse piles were placed here.

The two treatments were old and fresh refuse piles. To obtain old refuse piles, plants were inoculated in January and plots were infested in February (Table 3.1), and fresh refuse piles by inoculating plants in March and infesting plots in April (Table 3.1).

To check the infectivity of the refuse piles, they were sampled at planting time and in early summer (Table 3.1). An auger (diameter 1.6 cm) was used to take four

subsamples per pile (0 - 10 cm depth in refuse piles). Subsamples were mixed and 25 g of the mixture was macerated in 50 ml sterile water. Per refuse pile, one leaf on each of five test plants were inoculated with 0.03 ml suspension. Test plants were scored for absence/presence of symptoms three wk after inoculation. Koch's postulates were applied to symptomatic leaves.

Disease assessment. Black rot was assessed 4 or 5 times per summer until maturity of the crop was reached. In the plots, symptom expression was recorded by visual assessment of disease incidence (absence/presence of disease) and diseased leaf incidence (the number of diseased leaves) on each individual plant. Plots were visited only when leaves were dry to avoid possible mechanical dispersal of *X.c.* pv. *campestris* by the observer. Only one replicate could be observed on 25 July 1992 due to heavy rainfall.

Data analysis. Per plot, disease progress curves were analyzed by means of the Gompertz model, applied to incidence as well as diseased leaf incidence. Year effects on black rot development, the latter expressed as r x K values (Campbell and Madden, 1990), were tested using LSD ($P \leq 0.05$), with r = estimated infection rate (day^{-1}) and K = estimated final disease intensity. The values for r and K were estimated using the Genstat 5.1 nonlinear curve fitting procedures. Final observed disease intensities were tested for significant differences at 95% probability using Fisher's Least Significant Difference (LSD).

3.3 Results

No seed infection of the planting material was observed in the greenhouse tests, as none of the tested plants developed black rot symptoms. In 1993, only one trap plant (36 m from the nearest plot) developed black rot symptoms at day 251. No black rot symptoms were found in trap plants during 1992 and 1994.

Decomposition of plant material in refuse piles was observed for old and fresh refuse piles. Regrowth from chopped plant material was observed for old refuse piles sometimes resulting in a few flowering plants. All refuse piles contained viable *X.c.* pv. *campestris* (Table 3.2). No significant differences between old and fresh refuse piles were observed ($P \leq 0.05$). Application of Koch's postulates proved that all symptoms observed on inoculated test plants recognized as black rot symptoms were caused by *X.c.* pv. *campestris*.

Table 3.2. Infectivity of refuse piles. Number of diseased leaves observed 3 wk after inoculation with 0.03 ml *X.c.* pv. *campestris*-suspension extracted from refuse piles.

	First sampling [x]		Second sampling [x]	
Plot [w]	Old [y]	Fresh [y]	Old	Fresh
1992-A	5	5	5	4 [z]
1992-B	5	4	5	5
1993-A	4 [z]	4 [z]	5	5
1993-B	4 [z]	5	3 [z]	5
1994-A	5	5	4	4 [z]
1994-B	4	5	4 [z]	4

[w] A and B refer to replicates.

[x] First sampling: day 126 (1992), 133 (1993), and 129 (1992). Second sampling: day 180 (1992), 186 (1993), and 188 (1992).

[y] One leaf on each of five plants were inoculated per sample.

[z] Asymptomatic leaves with doubtful symptoms were not scored.

In the field, symptoms were detected in June in plots with fresh refuse piles, and in July in plots with old refuse piles. Age of the refuse piles had a significant effect on final disease incidence and diseased leaf incidence ($P \leq 0.05$) (Figure 3.1). In plots with fresh refuse piles, disease incidence and diseased leaf incidence were considerably higher than in plots with old refuse piles. Black rot foci appeared around fresh refuse piles (Figure 3.2). Largest distance between old refuse piles and diseased plants in the concerning plots was 0.5 m, 1.5 m, and 0.7 m in 1992, 1993, and 1994, respectively.

The Gompertz model adequately described variation of disease in time. Residuals were randomly scattered and R^2 values ranged from 0.97 to 0.99. Gompertz disease progress curves for incidence for 1992, 1993 and 1994 are shown in Figure 3.3.

A comparison among years was made for plots with fresh refuse piles. Significant differences in r x K values for disease incidence and diseased leaf incidence were observed among yr (Table 3.3). The r x K product was largest in 1994. Mean incidence values for r x K were 2.61, 1.89, and 4.59 for 1992, 1993, and 1994, respectively. Mean diseased leaf incidence values for r x K were 0.16, 0.08, and 0.30 for 1992, 1993, and 1994, respectively.

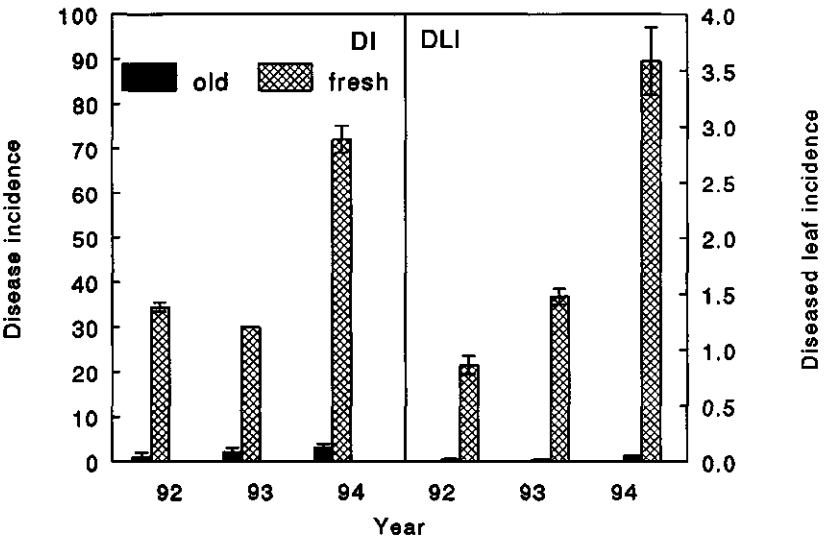


Figure 3.1. Final black rot incidence (DI) and diseased leaf incidence (DLI) of cabbage grown around old and fresh refuse piles in 1992, 1993 and 1994. Bars and error bars represent average of replicates and deviation from the average, respectively.

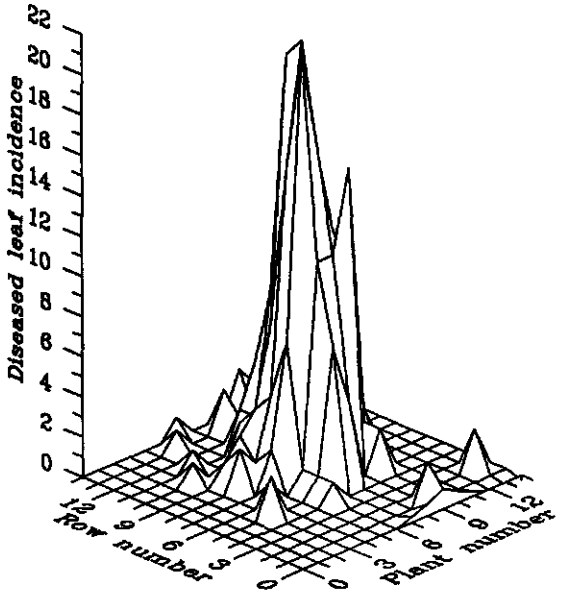


Figure 3.2. Typical black rot focus in cabbage plot. The graph shows diseased leaf incidence data on day 251, 1993 for a plot infested with a fresh refuse pile.

Frequency of rainfall between 06.00 and 09.00 h during May and June was 14, 13, and 22 days for 1992, 1993, and 1994, respectively. Black rot progress correlated positively with this rain frequency, with correlation coefficients of 0.96 and 0.94 for disease incidence and diseased leaf incidence, respectively, ($n=6$, $P \leq 0.05$).

3.4 Discussion

Outside sources of inoculum apparently were not involved in our experiments. The farm history records indicated that no cabbage had been grown for 40 years. Visual inspection of the farm and its surrounding did not reveal potential inoculum sources. The greenhouse tests and trap plant tests indicated that seed contamination of planting material and natural infection of transplanted material were absent or at least did not interfere with the results. In all plots, severe epidemics corresponded with young refuse piles and light epidemics with old refuse piles. Control plots

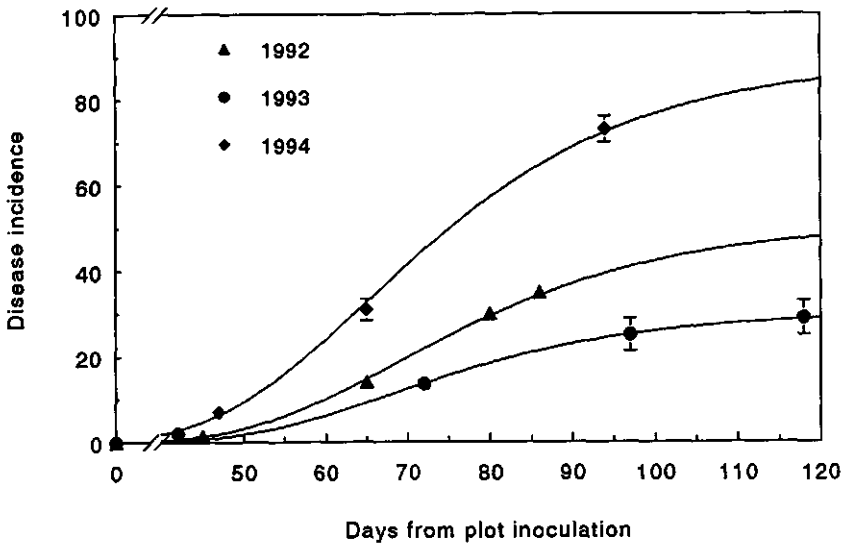


Figure 3.3. Gompertz progress curves for black rot disease incidence in plots with a fresh refuse pile (means of two replicates with standard error bar) for disease incidence in 1992, 1993, and 1994. Where markers do not have an error bar, the error bars are too small to plot.

Table 3.3. Seasonal effects on black rot development in cabbage. Estimated Gompertz rate parameters (r), estimated final disease intensities (K), and r x K values in the cabbage cultivar Perfect Ball grown around fresh refuse piles during 1992, 1993, and 1994.

	Disease incidence			Diseased leaf incidence		
Plot [y]	r	K	r x K	r	K	r x K
1992-A	0.05	51	2.55	0.15	1.15	0.17
1992-B	0.05	53	2.67	0.11	1.33	0.15
1993-A	0.03	46	1.38	0.03	2.45	0.07
1993-B	0.07	30	2.10	0.05	1.85	0.09
1994-A	0.05	89	4.81	0.07	4.39	0.31
1994-B	0.05	86	4.38	0.06	4.79	0.29
Mean values [z]						
1992			2.61 b			0.16 b
1993			1.89 a			0.08 a
1994			4.59 c			0.30 c

[y] A and B refer to replicates

[z] r x K values were compared by analysis of variance. Means were separated by LSD at $P \leq 0.05$. Values within a column followed by the same letter are not significantly different.

without refuse piles were absent but they would not have improved the evidence. We conclude that the black rot epidemics in the plots were due to inoculum from the refuse piles. The appearance of typical foci (Zadoks and Van den Bosch, 1994) around the refuse piles confirmed the conclusion that these refuse piles were the exclusive sources of initial inoculum in all plots.

At planting time, the old refuse piles consisted mainly of partly decomposed plant material whereas the fresh piles also contained green leaves and stems, with little decomposed plant material. Decomposition may have reduced the total amount of *X.c.* pv. *campestris* in the refuse piles. All refuse piles contained infective *X.c.* pv. *campestris* and showed to be infectious, but considerably more disease occurred in cabbage grown around fresh refuse piles. Data on populations of *X.c.* pv. *campestris* in old and fresh refuse piles are not available. We assume that the difference in

infectivity of the refuse piles is related to the differences in composition of the piles and in reduction of *X.c.* pv. *campestris*.

Black rot symptoms were detected in June in plots with fresh refuse piles, and in July in plots with old refuse piles. The time between infection and symptom expression (incubation period) is related to the initial density of bacteria in the leaf and the temperature. The incubation period will be longer when initial density is low and/or with low temperatures. We assume that the difference in first symptom expression among the old and fresh refuse piles is related to a difference in relative population of *X.c.* pv. *campestris* in the piles.

The Gompertz model was used to describe disease progress curves per plot since the Gompertz model is the better model for pathogens to which plants become less susceptible with age (Gottwald *et al.*, 1988). Bain (1955) and Hunter *et al.* (1987) demonstrated that susceptibility to black rot decreased with plant age. Using r x K values, significant year effects in black rot development could be demonstrated. Gottwald *et al.* (1988) suggested that the spread of inoculum from lesions oozing bacteria of *Xanthomonas campestris* pv. *citri* was related to wind speed and wind direction. In general, oozing of bacteria of *X.c.* pv. *campestris* occurs when guttation is present. Thus, water splash by rain during guttation is an important factor in the dispersal of black rot in cabbage. We expect early morning rain to be more important than rain at other times of the day since guttation is most likely during the early morning. High frequency of early morning rainfall during May and June was correlated with high r x K values, in accordance with Cook *et al.* (1952a) and Williams (1980), who found rain to be an important factor in black rot epidemics.

The present study shows that cabbage refuse piles can be important primary sources of black rot infection. Elimination of refuse piles may help to control *X.c.* pv. *campestris* and other pathogens such as *Mycosphaerella brassicicola* (Duby) Oudem. (Chupp and Sherf, 1960), *Plasmodiophora brassicae* Woron. (Sherf and MacNab, 1986), and *Phoma lingam* (Tode ex Fr.) Desm (Sherf and MacNab, 1986). The practice of sanitation should be of concern to all cabbage growers. It should include removal of refuse piles and removal of plant residues in addition to the control of cruciferous weeds and the use of pathogen-free seed.

The importance of refuse piles relative to other sources of infection may vary per region. In The Netherlands, where cabbage is grown on small fields by farmers using much manual labor, refuse piles occur frequently. Several of these piles are old. However, in case of storage of cabbage, a farmer sells some of his cabbage to auctions every day. Therefore, farmers prepare cabbage heads by removing diseased

leaves from the heads so that the piles are plenished daily. Farmers do not chop refuse as we did to construct experimental refuse piles. We suppose that chopping does not materially effect the difference between old and fresh refuse piles.

The senior authors did an exploratory survey during 1991-1993 (data not shown) and found refuse piles on 40% of the visited cabbage growing farms. Piles were found on farmyards, behind barns, in ditches, at field entrances, and close to nursing beds. In the Dutch situation of intensive cabbage cultivation, we consider refuse piles to be important potential sources of black rot infection, especially when they are close to plants in nursing beds or fields. In areas with large scale mechanized cabbage farming, as in parts of the USA, the relative importance of refuse piles may be far less than in the Netherlands and some other parts of Europe.

Chapter 4

Measuring Field Resistance of Cabbage Cultivars to Black Rot

C.G. Kocks and M.A. Ruissen
Euphytica: 91:45-54

Abstract: Disease progress and gradient curves of black rot on cabbage were evaluated in field plots of the cultivars Bartolo, Erdeno, Perfect Ball, and Roxy in The Netherlands during 1991 and 1992. Plots were inoculated by single sources in the centre of each plot. Individual plants were examined for diseased leaf incidence and severity. Disease progress was described by the Gompertz model. The overall measure of absolute rate (disease progress rate r multiplied with maximum disease intensity K) was used to compare cultivar effects on disease progress. Disease gradients were described by the negative exponential model. The percentile distance (distance from the source at which disease intensity reached 1% of the empirical maximum disease intensity) was used to compare cultivar effects on disease spread. Disease severity is more sensitive than diseased leaf incidence to calculate the disease progress and spread of black rot. Measures of progress and gradient were about equally effective to screen cultivars for field resistance to black rot. Perfect Ball was the most susceptible, Erdeno and Bartolo were intermediate and Roxy was the most resistant for leaf incidence and severity measures. Increased levels of field resistance reduced the development of black rot in time and in space. Field resistance of black rot is thought to be composed of several mechanisms. Microplots provide a good instrument for the assessment of small differences in field resistance, expressed equally well in disease progress as in disease gradient curves.

4.1 Introduction

Black rot of cabbage caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*X.c.* pv. *campestris*) is commonly found on cultivated cruciferous plants throughout the world (Williams, 1980). *X.c.* pv. *campestris* is seedborne, moves systemically through the plant after seed germination, and can spread to adjacent plants by rain splash and mechanical transmission (Williams, 1980). Invasion can occur through wounds (Williams, 1980) but hydathodes at the leaf margins are the primary site of entry (Cook *et al.*, 1952a; Ruissen and Gielink, 1994). Important ways to control black rot are elimination of sources of inoculum and breeding for resistance.

Some information on varietal resistance to black rot is available (Pillard *et al.*, 1991; Staub and Williams, 1972). Many breeders came to the conclusion that, in addition to the hypersensitive resistance which can apparently be overcome by a pathogen, a substantial degree of field resistance is required. Field resistance is

defined as any resistance which effects epidemics in the field but which is not immediately apparent in laboratory or glasshouse tests (Robinson, 1969). No information, however, was available on the effect of field resistance on temporal and spatial dynamics of black rot.

The purpose of this study is to *i*) compare cultivars as to progress of black rot epidemics, *ii*) compare cultivars as to disease spread of black rot, and *iii*) quantify effects of field resistance on disease progress in time and in space.

4.2 Materials and methods

Experimental plots. Black rot epidemics in cabbage were studied in Wageningen (coordinates 51.57N, 5.31E), 1991, and Emmen (coordinates 52.46N, 6.58E), 1992, The Netherlands. The white cabbage (*Brassica oleraceae* L. convar. *capitata* (L.) Alef var. *alba* DC) cultivars Bartolo, Erdeno and Perfect Ball were used in 1991. In 1992, the white cabbage cultivars Bartolo and Perfect Ball and the red cabbage (*B. oleraceae* L. convar. *capitata* (L.) Alef var. *rubra* DC) cultivar Roxy were used. The plants were grown in greenhouses until plants reached the six leaf stage and acclimatised two days before transplanting. Microplots (Zadoks and Schein, 1979) were planted on 17 May 1991 and 6 May 1992.

The experimental design in 1991 was a latin square with three by three treatments. A randomized block design with two by three treatments was used in 1992. Each plot comprised 14 x 14 plants in a square grid with interplant distances of 0.75 m in 1991 and 0.5 m in 1992. Plots were separated by 6 m borders of winter wheat (1991) or spring oats (1992).

Inoculum preparation. As proposed by Porta-Puglia (1994) and Parlevliet (1979), we used one isolate of the bacterium *X.c.* pv. *campestris* (isolate PD 714, Culture Collection Plant Protection Service, Wageningen, The Netherlands) for both years. Inoculum was prepared by suspending *X.c.* pv. *campestris* in distilled water (density approximately 10^8 cfu/ml).

In each plot, an inoculum source was established. In 1991, four week old plants (four leaf stage) were used as source plants. Four leaves per source plant were injected at the base of the leaf close to the petiole with about 0.03 ml inoculum. Inoculated plants were placed in the greenhouse to develop symptoms before transplanting. Sources were established by planting inoculated plants on 17 May.

Each source comprised six infected plants, two plants of each of the cultivars Bartolo, Erdeno and Perfect Ball. The four central plants of each plot were removed and a source was placed in the centre of each plot.

In 1992, four week old cabbage plants were inoculated with a *X.c.* pv. *campestris*-suspension (density approximately 10^8 cfu/ml) and placed in the greenhouse. After symptom appearance, plants were harvested and chopped to pieces to serve as inoculum. On 21 April, each plot was infested by placing 10 kg of chopped cabbage on the soil surface in its centre after removal of the central plants.

Disease assessment. Symptom expression was recorded by visual assessment of diseased leaf incidence and disease severity on each individual plant. Leaf incidence is defined here as the number of diseased leaves per plant and severity as the area (cm^2) of diseased leaf tissue per plant. Lesions were assessed in size classes 0, 0.5, 1, 2, 3, 5, 7.5, 10, 15, 20, 25, 50, 75, 100, 150, 200, 300, 400, and $\geq$ 500 cm^2 and severity was obtained by summarizing lesion sizes per plant. Black rot was assessed on 17 May, 4, 10, 17, and 24 July, 1, 7, 14, and 22 August in 1991 and on 5 May, 20 June, 11 July, 25 July and 1 August in 1992. Plots were visited only when leaves were dry to avoid possible mechanical spread of *X.c.* pv. *campestris* by the observers. Thus, only one replicate per cultivar could be observed on 25 July 1992 due to heavy rainfall.

Temporal analysis. Analytical models, such as the logistic and Gompertz models, can be used to assess field resistance of crops to disease for selection purposes (Zadoks and Schein, 1979). The Gompertz model is the better model for diseases in which infection sites become less susceptible with age (Headrick and Pataky, 1988). Since Bain (1952, 1955) and Hunter *et al.* (1987) demonstrated that cultivar resistance to black rot varied with plant age, the Gompertz model:

$$y = K \exp\ [-\ln (y_0 / (K-y_0)) \exp (-r\ t)] \qquad (4.1)$$

where y = disease intensity, K = maximum disease intensity, y_0 = initial disease intensity, r = infection rate (day^{-1}), and t = time in days (t = 0 at introduction of sources in 1991 and at planting time in 1992), was fitted to disease data per plot. Estimates of model parameters were obtained by nonlinear regression. Residuals were examined for goodness of fit, and the coefficient of determination (R^2) was determined. Since the maximum disease intensity (K) varied per plot, r x K was used as an overall measure of the absolute rate of disease progress (Campbell and

Madden, 1990). Statistical significance of differences in r x K values between cultivars was examined by analysis of variance. Means were separated using Fisher's Least Significant Differences (LSD) test ($P \leq 0.05$).

Spatial analysis. Spatial aspects of differences of varietal resistance to disease can be studied by gradient analysis (Zadoks and Schein, 1979). The causal agent of black rot in cabbage (*X.c.* pv. *campestris*) is a splash dispersed pathogen (Cook *et al.*, 1952a; Williams, 1980). Gradients of splash dispersed pathogens can be described by the negative exponential model (Fitt and Bainbridge, 1984). Therefore, the negative exponential model:

$$y = a \exp(-bx) \qquad (4.2)$$

where y = disease intensity, a = y intercept at $x = 0$, x = distance from the source (m), and b = steepness of the gradient (m^{-1}) was used in this study. Disease gradient data per plot were obtained by averaging disease intensity over plants for each distance from the source. Gradients were fitted to disease intensity assessed on 22 August 1991 and 1 August 1992. Goodness of fit was checked by the coefficient of determination (R^2) and by examining residuals. Percentile distances for diseased leaf incidence (PD_i) and severity (PD_s) were defined as the distance at which mean black rot intensity was 1% of the empirical maxima for leaf incidence and severity, respectively. The empirical maximum for diseased leaf incidence was 15 diseased leaves per plant (maximum observed in the experiments) and 500 cm^2 diseased leaf tissue per plant (highest summarized lesion size per plant observed in the experiments). Statistical significance of differences in percentile distance was examined by analysis of variance. Means were separated using Fisher's Least Significant Differences (LSD) test ($P \leq 0.05$).

4.3 Results

Disease progress in 1991. Symptoms were first observed 48 days after introduction of the sources and maximum symptom expression occurred after 99 days (Figure 4.1A). Mean observed maximum diseased leaf incidence was 0.16 for Bartolo, 0.23 for Erdeno, and 0.78 for Perfect Ball. Perfect Ball reached a higher maximum diseased leaf incidence then Erdeno and Bartolo ($P \leq 0.05$). The Gompertz model

(Table 4.1) adequately described variation of disease in time. Residuals were randomly scattered and R^2 values ranged from 0.80 to 0.99, except plot B2. Significant differences in r x K values were observed among cultivars (Table 4.2). Black rot leaf incidence increased fastest in Perfect Ball and slowest in Bartolo.

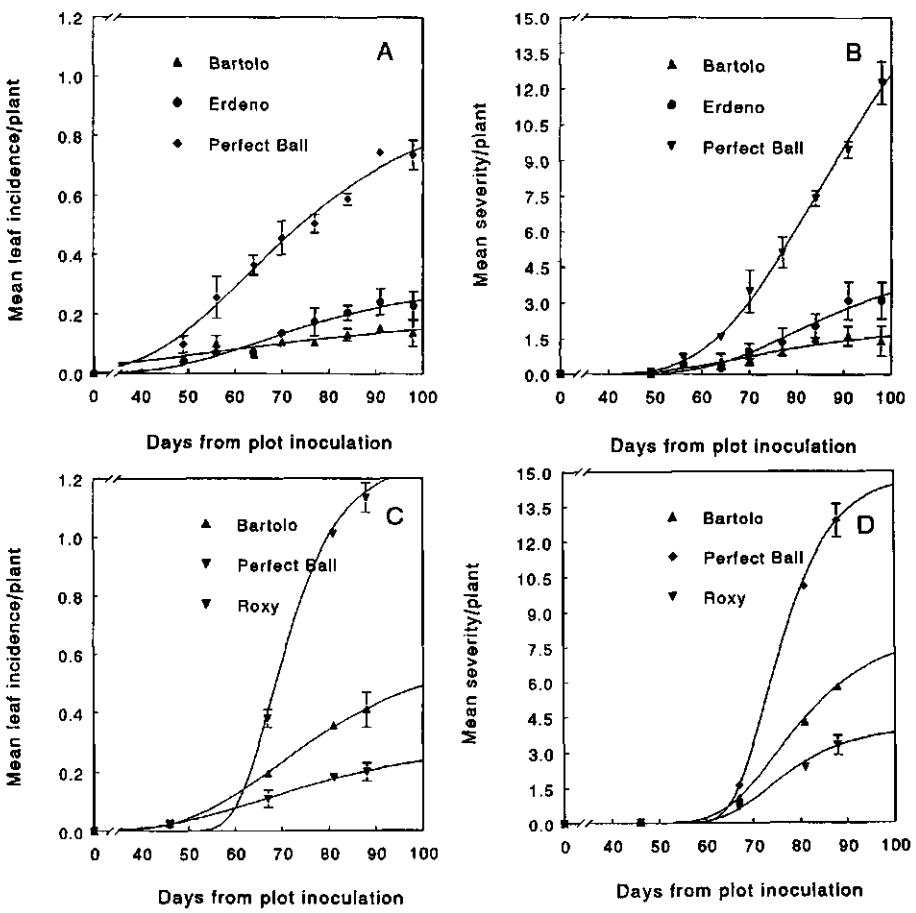


Figure 4.1A-D. Gompertz progress curves for black rot of cabbage cultivars. A: diseased leaf incidence in 1991, B: disease severity in 1991, C: diseased leaf incidence in 1992, and D: disease severity in 1992. Data points represent means of replicates.

Table 4.1. Estimated Gompertz rate parameters (r), final disease intensities (K), coefficients of determination of regression of black rot intensity with time (R^2), and r x K values in the cabbage cultivars Bartolo (B), Erdeno (E) and Perfect Ball (P) (1991).

	Diseased leaf incidence				Disease severity			
Plot [y]	r	K	R^2	r x K	r	K	R^2	r x K
B1	0.017	0.499	0.80	0.008	0.07	1.98	0.84	0.15
B2	0.083	0.102	0.58	0.008	0.12	1.11	0.54	0.14
B3	0.021	0.353	0.88	0.007	0.05	2.43	0.70	0.12
E1	0.068	0.193	0.90	0.013	0.06	2.96	0.93	0.18
E2	0.057	0.336	0.95	0.019	0.07	5.30	0.96	0.37
E3	0.083	0.156	0.95	0.013	0.03	5.50	0.79	0.14
P1	0.051	0.912	0.99	0.047	0.04	22.95	0.99	0.94
P2	0.031	1.301	0.98	0.040	0.06	14.58	0.99	0.82
P3	0.061	0.788	0.97	0.048	0.03	31.99	0.99	1.06
mean values [z]								
Bartolo				0.008 a				0.14 a
Erdeno				0.015 b				0.23 b
Perfect Ball				0.045 c				0.94 c

[y] 1, 2 and 3 correspond with the replicates

[z] r x K values were compared by analysis of variance. Means were separated by LSD ($P \leq 0.05$). Values within a column followed by the same letter were not significantly different.

Mean values for r x K were 0.008, 0.015, and 0.045 for Bartolo, Erdeno, and Perfect Ball, respectively.

Mean maximum disease severity observed on 22 August was 1.9 for Bartolo, from 3.2 for Erdeno, and from 11.8 for Perfect Ball. Perfect Ball had a significant higher maximum severity than the other cultivars ($P \leq 0.05$). The Gompertz model provided a good description of disease progress (Table 4.1 and Figure 4.1B). The R^2 values ranged from 0.70 to 0.99, except plot B2. Progress of disease severity differed significantly among cultivars and was fastest in Perfect Ball and slowest in Bartolo (Table 4.2). Mean r x K values for severity were 0.14 for Bartolo, 0.23 for Erdeno, and 0.94 for Perfect Ball.

Table 4.2. Estimated Gompertz rate parameters (r), final disease intensities (K), coefficients of determination of regression of black rot intensity with time (R^2), and r x K values in the cabbage cultivars Bartolo (B), Perfect Ball (P), and Roxy (R) during 1992.

	Diseased leaf incidence				Disease severity			
Plot [y]	r	K	R^2	r x K	r	K	R^2	r x K
B1	0.062	0.439	0.99	0.027	0.08	8.34	0.99	0.67
B2	0.045	0.809	0.99	0.036	0.09	7.97	0.99	0.72
P1	0.148	1.149	0.99	0.170	0.14	13.60	0.99	1.90
P2	0.109	1.328	0.99	0.145	0.12	16.08	0.99	1.93
R1	0.055	0.326	0.99	0.011	0.11	3.47	0.99	0.38
R2	0.036	0.291	0.99	0.016	0.10	4.60	0.99	0.46
mean values [z]								
Bartolo				0.032 a				0.70 b
Perfect Ball				0.158 b				1.92 c
Roxy				0.014 a				0.42 a

[y] 1 and 2 correspond with the replicates

[z] r x K values were compared by analysis of variance. Means were separated by LSD at ($P \leq 0.05$). Values within a column followed by the same letter were not significantly different.

Disease progress in 1992. Disease was first detected 46 days after planting (Figure 4.1C). On 1 August, mean diseased leaf incidence was 0.19 for Roxy, 0.41 for Bartolo, and 1.14 for Perfect Ball. Diseased leaf incidence differed significantly among cultivars and was highest in Perfect Ball and lowest in Roxy ($P \leq 0.05$). Parameter estimates for the Gompertz model are given in Table 4.2. Progress of diseased leaf incidence was faster in Perfect Ball than in Bartolo and Roxy ($P \leq 0.05$). Mean r x K values for diseased leaf incidence were 0.014, 0.032 and 0.158 for Roxy, Bartolo, and Perfect Ball, respectively.

Mean disease severity assessed on 1 August differed among cultivars ($P \leq 0.05$) and reached 3.3, 5.8, and 12.8 cm^2 diseased leaf tissue per plant for Roxy, Bartolo, and Perfect Ball, respectively. Cultivar effects on disease progress were evident

(Table 4.2 and Figure 4.1D). The r x K values for severity differed significantly among cultivars with mean values of 0.42, 0.70 and 1.92 for Roxy, Bartolo, and Perfect Ball, respectively.

Disease spread in 1991. The negative exponential model described the relationship of diseased leaf incidence and severity with distance from the inoculum source with R^2 values greater than 0.75 (Table 4.3), except for severity in plot B2. Spread of black rot symptoms was limited (Figure 4.2A and B). No symptoms were observed over 6.5 m from the sources. The percentile distances PD_i and PD_s for diseased leaf incidence and severity, respectively, were determined to compare cultivar effects on spread of symptoms. The highest distance was found in Perfect Ball, with a mean PD_i of 3.8 m (Table 4.3). Mean PD_i was low (2.0 m) in Bartolo and intermediate (3.0m) in Erdeno. Mean PD_s was 1.2 m, 1.9 m, and 2.7 m for Bartolo, Erdeno, and Perfect Ball, respectively.

Disease spread in 1992. Gradients of diseased leaf incidence and severity in 1992 were described adequately by the negative exponential model with R^2 values > 0.91 (Table 4.4 and Figs. 4.2C and D). No symptoms were observed over 7.0 m from the sources. PD_i and PD_s were highest in Perfect Ball and lowest in Roxy ($P \leq 0.05$). Mean PD_i was 4.4 m, 6.2 m, and 3.3 m, and mean PD_s was 2.8 m, 4.0 m, and 2.0 m for Bartolo, Perfect Ball and Roxy, respectively.

4.4 Discussion

Disease intensity measures. Diseased leaf incidence and disease severity were used to compare cultivars as to black rot development in time and space. Diseased leaf incidence has the advantage of being assessed rapidly and thus it may be preferred for epidemiological studies. Disease severity is a more accurate measure for comparing reactions of cultivars with moderate or low levels of resistance (Koch and Parlevliet, 1990). Whereas increase in leaf incidence is due to new infections, an increase in severity can be due to new infections and/or lesion growth. We propose to use disease severity measures for comparing varietal resistance since severity reflects the progress of black rot and predicts future crop loss better than leaf incidence.

Table 4.3. Estimated model parameters (*a* and *b*), coefficients of determination of regression of black rot on distance (R^2), and percentile distances of diseased leaf incidence (PD_i) and disease severity (PD_s) in the cabbage cultivars Bartolo (B), Erdeno (E), and Perfect Ball (P) in 1991.

	Diseased leaf incidence				Disease severity			
Plot [x]	a [y]	b [y]	R^2	PD_i	a	b	R^2	PD_s
B1	12.2	2.2	0.97	2.0	197.8	2.7	0.97	1.4
B2	3.4	1.2	0.78	2.6	19.3	0.9	0.58	1.5
B3	6.6	2.5	0.98	1.5	14.5	1.3	0.75	0.8
E1	3.2	1.1	0.88	2.8	29.8	0.9	0.81	2.0
E2	5.7	1.1	0.91	3.2	154.4	1.6	0.96	2.1
E3	5.6	1.1	0.94	3.1	290.3	2.8	0.97	1.5
P1	25.3	1.4	0.90	3.7	681.9	1.5	0.90	3.3
P2	19.0	1.5	0.94	3.3	602.2	2.3	0.93	2.1
P3	10.0	1.0	0.87	4.3	669.1	1.8	0.98	2.7
mean values [z]								
Bartolo				2.0 a				1.2 a
Erdeno				3.0 b				1.9 a
Perfect Ball				3.8 c				2.7 b

[x] 1, 2 and 3 correspond with the replicates

[y] parameters obtained from nonlinear regression of the negative exponential model, $y = a \exp(-bx)$, with y = disease, x = distance from the source in meters, a = y intercept at $x = 0$, and b = steepness of the gradient (m^{-1})

[z] Percentile distances were compared by analysis of variance. Means were separated by LSD at ($P \leq 0.05$). Values within a column followed by the same letter were not significantly different.

Modelling disease in time and space. Analysis of compound result of time and space was not possible because Jeger's premises (1983) were not fulfilled. Therefore, interest was focused on effects of varietal resistance on temporal and spatial development separately.

Disease progress was adequately described by the Gompertz model. Linearization of disease progress curves was thought to be inappropriate since *i*) epidemics would

be differentiated by *r* only, and *ii*) linearization requires a value for the potential maximal level of infection which is not clearly defined for black rot in cabbage and may vary among cultivars with different levels of resistance, which may in turn vary in time. The *r* x *K* value appeared to be a good parameter to reflect the overall effect of black rot epidemics since both final disease intensity and rate parameter were taken into account.

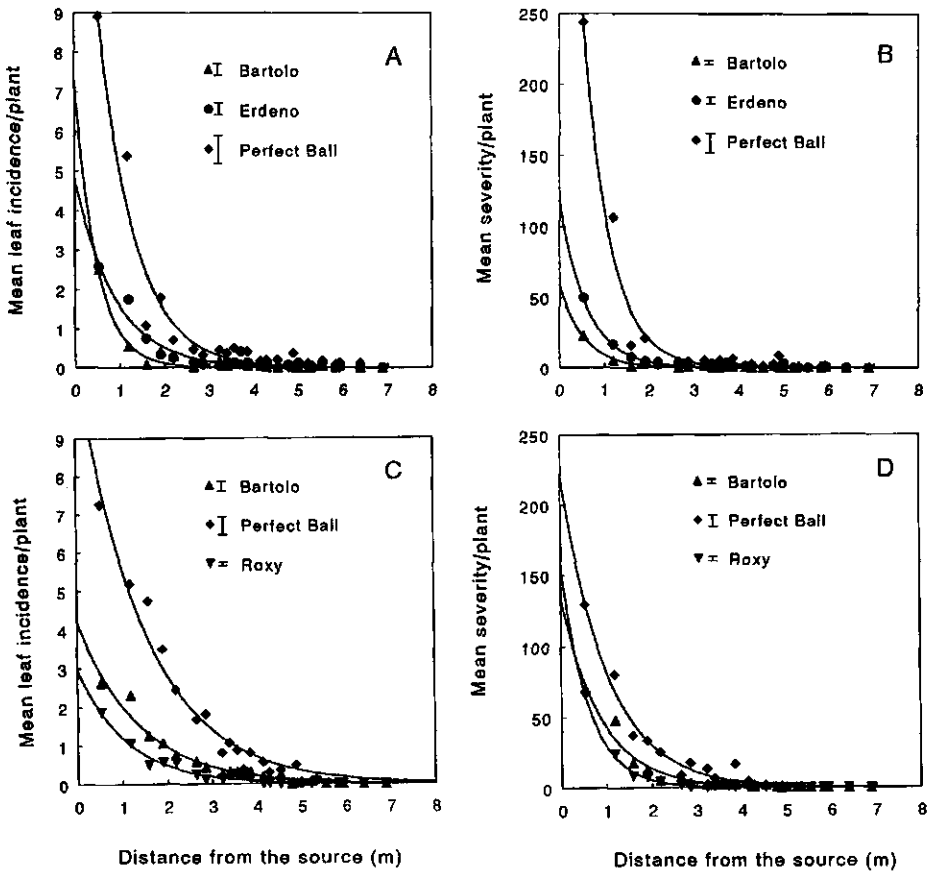


Figure 4.2A-D. Gradients of black rot of cabbage cultivars fitted by the negative exponential model. A: diseased leaf incidence on 22 August 1991, B: disease severity on 22 August 1991, C: diseased leaf incidence on 1 August 1992, D: disease severity on 1 August 1992. Data points represent means of replicates and error bars represent mean error of replicates.

Table 4.4. Estimated model parameters (*a* and *b*), coefficients of determination of regression of black rot on distance (R^2), and percentile distances of diseased leaf incidence (PD_i) and disease severity (PD_s) in the cabbage cultivars Bartolo (B), Perfect Ball (P), and Roxy (R) in 1992.

	Diseased leaf incidence				Disease severity			
Plot[x]	a^y	b^y	R^2	PD_i	a	b	R^2	PD_s
B1	4.3	0.9	0.94	3.8	148.9	1.2	0.96	2.8
B2	4.4	0.6	0.94	5.2	121.1	1.1	0.93	2.9
P1	12.6	0.8	0.95	5.8	260.1	1.0	0.95	3.9
P2	10.0	0.6	0.92	6.6	181.9	0.9	0.91	4.0
R1	3.7	1.1	0.97	2.8	296.8	2.4	0.99	1.7
R2	2.6	0.7	0.91	3.8	91.2	1.2	0.99	2.4
mean values[z]								
Bartolo				4.4 a				2.8 b
Perfect Ball				6.2 b				4.0 c
Roxy				3.3 a				2.0 a

[x] 1 and 2 correspond with the replicates

[y] parameters obtained from nonlinear regression of the negative exponential model, $y = a \exp(-bx)$, with y = disease, x = distance from the source in meters, a = y intercept at $x = 0$, and b = steepness of the gradient (m^{-1})

[z] Percentile distances were compared by analysis of variance. Means were separated by LSD at ($P \leq 0.05$). Values within a column followed by the same letter were not significantly different.

Disease spread was described by the negative exponential model. However, Figure 4.2C and D suggest that a logistic model could also describe disease gradients for Bartolo, but only in three out of fifteen gradients did the logistic model fitt better than the negative exponential model.

The negative exponential model tends to 0 when the distance tends to infinity. In our study, we determined the percentile distance PD_i and PD_s as a measure of spread, since our interest focused on low disease intensities at relative large distances from the source. PD_i and PD_s was measured as the average distance at which 1% of the empirical maximum was observed for diseased leaf incidence and

severity, respectively. PD_i and PD_s appeared to be good parameters to compare cultivars for disease spread of black rot.

Varietal resistance and black rot. During this study we focused on field resistance of a few cabbage cultivars, representative of a susceptible, an intermediate and a more resistant cultivar. Field resistance is defined as any resistance which effects epidemics in the field but which is not immediately apparent in laboratory or glasshouse tests (Robinson, 1969). Differences between varieties in field resistance were demonstrated for both progress and spread of disease. For both characteristics, cultivars were ranked from resistant to susceptible as Roxy, Bartolo, Erdeno and Perfect Ball. This ranking is consistent with the characterization of resistance in some of these cultivars reported by Pillard *et al.* (1991).

The cultivars Bartolo and Perfect Ball were studied for two years. The experiment of 1991 was repeated in 1992 but inoculations failed. Therefore, data from another experiment with Bartolo, Erdeno, Perfect Ball, and Roxy were used. No data were obtained from Erdeno since plants were heavily attacked by the larvae of *Delia brassicae* (Hoffm.). Although we used data from two experiments differing somewhat in experimental design, the ranking of Bartolo and Perfect Ball was the same.

Values for disease progress and spread were higher in 1992 than in 1991, possibly as a result of differences in inoculum type, weather and planting distance. Variation between experimental designs and years highlights a problem in measuring field resistance. Field resistance of any genotype can only be expressed relative to that of other genotypes within the same experiment. The solution is to compare black rot resistance to a set of reference cultivars and to express field resistance as deviations from these cultivars by means of a relative resistance index (Zadoks, 1972).

Field resistance to black rot. Field resistance to black rot can be determined by one or more of the following mechanisms: a) passive resistance, b) active resistance, c) disease escape, d) tolerance, and e) mature plant resistance.

Ad a: passive resistance is due to qualities such as an altered stomatal structure or few hydathodes, innate in the host prior to attack (Robinson, 1969).

Ad b: active resistance is due to reactions incited by pathogen attack (Robinson, 1969). Staub and Williams (1972) showed that bacteria can multiply and spread in resistant plants, but at a lower rate than in susceptible plants. Kamoun *et al.* (1992) demonstrated that hypersensitivity reactions can reduce symptom expression.

Ad c: disease escape is a particular environmental factor preventing infection and

development of disease (Robinson, 1969). Cook *et al.* (1952a) and Ruissen and Gielink (1994) showed the significance of the primary way of infection via hydathodes. Ruissen and Gielink (1994) described variation in guttation among cultivars. Guttation is the release of water at the end of leaf veins, which are hydathodes. Bartolo and Erdeno guttated less than Perfect Ball, and lesion development was positively correlated with guttation, Bartolo and Erdeno displayed a form of escape.

Ad d: a host shows tolerance if the pathogen is able to multiply but without symptoms appearing (Robinson, 1969). Symptom expressions is greatly influenced by the number of bacteria. The growth of the bacterial population is governed by temperature and moisture status of the leaf. Plants infected by *X.c.* pv. *campestris* may remain asymptomatic when the density of *X.c.* pv. *campestris* is not high enough to cause symptoms. According to Backman *et al.* (1989) and Sinclair (1991), it is likely that plants infected by secondary inoculum remain asymptomatic.

Ad e: mature plant resistance is the resistance apparent in mature plants but not in seedlings (Robinson, 1969). Microplots can be equated to growth stage bound incomplete resistance (Zadoks, 1972). Bain (1952, 1955) and Hunter *et al.* (1987) demonstrated that resistance to black rot differed with plant age and showed cultivar effects on resistance to black rot.

Field resistance to black rot as measured here represents the cumulative effects of the above mentioned (and possibly more) components of resistance. Field resistance is decidedly more complex than the resistance assessed by standard inoculation methods in the greenhouse because we were not able to reproduce the ranking of the varieties tested for field resistance in greenhouse experiments. When plants were artificially inoculated by injection of *X.c.* pv. *campestris*-suspensions (10^9 bacteria per ml) in leaves, resistant and susceptible cultivars showed the same rate of symptom expression (unpublished). Microplots as used in the present study are good though laborious instruments for fine-tuning field resistance measurements, allowing reliable statistical analysis of small but important differences in field resistance (Savary and Zadoks, 1990). Relatively small increases in resistance might help to control black rot by reducing the development of black rot in time and space.

Chapter 5

Spatio-temporal Response of Black Rot in Cabbage to Initial Inoculum Levels

C.G. Kocks, J.C. Zadoks, and M.A. Ruissen

submitted

Abstract: Black rot on cabbage was evaluated in replicated field experiments to compare effects of various initial inoculum levels on spatio-temporal disease development from artificial sources, one per plot. The results support the hypothesis that black rot is a potentially polycyclic disease. Black rot development was inoculum-dependant because the progress rate of black rot epidemics and the spatial spread were both positively correlated with the strength of the source. Fast disease development was related to the number of rain days. The spread of black rot, associated with the primary gradient, was primarily due to allo-infection. Later followed a phase during which allo-infection was complemented by auto-infection. Three-dimensional maps of diseased leaf incidence showed the dominance of the primary focus. Maximum distance of black rot symptoms to the source of the focus was limited to a few meters so that damage of cabbage by focal inoculum was limited to the plants close to the source. Spatio-temporal development and initial inoculum were related. High inoculum levels in point sources resulted in faster outward spread of black rot in cabbage, and significant differences between low and high levels were generally present. Under the conditions of the experiments, performed during three relatively dry seasons, a single source of infection measuring 0.5 x 0.5 m was not capable spread disease over all plants in a plot of 6.5 x 6.5 m. The results imply that severe full-field disease, as observed regularly in The Netherlands, can only originate from a large number of small foci per field.

5.1 Introduction

The bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*X.c.* pv. *campestris*) is the causal agent of black rot disease in cabbage (Williams, 1980). An important factor in the epidemiology of black rot is the initial inoculum. Soilborne (Alvarez and Cho, 1978; Ruissen *et al.*, 1990; Schaad and White, 1974b), seedborne (Schaad *et al.*, 1980) and weedborne (Schaad and Dianese, 1981) inoculum have been studied. The quantitative relationship between inoculum from infected plants present in fields and epidemic development of black rot in cabbage (foliar symptoms) under field conditions has not been established conclusively. Low initial densities of inoculum in infected plants may suffice to cause high levels of disease development since black rot is potentially polycyclic (Alvarez *et al.*, 1987).

The objectives of this study were to *i*) quantify temporal, spatial and spatio-temporal development of black rot from a point source, *ii*) compare the effect of

various initial inoculum levels to the spatio-temporal black rot development, and *iii*) evaluate the relevant importance of individual sources to black rot epidemics.

5.2 Materials and methods

Experimental plots. Black rot epidemics in cabbage were studied in Wageningen (1992 and 1994) and Lienden (1993), The Netherlands. The white cabbage cultivar Perfect Ball (susceptible to black rot) was used. Cabbage plants were grown in the greenhouse until plants reached the six leaf stage, and were hardened outdoors two (1992 and 1994) and three (1993) days before planting in the field. Planting was done on 18 May 1992, 7 May 1993 and 16 May 1994 (Table 5.1).

The experimental design in 1992 had three treatments without replicates. A randomized block design with three by three treatments was used in 1993 and 1994. Individual plots comprised 14 x 14 plants in a square grid with an interplant distance of 0.5 m. Plots were separated by 10 m of winter wheat.

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 was prepared by suspending 48-h-old *X.c.* pv. *campestris* colonies (grown on Yeast Peptone Glucose Agar at 27°C) in distilled water (density approximately 10^8 cfu/ml).

Inoculum sources. Source plants are cabbage plants inoculated with *X.c.* pv. *campestris* to be introduced in the plots as sources of inoculum. In 1992, four leaves per source plant (six leaf stage) were injected at the base of the leaf close to the petiole with about 0.03 ml inoculum. Inoculated plants were placed in the greenhouse to develop symptoms before planting in the plots.

In 1993 and 1994, the *X.c.* pv. *campestris* suspension was sprayed onto the foliage of source plants carrying guttation droplets. At inoculation time, source plants were placed outdoors far from the experimental plots. After spray inoculation, plants remained untouched for 4 hours, so that the bacterial cells in the guttation droplets could enter the hydathodes. When guttation droplets had disappeared, inoculated plants were placed in the greenhouse to develop symptoms. Two weeks after inoculation, plants with four symptomatic leaves were chosen to be planted in the plots as inoculum sources.

Table 5.1. Summary of experiments over 1992 to 1994. Dates are given in Julian days.

Year	Date of planting	Replicates	Date of source introduction	Date of guttation sampling	Date of black rot assessment
1992	139	1	141	184,197,213	141,167,177,184,190, 197,204,211,221,253
1993	128	3	141	182,200,215	141,161,182,190,200, 215,220,230,251
1994	137	3	144	181,218,244	144,161,166,173,181, 194,204,218,244,251

A source consisted of a number of inoculated source plants, confined to an area of 0.25 m^2, placed at the centre of each plot between the central four plants. Three inoculum levels (treatments) were applied, consisting of 4, 8 and 16 infected source plants (treatments A, B, and C, respectively) with 4 diseased leaves per plant. Sources were introduced on 20 May 1992, 20 May 1993, and 23 May 1994, and were not removed from the plots.

Disease assessment. Black rot symptom expression was recorded on each individual plant by visual assessment of its foliar incidence. Disease incidence is defined as the proportion of diseased plants per plot, and diseased leaf incidence as the proportion of diseased leaves per plot. Black rot was assessed ten times (1992 and 1994) and nine times (1993) per season (Table 5.1) until senescence of the leaves or interference by other diseases with black rot symptom assessment. To avoid mechanical spread of *X.c.* pv. *campestris* by the observer, plots were entered only when leaves where dry. Five plants per plot were assessed for the number of leaves per plant. Besides, numbers of dead (removed) leaves per plant were determined.

Reinfection in the field. To test Alvarez' statement (1987) that black rot disease is potentially polycyclic, guttation droplets were harvested three times per season (Table 5.1). Five to eight guttation droplets were taken per plant, from five carefully selected plants per plot. These plants were free from black rot symptoms and were about 1 m distant from the inoculum source. Guttation droplets were

placed separately on YPG medium in Petri dishes and incubated for 48 h at 27°C (one dish per sampled plant). Resulting colonies per Petri dish were bulked and suspended in sterile water. In the greenhouse, one test plant was inoculated with 0.03 ml of the suspension. Thus, one test plant was available for each plant sampled in the field. Test plants were scored for absence or presence of symptoms three weeks after inoculation. Koch's postulates were applied to leaves with symptoms.

Seed infection and natural field infection. Each year, 150 randomly selected hardened plants were returned to the greenhouse to be observed for black rot symptoms as a test for seed infection with *X.c.* pv. *campestris*. These test plants were scored for absence or presence of symptoms six weeks after return to the greenhouse. Koch's postulates were applied to symptomatic leaves.

One (1992) or two (1993 and 1994) control plots without treatments were included in the experiments to test for natural infection during the field trials.

Meteorological data. Meteorological data for each growing season were obtained from the weather station of the Department of Meteorology, Wageningen Agricultural University. The data consisted of average air temperature at +10 cm, relative humidity, wind speed at +2 m, and rainfall. The distance between the weather station and the location of the field experiments was about 0.5 km (1992 and 1994) and about 6 km (1993). Additionally, thermohygrographs, a leafwetness recorder (De Witt recorder), and a wind speed and direction recorder (De Woefle recorder) were operated in one control plot in all years. No weather data were available on 31 May and 25 July (1992), and 31 May and 10 June (1993). In addition, absence and presence of guttation was scored between 04.00 - 22.00 h from Julian day 159 to day 201 during 1994 (except on Sundays). Observations during 24 hours were realized on days 161, 162, 173, and 174 during 1994. Field observations showed that, when guttation was present, it generally appeared at 05.00 - 06.30 h and disappeared at 09.00 - 10.00 h (data not shown).

Temporal analysis. Logistic models were fitted to both disease incidence and diseased leaf incidence per plot to analyze disease progress. Residuals were examined for goodness of fit, and the coefficient of determination (R^2) was determined. The apparent infection rate (r) for disease development was calculated for each plot over all assessment dates using linear regression on transformed disease data. The diseased leaf incidence data were corrected for number of dead leaves and number of new leaves before transformation. In addition, apparent

infection rates were determined for intervals between successive assessment dates.

Spatial analysis. The spatial analysis was performed to diseased leaf incidence data only. 3D response surface maps were generated to find secondary foci and to study the directionality of disease spread within the plots. Disease maps were generated for each plot and treatment for a day early in the development of the epidemic, a day about halfway a season, and the last day of data collection. Two-dimensional contour maps were examined to see if disease intensity expanded regularly in concentric rings around the inoculum source. In addition, aggregation of diseased leaf incidence was examined by use of the Moran *I* statistic (Moran, 1950).

Gradient analysis was performed with untransformed disease data which were regressed on distance from the source. To obtain distance classes, concentric rings were drawn as a manyfold of 0.35m (smallest distance between plot plants and the centre of the source) around the centre of the source. The mean distance in a distance class was used as the distance from the source. Disease data within certain distance classes were calculated as weighted means of diseased leaf incidence.

Spatio-temporal analysis. The spatio-temporal analysis was applied to diseased leaf incidence data only. Jeger (1983) proposed eight models to describe the spatio-temporal development of epidemics. In a preliminary study, all eight models were analyzed for suitability to analysis of black rot epidemics. With each model, residuals were examined for goodness of fit, and the coefficient of determination (R^2) was determined. The log-logistic model provided the best fit (R^2 were substantially higher for this model and residuals had a random scatter, generally; data not shown). Therefore, description of the spatio-temporal development of black rot epidemics from a point source was based on the nonlinear model

$$y = 1 / (1 + Ax^{b} \exp [-r t]) \qquad (5.1)$$

where y is the proportion disease, A is the constant of integration, b is a parameter for development in space, r is a parameter for development in time (comparable to Van der Plank's apparent infection rate), x is distance, and t is time (Jeger, 1983; Madden *et al.*, 1990). Isopath movement given by $(r/b)x$ (Jeger, 1983; Madden *et al.*, 1990), was used to assess the spatio-temporal development of black rot. Values for $(r/b)x$ were calculated for each plot for all years. Isopaths were determined for each assessment date as the mean distances at which diseased leaf incidence was 0.05 ($x_{0.05}$). This level was observed in all plots and assessment dates.

Statistics. Differences in the parameters r, b, and $(r/b)x$ between treatments and among years were examined by analysis of variance. Means were separated using Fisher's Least Significant Difference (LSD) test ($P \leq 0.05$). In 1992, treatment effects on the mentioned parameters were examined using the standard errors found in 1993 and 1994.

5.3 Results

Checks. Seed infection was not detected in the greenhouse tests, as none of the tested plants developed black rot symptoms.

Black rot did not develop in non-inoculated control plots in 1992 and 1994, and diseased leaf incidence was 0.002 in 1993. Thus, plot-to-plot movement of black rot did not appear to greatly influence the observed diseased leaf incidence of black rot.

Black rot epidemics were potentially polycyclic since several harvested guttation droplets from plots outside the initial source contained contained viable *X.c.* pv. *campestris* as verified by application of Koch's postulates to symptoms observed on inoculated test plants (Table 5.2).

Temporal progress of black rot. At the time of source introduction, plots were free from black rot symptoms. First disease occurrence on plants near the source was on day 155 in treatment B (1992), on day 156 in treatment B (1993), and on day 158 in treatments B and C (1994).

Disease incidence. Maximum disease incidence per plot was found on the last assessment date of the season, and ranged from 0.26 (treatment A, 1992) to 0.48 (treatment C, 1992). Significantly highest disease incidence was found for treatment C in all three years ($P \leq 0.05$) (Table 5.3), indicating that high disease incidence of the plot is related to high initial level of the source. No significant year effects with regard to treatments were found ($P \leq 0.05$).

The disease incidence remained low for a long period in 1993 and 1994 whereas disease incidence on day 190 (1992) reached more than half of the maximum observed incidence (Figure 5.1). The disease development was noticeably related to rain (Figures 5.1 and 5.2). Rain during the first weeks of the experiment of 1992 initiated disease development. Number of days with rain to day 201 (half way the duration of the experiments) was 29 in 1992. In contrast, disease development

Table 5.2. Number of diseased plants observed 3 weeks after inoculation with 0.03 ml of bacterial suspension derived from guttation droplets of individually sampled field plants.

1992		1993		1994	
Day	Diseased[y]	Day	Diseased	Day	Diseased
184	3	182	3	181	1[z]
197	3	200	2[z]	218	1
213	5	215	4	244	4[z]

[y] Number of plants with black rot symptoms (sample size n=5).
[z] Plants with doubtful symptoms were not scored.

during 1993 and 1994 was delayed because it hardly rained during the first 60 days of the experiments. Numbers of rain days within 60 days after source introduction were 15 and 18 (1993 and 1994, respectively). Especially the high number of rain days during the first 20 days may have contributed to a rapid disease development early in 1992. Temperature during days 141 to 161 was lower in 1994 than in 1992 and 1993. Long intervals with relative high temperatures were found during days 181 to 221 in 1994, a period with only 6 rain days.

Differences between disease curves due to treatment effects appeared early in 1992. In 1993 and 1994, differences between curves were found in last part of the season only. Curves for 1992 suggest two major infection cycles, pointing at polycyclic development, around days 177 and 221. In 1993 and 1994, secondary infection cycles are not obvious (Figure 5.1), pointing to a monocyclic disease development.

The apparent infection rates, as obtained from nonlinear regression for the logistic model, are given in Table 5.3. Residuals generally had a random scatter and R^{*2} values (coefficient of determination for agreement between observed and predicted disease incidence, the latter determined after back-transforming predicted disease values) ranged from 0.94 to 0.97 (1993 and 1994). R^{*2} values for 1992 ranged from 0.70 to 0.81 since disease progress had two major infection cycles during the season. In 1992, disease progress was slowest in treatment A, moderate in treatment B, and fastest in treatment C ($P \leq 0.05$). In 1993 and 1994, disease

Table 5.3. Mean maximum disease incidence, mean apparent infection rate (day^{-1}) of logistic model, and R^{*2} as the coefficient of determination for agreement between observed and predicted disease intensity determined after back-transforming predicted disease values. Treatments denote sources consisting of 4, 8 and 16 infected plants (treatments A, B, and C, respectively).

Year	Treatment	Maximum disease incidence		Apparent infection rate[w]		R^{*2}
1992	A	0.26	a[x]	0.043 (0.003)[y]	a	0.81
	B	0.33	b	0.054 (0.004)[y]	b	0.70
	C	0.48	c	0.061 (0.005)[y]	c	0.73
1993	A	0.27 (0.001)[z]	a	0.041 (0.001)	a	0.96
	B	0.32 (0.002)	a	0.042 (0.002)	a	0.97
	C	0.41 (0.001)	b	0.044 (0.001)	b	0.96
1994	A	0.32 (0.004)	a	0.045 (0.003)	a	0.94
	B	0.34 (0.005)	a	0.046 (0.003)	ab	0.95
	C	0.39 (0.003)	b	0.049 (0.001)	b	0.94

[a] Apparent infection rate after linear regression with ln(y/(1.0-y)).

[b] Values within a column and within a year followed by the same letter are not significantly different ($P \leq 0.05$).

[c] Standard error for 1992 directly from regression analysis.

[d] Standard error (n = 3).

progress did not differ between any of the treatments. Year effects on disease progress within treatments were not significant ($P \leq 0.05$).

In 1992, the apparent infection rates (r) of intervals between successive assessment dates were high for interval 177-184 (treatment C) and interval 184-190 (all treatments) (Figure 5.3). Low r-values were, generally, found on the last four intervals. In 1993, r-values were irregular and higher r-values were found for interval 215-220. In 1994, high r was found for interval 194-204 and r-values were zero for interval 161-166 (treatment A), and for interval 166-173 (treatments A and C).

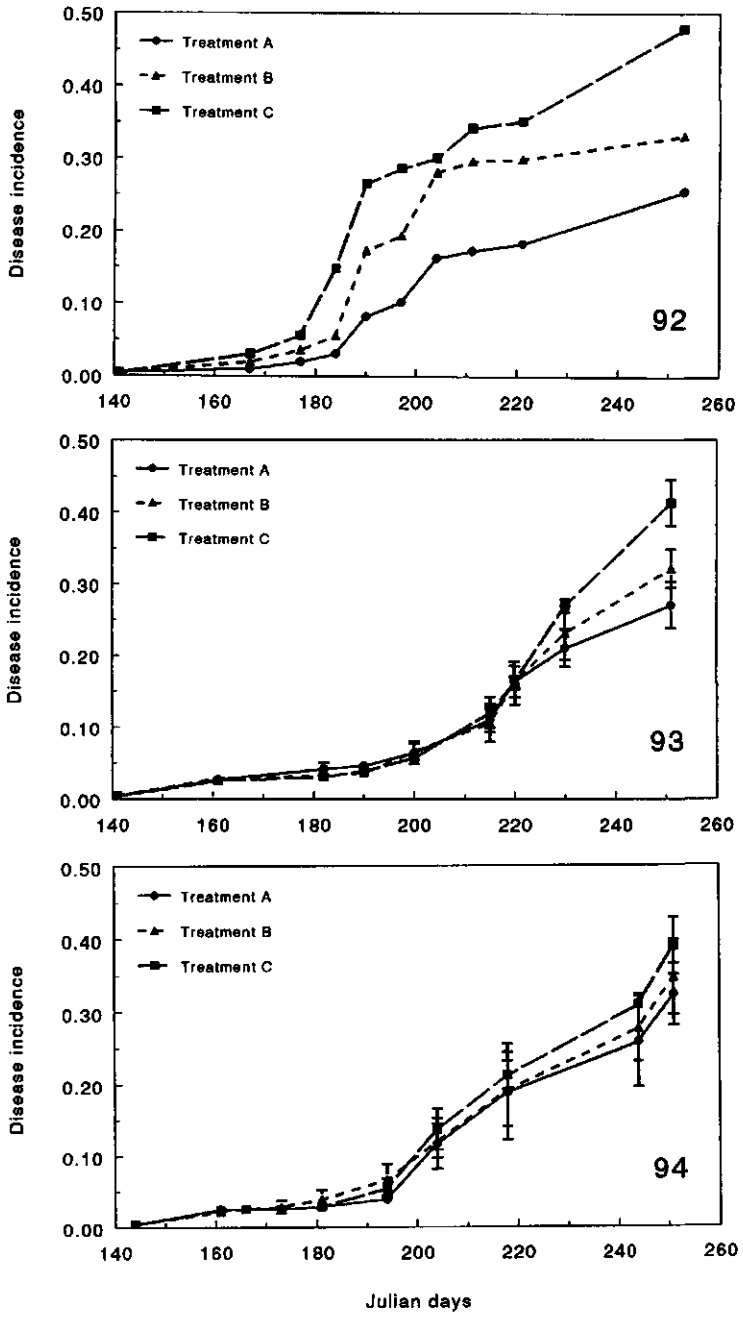


Figure 5.1. Black rot progress curves (disease incidence) for three initial inoculum levels (low = A, moderate = B, and high = C) in 1992, 1993, and 1994. Data points for 1993 and 1994 represent the averages of replicates (n=3) and error bars represent their standard errors.

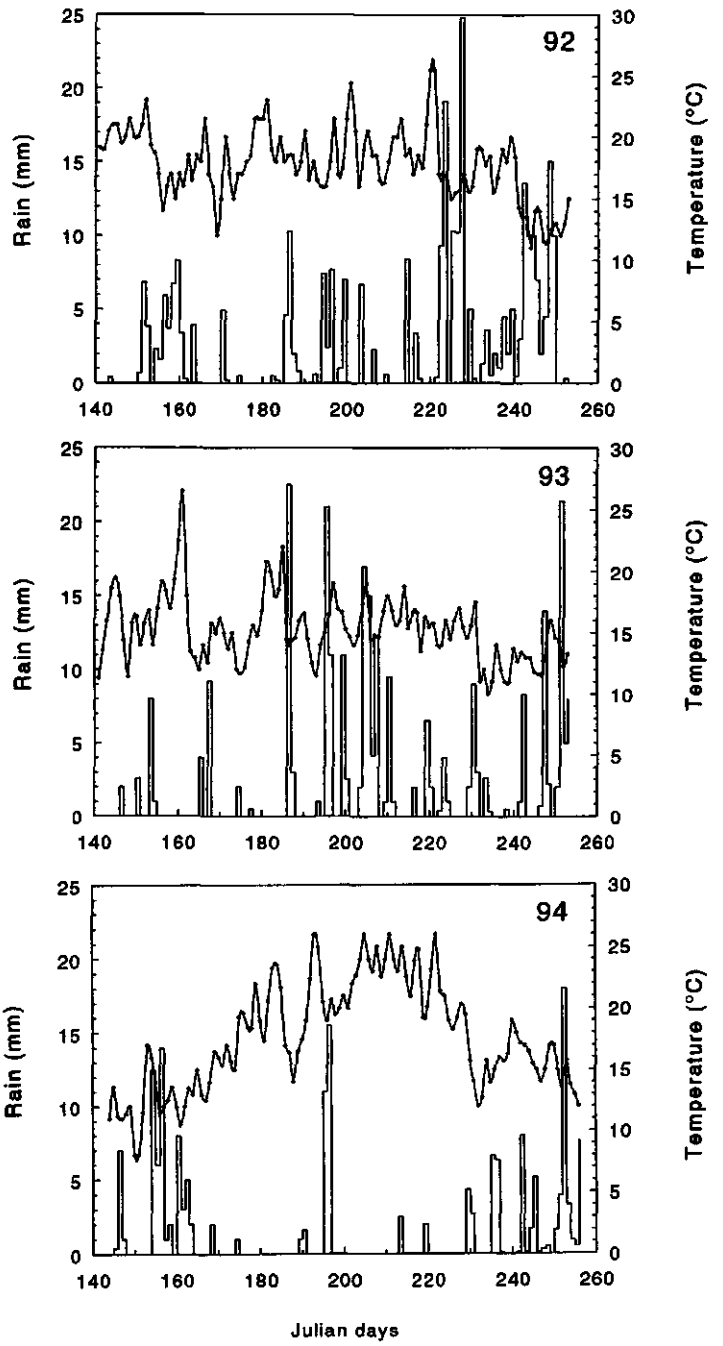


Figure 5.2. Rainfall (stacked bars) and average daily temperature (spline curve) for 1992, 1993, and 1994.

Table 5.4. Mean maximum diseased leaf incidence, mean apparent infection rate (day^{-1}) of logistic model, and R^{*2} as the coefficient of determination for agreement between observed and predicted disease intensity determined after back-transforming predicted disease values. Treatments denote sources consisting of 4, 8 and 16 infected plants (treatments A, B, and C, respectively).

Year	Treatment	Maximum diseased leaf incidence		Apparent infection rate[w]		R^{*2}
1992	A	0.033	a[x]	0.048 (0.003)[y]	a	0.84
	B	0.046	b	0.051 (0.004)[y]	b	0.72
	C	0.072	c	0.056 (0.007)[y]	c	0.71
1993	A	0.063 (0.004)[z]	a	0.054 (0.001)	a	0.98
	B	0.108 (0.005)	b	0.060 (0.001)	b	0.98
	C	0.137 (0.012)	c	0.061 (0.006)	b	0.97
1994	A	0.057 (0.008)	a	0.059 (0.002)	a	0.89
	B	0.117 (0.013)	b	0.065 (0.001)	b	0.92
	C	0.132 (0.007)	b	0.069 (0.001)	c	0.94

[w] Apparent infection rate after linear regression with ln(y/(1.0-y)).

[x] Values within a column and within a year followed by the same letter are not significantly different ($P \leq 0.05$).

[y] Standard error for 1992 directly from regression analysis.

[z] Standard error ($n = 3$).

Diseased leaf incidence. Maximum diseased leaf incidence per plot ranged from 0.033 (treatment A, 1992) to 0.137 (treatment C, 1993). Significant differences for maximum diseased leaf incidence between all treatments were found in 1992 and 1993 ($P \leq 0.05$) (Table 5.4), indicating that high diseased leaf incidence of the plot is related to high initial level of the source. No differences were found between treatments B and C in 1994. Within treatments, significant year effects were found between 1993 and 1992, and between 1993 and 1994 ($P \leq 0.05$).

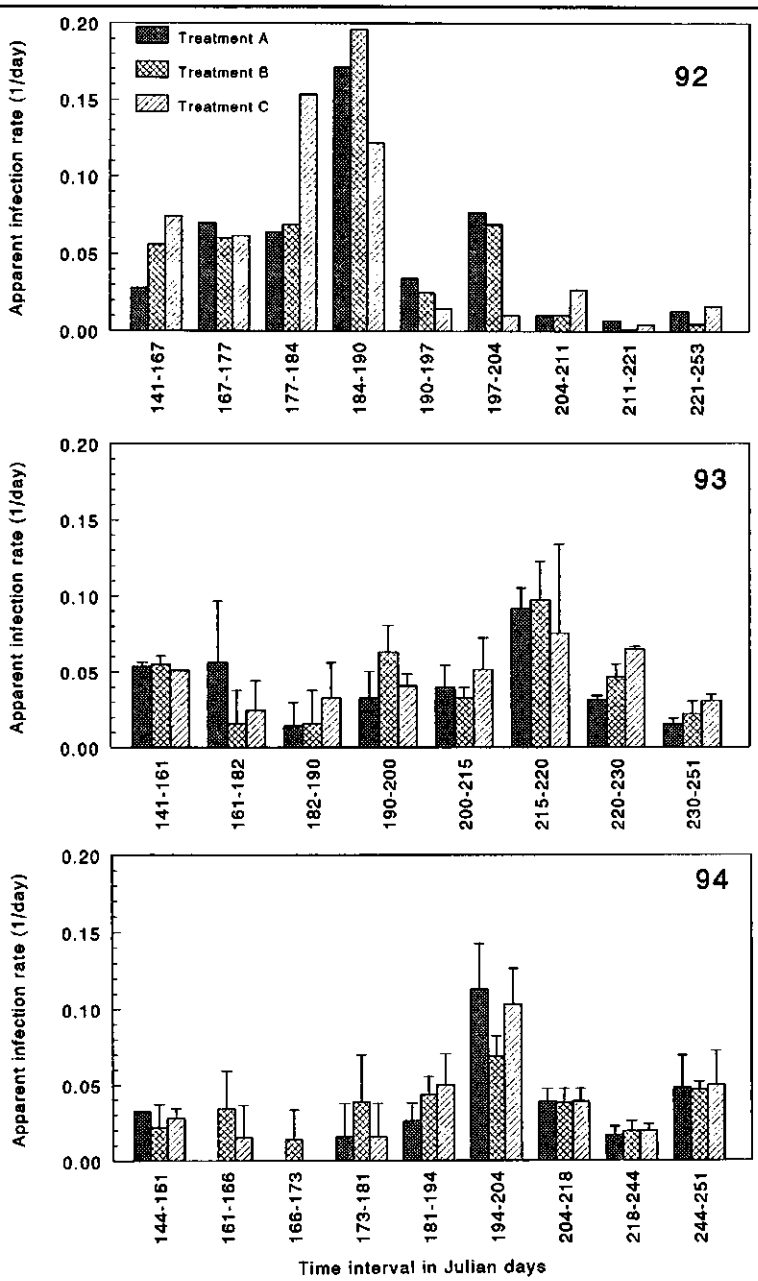


Figure 5.3. Apparent infection rate (r in day^{-1}) for disease incidence during intervals between the successive assessment days for three initial inoculum levels (low = A, moderate = B, and high = C) in 1992, 1993, and 1994. Columns show averages of replicates (n=3) and error bars represent their standard errors.

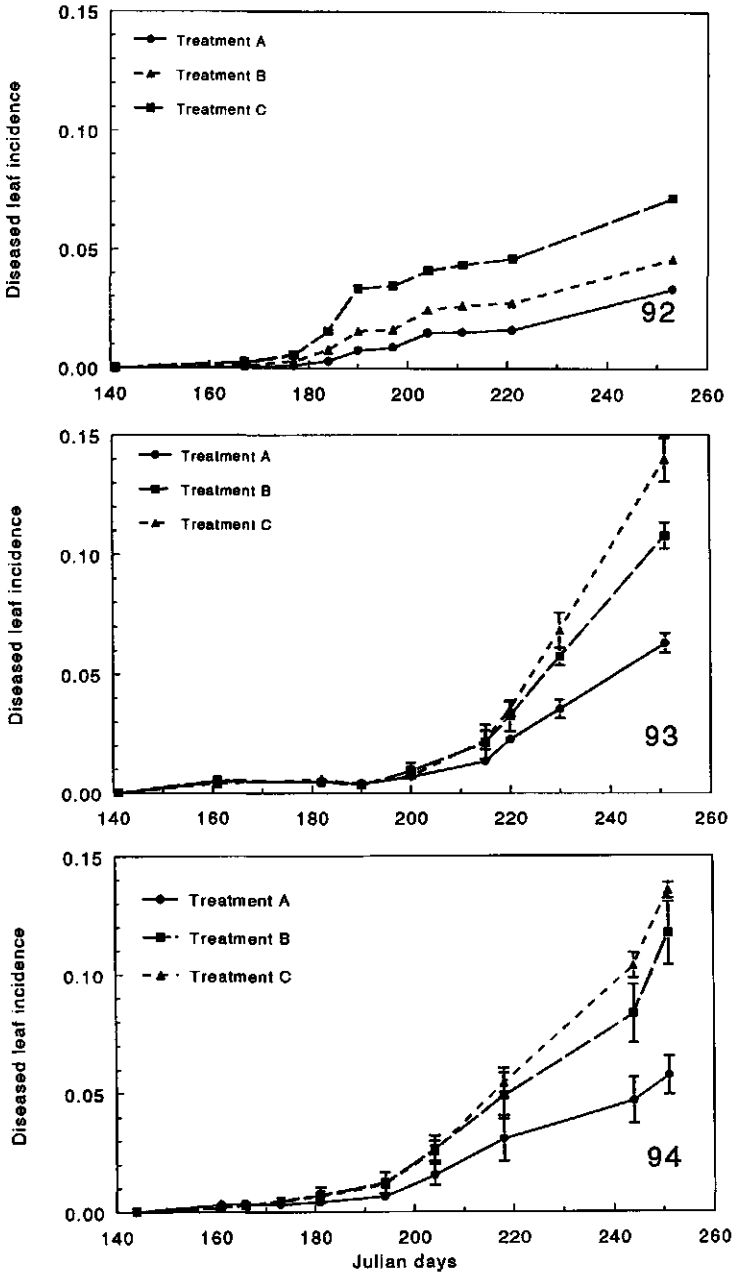


Figure 5.4. Black rot progress curves (diseased leaf incidence) for three initial inoculum levels (low = A, moderate = B, and high = C) in 1992, 1993, and 1994. Data points for 1993 and 1994 represent the averages of replicates (n=3) and error bars represent their standard errors.

Diseased leaf incidence remained low for a long period in 1993 and 1994, but not in 1992 (Figure 5.4). The apparent infection rates for diseased leaf incidence are given in Table 5.4. R^{*2} values ranged from 0.71 to 0.98. As for disease incidence, logistic fit to diseased leaf incidence data was less for 1992 than for 1993 and 1993, due to the irregular disease progress curves of 1992. In 1992 and 1994, disease progress was fastest in treatment C, moderate in treatment B, and slowest in treatment A ($P \leq 0.05$). In 1993, disease progress was significantly lowest in treatment A. Year effects on disease progress within treatments were not significant ($P \leq 0.05$).

The apparent infection rates of intervals between successive assessment dates (Figure 5.5) were corrected for the change in numbers of leaves per plant. Negative *r*-values are the consequence of low to zero increase in diseased leaf incidence and an increase in the number of leaves per plant during the respective interval. In 1992, *r*-values are high till day 197. The error bars point to a high variation within treatments. For interval 141-167 of 1992, *r* was zero.

Spatial development of black rot. A clear dominance of the initial focus (infection source) can be seen in the 3D plots (Figure 5.6). This dominance was present in all years and in all treatments. Infected plants and leaves were highly aggregated throughout the epidemics in all experiments and years as indicated by the Moran *I* statistic (Table 5.5). Generally, aggregation increased over time. Black rot disease increased both in the source itself and near the source. Diseased leaf incidence close to the source was up to 14 diseased leaves per plant. Plants had a maximum of 17 to 18 leaves per plant within a growing season. Diseased leaf incidence decreased very rapidly over the first 2 meters near the focus.

The 3D response surfaces of diseased leaf incidence show that secondary foci developed within 49 days in 1992, a time at which diseased leaf incidence was still limited and strongly aggregated around the source in 1993 and 1994. The first secondary foci were found on day 184 (1992). In contrast, secondary foci could first be recognized in 2D contour maps (not shown) from days 220 and 204 (1993 and 1994, respectively). Secondary foci are the peaks around the inoculum source (several are indicated by an arrow). The 3D response surfaces did not indicate predominant dispersal directions in 1992 and 1993, whereas in 1994 one plot of treatment C showed a predominant dispersal direction toward the North-East, in agreement with the South-West wind direction.

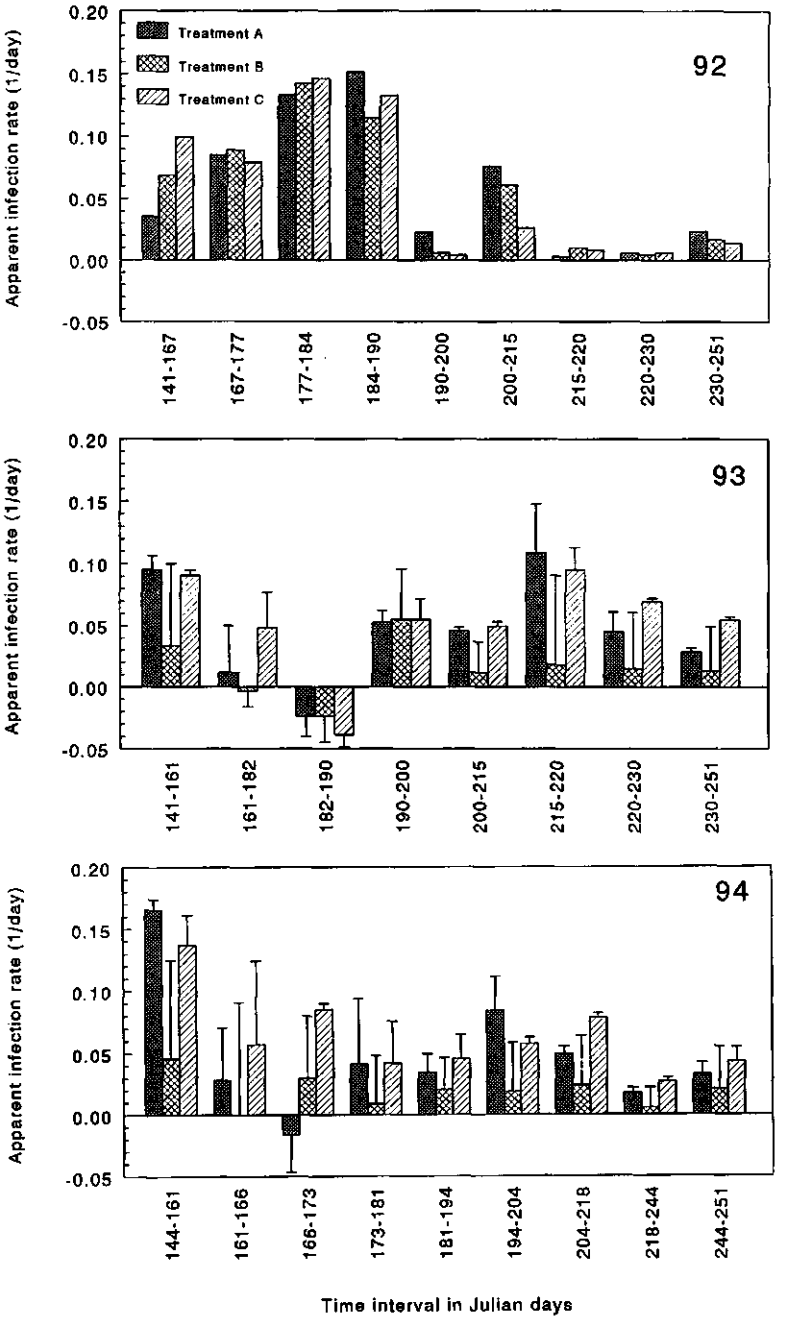


Figure 5.5. Apparent infection rate (r in day^{-1}) for diseased leaf incidence during intervals between the successive assessment days for three initial inoculum levels (low = A, moderate = B, and high = C) in 1992, 1993, and 1994. Columns show averages of replicates (n=3) and error bars represent their standard errors.

Table 5.5. Moran *I* statistics of black rot diseased leaf incidence. Treatments denote sources consisting of 4, 8 and 16 infected plants (treatments A, B, and C, respectively).

	Observation days[y]								
	1[z]	2	3	4	5	6	7	8	9
1992									
A	0.16	0.33	0.34	0.55	0.53	0.69	0.71	0.78	0.81
B	0.40	0.46	0.42	0.58	0.55	0.75	0.73	0.79	0.78
C	0.32	0.49	0.58	0.59	0.66	0.68	0.65	0.75	0.79
1993									
A	0.29	0.54	0.62	0.43	0.46	0.59	0.55	0.78	
B	0.39	0.25	0.43	0.49	0.51	0.71	0.77	0.81	
C	0.40	0.44	0.46	0.46	0.55	0.75	0.76	0.81	
1994									
A	0.50	0.54	0.33	0.43	0.50	0.60	0.75	0.79	0.82
B	0.37	0.25	0.37	0.49	0.52	0.71	0.75	0.78	0.79
C	0.35	0.44	0.44	0.46	0.53	0.75	0.79	0.80	0.82

[y] Observation days 1, 2, 3, 4, 5, 6, 7, 8, and 9 correspond with Julian days 167, 177, 184, 190, 197, 204, 211, 221, and 253 (1992); 161, 182, 190, 200, 215, 220, 230, and 251 (1993); and 161, 166, 173, 181, 194, 204, 218, 244 and 257 (1994).

[z] Standard error vary from about 0.1 on observation day 1 to about 0.02 on observation day 9.

Gradients were described by non-linear regression (Table 5.6). Different widths of the concentric rings were evaluated but did not influence the conclusions made [unpublished]. Data from the last eight (1992) and five (1993 and 1994) observation dates per season were used for gradient analysis, since disease was present only on plants at 0.35 m from the source on earlier dates. Residuals generally had a random scatter and R^{*2} values ranged from 0.87 to 0.98 (Table 5.6). Diseased leaf incidence declined with distance from the inoculum source for all treatments in all years. The estimated parameter *b* changed with time. Initially, disease gradients were steep (i.e. high *b*).

Generally, disease gradients flattened with time, which indicated spread of the disease, at least in part by formation of secondary foci (Figure 5.6). On day 251 of 1993, *b*-values were high due to increase of disease on plants close to the source (increase of *a*). Generally, estimates of *b* were not significantly different between observation days within treatments in any year ($P \leq 0.05$). Where differences were significant, high initial levels were related to low *b*-parameters.

Spatio-temporal response of black rot to inoculum level. The outward spread of disease from infection foci was calculated as $v = (r/b)x$ with x as a variable distance from the focus. For practical reasons, x was put to 1 m. Values for v were calculated for intervals between successive assessment dates using the appropriate apparent infection rates and *b*-parameters. The spatio-temporal development varied over time (Table 5.7). High v-values were found early in the season of 1992 (days 184 and 190). In contrast, high v-values were found late in 1993, day 220. When differences were significant, spatio-temporal development was highest for treatment C ($P \leq 0.05$).

The average r and average b were used to obtain an average v for the spatio-temporal development of black rot over a growing season. Mean b was based on the last seven (1992) and five (1993 and 1994) *b*-values. The rate of disease spread (v) was significantly higher for treatment C than for treatment A (avr-values in Table 5.7). Mean v-values for treatment B were intermediate in all three years.

Values for $x_{0.05}$ were used to compare black rot spread. In 1992, treatment effects were significant when based on isopaths $x_{0.05}$ (Wilcoxon Signed Rank test, $P \leq 0.05$) (Figure 5.7). The $x_{0.05}$-values were lowest for treatment A and highest for treatment C, indicating that high initial inoculum levels result in more disease from the focus. In 1993, significant differences between treatments were found only at the end of the season. At days 220 and 230, $x_{0.05}$ was significantly higher in treatment C than in treatment A. At day 244, $x_{0.05}$ was significantly highest for treatment C, intermediate for treatment B, and lowest for treatment A (students t test, $P \leq 0.05$). In 1994, $x_{0.05}$ was significantly lowest for treatment A. Significant differences between treatment B and C were found for day 251 (1994).

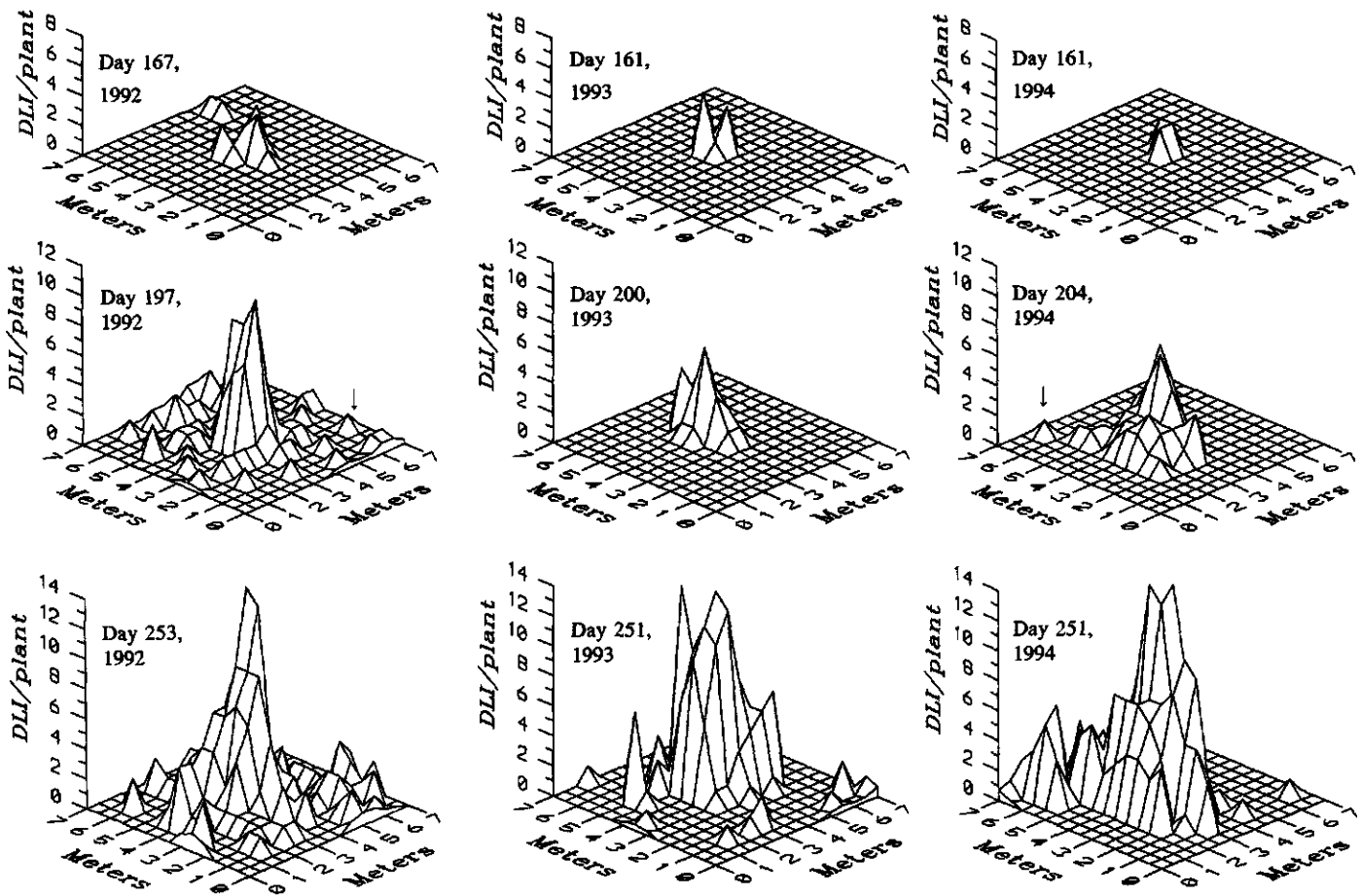


Figure 5.6. Three-dimensional response surfaces of black rot development and spatial distribution in treatment C for cabbage plots per year on the indicated days after inoculation for 1992, 1993, and 1994. Arrows point to secondary foci.

Table 5.6. Estimated parameters of the log-logistic model fitted to gradient data of black rot diseased leaf incidence and R^{*2} as the coefficient of determination for agreement between observed and predicted diseased leaf incidence determined after back-transforming predicted disease values (standard errors are given between brackets).

Time[x]	A			B			C		
	b[y]		R^{*2}	*b*		R^{*2}	*b*		R^{*2}
1992									
177	2.52 (0.18)	a[z]	0.91	2.00 (0.28)	a	0.92	2.15 (0.12)	a	0.93
184	1.93 (0.22)	a	0.95	1.74 (0.11)	a	0.94	1.49 (0.09)	b	0.93
190	1.56 (0.17)	a	0.94	1.74 (0.07)	a	0.98	1.75 (0.18)	a	0.94
197	1.70 (0.21)	a	0.93	1.68 (0.08)	a	0.98	1.66 (0.18)	a	0.93
204	2.26 (0.13)	a	0.98	1.81 (0.12)	b	0.94	1.66 (0.06)	b	0.98
211	2.00 (0.12)	a	0.98	1.64 (0.07)	b	0.98	1.63 (0.08)	b	0.98
221	1.97 (0.11)	a	0.97	1.64 (0.02)	b	0.97	1.62 (0.05)	b	0.97
253	1.60 (0.18)	a	0.93	1.73 (0.09)	a	0.95	1.66 (0.06)	a	0.98
1993									
200	2.25 (0.13)	a	0.94	1.98 (0.20)	ab	0.98	1.52 (0.06)	b	0.87
215	1.58 (0.06)	a	0.89	1.67 (0.04)	a	0.93	1.62 (0.09)	a	0.94
220	1.81 (0.20)	a	0.93	1.41 (0.03)	b	0.88	1.69 (0.11)	ab	0.94
230	1.77 (0.22)	a	0.93	1.58 (0.12)	a	0.89	1.72 (0.04)	a	0.95
251	2.24 (0.21)	a	0.89	1.81 (0.10)	b	0.91	1.93 (0.07)	ab	0.97
1994									
194	2.88 (0.56)	a	0.93	2.37 (0.36)	a	0.94	2.67 (0.08)	a	0.95
204	2.56 (0.01)	a	0.98	2.13 (0.27)	a	0.93	2.38 (0.10)	a	0.93
218	1.65 (0.10)	a	0.95	1.69 (0.13)	a	0.95	1.54 (0.06)	a	0.94
244	1.50 (0.11)	a	0.93	1.65 (0.15)	a	0.96	1.67 (0.04)	a	0.97
251	1.65 (0.02)	a	0.95	1.70 (0.16)	a	0.88	1.64 (0.06)	a	0.96

[x] Time is in Julian days.

[y] *b* is a parameter for development in space (m).

[z] Values within rows followed by same letters are not significantly different ($P \leq 0.05$).

Table 5.7. Rates of spatio-temporal development (v) at different times, at 1 m distance from the centre of the focus with three inoculum levels of *X.c.* pv. *campestris*, 4, 8 and 16 infected plants (treatment A, B, and C, respectively).

Time[w]	A		B		C	
	v[x]		v		v	
1992						
177	0.033		0.044		0.036	
184	0.069		0.082		0.098	
190	0.097		0.071		0.081	
196	0.013		0.003		0.002	
204	0.033		0.033		0.015	
211	0.001		0.006		0.005	
221	0.002		0.002		0.003	
254	0.014		0.009		0.008	
avr[y]	0.024		0.029		0.032	
1993						
200	0.024 (0.007)	a[z]	0.035 (0.039)	a	0.036 (0.039)	a
215	0.029 (0.001)	a	0.034 (0.003)	a	0.047 (0.001)	b
220	0.068 (0.041)	a	0.074 (0.017)	a	0.062 (0.017)	a
230	0.028 (0.004)	a	0.038 (0.004)	b	0.040 (0.004)	b
251	0.013 (0.003)	a	0.021 (0.002)	a	0.019 (0.002)	a
avr	0.028 (0.002)	a	0.036 (0.003)	b	0.036 (0.001)	b
1994						
194	0.012 (0.005)	a	0.020 (0.004)	a	0.017 (0.009)	a
204	0.033 (0.012)	a	0.039 (0.002)	a	0.035 (0.002)	a
218	0.029 (0.001)	a	0.028 (0.002)	a	0.036 (0.001)	b
244	0.012 (0.002)	a	0.024 (0.001)	b	0.022 (0.002)	b
251	0.020 (0.007)	a	0.033 (0.005)	a	0.024 (0.008)	a
avr	0.032 (0.001)	a	0.038 (0.007)	ab	0.039 (0.001)	b

[w] Time is in Julian days.

[x] v is the spatio-temporal development based on the log-logistic model (standard errors are between brackets) as $v = (r/b)x$ with $x = 1$ m with apparent infection rate for the interval following the indicated observation date.

[y] avr: v based on mean b over the b-values of Table 6 and apparent infection rate from plot inoculation till last observation date.

[z] Values within a row followed by the same letter are not significantly different ($P \leq 0.05$).

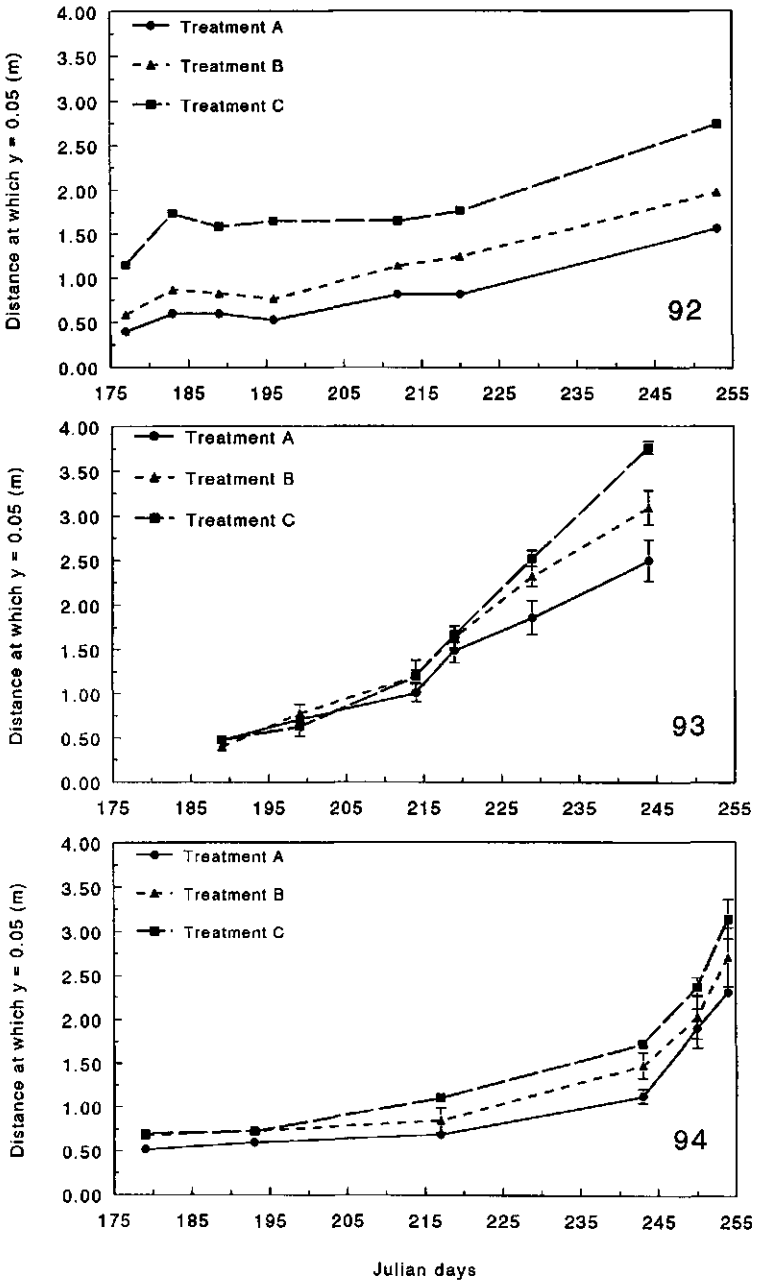


Figure 5.7. Progress of the $x_{0.05}$ isopath in the years 1992, 1993, and 1994. A, B, and C represent initial inoculum sources of 4, 8 and 16 diseased plants, respectively. Data points represent averages of replicates (n=3) and error bars represent their standard errors.

Discussion

Obviously, seed infection and outside sources of infection were not involved in our experiments. Therefore, we consider the black rot development in the plots as the exclusive result of infection from the artificial inoculum sources (Kocks and Zadoks, 1996). The appearance of typical foci (Zadoks and Van den Bosch, 1994) around the inoculum sources confirmed this conclusion.

Black rot epidemics. Several harvested guttation droplets contained viable *X.c.* pv. *campestris*, as confirmed by application of Koch's postulates, so that secondary inoculum was detected in our study. Inoculations of cabbage plants in the greenhouse with this secondary inoculum caused black rot, indicating that the secondary inoculum produced in the field is capable of causing infections on other cabbage plants. It was not possible to discriminate primary symptoms from secondary symptoms when looking at symptom expression. Likely, plants infected by secondary inoculum remained asymptomatic (latent infections) (Backman *et al.*, 1989; Kuan *et al.*, 1986; Sinclair, 1991). The bacterial density in such plants is not sufficient to induce symptom expression and infected plants may remain symptomless for the complete growing season. Figures 1 and 4 suggest the existence of two infection cycles during 1992, pointing at the possibility of polycyclic disease development. However, irregularities in the disease curves may be caused by unfavourable circumstances for bacterial growthand further disease development. So far, our results do not contradict Alvarez' statement (1987) that black rot disease is potentially polycyclic.

The combination of the assessment of disease incidence and diseased leaf incidence gave the opportunity to analyse disease progress for auto-infection and allo-infection at plant level (Robinson, 1976). The spread of black rot, associated with the primary gradient, was primarily due to allo-infection. Later in the season, increase of black rot near the foci was caused by auto-infection. Auto-infection was higher in treatments C than in treatments A because of the higher initial density of inoculum in treatments C. We conclude that, after initial spread, disease development was the compound effect of auto-infection and allo-infection since both increased with time. Diseased leaf incidence better reflected treatment effects than disease incidence. The combination of disease incidence and diseased leaf incidence gave the possibility to discriminate between auto-infection and allo-infection.

Black rot disease in time. In our experiments, high inoculum levels revealed a fast temporal development of black rot and high disease intensities. Van der Plank (1963, 1975) classified plant diseases into inoculum-dependent and infection rate-dependent diseases. Black rot, as developed in our plots, fits the definition of an

inoculum-dependent disease. Diseases can be classified as single and multiple cycle diseases in part according to the dependence of their epidemic development on initial inoculum levels (Van der Plank, 1963). Black rot, identified as a polycyclic disease (Alvarez *et al.*, 1987), may have several generations within a season and thus the potential to cause compound interest disease (Van der Plank, 1963; Zadoks and Schein, 1979). Therefore, the artificially introduced low levels of inoculum at the start of the experiments were expected to cause severe epidemics. Instead, black rot disease did not have the kinetics expected for multiple secondary infections. Since black rot is considered a potentially polycyclic disease, we believe that the number of multiplication cycles was limited by unfavourable circumstances for bacterial growth and dispersal.

Optimum temperature for growth of *X.c.* pv. *campestris* is 27-28°C (Pinches and Pallent, 1986; Shu and Yang, 1990). Ruissen *et al.* (1993) found growth of *X.c.* pv. *campestris* at variable temperatures to be slower than under constant temperatures, a conclusion drawn by Xu (1996) and Scherm and Van Bruggen (1994) for fungal diseases (1996). Since temperatures under field conditions varied between -2°C and +36°C and rarely were 27-28°C, we surmise that field temperature for bacterial growth was less than optimal. Another factor for bacterial growth is the availability of free water. In 1993 and 1994, leaves lost turgescence occasionally, due to water deficiency. Growth of *X.c.* pv. *campestris* may have been reduced in wilting leaves and therewith symptom expression delayed.

Dispersal of black rot in cabbage is governed by rain splash during guttation on diseased leaves (Cook *et al.*, 1952b), occurs especially during rainfall (Williams, 1980), and can be stimulated by overhead irrigation (Grimm and Vogelsanger, 1990). Kuan *et al.* (1986) sampled airborne *X.c.* pv. *campestris* and found bacteria populations in presence of rain and also in absence of rain when leaves were wet. We suppose therefore that dispersal of *X.c.* pv. *campestris* is not limited to morning rain events when circumstances are optimal for guttation but also can occur during rain in afternoon or evening. The high disease development during early 1992 was related to the number of rain days in the corresponding period, in accordance with Cook *et al.* (1952b), Williams (1980), and Grimm and Vogelsanger (1990).

Black rot disease in space. 3D-maps of diseased leaf incidence provided an excellent means to study the spatial location, size and intensity of primary and secondary foci. Most secondary foci developed within 4 meters from the source. The dominance of the primary focus was obvious and this dominance remained in the presence of secondary foci. High disease intensities near the foci and lower intensities further away suggest that splash dispersal decreased with distance, probably by dilution of inoculum (Butterworth and McCartney, 1991, 1992).

Gradients were fitted by non-linear regression using untransformed data. Disease gradients plotted gradients frequently flatten over time. Various workers (Gregory,

(Gregory, 1968; Van der Plank, 1963) have interpreted this flattening to mean that disease was proceeding faster at the focal periphery than at the centre. Only in plots with very noticeable secondary foci did the disease gradients flatten over time. Black rot symptoms were initially found only on the plants close to the inoculum source. Fluctuation of diseased leaf incidence near the focus affected disease gradient analysis. These fluctuations occurred in several plots since datasets comprised many zero-values for diseased leaf incidence. We think that the three initial inoculum levels were too close together to find clear inoculum effects on steepness (*b*-parameter) of gradients, although some significant effects were found.

Disease development generally did not depend on direction, except in one replicate of treatment C (1994). Raindrops hitting a guttation droplet containing *X.c.* pv. *campestris* bacteria would fragment into many smaller droplets. These droplets have to settle on the infection sites of a leaf to cause infection. During rain with wind, droplets would be carried predominantly downwind to neighbouring plants, as in the plot C of 1994. Since directional spread of disease was absent in all other plots of the experiments, the observed directionality of spread in 1994 seems to be an incident rather than a support of windborne spread. Splash dispersal with establishment of secondary foci close to the original focus apparently dominated over wind dissemination of *X.c.* pv. *campestris*, and thus determined high aggregation (Lipps, 1988; Reynolds *et al.*, 1988).

Spatio-temporal development. Although Kocks and Ruissen (1996) suggested the Gompertz model to analyze temporal progress of black rot, we chose a log-logistic model here. Our results indicated that spatio-temporal development and initial inoculum are related. High inoculum levels in point sources resulted in faster outward spread of black rot in cabbage, and significant differences between low and high levels were generally present. Differences in spread caused by initial inoculum level are important with regard to later damage to the crop. Maximum distance travelled by the $x_{0.05}$ isopath was 3.7 m (Figure 5.7) so that damage to cabbage by focal inoculum is limited to the plants closest to the source. No symptoms were observed over 6 m from the foci. In the present study, one single source of infection was not capable to infect (resulting in symptom expression) one single plot completely. Such a limited infection potential was also found with cabbage refuse piles (Kocks and Zadoks, 1996) and with single source inoculations to test field resistance of cabbage against black rot (Kocks and Ruissen, 1996). Nevertheless, farmers are confronted with completely infected cabbage crops. The contrast between our experimental results and farmers' experiences points to a serious impact of the spatial distribution of inoculum sources on disease development. We suggest that infection of large fields possibly is the result of seed infection, infection of seedlings, or multiple source infection or unusually favourable circumstances for growth and dispersal of *X.c.* pv. *campestris*.

Chapter 6

Response of Black Rot in Cabbage to Spatial Distribution of Inoculum

C.G. Kocks, J.C. Zadoks, and M.A. Ruissen

submitted

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 (incidence and diseased leaf incidence) was plotted over time, two and three dimensional maps were made, and disease aggregation was studied by means of geostatistics, black-black counts and Moran's *I* statistic. The results show that black rot progress is 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. Focal anisotropy occurred incidentally. Disease proceeds faster in plots with multi-focal inoculation than in those with uni-focal inoculation. The results suggest that serious epidemics in Dutch cabbage fields originate from large numbers of foci.

6.1 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, 1974b), 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), Kocks and Zadoks (1996), and Chapter 5 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.

Table 6.1. Summary of experiments over 1992 to 1994. Dates are given in Julian days.

Year	Planting date	Replicates	Date of source introduction	Date of black rot assessment
1992	136	1	140	140,155,167,177,183,189,196,212,220,253
1993	130	4	140	140,173,182,215,221,229,246
1994	136	4	143	143,161,166,173,181,194,218,251

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.

6.2 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 (Table 6.1).

Each year, 150 randomly selected plants were 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 x 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 in Chapter 5.

Inoculum sources. Initial disease intensity per plot was 16 plants with each 4 diseased leaves per plant. The spatial distributions of source plants were *i*) 16 source plants in a quadrat at the centre of a plot (size 2 x 2 m) (uni-focal inoculation), and *ii*) a regular spatial distribution of 16 source plants through a plot (multi-focal inoculation) (treatments A and B, respectively) (Figure 6.1). 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 disease incidence (proportion diseased plants per plot) and diseased leaf incidence (proportion diseased leaves per plot). In 1992, 1993, and 1994, black rot was assessed 10, 7, and 8 times, respectively, Table 6.1)

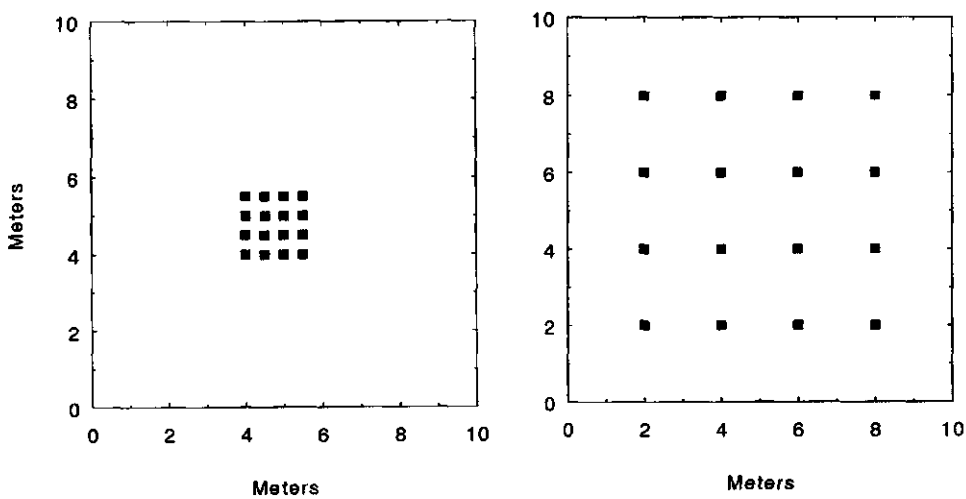


Figure 6.1. Spatial distribution of source plants. Left: 16 source plants in a quadrat at the centre (uni-focal source, treatment A). Right: regular spatial distribution of 16 source plants (multi-focal source, treatment B).

per summer until senescence of the crop or other disease symptoms 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 observer.

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 minutes 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 dimension of AUDPC is proportion × day. Treatment effects were examined by analysis of variance and means were separated using Fisher's Least Significant Difference (LSD) test at $P \leq 0.05$.

The present experiment 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

$$y = \frac{I + 0.5}{N + 1} \tag{6.1}$$

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

Spatial analysis. Geographic trends of disease were examined by the use of 2D-indicator maps (incidence data) and by 3D-response surface maps (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 computed per plot in the 0° and 90° directions, with 0° as the prevailing wind direction. Comparison of the 0° and 90° semivariograms revealed information on presence or absence of anisotropy.

Nicot *et al.* (1984) stated that frequency distribution does not adequately discriminate among random, aggregated, or regular dispersion of disease over the field, but that methods that take into account the location of the samples allows 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 indicates the number of times that two 'black'-units are neighbours in a plot with 'white' and 'black' units. In our study 'black' and 'white' means a disease incidence 1 and 0, respectively. The black-black counts were calculated as described by Sokal and Oden (1978a,b). High values of the black-black counts indicate high degrees of aggregation in spatial patterns, while 0 or values close to zero indicate randomness 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 (Cliff and Ord, 1973; 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 is 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. Practically, 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 (aggregated spatial pattern). 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* is close to zero if no trend is present in the spatial pattern (randomness).

6.3 Results

Checks. Seed infection was not detected in the greenhouse tests, as 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. Thus, plot-to-plot movement of black rot did not appear to influence black rot development. 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 the other cases, no symptoms developed after injection in the petiole of a healthy cabbage plant and one injected leaf of the cabbage plant died within three days.

Source plants. Source plants were introduced on 20 May 1992, 20 May 1993 and 23 May 1994, and were left in the plots. They recovered well from inoculation and transplanting and became, apart from infection, indistinguishable from original plants. In 1994, a few source plants were damaged by pigeons which 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.

In 1992, maximum, minimum, and mean temperature, mean RH, and number of days with rain were 34.4°C, 3.6°C, 17.6°C, 72%, 60 days, respectively. In 1992, highest disease incidence (0.74) and diseased leaf incidence (0.11) was found in treatment B (multi-focal inoculation) (Figures 6.2 and 6.3). AUDPC (Table 6.2) in treatment A for disease incidence and diseased leaf incidence reached 5.70 and 1.02 proportion × days, respectively, which was lower than AUDPC's in treatment B (12.01 and 2.33 proportion×days for 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 too close to the experimental plots (Figures 6.2 and 6.3). Two replicates of treatment A and B 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). Maximum, minimum, and mean temperature, mean RH, and number of days with rain were 31.4°C, -0.6°C, 15.3°C, 77%, 43 days, respectively. On sunny days, generally, mean temperature and mean RH were, respectively, higher

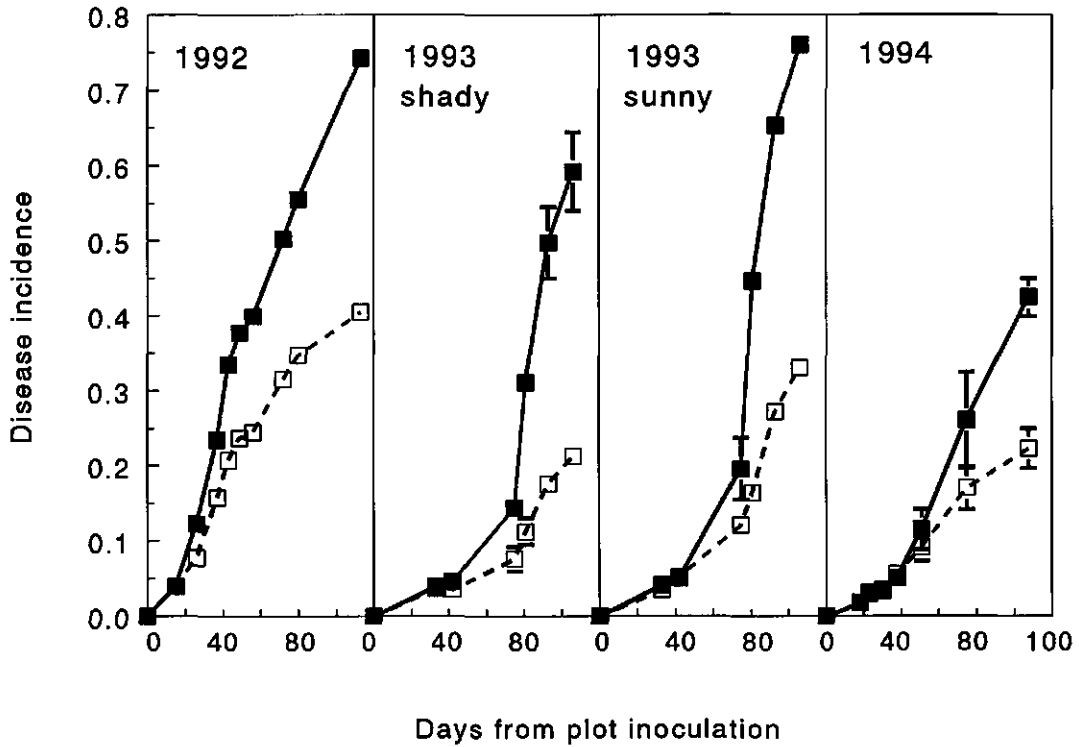


Figure 6.2. Black rot disease incidence for uni-focal (treatment A) and multi-focal (treatment B) sources in 1992, 1993, and 1994. Open squares and broken lines represent data of uni-focal inoculation, closed squares and drawn lines represent data of multi-focal inoculation. Markers in 1992 represent non-replicated plots. Markers in 1993 represent means of two replicates and markers in 1994 represent means of four replicates. Error bars represent standard deviation from each mean. Where markers do not have an error bar, the error bars are too small to plot.

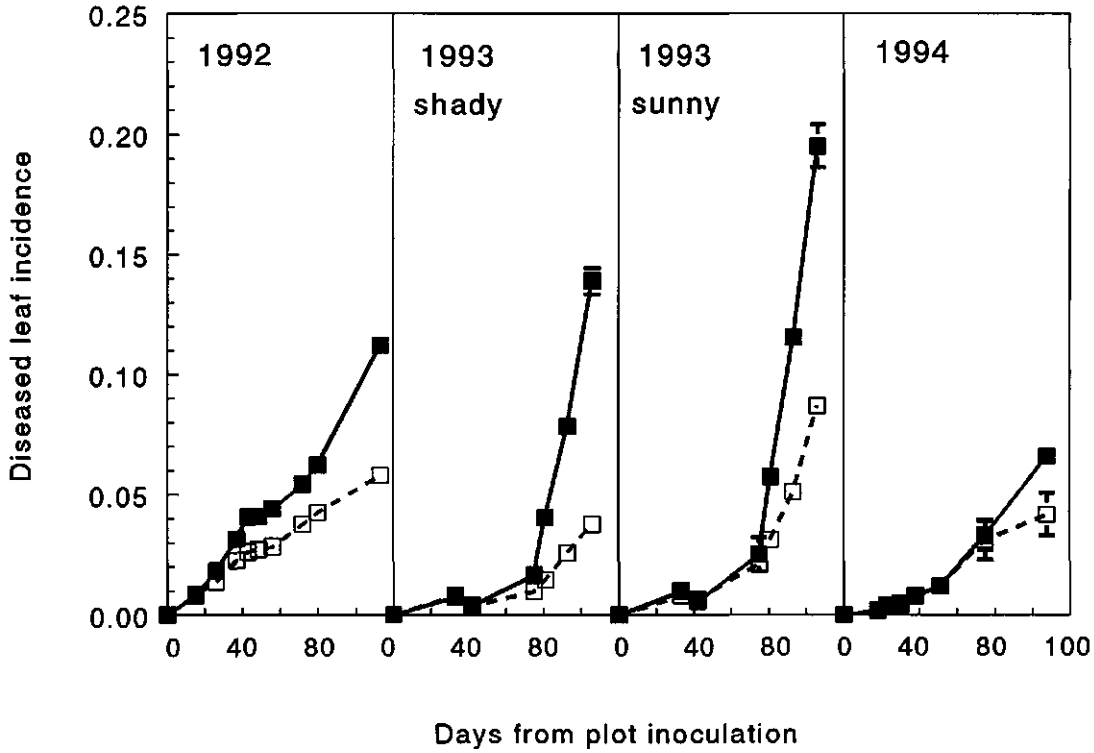


Figure 6.3. Black rot diseased leaf incidence for uni-focal (treatment A) and multi-focal (treatment B) sources in 1992, 1993, and 1994. Open squares and broken lines represent data of uni-focal inoculation, closed squares and drawn lines represent data of multi-focal inoculation. Markers in 1992 represent non-replicated plots. Markers in 1993 represent means of two replicates and markers in 1994 represent means of four replicates. Error bars represent standard deviation from each mean. Where markers do not have an error bar, the error bars are too small to plot.

Table 6.2. AUDPC for disease incidence and diseased leaf incidence from experiments over 1992 to 1994 for treatments A (uni-focal inoculation) and B (multi-focal inoculation). Units are give in proportion×day.

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

[x] Values within a disease assessment method and within a year followed by the same letter are not significantly different ($P \leq 0.05$).

[y] Standard deviation, n = 2 for 1993-sha and 1993-sun, and n = 4 for 1994.

[z] sha: plots shaded till 09.30-10.00 h. Sun: plots shaded till 08.00-8.30 h.

and lower in the sunny plots than in the shady plots. Highest disease incidence (0.77) and diseased leaf incidence (0.195) were found in the sunny plots with multi-focal treatments (Figures 6.2 and 6.3). Treatments differed significantly in AUDPC's for both disease incidence and diseased leaf incidence (Table 6.2, $P \leq 0.05$). AUDPC was significantly lowest for uni-focal inoculation. Significant shade effects were found in both treatments, as disease incidence revealed higher AUDPC's in the sunny plots than in the shady ones. 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, maximum, minimum and mean temperature, mean RH, and number of days with rain were 36.3°C, -1.7°C, 17.1°C, 75%, 37 days, respectively. Disease incidence and diseased leaf incidence reached higher levels in treatment B (0.43 and 0.066 for incidence and diseased leaf incidence, respectively) than in treatment A (0.22 and 0.042 for incidence and diseased leaf incidence, respectively, $P \leq 0.05$) (Figure 6.2). AUDPC's for both disease intensity measures over four replicates were significantly affected by inoculation treatments (Table 6.2, $P \leq 0.05$), clearly showing that the multi-focal inoculation resulted in a faster disease development than the uni-focal inoculation.

Table 6.3. Repeated measures ANOVA for disease incidence and diseased leaf incidence for black rot in cabbage with uni-focal and multi-focal inoculations (treatments A and B, respectively) in 1994.

Source of variation	Disease incidence			Diseased leaf incidence		
	df	MS	*P*	df	MS	*P*
Replicate plots	3	0.01	0.987	3	<0.01	0.916
Treatment	1	0.61	<0.001	1	0.64	<0.001
Residual	3	0.03		3	0.04	
Time	7	0.10	<0.001	7	0.10	<0.001
Time×Treatment	7	<0.01	0.002	7	0.01	0.022
Residual	43	<0.01		43	<0.01	

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). In addition, significant treatment, time, and time×treatment effects were found in 1994 (Table 6.3, $P \leq 0.05$). In 1993, significantly treatment and shade effects were found for incidence and diseased leaf incidence, but treatment×shade effects were less clear (Table 6.4). Time and time×treatment effects were significant for incidence and diseased leaf incidence. Significant time×shade and time × treatment × shade effects were not found. Obviously (Figures 6.2 and 6.3), time was the important determinant in the development of disease intensity. Inoculation treatment and shade were, respectively, the second and third determinant. Besides, effect of time was dependent on the spatial distribution of inoculum as indicated by the significant time×treatment interaction.

Spatial analysis. The final spatial pattern of diseased leaf incidence of black rot with regard to the spatial distribution on Julian day 253 (1992) after uni-focal inoculation is shown in Figure 6.4. Little disease was found on plants at the edges of the plots, possible due to border effects. Incidentally it was found that guttation at the border plants was less than at other plants of the plots. The 3D maps of diseased leaf incidence show that secondary foci developed but that diseased leaf incidence was still strongly aggregated around the initial source.

Table 6.4. Repeated measures ANOVA for disease incidence and diseased leaf incidence of black rot in cabbage with uni-focal and multi-focal inoculations (treatments A and B, respectively) and shade effects, 1993.

Source of variation	Treatment and shade effects				Time effects		
	df	MS	*P*		df	MS	*P*
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	0.70					

The average treatment effects over replicates and years for final disease incidence and final diseased leaf incidence are shown in Figures 6.5A and 6.5B, 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 treatment A was structured and strongly aggregated around the centre of the plot (4 x 4 source plants). For treatment B, the observed symptoms were 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 only since visual inspection of the 3D-response surface maps revealed that, generally, disease development did not depend on direction. Results of semivariogram analysis of data from the last four assessment dates showed some directionality of disease development (Table 6.5). 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

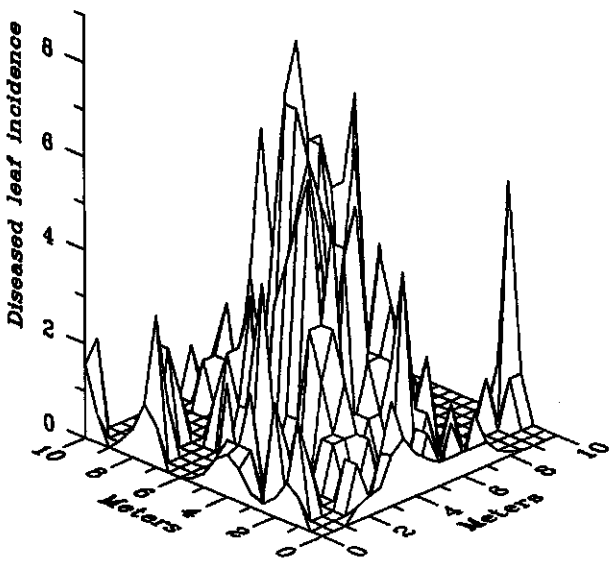


Figure 6.4. Three-dimensional response map of black rot diseased leaf incidence on Julian day 253, 1992, uni-focal inoculation.

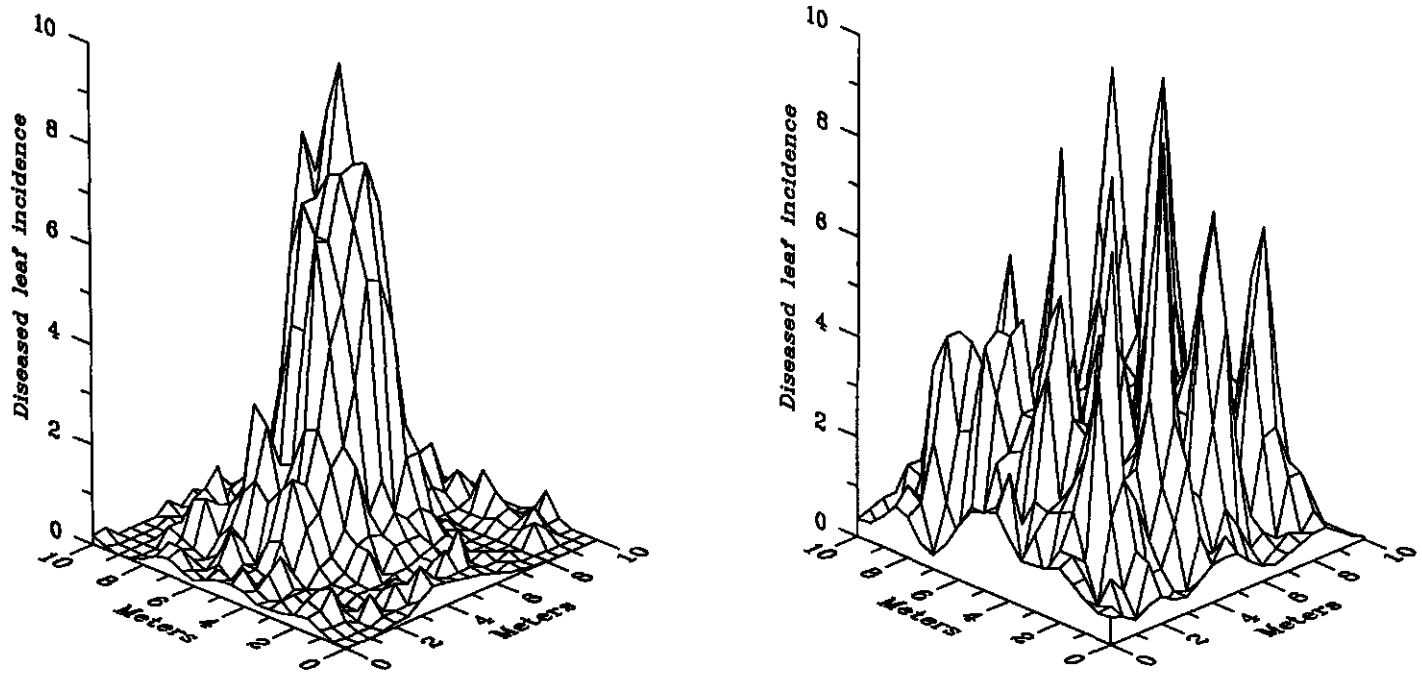


Figure 6.5. Three-dimensional response maps for diseased leaf incidence of black rot on cabbage. Left: average final diseased leaf incidence over all replicates and years, uni-focal inoculation (n=9). Right: average final diseased leaf incidence over all replicates and years, multi-focal inoculation (n=9).

Table 6.5. Parameters of spherical semivariograms for diseased leaf incidence data in the 0° and 90° directions to detect directionality of black rot development, with 0° as the prevailing wind direction.

Julian Day	Treatment A				Treatment B			
	0°		90°		0°		90°	
	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

(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 (0°) and cross (90°) for treatment A, but ranges were higher for 0° than for 90° direction. Directional effects in treatment B 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 was observed for several days for treatment A, but were rarely for treatment B, but the directional effects were of minor importance.

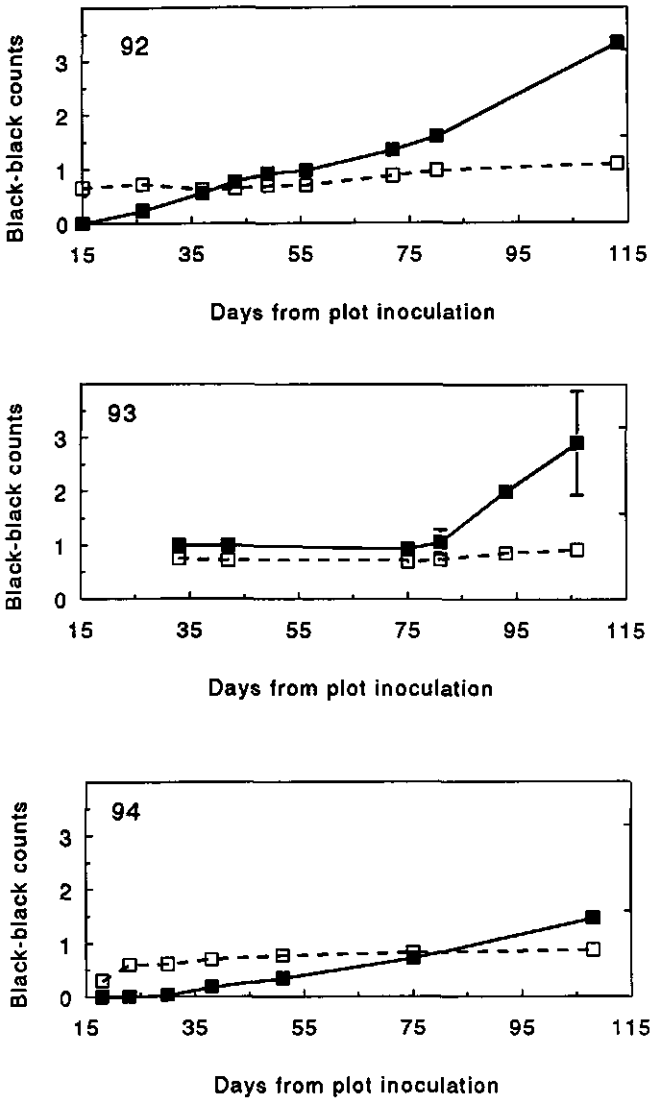


Figure 6.6. Black-black counts plotted against time from cabbage plots inoculated with black rot. Counts refer to disease incidence in 1992, 1993, and 1994. Open squares and broken lines represent data on uni-focal inoculation, closed squares and drawn lines represent data on multi-focal inoculation. Markers in 1993 and 1994 represent means of four replicates and error bars represent standard deviation from each mean. Where markers do not have an error bar, the error bars are too small to plot.

Spatial patterns, as examined by black-black counts on disease incidence varied over time (Figure 6.6). In most plots, black-black counts were similar within replicates, as indicated by the absence of error bars but different between treatments. Depending on assessment date, either randomness (values 0 or close to 0) or aggregation (values significantly different from 0) was indicated. Despite the variability, a general process can be described with only minor exceptions for all three years. At the earliest times of the epidemics, spatial autocorrelations indicated aggregation in treatment A (uni-focal treatment) and randomness in all years for treatment B (multi-focal inoculation). Aggregation did not increase with time in treatment A. In contrast, the spatial pattern of black rot changed from random to aggregated in treatment B. Aggregation reached a maximum at the last assessment date in treatment B. 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 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 treatment A but increased in treatment B. The low values during the 26 days after plot inoculation (1994) were 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 (treatment A, Figure 6.7). Positive values of I indicate adjacent plants with similar values for diseased leaf incidence (positive correlation or clustering), I is negative if high diseased leaf incidence values tend to be located near low values of diseased leaf incidence and vice versa (regular spatial pattern), and I approximately equals 0 if no trend is present in the spatial pattern of disease (random spatial pattern or absence of autocorrelation). Thus, the initial I-value in treatment A indicated similar disease intensities on adjacent plants. During the development of black rot only minor changes in Moran's I were observed in treatment A, pointing at low variation in aggregation level. In treatment B, Moran's I was about zero at the beginning of the season, which indicates 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. In contrast to 1992 and 1993, aggregation of diseased leaf incidence in treatment A of 1994 was initially low, probably due to pigeons which damaged several diseased leaves on source plants.

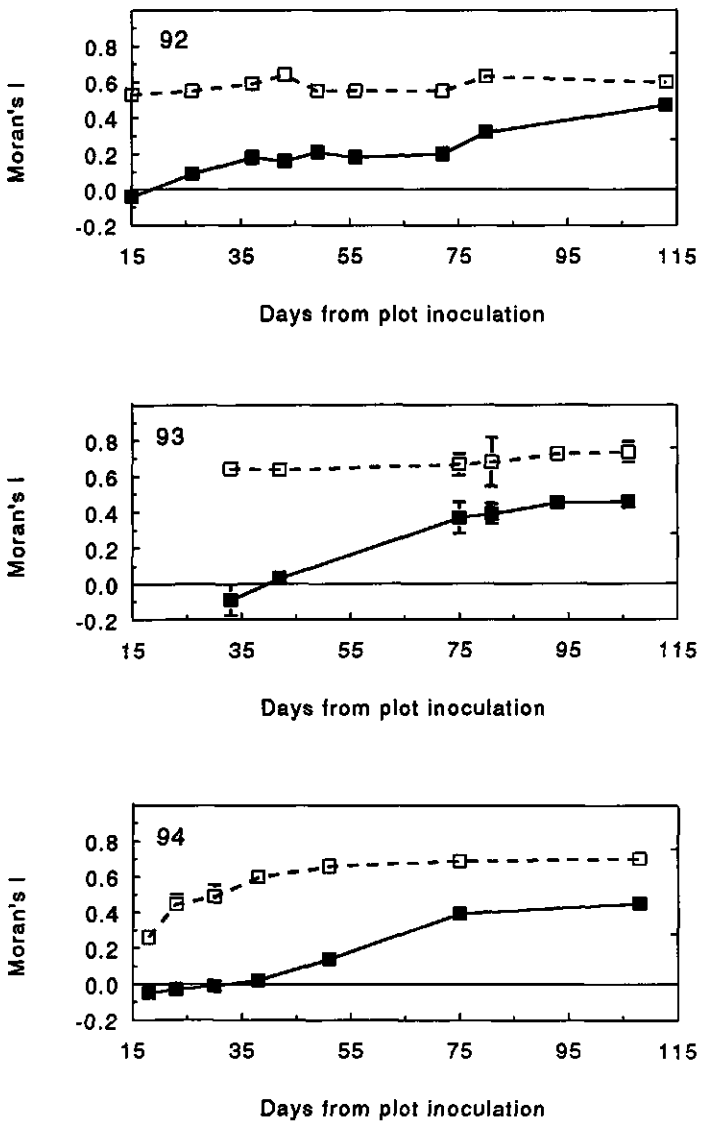


Figure 6.7. Moran's *I* for black rot diseased leaf incidence in cabbage plotted against time from plots inoculated in 1992, 1993, and 1994. Open squares and broken lines represent data on uni-focal inoculation, closed squares and drawn lines represent data on multi-focal inoculation. Markers in 1993 and 1994 are means of four replicates and error bars represent standard deviation from each mean. Where markers do not have an error bar, the error bars are too small to plot.

6.4 Discussion

The greenhouse checks, trap plant tests and control plots indicated that seed contamination of planting 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 minor errors only due to undesirable cross-contamination. The results indicate that the bacterial isolate chosen was infectious and adequate for the purpose of the experiment.

The experiment of 1993 showed 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 revealed 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 the influence of temperature was more important for black rot progress than the possibility of a guttation period prolonged by shading.

Visual examination of the 3D-maps and spatial pattern analysis by use of black-black counts (Cliff and Ord, 1973) and Moran's *I* statistic of autocorrelation (Moran, 1950), showed that 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, generally. Black rot symptoms were found close to the source plants (Figure 6.5), 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; Chapter 5) 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 revealed higher disease levels with time but also a certain progress in 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 showed clearly that the aggregation of inoculum had a major influence on disease development and suggest that black rot normally has few multiplication cycles, as suggested earlier in Chapter 5.

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)). 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 present paper shows that dispersal of black rot in cabbage under Dutch conditions is generally a short distance dispersal, resulting in increase of focus size, and that development of secondary foci by long distance dispersal seems to be of minor importance due to the sharp decrease of density of *X.c.* pv. *campestris* with distance 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 (Chapter 5), 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.

Several studies have shown that dispersal of black rot from various inoculum sources is limited to a few meters only under the Dutch field conditions.

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

Chapter 7

Survival and Extinction of *Xanthomonas campestris* pv. *campestris* in Soil

C.G. Kocks, M.A. Ruissen and J.C. Zadoks

submitted

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 and 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, and over three years, infection foci of black rot disease were established. At harvest time, all plants were chopped and resulting plant debris was rotovated. The resulting soil infestation was sampled and showed clear infestation foci of the soil 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. Comparisons are made between the polyetic situation in Russia and in The Netherlands and some practical conclusions are drawn.

7.1 Introduction

A focus is a patch of crop with disease limited in space and time (Anonymous, 1953). Formation of infection foci has been described for several diseases, among which *Puccinia striiformis* Westend. (Zadoks, 1961), *Phytophthora infestans* (Mont.) de Bary (Van der Zaag, 1956), *Puccinia arachidis* Speg. (Savary, 1987), and *Phytophthora porri* Foister (Smilde, 1996). 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). The studies mentioned 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). Schaad and White (1974b) 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 (Chapter 5), inoculum levels of *X.c.* pv. *campestris* in the soil at planting time may play a critical role in polyetic disease development.

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 dwindling away of the soil's black rot infestation during the winter, and *iv*) the polyetic development of black rot in cabbage.

7.2 Materials and methods

Recovery experiment (Experiment I)

An experiment was performed to test the recovery of *X.c.* pv. *campestris* from artificially infested soil. A sample of 600 gram fresh soil was taken from a clay soil where no cruciferous crops had been grown and divided in samples A, B, and C of 200 gram 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 (density approximately 10^3 or 10^8 cfu/ml). Estimates of the actual densities were obtained from plate counts.

All treated samples were blended for 45 seconds (Braun blender, low speed). After 15 minutes to sediment soil and plant debris, the suspension was sub-sampled, collecting the upper ml of supernatant by means of 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. Dilutions were stored 5-10 minutes at 20-25°C in plastic Erlenmeyers (2.5 ml, SIGMA). Quantification of *X.c.* pv. *campestris* was done by bioassay and MPNs.

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 cultivar Perfect Ball (susceptible to black rot) were used for the bioassay. Cabbage plants were not watered during the two days before inoculation to facilitate the inoculation.

Erlenmeyers containing inoculum were thoroughly shaken for 30 seconds to prevent bacteria to adhere to the inner surface of the Erlenmeyers. Inoculation was performed by injection of 0.03 ml inoculum at the base of a leaf close to the leaf stalk by means of a Hamilton microliter 1 ml syringe (1710RN) mounted with a sterile disposable needle (BD Plastipak 30G ½). Per dilution, a new sterile needle was used. Four plants were inoculated per dilution, inoculating only the second oldest leaf per plant. 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.

Data analysis. The bacterial density was assessed by use of Most Probable Numbers (MPNs). The method of MPNs for the numerical interpretation of dilution data was 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 find the bacterial densities in the sub-sample, and were converted to cfu/g fresh soil or as log cfu/g fresh soil.

When no symptoms appear in the bioassay, MPN is -0.707 (Fisher and Yates, 1963). Taking into account the 0.03 ml inoculated suspension, 0.34 cfu/g fresh soil (or log -0.47 cfu/g fresh soil) may be present in the infested soil. This level is the detection level of the present bioassay.

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

Sub-sample experiment (Experiment II)

Experiment II tested the influence of the volume of the sub-sample after blending. It was performed with one sample of suspension B (low inoculation level) and one of suspension C (high inoculation level) of the fourth recovery test of experiment I. 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 new top of 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 influence of volume of sub-sample on recovery at low and high 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 the low and high inoculation samples of experiment II. 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 seconds in 10 ml distilled water. After 1 minute, 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.

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 kilogram of black rot infected cabbage plants (with 8-11 diseased leaves per plant) were chopped to 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 x 15 x 12 cm) with 4.5 kg each. Each box was incubated at a different constant temperature (-12°C, 0°C, 5°C, 10°C, 15°C, or 20°C) for 20 weeks. Four-weekly samples of 600 g, taken from every box, were divided into three replicates of 200 g. Distilled water (200 ml) was added to each replicate. Suspensions, dilution series, 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. Per 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 of populations of *X.c.* pv. *campestris* in soil and the dwindling of infestation foci under natural conditions.

History of the plots. Experiments to study foliar black rot development, performed in Wageningen during 1992, 1993, and 1994, have been documented by Kocks *et al.* (1998). Black rot epidemics were initiated by placing artificial sources in the centres 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 7.1).

Preparation of the plots. After the final disease assessment (Table 7.1), one (1992) or two plots (1993 and 1994) were prepared. Plants (including heads) and soil were chopped by a rotary cultivator (Sandri TCR-220) to a depth of at least 10 cm. All cultivator runs were made in the same order to obtain a minimal and uniform

Table 7.1. History of the plots and summary of the soil experiment.

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

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 7.1. A sample location corresponded to an area of 0.5 x 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 randomly taken cores 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. Thirtyseven composite soil samples were taken per plot per sampling date (Table 7.1).

Two sub-samples of 200 g were taken from each composite soil sample and treated as replicates. A replicate 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.

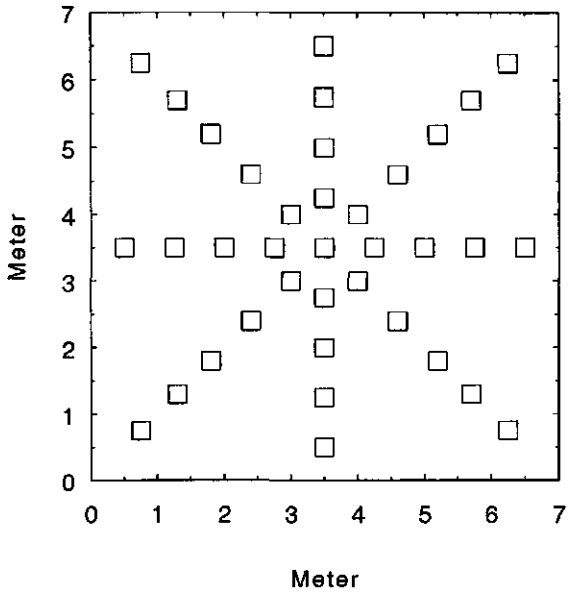


Figure 7.1. Experiment V, sample locations per plot with sample location corresponding to an area of 0.5 x 0.5 m (indicated by an open square).

Meteorological data. The average daily soil temperature at -5 cm 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. The bacterial densities in composite soil samples were assessed by MPN. The relative rate of extinction (e) per sampling time interval per plot was determined as

$$e = -\frac{(\log y_2 - \log y_1)}{t_2 - t_1} \qquad (7.1)$$

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 replicates 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 soil-borne inoculum. The years following the field experiments described above, seedlings of the white cabbage cultivar Perfect Ball (five leaf stage) were planted as the susceptible crop about mid May. Individual plots (7 x 7 m) had 196 plants with an interplant distance of 0.5m. Three-weekly observations

Table 7.2. 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*.

	Sample A[v]	Sample B[w]		Sample C[x]	
Test	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 obtained by plate counts.
[z] Avg = average over five tests.

were made of all individual plants and black rot incidence was recorded. Per plot, 50 seedlings were placed in greenhouses to check for seed infection.

7.3 Results

Recovery

Experiment I. The bioassay showed that the bacterial density of *X.c.* pv. *campestris* in the soil was below the detection level (Table 7.2, 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 an average of 52%. At high infestation level, recovery ranged from 51 to 71%, with an average of 63%. The difference between these averages was not significant (paired *t*-test, P=0.20; independent *t*-test, P=0.10). The grand average for recovery was about 58%.

Experiment II. We compared samples from the supernatant of 1, 1.9, and 9.8 ml. No significant difference in bacterial densities was found between sampling 1 ml or 1.9 ml (both 1.33 x 10^9 cfu/g fresh soil). Bacterial density decreased to 3.1 x 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. *X.c.* pv. *campestris* in the sediment yielded a density of 9.2 x 10^3 cfu/g fresh soil at the high infestation level. At low infestation level, no *X.c.* pv. *campestris* could be found. The difference of about 10^5 between supernatant and sediment is so impressive that the supernatant (top 1 ml) can be considered as representative for the assessment of the bacterial density in soil samples.

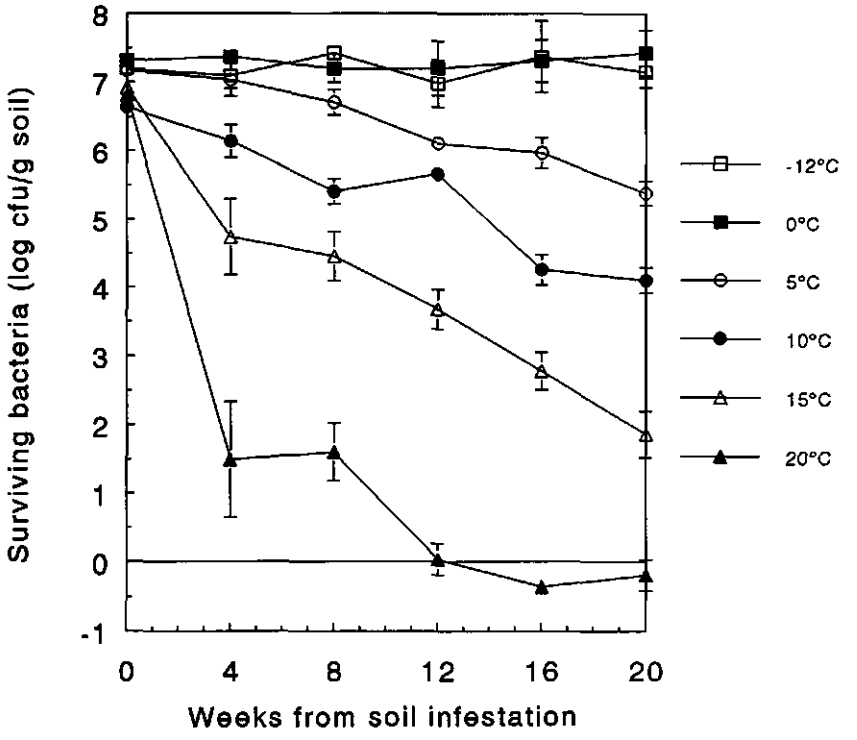


Figure 7.2. Experiment IV, extinction of *X.c.* pv. *campestris* in soil artificially infested with debris from black rot diseased cabbage and stored at six various temperatures. Bacterial densities (log cfu/g soil) were obtained by bioassay. Markers represent means of three replicates. Error bars represent standard deviations of each mean. Where error bars fail, they were too small to plot.

Table 7.3. 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 replicates per treatment, dilution ratio 10, and 4 replicates per dilution.

Temperature	a^w	e^x	R^2
-12°C	7.65 ± 0.29	-0.001 ± 0.011 a[y]	0.26[z]
0°C	7.74 ± 0.31	-0.003 ± 0.010 a	0.30[z]
5°C	7.77 ± 0.20	-0.089 ± 0.007 b	0.91
10°C	7.10 ± 0.36	-0.123 ± 0.012 c	0.84
15°C	6.77 ± 0.55	-0.217 ± 0.019 d	0.89
20°C	5.05 ± 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.

Temperature experiment (Experiment IV)

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

The density of *X.c.* pv. *campestris* did not decline within 20 weeks at -12°C or 0°C (Figure 7.2). Populations of *X.c.* pv. *campestris* declined with time when temperatures were 5°C or higher. Extinction went was fastest at 20°C. After 12 weeks at 20°C, low bacterial densities were detected. Differences in *e* between temperatures were significant except between -12°C and 0°C ($P \leq 0.05$, Table 7.3). The linear correlation coefficient between *e* and temperature was -0.93 ($P = 0.001$, $n=18$).

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°C or 0°C. The leaf tissue was intact and no signs of rotting were observed. After 20 weeks at 5 and 10°C, leaf tissue was partly decomposed. After 20 weeks at 15°C most leaf tissue was decomposed but petioles were still present. After 12 weeks of storage at 20°C, leaf tissue could hardly be distinguished from soil, and after 20 weeks petioles were partly decomposed but stem parts were still recognisable.

Soil experiment (Experiment V)

Densities of *X.c.* pv. *campestris* in soil declined during the winter (Figure 7.3). *X.c.* pv. *campestris* declined with time until log cfu/g soil reached -0.47 (no symptoms in the bioassay). Bacterial density in interval 296-363 (winter '92/'93) was higher than in interval 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 7.1). On Julian days 69 ('92/'93), 48 ('93/'94) and 87 ('94/'95) surviving bacteria had 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 are given in Figures 7.4 and 7.5, 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 average soil temperatures over these intervals were 6.7°C and 5.1°C, respectively.

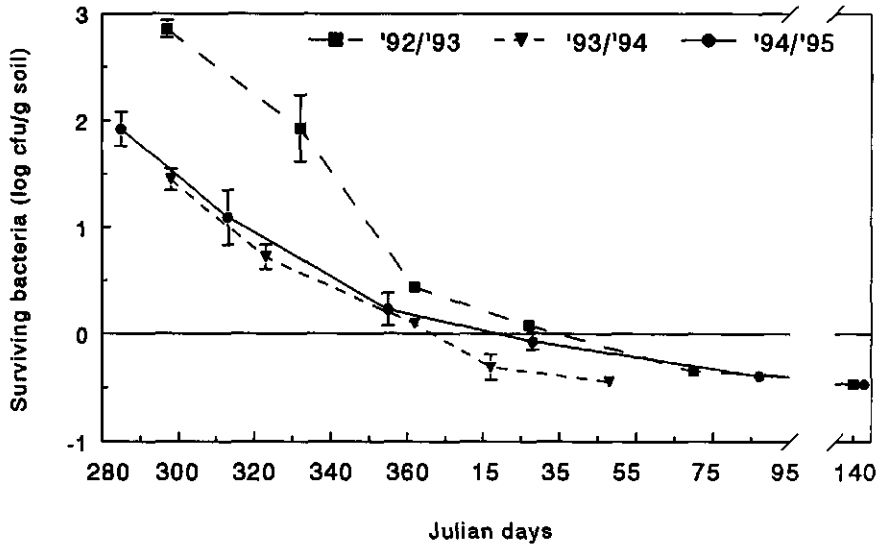


Figure 7.3. Experiment V, density of *X.c.* pv. *campestris* in soil (log cfu/g soil). Densities are obtained by bioassay. Markers represent means of two replicates from one plot ('92/'93) or means of two plots ('93/'94 and '94/'95). Error bars represent standard deviations of means. Where error bars fail, they were too small to plot.

The *e* dropped to 0.012/day for interval 363-26, an interval with an average 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 average soil temperature of 2.4°C and 12 days of soil temperatures below 0°C. The *e* for 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 average 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. Average 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 7.4 and 7.5).

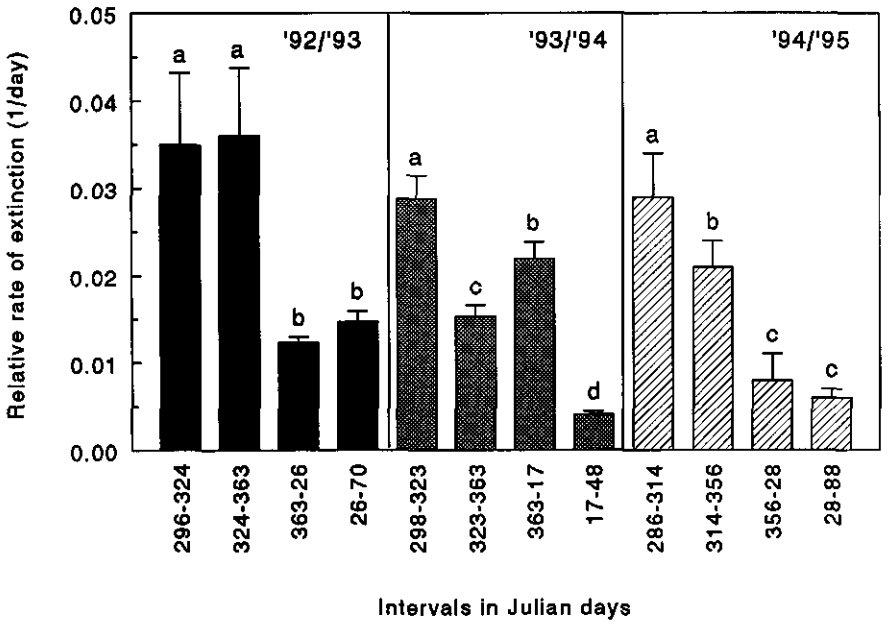


Figure 7.4. Experiment V, relative rate of extinction (*e* in 1/day) for *X.c.* pv. *campestris* in soil obtained by bioassays. Columns show means of two replicates from one plot ('92/'93) or means of two plots ('93/'94 and '94/'95). Error bars represent standard deviations of means. Columns within a year coded with the same letter are not significantly different (P=0.05).

Values of *e* were correlated with average 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 7.6. The figures 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 7.7, upper row) was compared visually with the spatial distribution of foliar disease in Figure 7.6. 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 7.7 were smoothed due to intrapolation and the irregularities in Figure 7.6 were levelled.

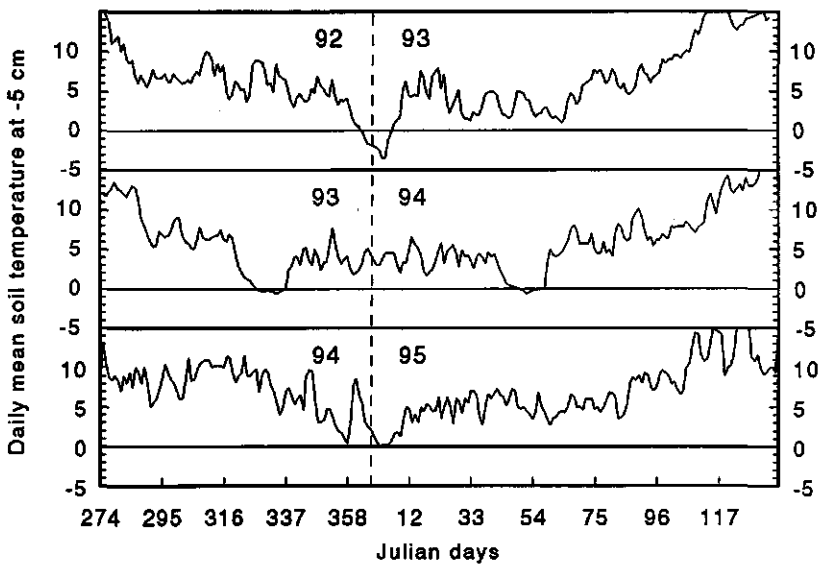


Figure 7.5. Experiment V, average daily soil temperature at -5 cm for the meteorological station during the winters '92/'93, '93/'94, and '94/'95.

Looking along the columns of Figure 7.7. from top to bottom, the disappearance or dwindling of the infestation foci of *X.c.* pv. *campestris* in soil during winter jumps to the eye. 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 7.4). Residuals generally had a random scatter and R^2 values ranged from 0.75 to 0.99. The gradients were initially steep ($b < -4.2$ m^{-1}) and gradually flattened at the end of the winter ($b > 1.3$ m^{-1}).

Radial rates of focus expansion c ($cm.d^{-1}$) were negative and varied according to time interval. Since rates were negative, we speak of the radial rate of focus contraction, negative expansion rates corresponding with positive contraction rates (Table 7.4). Generally, c 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 (Table 7.5), 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 (Table 7.5).

7.4 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

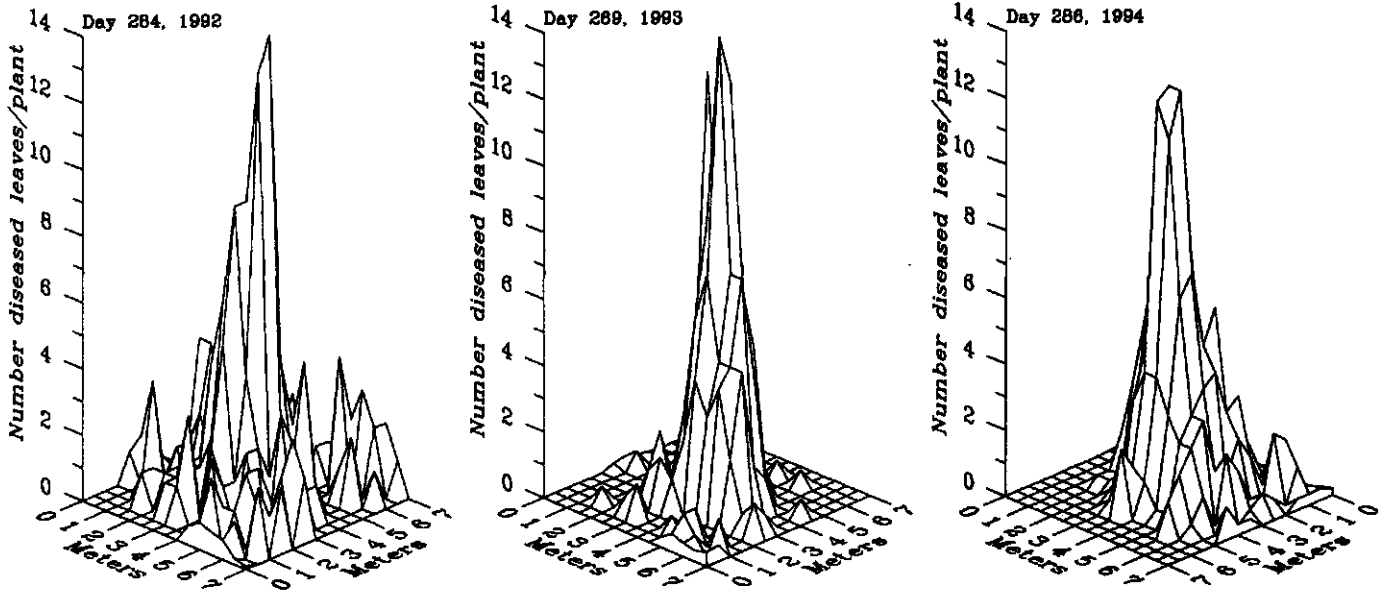


Figure 7.6. Experiment V, disease intensity of black rot in cabbage at date of chopping in 1992, 1993, and 1994. Intensity is expressed by number of diseased leaves per plant. Maps are derived from 196 plants with interplant distances of 0.5 m.

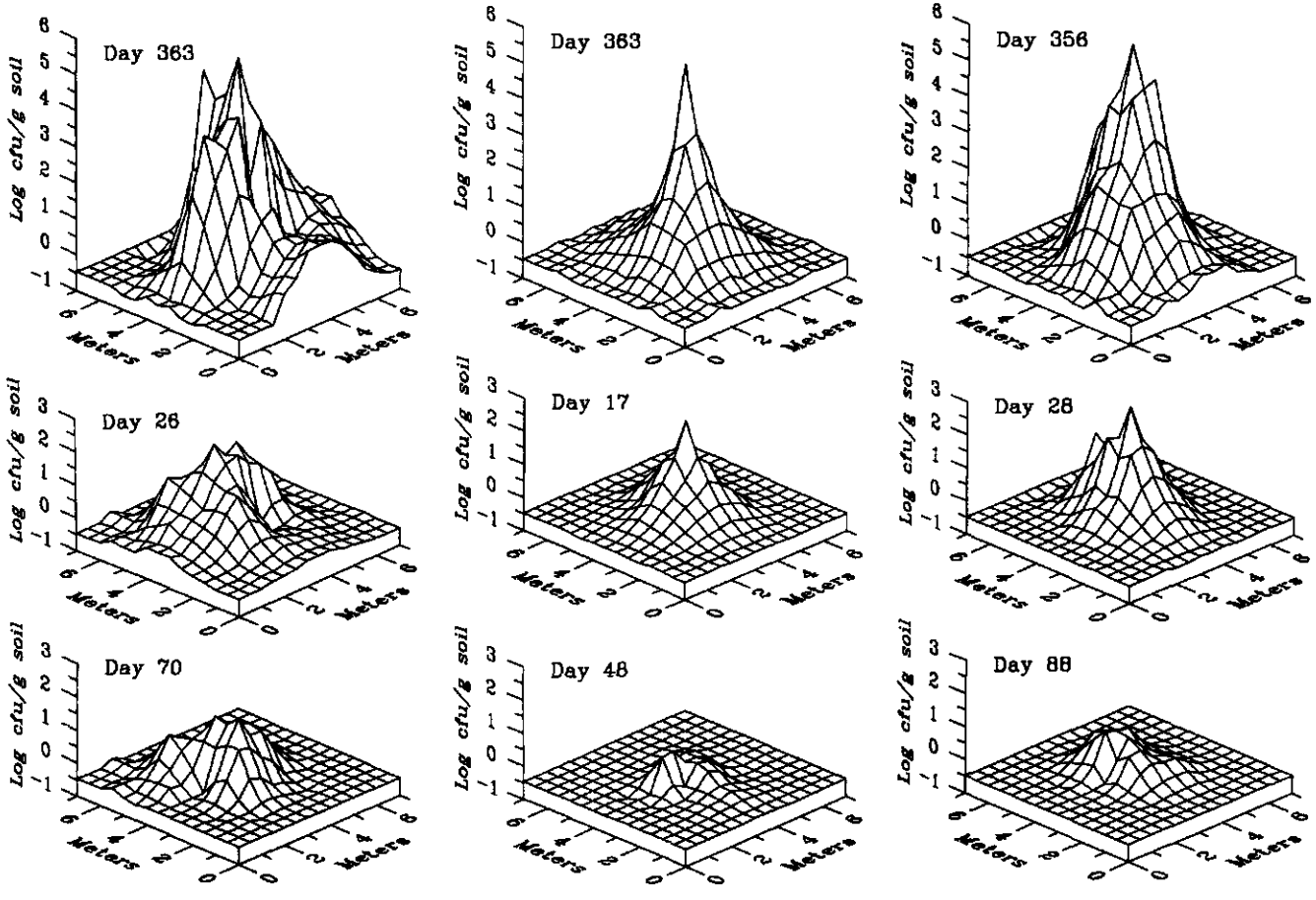
Table 7.4. Experiment V, estimated parameters for the linearized negative exponential model fitted to mean gradients per replicate and date of soil sampling, and time interval and rate of focus contraction for concerning time interval. *a* = intercept and standard deviation, *b* = slope and standard deviation; R^2 = the coefficient of determination for agreement between observed and predicted disease density; *c* = radial rate of focus contraction.

Date of sampling	*a*	*b*	R^2	time interval	*c* (cm.day^{-1})[z]
'92/'93					
296	18.43 ± 1.08	-4.57 ± 0.45	0.97		
324	14.83 ± 1.13	-3.85 ± 0.48	0.96	296-324	1.86 ± 0.14
363	7.09 ± 0.15	-1.61 ± 0.07	0.99	324-363	3.97 ± 0.63
26	5.53 ± 0.34	-1.28 ± 0.14	0.96	363-26	3.12 ± 0.22
'93/'94					
298	14.38 ± 1.10	-4.26 ± 0.47	0.97		
323	10.83 ± 0.88	-3.18 ± 0.37	0.88	298-323	1.83 ± 0.36
363	7.23 ± 1.01	-2.07 ± 0.43	0.89	323-363	2.31 ± 0.23
17	3.99 ± 0.71	-0.88 ± 0.30	0.75	363-17	3.86 ± 0.38
'94/'95					
286	15.89 ± 1.22	-4.52 ± 0.51	0.96		
314	11.19 ± 0.96	-3.14 ± 0.40	0.96	286-314	2.09 ± 0.22
356	7.65 ± 0.91	-2.12 ± 0.39	0.91	314-356	2.05 ± 0.40
28	4.40 ± 0.35	-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.

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 remnants contributed to the destruction of the inoculum so that a follow-up cabbage crop remained virtually uninfected.

An exploratory survey on farmers' practices during 1991-1993 (data not shown) 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


Day 363
Log cfu/g soil
Meters
Day 26
Day 70
Day 363
Day 17
Day 48
Day 356
Day 28
Day 88
'92/'93
'93/'94
'94/'95

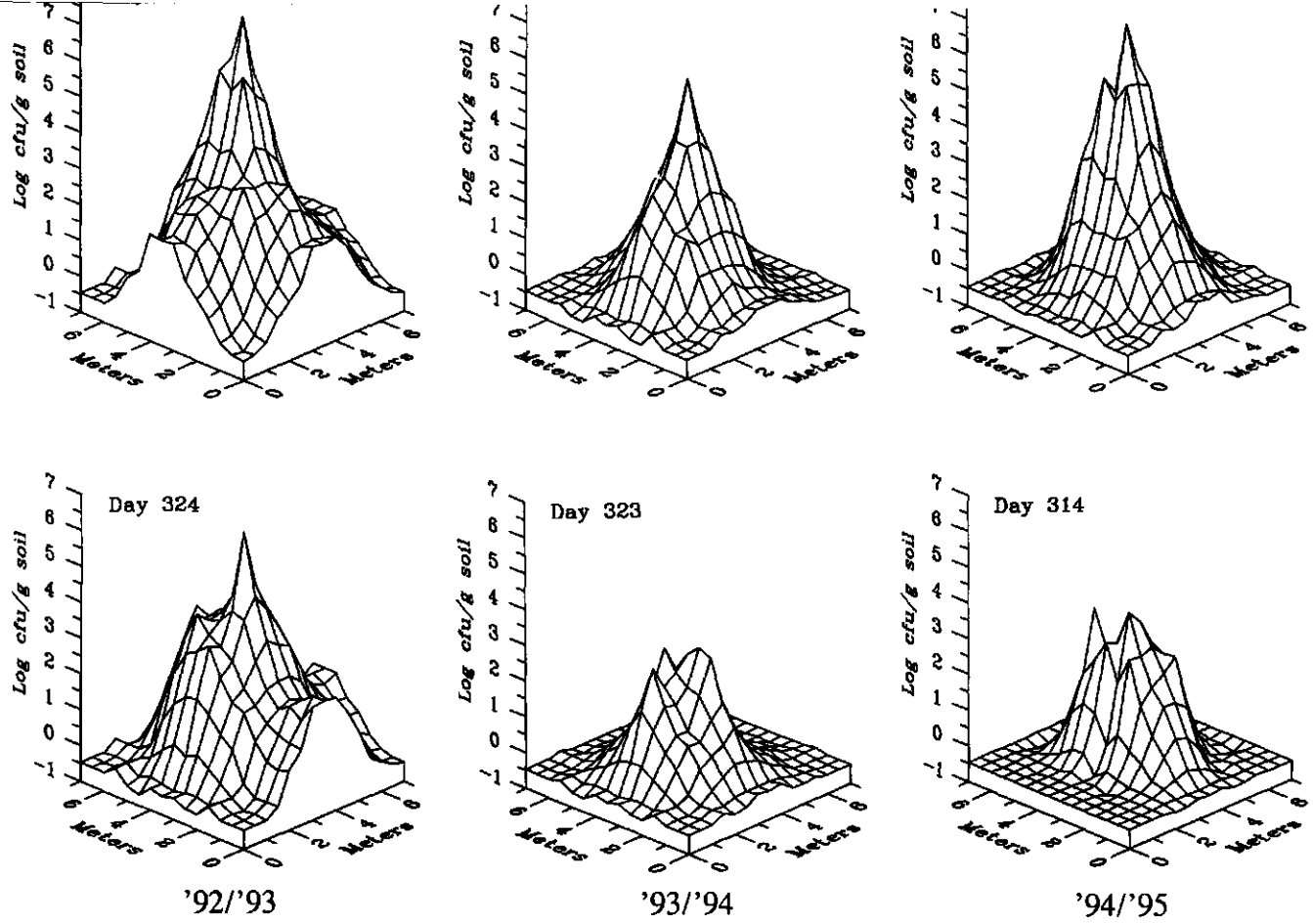


Figure 7.7. Experiment V, spatial distribution of the bacterial density of *X.c.* pv. *campestris* from plots previously cropped with diseased cabbage. Bacterial densities are obtained by bioassay and expressed as log cfu/g soil. Maps are derived from 37 composite soil samples. Detection threshold for bacterial density is -0.47 log cfu/g soil.

Table 7.5. Experiment VI, results of the survival experiment to study polyetic development of black rot in cabbage.

Year	Greenhouse checks	MPN at planting	MPN at harvest	Disease incidence	
				year of interest	previous year
1993[x]	0	0	0	0.01[y]	0.51
1994[x]	0	0	0	0	0.56
1995[x]	0	0	-[z]	0	0.47

[x] Year 1993, 1994 and 1995 were successive cropping years.
[y] One plant was infected. The source of infection could not be identified.
[z] Not measured.

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.

Bioassay

Schaad and White (1974a) developed a selective medium to quantify *X.c.* pv. *campestris* in soil with a detection level of 10^2 cfu/g soil and a recovery of 10%. Because this recovery was too low for our purpose, we used a bioassay to estimate densities of *X.c.* pv. *campestris* in soil. Mean recovery was about 58%. Our extraction method and bioassay permitted the assessment of the densities of *X.c.* pv. *campestris* in the soil without confounding by other pathogens.

Quantitative assessment of *X.c.* pv. *campestris* 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°C or >28°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 in stead 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°C. Higher temperatures also stimulated decomposition of plant debris. Schaad and White (1974b) 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 dies faster.

Field experiment

The 3D response surfaces (Figure 7.6) 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 7.7, upper row). Figure 7.7 (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 fast 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, 1974b) (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 average soil temperature at -5 cm was -0.49. A high e corresponded to an average daily soil temperature of $> 5°C$ whereas a low e was found for intervals having days with frost and with intervals with average soil temperature $< 3°C$ (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 due to temperature effects only.

Survival experiment

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. This carry-over 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, 1974b) 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 disintegrate. 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).

Comparison The Netherlands-Russia

In conclusion, *X.c.* pv. *campestris* would most likely not survive the winter in soil under conditions in The Netherlands. In contrast to the Dutch situation, soil infestation is of major importance for black rot development in Russia. The senior author worked in the Moscow Region and Kolomna Region and found some clear differences with the Dutch situation. In the Moscow and Kolomna Regions, winters have many days with temperatures below 0°C, equipment is poor and tillage is mainly done in spring. The combination of long winters with temperatures below 0°C and tillage in spring contributes little to the decomposition of plant residues

during winter. Hence, crop residue management is definitely inadequate. As a result, *X.c.* pv. *campestris* and other pathogens such as *Mycosphaerella brassicicola* (Fr.) Lindan and *Peronospora parasitica* (Fr.) Tul. can survive the winter and in spring can infect the next cabbage crop from plant debris on and in the soil. As in The Netherlands, we recommend in Russia to chop plant debris and plough it under in the fall, soon after harvest.

Chapter 8

Sampling and Pattern Analysis of Cabbage Plants Naturally Infected with Black Rot

C.G. Kocks and M.A. Ruissen

Abstract: Geostatistics was used to analyze patterns of black rot in cabbage, to optimize sampling plans in combination with observation methods, and to map the patterns based on samples of reduced sizes. For all plants in plots, natural infection by *Xanthomonas campestris* pv. *campestris* was recorded by assessing disease incidence (healthy or diseased) and the diseased leaf incidence (number of diseased leaves per plant) during 1990 and 1991 on at least 6 days. Spatial variability was analyzed by determining semivariograms. Semivariograms were influenced by the observation method and the sampling distance which gave changes in model types and parameter values. In some cases semivariograms revealed anisotropy with preference for the northeast direction, in accordance with the prevalence of southwestern winds during rainfall. Kriging visualized the features of spatial heterogeneity, and the main characteristics of the pattern, including higher disease intensities along wind-exposed directions and the location of foci. The range of influence was 2.5 m for disease incidence and 3.5 m for the diseased leaf incidence. According to these results the sampling intensity could be reduced by 80% and 86% for disease incidence and diseased leaf incidence respectively. Geostatistics was successfully applied to reduce field sampling needed to obtain insight in the disease patterns.

8.1 Introduction

Black rot in cabbage crops is caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*X.c.* pv. *campestris*). It is considered to be an important disease of crucifers (Cook *et al.*, 1952; Schaad, 1980; Williams, 1980). *X.c.* pv. *campestris* is seed-borne (Cook *et al.*, 1952; Klisiewicz and Pound, 1961; Schaad, 1980), and infected seed is an important source of inoculum (Walker, 1941). Other inoculum sources are cruciferous weeds, plant residues, contaminated soil, volunteers, and non-host plants (Alvarez and Cho, 1978; Klisiewicz and Pound, 1961; Monteith, 1921; Schaad and Dianese, 1981, Schaad and White, 1974; Williams, 1980). The disease is dispersed rapidly with wind and rain (Hunter *et al.*, 1975; Williams, 1980), but how the pattern of disease in a field develops with time is not fully understood. Usually, the disease is not homogeneously distributed in a field. Thus problems arise as to the sampling method for disease assessment, since many sampling schemes assume a random distribution of the character of interest (Brus, 1993; Chiarappa, 1970; Journel and Huijbregts, 1978). Geostatistical techniques do not propose a random distribution. In geostatistics, the intrinsic

hypothesis is important meaning that an expectation of a variable exists in independence of location, and that a finite variance exists (Olea, 1991). Trangmar *et al.* suggested that geostatistics could be applied successfully to analyze patterns of pests and diseases in crops (1985). Several authors applied geostatistics to characterize patterns of disease (Chellemi *et al.*, 1988; Lannou and Savary, 1991; Lecoustre *et al.*, 1989; Nelson *et al.*, 1994; Stein *et al.*, 1994; Van de Lande, 1993).

Geostatistics are used to optimize sampling patterns or to map properties by interpolating values from samples of reduced size. The spatial dependence of the character of interest can be calculated by comparing the variation among samples separated by the same distance which is visualized with semivariograms. From the shape of the semivariogram and its parameters, one can infer whether the pattern of spread is random or spatially dependent (Chellemi *et al.*, 1988; Journel and Huijbregts, 1978; Lecoustre *et al.*, 1989; Munkvold *et al.*, 1993; Van de Lande, 1993). Another feature of geostatistics is the kriging procedure. Kriging is a method for optimal local interpolation to predict values at unobserved locations. These predictions are based on neighbouring observations, the spatial configuration of these observations and the semivariogram (Journel and Huijbregts, 1978). This way of spatial interpolation is widely used for mapping in geology (Trangmar *et al.*, 1985). Monitoring diseases requires intensive and repeated observations which are time-consuming and expensive. Therefore, kriging would be very useful for spatial studies.

Another aspect to consider in characterizing patterns is the method of disease assessment. The disease intensity in a plot can be measured in various ways such as the number of lesions, the lesion area, the number of diseased leaves, the percent of defoliation, or the percent incidence. The method of disease assessment might influence the results of pattern analysis of black rot in cabbage and their interpretation.

The objectives of this paper were *i*) to study the pattern of cabbage plants naturally infected by the black rot pathogen, *ii*) to evaluate the sampling pattern, *iii*) to evaluate the efficiency of kriging to reproduce observed patterns using a reduced number of samples, and *iv*) to compare methods of disease assessment and their effect on pattern analysis.

8.2 Materials and methods

Field plots and disease assessment. Spontaneous black rot epidemics in field grown cabbage were studied at Wageningen (coordinates 51.57N, 5.31E) during 1990 and 1991. The white cabbage (*Brassica oleraceae* L. convar. *capitata* (L.) Alef var. *alba* DC) cultivar Erdeno was used in 1990 and the red cabbage (*B. oleraceae* L. convar. *capitata* (L.) Alef var. *rubra* DC) cultivar Vesta was used in 1991. The cultivar Erdeno was planted on 7 May 1990 in a field with 17 x 90 plants. Three plots (90A, 90B and 90C) were chosen, each of 15 x 10 plants. These plots were separated by 6 meters borders of non-plot cabbage plants. The cultivar Vesta was planted on 22 May 1991 in two fields (91A and 91B), each with 48 x 48 plants. These plots were separated by 6m of field beans. The plants were planted in a square grid with a distance between plants of 0.5 m for both 1990 and 1991. Wind-direction and rainfall were recorded in both years.

Black rot appeared spontaneously and it was recorded for each individual plant by visual assessment of both disease incidence and diseased leaf incidence, the latter defined as the number of diseased leaves. These observations were made on 27 July, 1, 7, 10 and 21 August, and 3 September in 1990 and on 6, 17, 23 and 30 July and on 6, 13, 21 and 29 August in 1991.

Pattern analysis. The first step was to calculate semivariograms from the field data. Theoretical models were fitted by weighted least squares regression with weights equal to the number of pairs of observations in a distance class. The coefficient of correlation between calculated semivariogram values and the values of the regression model (R^2) was used to evaluate the model type. A *t*-test was used to test slopes ($P < 0.05$) in the linear semivariograms for a significant difference from zero. If the spatial dependence varies according to direction, the variable is said to be anisotropic. Therefore, non-directional and directional semivariograms were studied. Semivariograms were calculated in four directions, 0°, 45°, 90° and 135° (north, northeast, east and southeast) to analyze the influence of wind direction during rainfall.

The second step was to determine the sampling distance. The sampling distance is defined as the distance in meters between two sample units. A sample unit consists of one single plant. Since we were interested in reducing sampling time during field studies, the effect of reducing the sample units at any observation day was studied. We increased the sampling distance from 0.5 m to 1.0 m and 1.5 m (1990) and from 0.5 m to 1.0 m, 1.5 m, 2.0 m, 2.5 m, 3.5 m, 5.0 m, 7.5 m, and

Table 8.1. Disease incidence and semivariogram models at different dates for black rot in cabbage, 1990. L, P, S represent semivariogram models, L = linear, P = pure nugget, S = spherical. Pure nugget semivariogram when the slope of the linear model did not differ significantly form zero ($P<0.05$).

	90A [x]				90B				90C			
Date	DI [y]	a[z]	b	c	DI	a	b	c	DI	a	b	c
27/7	3	P	S	P	15	P	P	P	18	S	S	P
01/8	7	S	S	P	51	L	L	P	42	L	S	P
07/8	13	P	S	P	54	L	L	L	57	L	L	P
10/8	23	P	P	P	65	L	S	S	59	L	L	P
21/8	24	P	L	P	59	L	L	P	51	L	L	L
03/9	11	P	P	P	39	L	S	S	48	L	L	L
	DLI [y]	a	b	c	DLI	a	b	c	DLI	a	b	c
27/7	0.1	L	S	P	0.3	S	P	P	0.5	S	S	P
01/8	0.1	L	S	S	1.3	L	P	P	1.1	S	S	L
07/8	0.2	P	P	S	1.5	L	P	P	1.7	S	S	L
10/8	0.3	L	L	L	1.7	S	S	P	1.9	S	S	L
21/8	0.3	L	S	P	1.4	S	S	L	1.6	L	L	P
03/9	0.1	L	P	L	0.6	S	S	P	1.1	L	L	P

[x] 90A, 90B and 90C = black rot in cabbage during 1990, plots A, B and C.
[y] DI = disease incidence per plot, DLI = diseased leaf incidence per plot.
[z] Sampling distance: a = 0.5 m, b = 1.0 m, c = 1.5 m.

Kriging black rot in 1990. Table 8.2 represents the kriging results of sampling distances 1.0 m and 1.5 m for disease incidence. The observed and calculated disease incidence and the mean squared difference V_D are presented. A distinction was made between the percentage of correctly calculated healthy plants and diseased plants. A sampling distance of 1.0 m resulted in a clear difference in the calculation of disease incidence in plot 90A. The pattern of disease did not show a pronounced structure and no good relation between observed and calculated disease incidence was found. Kriging underestimated disease incidence for all observation days. The V_D increased with increasing disease intensity indicating a decrease in the accuracy of kriging.

Table 8.2. Observed (obs) and calculated (cal) disease incidence, percentage of correctly calculated healthy plants (hea) and percentage of correctly calculated diseased plants (dis) in plot 90A, 90B and 90C with kriging using sampling distance of 1.0 m and 1.5 m.

		1.0 m				1.5 m			
Date	obs	cal	V_D[z]	hea	dis	cal	V_D	hea	dis
90A									
27/7	3	1	0.01	100	25	1	0.01	100	25
01/8	7	3	0.05	100	39	3	0.05	100	39
07/8	13	4	0.09	100	30	5	0.09	100	35
10/8	23	7	0.18	100	29	7	0.18	99	26
21/8	24	13	0.19	95	39	11	0.19	99	25
03/9	11	3	0.07	100	25	4	0.07	100	38
90B									
27/7	15	13	0.07	97	68	7	0.07	100	50
01/8	51	54	0.19	78	84	59	0.15	77	92
07/8	54	56	0.17	80	86	64	0.19	68	91
10/8	65	70	0.05	76	99	72	0.15	70	94
21/8	59	63	0.16	76	90	63	0.27	63	87
03/9	39	35	0.13	92	78	37	0.22	84	69
90C									
27/7	18	15	0.00	98	87	17	0.08	97	65
01/8	42	41	0.15	87	81	38	0.23	82	70
07/8	57	65	0.15	72	94	66	0.24	65	86
10/8	59	55	0.23	77	76	64	0.29	57	80
21/8	51	47	0.22	82	74	55	0.15	81	88
03/9	48	54	0.19	76	86	47	0.24	78	74

[z] V_D is the mean squared difference

The kriging results were divided into healthy and diseased. The percentage of correctly calculated healthy plants gives the percentage of healthy plants observed which were correctly calculated to be healthy. The same was done with regard to the diseased plants. The calculation of the healthy plants was successful since the percentage correctly calculated healthy plants was high (range 95% to 100%). However, calculation of diseased plants gave problems since only 25-39% was

Table 8.3. Observed (obs) and calculated (cal) diseased leaf incidence, correctly calculated percentage of healthy plants (hea) and correctly calculated percentage of diseased leaf incidence per plant with kriging using sampling distances of 1.0 m and 1.5 m.

		1.0 m				1.5 m			
Date	obs	cal	V_D[1]	hea	dis	cal	V_D	hea	dis
90A									
27/7	0.1	<0.1	0.09	100	25	<0.1	0.09	100	25
01/8	0.1	<0.1	0.13	100	39	0.1	0.11	99	69
07/8	0.2	0.1	0.17	98	30	0.1	0.2	94	45
10/8	0.3	0.2	0.41	93	40	0.2	0.43	91	37
21/8	0.3	0.2	0.38	92	42	0.2	0.31	96	34
03/9	0.1	0.1	0.41	96	48	0.1	0.10	95	50
90B									
27/7	0.3	0.3	0.29	89	64	0.2	0.39	96	36
01/8	1.3	1.4	1.77	67	61	1.1	2.13	55	56
07/8	1.5	1.7	2.07	65	61	1.4	2.84	51	52
10/8	1.7	1.8	2.03	55	57	1.5	2.69	49	44
21/8	1.4	1.4	3.87	58	53	1.4	3.55	59	46
03/9	0.6	0.8	2.43	60	54	0.7	1.77	55	43
90C									
27/7	0.5	0.4	0.43	67	22	0.4	0.71	86	48
01/8	1.1	1.1	1.32	71	54	1.0	2.43	60	43
07/8	1.7	1.8	1.95	63	64	1.7	3.06	48	49
10/8	1.9	1.9	1.84	67	66	2.0	3.11	43	52
21/8	1.6	1.6	1.78	64	57	1.9	3.02	34	52
03/9	1.1	1.2	1.17	65	67	1.2	1.86	55	51

[1] V_D is the mean squared difference

correctly calculated to be diseased in plot 90A, using a sampling distance of 1.0 m. Further increase of the sampling distance from 1.0 m to 1.5 m gave only small differences. Kriging resulted in predictions approximating the real situation for plots 90B and 90C (Table 8.2). A close relation was found between observed and calculated values.

Kriging was used effectively to evaluate the patterns with time for plots 90B and 90C. The V_D increased with increasing disease incidence for plots 90B and 90C. Increasing the sampling distance decreased the quality of spatial interpolation, as shown by the increase of V_D and the decrease in the percentages of correctly calculated healthy or diseased plants.

Three major differences between plot 90A and plots 90B and 90C can be mentioned, *i*) the V_D value was much lower for 90A, *ii*) the percentage of correctly calculated healthy plants was much higher for 90A, and *iii*) the percentage of correctly calculated diseased plants was much lower for 90A.

Kriging was also performed to calculate the diseased leaf incidence (Table 8.3). For plot 90A, kriging underestimated the diseased leaf incidence per plant. This underestimation was found for all observation days and for both sampling distances. The V_D increased with increasing disease intensity. The calculation of healthy plants was successful since the percentage correctly calculated healthy plants was high. However, calculation of diseased leaves per plants was unsuccessful in plot 90A, using a sampling distance of 1.0 m. In plots 90B and 90C, increasing the sampling distance to 1.0 m or 1.5 m also gave a difference in the interpolation but the calculated diseased leaf incidence approximated the actual situation. For all plots, increasing the sampling distance resulted in a poorer interpolation, as shown by the increase of V_D.

Pattern analysis of black rot in 1991. The highest disease incidence in 1991 was 60% (91A) and 46% (91B), reached on the last observation date (Table 8.4). A chi-square test applied to wind direction during rainfall indicated that a southwesterly wind direction was strongly related to rainfall (Table 8.5, $\chi^2 = 18.5$, $P < 0.001$). The semivariograms for plots 91A and 91B indicated a spatial dependence of black rot disease incidence, since semivariograms were linear or spherical, except for 6 July in 91A and for 6 and 17 July in 91B. The patterns of these early spontaneous infections were random. Directional semivariograms showed anisotropy for several dates (Table 8.5). The spatial dependence had a preference for 45° in both plots.

Increasing sampling distance gave a change of model type (Table 8.4) and parameter values (not shown) of semivariograms. In general, semivariograms only showed pure nugget effects when sampling distance was >2.5 m.

The results of the pattern analysis using diseased leaf incidence are shown in Table 8.6. The highest diseased leaf incidence was 1.46 in plot 91A and 0.80 (leaves per plant) in plot 91B. The diseased leaf incidence showed spatially dependence, except on 6 July (91A and 91B, pure nugget effect). The spatial

Table 8.4. Disease incidence, anisotropy and semivariogram model at different dates for black rot in cabbage, 1991. L (linear), P (pure nugget), S (spherical) represent semivariogram models. Pure nugget semivariogram when the slope of the linear model did not differ significantly form zero ($P<0.05$).

Date	DI[x]	G[y]	a[z]	b	c	d	e	f	g	h	i
91A											
06/7	6	-	P	S	P	P	P	P	P	P	P
17/7	21	-	S	S	S	S	S	P	P	P	P
23/7	31	0°	S	S	S	S	S	P	S	P	P
30/7	43	45°	L	L	S	L	L	P	P	P	P
06/8	50	45°	S	S	S	S	L	S	P	P	P
13/8	54	45°	S	S	S	S	L	S	P	P	P
22/8	60	45°	L	L	S	S	P	S	P	P	L
29/8	60	45°	L	L	S	S	P	S	P	P	P
91B											
06/7	5	-	P	S	S	L	P	P	P	P	P
17/7	5	-	P	S	S	L	P	P	P	P	P
23/7	13	-	S	S	S	L	P	P	P	P	P
30/7	27	-	S	P	S	S	S	P	P	P	P
06/8	32	-	S	S	S	S	P	P	P	P	P
13/8	43	45°	S	S	S	S	L	P	L	P	P
22/8	47	45°	S	L	S	S	S	P	L	P	L
29/8	47	45°	S	L	L	S	S	P	L	P	P

[x] DI = disease incidence.

[y] G = direction of anisotropy, - : no anisotropy was found.

[z] Sampling distance a = 0.5 m, b = 1.0 m, c = 1.5 m, d = 2.0 m, e = 2.5 m, f = 3.5 m, g = 5.0 m, h = 7.5 m, and i = 10.0 m.

dependence had a preference for 45° in anisotropic patterns (Table 8.6).

Increasing the sampling distance influenced the results of semivariogram analysis. Generally, non-directional semivariograms turned into pure nugget semivariograms when sampling distance was >3.5 m.

Table 8.5. Wind direction and rainfall from 17 May until 29 August 1991.

	N [y]	NE	E	SE	S	SW	W	NW
observed frequency[z]	2	3	5	2	3	17	5	3
zero hypothesis	5	5	5	5	5	5	5	5

[y] wind direction

[z] frequency of the combination wind direction and rain appeared.

Table 8.6. Diseased leaf incidence, anisotropy and the semivariogram model at different dates for black rot in cabbage, 1991. L (linear), P (pure nugget), S (spherical) represent semivariogram models. Pure nugget semivariogram when the slope of the linear model did not differ significantly form zero ($P < 0.05$).

Date	DLI [x]	G [y]	a [z]	b	c	d	e	f	g	h	i
91A											
06/7	0.1	-	P	P	P	S	P	P	P	P	P
17/7	0.3	-	S	S	S	S	S	S	L	P	P
23/7	0.4	-	S	L	S	S	S	S	P	P	P
30/7	0.7	45°	S	L	S	L	L	P	P	P	P
06/8	0.9	45°	S	L	L	L	L	L	P	P	P
13/8	1.1	45°	S	L	S	S	L	L	P	S	P
22/8	1.5	45°	S	L	S	S	L	P	P	S	P
29/8	1.5	45°	S	S	S	S	L	S	P	P	P
91B											
06/7	0.1	-	P	S	S	S	P	P	P	P	P
17/7	0.1	-	L	P	S	S	P	P	P	P	P
23/7	0.2	-	S	S	S	S	S	S	S	P	P
30/7	0.4	45°	S	S	S	S	L	L	S	S	P
06/8	0.5	-	S	S	S	S	P	P	L	P	P
13/8	0.7	-	S	S	S	S	S	S	P	P	P
22/8	0.8	45°	S	S	S	S	P	P	P	P	S
29/8	0.8	45°	L	L	S	S	L	S	P	P	P

[x] DLI = diseased leaf incidence.

[y] G = direction of anisotropy, - : no anisotropy was found.

[z] Sampling distance a = 0.5 m, b = 1.0 m, c = 1.5 m, d = 2.0 m, e = 2.5 m, f = 3.5 m, g = 5.0 m, h = 7.5 m, and i = 10.0 m.

Table 8.7. Observed (obs) and calculated (cal) disease incidence, correctly calculated percentage of healthy plants (hea) and correctly calculated percentage of diseased plants (dis) in plot 91A and 91B with kriging using sampling distances of 1.0 m, 2.5 m, and 10.0 m.

		1.0 m				2.5 m				10.0 m			
Date	obs	cal	V_D[z]	dis	hea	cal	V_D	hea	dis	cal	V_D	hea	dis
91A													
06/7	6	5	0.04	98	56	2	0.05	100	38	1	0.06	100	10
17/7	21	19	0.12	84	67	9	0.17	97	31	2	0.19	99	9
23/7	31	32	0.16	87	77	22	0.23	89	49	17	0.31	88	28
30/7	43	45	0.15	84	86	38	0.26	82	65	41	0.35	71	57
06/8	50	50	0.20	80	80	51	0.29	70	73	54	0.36	68	60
13/8	54	57	0.15	79	89	58	0.30	63	77	58	0.35	69	58
22/8	60	64	0.15	76	91	68	0.28	55	84	71	0.34	81	43
29/8	60	64	0.15	76	91	69	0.28	54	84	68	0.35	77	45
91B													
06/7	5	4	0.03	99	57	1	0.04	100	19	1	0.04	100	10
17/7	5	4	0.03	99	57	1	0.04	100	19	1	0.04	100	10
23/7	13	10	0.07	98	59	4	0.11	99	22	1	0.12	100	8
30/7	27	27	0.14	91	73	14	0.22	94	36	5	0.26	97	11
06/8	32	34	0.17	86	77	30	0.26	82	57	16	0.32	88	26
13/8	43	46	0.18	81	82	41	0.31	75	62	35	0.39	72	45
22/8	46	46	0.21	81	77	48	0.32	68	67	40	0.41	67	49
29/8	47	46	0.21	81	77	48	0.32	68	67	40	0.41	67	49

[z] V_D is the mean squared difference

Kriging black rot in 1991. Table 8.7 shows the kriging results for disease incidence with sampling distances of 1.0 m, 2.5 m, and 10.0 m. An increase of the sampling distance to 1.0 m resulted in a minor change of calculated disease incidence. V_D increased with increasing disease intensity and also increased when the sampling distance was increased. The calculation of healthy and diseased plants was successful in terms of percentage correctly calculated disease status of the plants (Table 8.7). Kriging reproduced the observed disease incidence with high precision as illustrated in Figure 8.2. Figure 8.2 contains a selection of maps of the observed

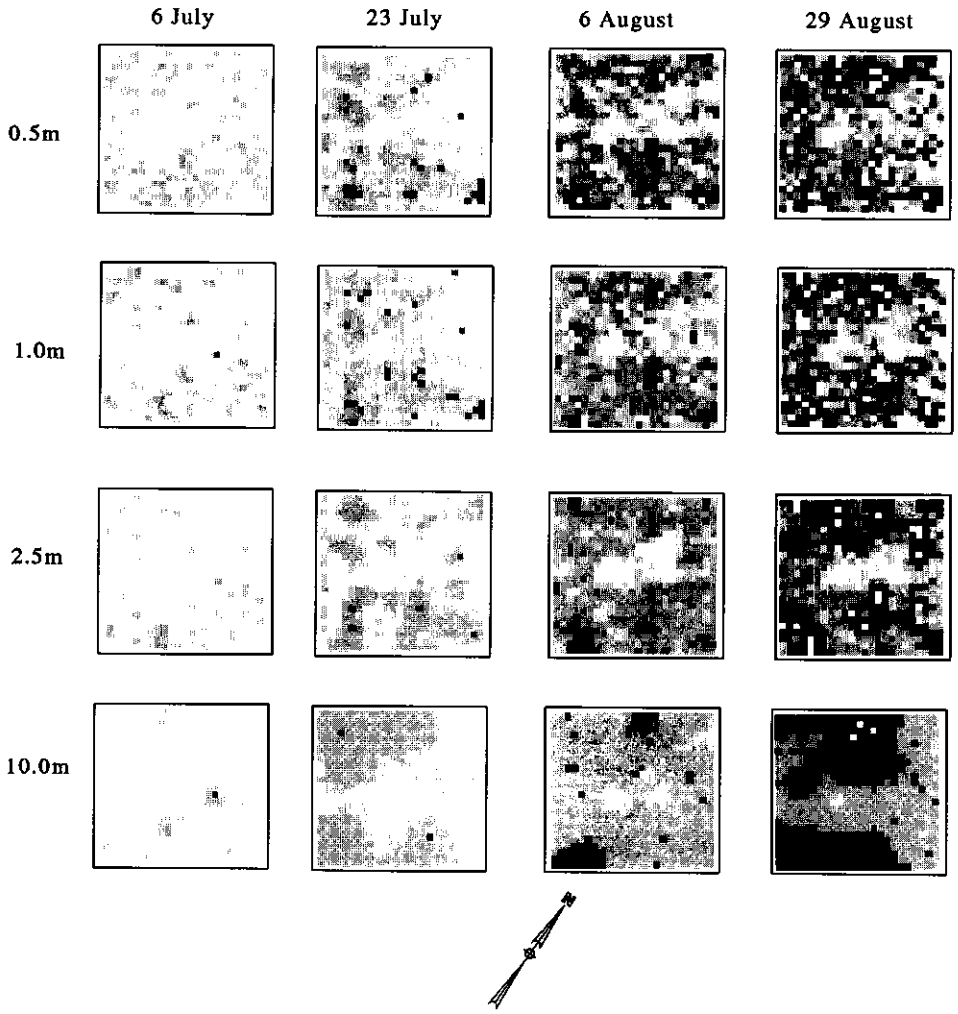


Figure 8.2. Black rot of cabbage in 1991, patterns of disease incidence in plot 91A for 6 July, 23 July, 6 August and 29 August (from left to right), sampling distance 0.5 m, 1.0 m, 2.5 m and 10.0 m (from top downwards). One square represents a quadrat of 2x2 plants. The intensity of shading indicates the cumulative disease incidence. Shading categories: 0(not shaded), no diseased plants; 1, 1 diseased plant; 2, 2 diseased plants; 3, 3 diseased plant; and 4 (heavily shaded), 4 diseased plants.

patterns of disease incidence and of the calculated patterns with sampling distances 1.0 m, 2.5 m and 10.0 m (plot 91A). Each square represents the cumulative disease incidence per 4 plants, a reduction applied only to simplify the figures. The maps of disease incidence in plot 91A show that the initial pattern was random (6 July). The disease incidence increased over time and secondary foci developed after a few weeks (23 July and 6 August). Diseased plants were generally found at the southeastern half of the plot, which had a higher disease incidence than the centre. Comparison of the maps of the observed and calculated data shows that kriging based on sampling distance 1.0 m provided good spatial interpolations. Kriging reproduced the higher incidence at the borders and also indicated the foci. For 6 July with sampling distance 1.0 m, a focus was indicated in the southwest sector. Comparison with the observed data showed that this was an overestimation of the disease incidence in this sector of the plot. Increase of the sampling distance to 2.5 m gave larger deviations, especially during the beginning of the epidemic. These larger deviations were caused by lack of detectable disease on plants using the larger sampling distance. However, the kriging maps still provided appropriate representations of the observed field situations. The calculated patterns were more uniform than the observed patterns but foci were still detectable. A sampling distance of 10.0 m gave unacceptable maps. For 6 and 23 July, the pattern was clearly wrong interpolated. Disease was hardly detected on 6 July and three sharp foci were indicated on 23 July. For 6 and 29 August, kriging suggested a highly structured pattern which no longer reflected the real situation.

Kriging the diseased leaf incidence resulted in calculations approaching the observed values (Table 8.8). Deviation in calculated values from the observed situation increased with sampling distance as shown by V_D values. Figure 8.3 shows a selection of maps of observed and calculated patterns of diseased leaf incidence in plot 90A with sampling distances 1.0 m, 2.5 m and 10.0 m. Each square represents the cumulative diseased leaf incidence per 4 plants. The calculated pattern, although reproducing the average disease intensity, underestimated the random variation. Diseased leaf incidence per individual plant was sometimes overestimated or underestimated, as reflected by the relative low percentage of correctly calculated cases. Kriging provided appropriate representations of the observed field situations, as exemplified by Figure 8.3. The patterns varied over time with either randomness or some aggregation. Despite the variability, a trend can be described. In the early epidemics, randomness was found, which could be reproduced using sampling distance 1.0 m. With fewer sample units (larger sampling distances), we missed the early characteristic of the pattern (maps with sampling distances 2.5 m and 10.0 m,

Table 8.8 Observed (obs) and calculated (cal) diseased leaf incidence, correctly calculated percentage of healthy plants (hea) and correctly calculated percentage diseased leaf incidence per plant with kriging using sampling distances of 1.0 m, 2.5 m and 10.0 m.

		1.0 m				2.5 m				10.0 m			
Date	obs	cal	V_D[z]	dis	hea	cal	V_D	hea	dis	cal	V_D	hea	dis
91A													
06/7	0.1	0.1	0.08	97	58	<0.1	0.10	99	24	<0.1	0.13	98	10
17/7	0.3	0.2	0.20	92	61	0.2	0.30	89	39	0.2	0.41	83	27
23/7	0.4	0.4	0.30	86	69	0.4	0.46	73	52	0.5	0.71	44	52
30/7	0.7	0.7	0.53	79	69	0.7	0.90	53	56	0.8	1.16	30	55
06/8	0.9	0.9	0.62	74	68	0.9	1.04	41	56	1.0	1.25	15	52
13/8	1.1	1.1	0.92	71	66	1.2	1.52	34	53	1.2	1.87	12	46
22/8	1.5	1.4	1.35	68	64	1.5	2.19	25	48	1.5	2.78	10	37
29/8	1.5	1.5	1.33	69	63	1.5	2.17	24	47	1.5	2.84	9	36
91B													
06/7	0.1	<0.1	0.05	99	53	<0.1	0.06	100	18	<0.1	0.07	100	10
17/7	0.1	0.1	0.08	97	57	<0.1	0.09	99	26	<0.1	0.11	100	9
23/7	0.2	0.1	0.10	97	61	<0.1	0.17	99	20	<0.1	0.19	100	8
30/7	0.4	0.3	0.24	90	66	0.2	0.48	87	35	0.2	0.60	88	17
06/8	0.5	0.5	0.37	84	67	0.4	0.64	72	46	0.4	0.87	65	34
13/8	0.7	0.6	0.48	77	70	0.7	0.75	44	60	0.5	0.99	55	41
22/8	0.9	0.8	0.55	71	70	0.8	0.94	35	59	0.7	1.19	46	41
29/8	0.8	0.8	0.59	70	70	0.8	0.98	40	55	0.7	1.19	46	41

[z] V_D is the mean squared difference

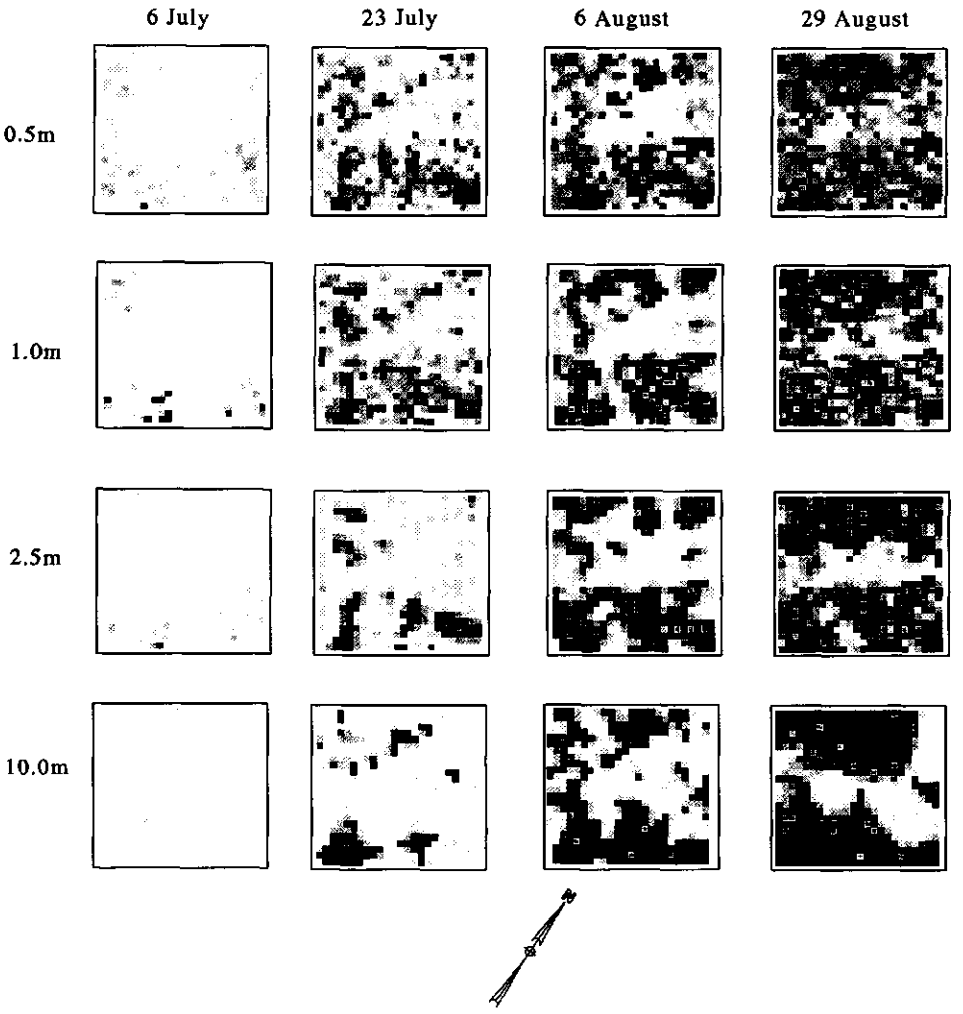


Figure 8.3. Black rot of cabbage in 1991, patterns of diseased leaf incidence in plot 91A for 6 July, 23 July, 6 August and 29 August, (from left to right), sampling distance 0.5 m, 1.0 m, 2.5 m and 10.0 m (from top downwards). One square represents a quadrat of 2x2 plants. The intensity of shading indicates the cumulative number of diseased leaves. Shading categories: 0(not shaded), no diseased leaves; 1, 1 diseased leaf; 2, 2-3 diseased leaves; 3, 4-6 diseased leaves; 4, 7-10 diseased leaves; 5 (heavily shaded), more than 10 diseased leaves.

6 July). The calculated maps using a sampling distance of 1.0 m or 2.5 m were well suited for spatial interpretation of the black rot epidemics. Kriging reproduced the higher diseased leaf incidence at the borders and foci. Increase of the sampling distance to 10.0 m gave large deviations at all dates due to reduced detection of disease at this large sampling distance.

8.4 Discussion

Spatial dependence and anisotropy. Our objectives were *i*) to study the pattern of cabbage plants naturally infected by the black rot pathogen, *ii*) to evaluate the sampling pattern, *iii*) to evaluate the efficiency of kriging to reproduce observed patterns using a reduced number of samples, and *iv*) to compare methods of disease assessment and their effect on pattern analysis. The pattern analysis was performed by interpreting semivariograms as to absence and presence of spatial dependence and anisotropy. Notable was the high number of linear models in 1990. Linear semivariograms suggest that the disease pattern is determined by strong spatial dependence without a limiting value (Lecoustre *et al.*, 1989). We expected to find spherical models since we assumed the influence of disease decreases with distance until a limiting distance where no influence exists. The plots had a rather small size in 1990 and, maybe, therefore no sill could be detected. In 1991, many spherical models were found, possibly because the plots were larger than in 1990 so that a sill and a range could be detected. We conclude that plot size may affect the choice of model to describe a semivariogram.

An aggregation in the direction of the prevailing wind-direction was expected since wind driven rain splash disperses *X.c.* pv. *campestris* downwind (Cook *et al.*, 1952; Williams, 1980). In 1990, anisotropy could hardly be found, possibly due to infection from outside the plots. In 1991, directional semivariograms pointed for strong anisotropy with preference to the northeast direction, the wind direction during rainfall. We conclude that wind driven rain splash may affect disease patterns, in agreement with the results of Hunter *et al.* (1975) and Strandberg (1973).

Sampling plan. An increase of the sampling distance sometimes resulted in a shift from structured models of semivariograms into pure nugget models. Pure nugget semivariograms either indicate absence of spatial dependence or suggest that

Table 8.9. Observed (obs) and calculated (cal) disease incidence, correctly calculated percentage of healthy plants (hea) and correctly calculated percentage of diseased plants (dis) in plot 90A with kriging using sampling distances of 1.0 m and 1.5 m, based on even sampling locations.

		1.0 m			1.5 m		
Date	obs	cal	hea	dis	cal	hea	dis
27/7	3	2	100	75	1	100	50
01/8	7	9	96	77	6	100	69
07/8	13	13	96	70	13	93	65
10/8	23	26	90	77	29	83	69
21/8	24	22	91	69	25	82	57
03/9	11	'11	96	75	6	98	48

sampling distances were too large to detect spatially dependent structures (Journel and Huijbregts, 1978). The latter is the case in 1991 with sampling distances over 2.5 m (Table 8.4) and 3.5 m (Table 8.6). Therefore, a sampling distance up to 2.0 m and to 3.0 m for respectively disease incidence and diseased leaf incidence is adequate to study small-scale patterns of black rot disease in small cabbage fields.

Spatial interpolations. Kriging with a sampling distance of 1.0 m was less successful for plot 90A with grid sampling, a method of spatial sampling suitable for kriging (Corsten and Stein, 1994). The first plant in the edge of a plot was taken as the first sample location. Curiously, kriging performed much better for plot 90A by taking the second plant as starting point (Table 8.9). Inspection of the original data showed that black rot occurred mainly on the even sample units. Here, we stumbled upon an extreme example of an unusual localisation of the disease. For this particular case, random sampling it would have been better than grid sampling.

Results of 1991 showed that kriging was successfully applied to visualize several features of spatial heterogeneity of the small-scale black rot patterns, including foci and higher disease intensity at the borders, when sampling distances did not exceed a certain limit, the range. In our experiments, the range was 2.5 m for disease incidence and 3.5 m for the diseased leaf incidence. Sampling based on these results would result in a reduction of 80% and 86% of the number of sample units, respectively. Lecoustre *et al.* (1989) stated that a sample of 7% was sufficient to

assess the pattern of African cassava mosaic virus (ACMV) (1989) which suggest that the upper limit to sampling distance depends on the pathosystem under study. Tables 8.2, 8.3, 8.7, and 8.8 show that the calculation of rare events, such as a particular plant being diseased, is difficult at low disease incidence in pure nugget situations as reflected by the low percentage of correctly calculated diseased plants. Not surprisingly, the calculation of the healthy plants performed well. When disease incidence is low, it is easy to calculate a particular plant to be healthy. We conclude that it is difficult to calculate rare events when they are not detected by samples of reduced size.

Quality of kriging. We compared the quality of kriging for the different sampling distances by calculating the mean square difference V_D per distance (Ahmed and De Marsily, 1992). V_D increased with increasing sampling distance (Tables 8.2, 8.3, 8.7, and 8.8). Our conclusion is that a decrease in the number of sample units decreases the quality of kriging. V_D also increased with increasing mean disease intensity (Tables 8.2, 8.3, 8.7, and 8.8) which would suggest that kriging performed less adequately when the disease intensity is higher. In our opinion, this would be an incorrect conclusion since the maps of Figures 8.2 and 8.3 illustrate the opposite. Therefore, we tried to correct the V_D for the mean disease intensity by calculating a coefficient of difference. This coefficient of difference is the quotient of squared root of V_D and the mean disease intensity (analogous the coefficient of variation). Results indicates that the coefficient of difference decreased when the disease intensity increase so that kriging performs better when mean disease intensity increases (data not shown). The coefficient of difference also increases when the sampling distance increases, suggesting a decrease in the quality of kriging wit increasing sampling distances. Both tentative conclusions are in accordance with the interpretations of Figures 8.2 and 8.3. The proposed calculation of the coefficient of difference looks promising, but more research is needed to avoid misinterpretation.

We calculated the average disease intensity and the pattern of disease. If only the first is the objective, it seems preferable to apply random sampling (Brus, 1993; Cochran, 1977). Strandberg proposed a modified sequential sampling method to obtain an average disease intensity of black rot in cabbage (1973). Although this method reduced sampling time by about one-third, it allow for information about the mean disease intensity only. We conclude that it is more efficient to use geostatistics since it can reduce the amount of sampling time, gives the mean disease intensity and provides spatial information about the pattern of disease.

Disease assessment method. Disease incidence and diseased leaf incidence data were both suitable to analyze the patterns of black rot. Gathering plant incidence data takes less time than counting the number of diseased leaves per plant (diseased leaf incidence) but diseased leaf incidence provides more detailed information about disease progress during later stages of disease development. We compared these two methods with regard to semivariogram analysis and to kriging. Theoretical maximum semivariance in a 2-class system (0 and 1,[incidence]) is 1^2, in a 9-class system (0-8,[diseased leaf incidence]) is 9^2. The discriminative capacity of a multi-class system increases with the square of the class number. Thus, diseased leaf incidence provides better discrimination of patterns and more nuance in kriging maps than incidence, at the expense of more sampling time. The dissimilarity of observation methods can result in different interpretations of spatial patterns. For example, we would conclude that using incidence data, black rot was spatially independent in plot 90A, whereas black rot was spatially dependent when using the number of diseased leaves. Another dissimilarity in semivariogram analysis due to the difference in assessment methods was shown in Tables 8.5 and 8.6. Semivariograms turned into pure nugget effects when sampling distance was >2.5 m and >3.5 m using disease incidence and diseased leaf incidence respectively thus illustrating the discriminative capacity of either assessments of disease intensity. We conclude that the intensity measure used affects the outcome of pattern analysis by geostatistics. Diseased leaf incidence provides more detail then incidence, but for a first approximation or a rapid survey incidence is satisfactory in the case of black rot of cabbage.

Effects of scale. This study showed, once again, that geostatistics can be useful to analyze and map patterns. Geostatistics can help to reduce the number of sample units and still visualize the real situation when the sampling distance does not exceed the range of spatial dependence of the studied pathogen. This range can change each year. Van de Lande found the range for spear rot in oil palm differing between 94 m and 275 m in 1986 to 1988 (Van de Lande, 1993). Dissimilar semivariograms for different years were also found by Munkvold *et al.* (1993). However, the pathogen and scale effects are of importance too. Nelson *et al.* found that the range varied between 17 and 20 km for a multiple virus system (1994) while Chellemi *et al.* found a range of 0.9 m for *Phytophthora nicotianae* var. *parasitica* (1988). Another point in these articles is the scale of observation. Nelson *et al.* (1994) discussed a large scale experiment since they used sample units of 5 x 5 km squared while Chellemi *et al.* (1988) analyzed small scale experiments with

sample units 0.3 x 0.3 m. We also performed small scale experiments. The optimum sampling distance of 3.5 m gives a notable reduction of needed sample units. However, such a reduction is not good enough for large scale black rot surveys. We conclude that the applicability of geostatistics may vary according to pathosystem, for year and scale of experimentation.

Chapter 9

General Discussion

Cabbage can be damaged by black rot, caused by the bacterium *X.c.* pv. *campestris*. Damage by black rot in cabbage in The Netherlands was first described in 1900 by Van Hall. Black rot is still a threat to cabbage production. Various aspects of the ecology and epidemiology of black rot in cabbage have been analyzed but quantitative information on black rot development in time and space is poor. In view of the limited possibilities to control black rot, more quantitative ecological knowledge is of scientific, economic and social importance.

The study reported here intends to obtain knowledge about sampling black rot on cabbage, to study black rot development in time, space and time-space taking into regard various inoculum sources and field resistances, and to evaluate the results in order to improve black rot control in The Netherlands.

9.1 Sampling and analysis of black rot on cabbage

Disease intensity measures. The course of an epidemic may be studied by monitoring the incidence or severity of disease in experimental units or in representative portions thereof (Van der Plank, 1963). The assessment unit (leaf, plant, group of plants, etc.) is a choice left to the experimenter. In the field experiments described in Chapters 3 to 8, all individual plants of each plot were monitored. Assessments of black rot were based on counts of the number of diseased plants per plot (disease incidence), counts of the number of diseased leaves per plant (diseased leaf incidence), and on the diseased area (cm^2) per plant (disease severity). Whereas an increase in disease incidence is only due to newly infected plants, an increase in diseased leaf incidence is due to new infections on either new plants or already infected plants. An increase in disease severity may also be due to lesion growth. Diseased leaf incidence provides more detailed information about disease progress during later stages of disease development. Although the diseased leaf incidence is not often used for plant diseases (Smilde, 1996), diseased leaf incidence appeared to be a good measures for analysing progress and spatial patterns of black rot, being a compromise between time-consuming severity measures and less informative plant incidence measures.

Temporal analysis. Black rot in cabbage was studied in field plots comprising various amounts of plants per plot (196 to 2500 plants per plot) caused by artificial uni-focal, artificial multi-focal, or natural infection. Disease intensity did not reached 100% in any of the plots (Table 9.1) and maximum disease intensity (K)

Table 9.1. Summary of experiments on type of infection, plot size and subsequent maximum disease intensity reached in the plots.

Year	Chapter	Type of infection[y]	Plot size (m)	Maximum disease incidence[z]	Maximum diseased leaf incidence[z]	Maximum disease severity[z]
'92/'93/'94	3	AUF	7 x 7	0.70	0.194	-
'91/'92	4	AUF	10.5 x 10.5	-	0.796	13.5
		AUF	7 x 7	-	1.181	13.8
'92/'93/'94	5	AUF	7 x 7	0.48	0.137	-
'92/'93/'94	6	AUF	10 x 10	0.41	0.088	-
		AMF	10 x 10	0.77	0.195	-
'90/'91	8	N	24 x 24	0.60	0.083	-
		N	7.5 x 5	0.59	0.106	-

[y] AUF = artificial uni-focal inoculation, AMF = artificial multi-focal inoculation, N = natural infection.

[z] Maximum disease incidence, diseased leaf incidence or disease severity found in the corresponding year. -: No intensity measures used.

varied per plot, so that r x K was used as an overall measure of the absolute rate of disease progress (Campbell and Madden, 1990). The r x K value appeared to be a good parameter to reflect the overall effect of black rot epidemics since both final disease intensity and rate parameter were taken into account (Chapters 3 and 4).

In the present studies, single sources of high infection level nor multi-focal sources were capable to infect (resulting in symptom expression) all plants of a single plot. Such a limited infection potential of foci was found for cabbage refuse piles (Chapter 3), for single source inoculations to test field resistance of cabbage against black rot (Chapter 4), and for natural infection (Chapter 8).

Spatial pattern analysis. Strandberg (1973) recognized the relevance of assessing the pattern of black rot to design experiments, sampling plans, and disease management studies. His spatial analysis was based on the use of the negative binomial distribution. Use of this distribution implies the assumption of independence of sampling points, an assumption which is often disregarded. Therefore, many classical methods are not adequate in describing spatial structures

(Nicot *et al.*, 1984). The introduction of geostatistics in phytopathology allowed to perform spatial analysis with interdependence of sampling points for small-scale (Lannou and Savary, 1991; Lecoustre *et al.*, 1988) and large-scale (Nelson *et al.*, 1994) experiments. Geostatistics appeared to be useful to design optimal sampling strategies and to perform spatial interpolation (Chapter 8). In addition, gradient-models (Gregory, 1968; Chapters 3, 4, 5, and 7), auto-correlation (Gottwald et al., 1992; Madden et al., 1987; Chapters 5 and 6), and geostatistics (Chapters 6, 7, and 8) have been used to study, describe, analyse, and/or discriminate spatial patterns of disease.

Three-dimensional maps and gradient analysis of disease provided excellent means to study the location, size and intensity of primary and secondary foci. Most secondary foci developed within 4 meters from the source. The dominance of the primary focus was obvious and this dominance persisted in the presence of secondary foci. Black rot symptoms were found close to the source plants, pointing at black rot dispersal over small distances. These observations confirm earlier work on spread of black rot of cabbage (Walker and Tisdale, 1920) and cauliflower (Clayton, 1929) and support the conclusions of Strandberg (1973) and Yuan *et al.* (1987) that infection of new plants is highly dependent upon their proximity to infected plants.

Space-time analysis. The spatio-temporal black rot development was related to initial inoculum level (Chapter 5) and spatial distribution of initial inoculum (Chapter 6). High inoculum levels in both uni-focal and multi-focal inoculation resulted in faster black rot progress in cabbage.

Van den Bosch *et al.*, (1988), Zadoks and Kampmeijer (1977), and Zadoks and Van den Bosch (1994) reasoned that disease gradients from infection foci will move outwards at a constant velocity. This theory was not confirmed with regard to the spatio-temporal development of black rot in cabbage (Chapter 5). Gradients of foliar symptoms did not move at a constant rate, a result also found for *Phytophthora infestans* of leek (Smilde, 1996). Neither was the theory confirmed with regard to the spatio-temporal development from infection foci (Chapter 5) or with regard to the decline of infestation foci due to bacteria in soil and plant debris therein (Chapter 7).

9.2 Epidemics

The artificial or natural inoculum sources studied here were not capable to spread disease over all plants of a plot, an experience which led to considerations concerning monocyclic and polycyclic development of black rot. Several harvested guttation droplets contained viable *X.c.* pv. *campestris*, so that secondary inoculum was detected in our study. Furthermore, Figure 5.1 suggests the existence of two infection cycles during 1992. Both results point at the possibility of polycyclic disease development. If black rot disease progress in cabbage would be polycyclic, the influence of initial aggregation of inoculum and initial level of inoculum should have been overcome, at least partially, by multiple cycles of inoculum production and dispersal and, evidently, to levelling of the disease intensity over the whole plot. Since disease development was limited, black rot is suggested to have few multiplication cycles, at least in comparatively dry seasons as in this study.

9.3 Black rot: incubation time and dispersal

Artificial or natural inoculum sources, under the conditions of the experiments, were not capable to spread disease over all plants of a plot (Table 9.1). Therefore, questions arise concerning incubation time, dispersal gradients, and the relation between inoculum density and incubation time. The influence of temperature and inoculum density on incubation time of black rot is analyzed in experiments A and B, and results are integrated with the evaluation of bacterial dispersal and disease development.

Experiment A. Cabbage plants of cv. Perfect Ball (three to five leaf stage) were watered thoroughly and placed in a plastic dew chamber (182x72x38 cm) with 15/9 h light/dark photoperiod, 20°C/12.5°C light/dark temperature and >80% RH for 24 h to induce the formation of guttation droplets. 48-h-old *X.c.* pv. *campestris* (PD 174) colonies, cultured on Yeast-Peptone-Glucose plates at 27°C, were diluted in distilled water to obtain a dilution series (about 10^2, 10^5, and 10^8). A hydathode inoculation was used which facilitated a reliable inoculation of *X.c.* pv. *campestris* into cabbage, in a manner which simulates a natural process of penetration.

Plants offered suitable inoculation sites when guttation droplets were present at the leaf margins. A single droplet of inoculum (2 μl) was delivered into each target

guttation droplet with a 100 μl Hamilton microliter syringe 1710RN. Five guttation droplets per leaf of the three youngest leaves per plant were inoculated. After inoculation and several hours of incubation in order to facilitate entry of bacteria into the leaf, plants were placed in growth chambers. Six to seven inoculated plants per density were placed in growth chambers at 10°C, 22.5°C, or 26°C with a 15/9 light/dark regime at 85% RH. Symptom expression was observed daily and scored over a period of three to four weeks. The incubation time was recorded for each inoculation site.

Incubation time was transformed to inverse incubation time. Healthy sites, having infinite value for incubation time, were set to zero. The average inverse incubation time was calculated for individual plants. Differences in the inverse incubation time between treatments were examined by analysis of variance ($P \leq 0.05$) (Genstat 5.01).

Experiment B. Four leaves per plant (white cabbage, cv. Perfect Ball, four to five leaf stage) were inoculated with various dilutions of *X.c.* pv. *campestris*. 48-h-old *X.c.* pv. *campestris* (PD 174) colonies, cultured on Yeast-Peptone-Glucose plates at 27°C, were diluted in distilled water to obtain a dilution series. Inoculation of dilution series was performed by injection of 0.03 ml inoculum at the base of a leaf close to the leaf stalk using a 1 ml syringe with sterile disposable needles (BD Plastipak 30G ½). Inoculated plants were placed in the greenhouse at 25°C temperature and 75% relative humidity (RH). Plants were scored for absence or presence of black rot symptoms. The experiment was performed three times (test I, II, and III). Data were obtained and analysed as described in experiment A.

Results and discussion. The inverse incubation time was influenced by inoculum density and by temperature (Table 9.2). The interaction of inoculum density × temperature was significant ($P = 0.02$). This interaction means that short incubation times were obtained at high bacterial densities at optimal temperatures for bacterial growth. Optimum temperature for growth of *X.c.* pv. *campestris* is 27-28°C (Pinches and Pallent, 1986; Shu and Yang, 1990). Thus, Table 9.2 supports the conclusions of Chapter 5 stating that the rate of black rot symptom development increases when temperature increases until the optimum temperature for bacterial growth is reached.

Table 9.2. Average inverse incubation time of black rot on cabbage at three temperatures and three inoculum densities, averaged over two inoculation days (Van Kesteren, 1993).

Temperature (°C)	Inoculum density (cfu/ml)[x]			
	Low[yz]	Medium	High	LSD[y]
10	0.018	0.023	0.026	0.004
22.5	0.063	0.079	0.074	0.011
26	0.074	0.106	0.093	0.017

[x] Bacterial densities are 1.75×10^2 and 1.17×10^3 (low), 1.75×10^5 and 8.81×10^7 (medium), and 7.65×10^8 and 3.97×10^9 cfu/ml (high).

[y] LSD within rows ($P = 0.05$)

[z] LSD within columns for inoculum density was 0.013 ($P = 0.05$)

The effect of inoculum density on incubation time of black rot was clear, notwithstanding the differences between the three tests (Figure 9.1). The inverse incubation time increased significantly at higher bacterial densities ($P = 0.006$, test I; $P = 0.003$, test II; $P = 0.004$ for test III), which means that long incubation times are due to low bacterial densities, as in Table 9.2.

As mentioned in several chapters, dispersal of black rot from various inoculum sources is limited to a few meters under Dutch field conditions, at least in relatively dry summers. The limited spread of *X.c.* pv. *campestris* in cabbage can be explained in several ways. The first considers dilution of inoculum with distance from the source. Butterworth and McCartney (1991) found that numbers of bacteria from strains of *Pseudomonas syringae*, *Klebsiella planticola* and *Bacillus subtillis* var. *niger* decreased rapidly with distance from sources, and that dispersal patterns resembled those previously found with splash-dispersed fungal plant pathogens (Fitt and McCartney, 1986). Similar dilution of inoculum was found for *X.c.* pv. *campestris* (Butterworth and McCartney, 1992). Dilution of inoculum decreases the number of bacteria available for infection of a leaf further away from an inoculum source. The subsequent rate of symptom development will be reduced since incubation time increases with decreasing amount of bacteria infecting a leaf. Plants more distant from the source may carry latent infections.

A second way considers that raindrops hitting a guttation droplet containing *X.c.*

pv. *campestris* would fragment into many, much smaller droplets. To cause infection of a leaf, it is necessary that bacteria settle on the infection sites of a leaf. Stomata are relative unimportant ports of entry for infection since openings need to be filled with water instead of air (Alvarez *et al.*, 1987). Under field conditions, stomata are rarely filled with water while hydathodes are regularly filled with guttation fluid. Therefore, hydathodes must be seen as the major ports of entry for secondary infection. Since hydathodes are relative few and small, only small amounts of dispersed bacteria will settle on the hydathodes.

The third way evaluates the dispersal conditions. Dispersal of black rot in cabbage is governed by rain splash (Williams, 1980) during guttation on diseased leaves (Cook *et al.*, 1952). Kuan *et al.* (1986) sampled airborne *X.c.* pv. *campestris* and found bacterial populations in presence of rain and also in absence of rain when leaves were wet (13.68 and 0.84 of viable *X.c.* pv. *campestris* per cubic meter of air, respectively). Thus, dispersal of *X.c.* pv. *campestris* is mainly governed by rain. As found in chapter 5, disease development and number of rain days were positively related, which agrees with results of Cook *et al.* (1952), Williams (1980), and Grimm and Vogelsanger (1990).

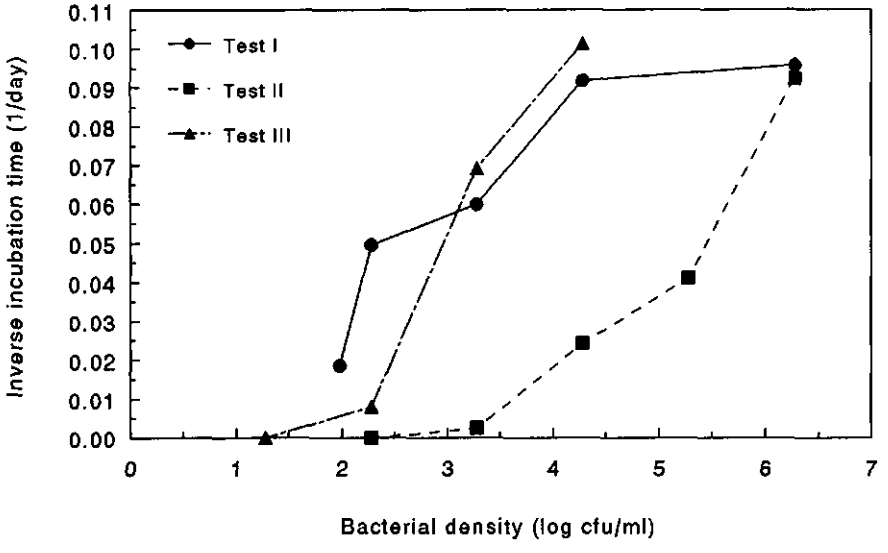


Figure 9.1. Effects of bacterial densities of *X.c.* pv. *campestris* on inverse incubation time (1/day) (Lengkeek and Etteger, 1992).

Necessary elements for development of plant disease epidemics are infection, colonization, inoculum production, dispersal, and survival (Madden, 1992). Bacteria and spores of many fungi are disseminated to new infection sites by water, which runs over plant surfaces, drops to lower plant parts, or is dispersed by splash droplets. Rain splash in the dispersal *X.c.* pv. *campestris* may be the critical component in black rot epidemics in cabbage crops. In overview, the dispersal of bacteria can be divided into three successive events, liberation (take-off), transport (flight), and deposition (landing). Compared with dissemination by wind, transport is short in duration and distance for splash-dispersed propagules (Campbell and Madden, 1990). However, a fuller understanding of splash dispersal requires an understanding of factors that influence transport and deposition of droplets carrying bacteria. Such understanding can be achieved by determining raindrop impaction and interaction with infection sites. These items are addressed by e.g. Madden (1992).

9.4 Explanatory survey related to experiments with black rot

Sanitation is an important component in the control of black rot (Williams, 1980). The importance of weedborne inoculum (Schaad and Dianese, 1981), soilborne inoculum (Ruissen *et al.*, 1990; Schaad and White, 1974), plant refuse piles (Chapter 2), and seedborne inoculum (Schaad *et al.*, 1980) has been described, considering sanitation of black rot in cabbage. Cabbage growers should reconsider the practices mentioned to prevent cabbage crops from black rot infection.

Weedborne inoculum. Weed control in ditches surrounding cabbage fields is done by order of the regional waterway management authority during autumn and winter (Chapter 2). If black rot epidemics in The Netherlands would originate from weeds surrounding the fields, infection of cabbage crop could occur during the complete cropping season, starting near ditches. If so, cabbage crops would be heavily infected. In the surveys, black rot infection could not be related to infection from weeds (although cruciferous weeds were present). Thus, infection from weeds was of minor importance with regard to the black rot levels found during the surveys in The Netherlands.

Soilborne and rootborne inoculum. Plant residue management is done in various ways (Table 2.7), but none of the practised plant residue management methods completely prevented black rot development. The result could mean that none of the methods is good enough to protect cabbage from black rot infection. However, plants may be infected in the nursery beds, infection may have come from other inoculum sources, or seeds may have been infected.

High black rot levels may be related to high disease levels in previous crops (Alvarez and Cho, 1978). In one case of the exploratory survey, a red cabbage crop (cv. Roxy) was completely infected by *X.c.* pv. *campestris* and heads were not harvested due to black rot. The crop was chopped and ploughed under in week 46. In week 11 of the next year, a cauliflower crop (Sernio) was planted which became infected in low amounts (black rot level 1; 1-10% diseased plants; 1992). These survey data and results of Chapter 7 suggest that, although soilborne inoculum may play a role under certain conditions, soilborne inoculum is not the major cause of full-field infections as found regularly in The Netherlands.

Plant refuse piles. Farmers in Heerhugowaard usually destroyed plant refuse by feeding it to cattle or by delivering it to commercial composers (Figure 2.7). Remaining refuse piles were found on farmyards, behind barns, in ditches, at field entrances, and close to nursery beds. As shown in Chapter 3, fresh refuse piles may infect cabbage crops, but complete field infection is hardly possible since black rot did not appear over distances larger than 4-7 m. Refuse piles may be a very serious problem when located near to the place where seedlings are grown. Seedlings are grown close together on small areas. Dispersal of *X.c.* pv. *campestris* over several meters may infect the seedling crop and subsequently result in field infection when the seedlings have been transplanted to the field and incubation time has elapsed. Thus farmers should be aware of refuse piles especially when they are located close to the cabbage nursery.

Although destruction of refuse piles may protect fields and seedlings from black rot infection, still some 50% of the fields in De Streek, where destruction was the usual method, were found to be infected. This result suggest that refuse piles were not the only infection source of black rot.

Seedborne inoculum. The use of healthy seeds is a major way to control black rot. Healthy seeds may be obtained by application of seed treatments. However, treatment may not be fully effective in eliminating the pathogen from the seed, and they may seriously affect the germination because of phytotoxicity (Humaydan *et*

al., 1980). Following seed treatment, a check on the efficacy of the treatment and the germination of the seed lot is always necessary.

Seed contamination with *X.c.* pv. *campestris* is an important source of primary inoculum for black diseases. Stock seed is imported annually from all over the world and the pathogen may have been introduced repeatedly in this manner. In several cases, full-field infection of cabbage could be related to a specific seed company.

The use of healthy crucifer seed is a major method to control the black rot disease. Therefore, good methods for detecting *X.c.* pv. *campestris* in crucifer seed lots are needed. Several methods are summarized or described by Franken (1992).

Resistance to black rot. A difference in resistance between cultivars may exist (Table 2.9 and Chapter 4). The availability of good resistance genes is limited. There is a need to find new sources of resistance, because the use of the few resistance genes available has undoubtedly narrowed the genetic base of hybrid crucifers (Williams, 1980). Moreover, there is a need for resistance at the seedling stages in addition to the mature plant resistance found in some cultivars (Hunter *et al*, 1987).

9.5 Final remark

Arguments for ecological and epidemiological research have been described in Chapter 1. The first argument concerned crop management practices at farm level. The survey performed to document cropping practices with regard to black rot indicated that a complex of different infection routes may contribute to black rot development in The Netherlands. Furthermore, the survey gave the opportunity to refine the research agenda.

Quantitative information is available on plant refuse piles as a factor in the origin of black rot epidemics (Chapter 3). Although infection can occur from refuse piles, they are not the major missing piece in the black rot puzzle.

Several authors estimated the survival of *X.c.* pv. *campestris* in soil and in host plant debris (Dzhalilov and Tiwari, 1995; Schaad and White, 1974b), but did not consider the appearance of foci, the disappearance of focal soil infestation and the spatial distribution of soilborne inoculum. The spatial distribution of soilborne inoculum could be reproduced clearly by soil sampling (Chapter 7). Dynamics of soil infestation by *X.c.* pv. *campestris* were followed during the winters and showed

a clear decline of bacterial populations with time. Build-up of new foci was not found since foci became extinct during these winters. Therefore, polyetic development of black rot through soil infestation did not occur.

Quantative assessment of the influence of initial inoculum and the effect of spatial distribution of primary inoculum on spatio-temporal black rot development are given in Chapters 5 and 6, respectively. Integration of both chapters indicate that full-field infection in The Netherlands can only originates from the presence of many foci in the field.

The effect of varietal resistance on temporal and spatial dynamics of black rot was clear (Chapter 4). Temporal and spatial development of black rot was governed by susceptible cultivars. Thus, increased levels of resistance can help to control black rot by reducing the development of the disease in time and space.

Cabbage fields sometimes become completely diseased by black rot in The Netherlands (Van de Ven, 1984; Chapter 2). The themes integrated in this chapter support the conclusion 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). In the latter case, infection of large fields seems to be possible only under unusually favourable circumstances for survival, infection, multiplication and dispersal of *X.c.* pv. *campestris*. Continued quantitative research on these topics should provide the necessary information on effects of seed infection and seedling infection on subsequent black rot development in cabbage.

References

Ahmed S and De Marsily G. 1987. Comparison of geostatistical methods for estimating transmissivity using data on transmissivity and specific capacity. Water Resource Research, 23(9):1717-1737.

Alvarez AM and Cho JJ. 1978. Black rot cabbage in Hawaii: inoculum source and disease incidence. Phytopathology 68:1456-1459.

Alvarez AM, Cho JJ and Hori TM. 1987. Black rot of cabbage in Hawaii. Research Series 051/ Hawaii Institute of Tropical Agriculture and Human Resources ISSN 0197-9310.

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

Backman PA, McGee DC and Morgan-Jones G. 1989. Stem canker. In: Compendium of Soybean Diseases, 3rd ed. Sinclair JB and Backman PA, eds. American Phytopathological Society, St.Paul, MN. pp. 41-43.

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.

Beyerlee D and Collins M. 1980. Planning technologies appropriate to farmers. Concepts and procedures. Centro Internacional del Mejoramiento de Maiz y Trigo (CIMMYT), Mexico. 71 pp.

Bonde R and Schultz ES. 1943. Potato refuse piles as a factor in the dissemination of late blight. Maine Agricultural Experiment Station Bulletin 416:230-246.

Brus DJ. 1993. Incorporating variation in sampling strategies for soil. PhD-thesis, Agricultural University Wageningen. 211 pp.

Buchanan RE and Gibbons NE. 1974. Bergey's manual of determinative bacteriology. Williams and Eilkins Co., Baltimore. pp. 243-244.

Butterworth J and McCartney JB. 1991. The dispersal of bacteria from leaf surfaces by water splash. Journal of Applied Bacteriology 71:484-496.

Butterworth J and McCartney JB. 1992. The removal and dispersal of foliar bacteria by rain splash. In: The release of genetically modified organisms. Stewart-Tull DES and Sussman M, eds. Plenum Press, New York. pp. 187-189.

Campbell CL and Madden LV. 1990. Introduction to plant disease epidemiology. John Wiley & Sons, New York. 532 pp.

Chellemi DO, Rohrbach KG, Yost RS and Sonoda RM. 1988. Analysis of the spatial pattern of plant pathogens and diseased plants using geostatistics. Phytopathology 78:221-226.

Chiarappa L (editor). 1970. Crop loss assessment methods. FAO manual on the evaluation and prevention of losses by pests, diseases and weeds. AGP:CP/22. Food Agric. Organ. UN., Rome, Italy.

Chupp C and Sherf AF. 1960. Vegetable Diseases and their Control. Ronald Press Company, New York. 698 pp.

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.

Clayton EE. 1929. Studies of the black-rot or blight disease of cauliflower. N.Y. State Agricultural Experiment Station Bulletin.

Cliff AD and Ord JK. 1973. Spatial autocorrelation. Pion Ltd., London. 178 pp.

Cochran WG. 1977. Sampling techniques. 3nd edn. Wiley New York. 248 pp.

Cook AA, Larson RH and Walker JC. 1952a. Relation of the black rot pathogen to cabbage seed. Phytopathology 42:316-320.

Cook AA, Walker JC and Larson RH. 1952b. Studies on the disease cycle of black rot of crucifers. Phytopathology 42:162-167.

Corsten LCA and Stein A. 1994. Nested sampling for estimating spatial semivariograms compared to other designs. Applied stochastic models and data analysis. Vol.10:103-122.

Drechsler C. 1919. Cotyledon infection of cabbage seedlings by *Pseudomonas campestris*. Phytopathology 9:275-282.

Dzhalilov FS and Tiwari RD. 1995. Soil and cabbage plant debris as infection sources of black rot. Archives of Phytopathology and Plant Protection 29:383-387.

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

Fitt BD and Bainbridge A. 1984. Effect of cellulose xanthate on splash dispersal of *Pseudocercosporella herpotrichoides* spores. Transactions of the British Mycological Society 82:570-571.

Franken AAJM. 1992. Immunofluorescence microscopy and dilution-plating for the detection of *Xanthomonas campestris* pv. *campestris* in crucifer seeds - methods to determine seed health and seed infection. PhD-thesis. Wageningen Agricultural University. pp. 165.

Gill JL. 1978. Design and analysis of experiments in the animal and medical sciences. Vol. 2. Iowa State University Press, Ames.

Gottwald TR, McGuire RG and Garran S. 1988. Asiatic citrus canker: spatial and temporal spread in simulated planting situations in Argentina. Phytopathology 78:739-745.

Gottwald TR, Reynolds KM, Campbell CL and Timmer LW. 1992. Spatial and spatiotemporal autocorrelation analysis of citrus canker in citrus nurseries and groves in Argentina. Phytopathology 82:843-851.

Graman H. 1981. A bacterial disease of cabbage. Botanical Gazette 16:265.

Gregory PH. 1968. Interpreting plant disease dispersal gradients. Annual Review Phytopathology 6:189-212.

Grimm R and Vogelsanger J. 1990. Black rot disease on cabbage, irrigation and spreading. Proceedings of the 7th International conference on Plant Pathogenic Bacteria, Budapest, Hungary, June 11-16, 1989. pp. 225-229.

Hau FC, Campbell CL and Beute MK. 1982. Inoculum distribution and sampling methods for *Cylindrocladium crotalariae* in a peanut field. Plant Disease 66:568-571.

Headrick JM and Pataky JK. 1988. Spatial and temporal development of common rust in susceptible and partially resistant sweet corn hybrids. Phytopathology 78:227-233.

Helm J. 1963. Morphologisch-taxonomische Gliederung der Kultursippen von Brassica olearacea. Kulturpflanze 11:92-210.

Humaydan HS, Harman GE, Nedrow BL and DiNito LV. 1981. Eradication of *Xanthomonas campestris* pv. *campestris*, the causal agent of black rot, from *Brassica* seeds with antibiotics and sodium hypochlorite. Phytopathology 70:127-131.

Hunter JE, Abawi GS and Becker RF. 1975. Observations on the source and spread of *Xanthomonas campestris* in an epidemic of black rot in New York. Plant Disease Reporter 59:384-387.

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.

Jeger MJ. 1983. Analyzing epidemics in time and space. Plant Pathology 32:5-11.

Journel AJ and Huijbregts CJ. 1978. Mining geostatistics. Academic Press, New York. 600 pp.

Kamoun S, Kamdar HV, Tola E and Kado CI. 1992. Incompatible interactions between crucifers and *Xanthomonas campestris* involve a vascular hypersensitive response: role of the hrpX locus. Molecular Plant-Microbe Interactions 5(1):22-33.

Kesteren FGHH. 1993. De invloed van inoculumdichtheid, temperatuur en relatieve

luchtvochtigheid op de ontwikkeling van zwartnervigheid. Afstudeerdverslag Landbouwuniversiteit Wageningen. 41 pp.

Klisiewicz JM and Pound GS. 1961. Studies on control of black rot of crucifers by treating seed with antibiotics. Phytopathology 51:495-500.

Koch MF and Parlevliet JE. 1990. Assessment of quantitative resistance of rice cultivars to *Xanthomonas campestris* pv. *oryzae*: A comparison of assessment methods. Euphytica 51:185-189.

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

Kocks CG, Ruissen MA and Zadoks JC. 1998. Spatio-temporal response of black rot in cabbage to initial inoculum levels. Submitted

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

Kuan TL, Minsavage GV and Schaad NW. 1986. Aerial dispersal of *Xanthomonas campestris* pv. *campestris* from naturally infected *Brassica campestris*. Plant Disease 70:409-413.

Lannou C and Savary S. 1991. The spatial structure of spontaneous epidemics of different diseases in a groundnut plot. Netherlands Journal of Plant Pathology 97:355-368.

Lecoustre R, Fargette D, Fauguet C and Reffye P. 1989. Analysis and mapping of the spatial spread of African cassava mosaic virus using geostatistics and the kriging technique. Phytopathology 79:913-920.

Lengkeek AG and Van Etteger M. 1992. De afname van *Xanthomonas campestris* pathovar *campestris* in de bodem. Afstudeerdverslag Landbouwuniversiteit Wageningen. 35 pp.

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

Lipps PE. 1988. Spread of corn anthracnose from surface residues in continuous corn and corn-soybean rotation plots. Phytopathology 78:756-761.

Madden LV. 1986. Statistical analysis and comparison of disease progress curves. In: Plant disease epidemiology: Population dynamics and management. Leonard KJ and Fry WE, eds. Macmillan Publishing Company, New York. pp. 38-84.

Madden LV. 1992. Rainfall and the dispersal of fungal spores. Advances in Plant Pathology 8:39-79.

Madden LV, Knoke JK, Louie R. 1990. Spread of maize chlorotic dwarf virus in maize fields by its leafhopper vector, *Graminella nigrifrons*. Phytopathology 80:291-298.

Madden LV, Pirone TP and Raccah B. 1987. Analysis of spatial patterns of virus-diseased tobacco plants. Phytopathology 77:1409-1417.

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.

Matheron G. 1962. Traité de géostatistique appliqué. Tome 1. Éditions Techniques, Paris

Mitchell DJ and Kannwischer-Mitchell ME. 1983. Relationship of inoculum density of *Phytophthora* species to disease incidence in various hosts. In: Phytophthora: Its biology, taxonomy, ecology and pathology. Erwin DC, Bartnicki-Garcia S and Tsao PH, eds. American Phytopathological Society, St. Paul, MN. pp. 259-269.

Monteith JR. 1921. Seed transmission and overwintering of cabbage black rot. Phytopathology 11:53-54 (Abstr.).

Moran PAP. 1950. Notes on continuous stochastic phenomena. Biometrika 37:17-23.

Munkvold GP, Duthie JA and Marois JJ. 1993. Spatial patterns of grapevines with Eutypa Dieback in vineyards with or without Perithecia. Phytopathology 83:1440-1448.

Munz PA. 1963. Cruciferae. Pages 210-217 in California Flora. University California Press, Berkeley. 1,681 pp.

Nelson MR, Felis-Gastelum R, Orum TV, Stowel LJ and Meyers DE. 1994. Geographical information system and geostatistics in the design and validation of regional plant virus management progress. Phytopathology 84:898-905.

Nicot PC, Rouse DI and Yandell BS. 1984. Comparison of statistical methods for studying spatial patterns of soilborne plant pathogens in the field. Phytopathology 74:1399-1402.

Olea RA. 1991. Geostatistical glossary and multilingual dictionary. Oxford University Press, New York. 177 pp.

PAGV. 1993. Teelt van bloemkool. PAGV, Lelystad. 165 pp.

PAGV. 1995. Kwantitatieve informatie voor de akkerbouw en de groenteteelt in de vollegrond 1995. PAGV, Lelystad. 222 pp.

Parlevliet JE. 1979. Components of resistance that reduce the rate of epidemic development. Annual Review of Phytopathology 17:203-222.

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.

Porta-Puglia A, Bernier CC, Jellis GJ, Kaiser WJ and Reddy MV. 1994. Screening techniques and sources of resistance to foliar diseases caused by fungi and bacteria in cool season food legumes. Euphytica 73:11-25.

Pillard HR, Cobb AC and Moore CJ. 1991. Biological and cultural tests for control of plant diseases. 6:7.

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.

Radford AE, Ahiles HE and Bell CR. 1968. Brassicaceae. Pages 486-511 in: Manual of the vascular flora of the Carolinas. AE Radford et al., eds. University NC Press, Chapel Hill. 1,183 pp.

Reynolds KM, Madden LV and Ellis MA. 1988. Spatio-temporal analysis of epidemic development of leather rot of strawberry. Phytopathology 78:246-252.

Richardson JK. 1945. Black rot of rutabagas. Sci. Agric. 25:415-425.

Robinson RA. 1976. Plant Pathosystems. Berlin, Springer. 184 pp.

Robinson RA. 1969. Disease resistance terminology. Review of Applied Mycology 48:593-605.

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. 248 pp.

Ruissen MA and Gielink AJ. 1994. The development of black rot in cabbage as a result of differences in guttation between cultivars, and the relation of guttation to infectiousness. Proceedings of the 8th International conference on Plant Pathogenic Bacteria, Versailles, France, June 9-12, 1992. pp. 767-774.

Ruissen MA, Van der Gaag M and Toruno Lua. 1990. Release of soil-borne *Xanthomonas campestris* pv. *campestris* in the phyllospere of cabbage plants. Proceedings of the 7th International conference on Plant Pathogenic Bacteria, Budapest, Hungary, June 11-16, 1989. pp. 299-303.

Ruissen MA, Van der Vossen RTM, 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.

Ruissen MA and Van Tol RWHM. 1988. Growth and survival of *Xanthomonas campestris* pv. *campestris* in the rhizosphere of host and non-host plants. Acta Botanica Neerlandica Vol. 37: 544-545 (Abstr.)

Russel HL. 1898. A bacterial rot of cabbage and allied plants. Wisconsin Agricultural Experimental Station. Bulletin 65.

Savary S. 1987. Decrease by plant development and leaf stage of susceptibility of groundnut to rust (*Puccinia arachidis* Speg.) in a susceptible cultivar. Netherlands Journal of Plant Pathology 93:25-31.

Savary S and Zadoks CJ. 1990. Analyse des composantes de l'interaction hôte-parasite chez le rouille de l'arachide, II. Mesure de la résistance intermédiaire par un essai en micro-parcelles. Oléagineux 44:215-224.

Schaad NW. 1976. Control of black rot of cabbage. Univ. Gazette Research Bulletin 187. 13 pp.

Schaad NW. 1980. Relationship of incidence of seedborne *Xanthomonas campestris* to black rot of crucifers. Plant Disease 64:91-92.

Schaad NW and Dianese JC. 1981. Cruciferous weeds as sources of inoculum of *Xanthomonas campestris* in black rot of crucifers. Phytopathology 71:215-1220.

Schaad NW, Sitterly WR and Humaydan H. 1980. Relationship of incidence of seedborne *Xanthomonas campestris* to black rot of crucifers. Plant Disease 64:91-92.

Schaad NW and White WC. 1974a. A selective medium for soil isolation and enumeration of *Xanthomonas campestris*. Phytopathology 64:876-880.

Schaad NW and White C. 1974b. Survival of *Xanthomonas campestris* in soil. Phytopathology 64:1518-1520.

Scherm H and Van Bruggen AHC. 1994. Global warming and nonlinear growth: how important are changes in average temperature? Phytopathology 84:1380-1384.

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.

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

Schuster ML and Coyne DP. 1975. Survival factors of plant pathogenic bacteria. Nebraska Agricultural Experimental Station Research Bulletin 268. 53 pp.

Sinclair JB. 1991. Latent infection of soybean plants and seeds by fungi. Plant Disease 75:220-224.

Sherf AF and MacNab AA. 1986. Vegetable Diseases and Their Control. John Wiley & Sons, New York. 728 pp.

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.

Smilde WD. 1996. *Phytophthora porri* in leek: epidemiology and resistance. PhD-thesis. Wageningen Agricultural University. pp. 117.

Sokal RR and Oden L. 1978a. Spatial autocorrelation in biology. 1. Methodology. Biological Journal of the Linnaean Society 10:119-228.

Sokal RR and Oden L. 1978b. Spatial autocorrelation in biology. 2. Some biological implications and four applications of evolutionary and ecological interest. Biological Journal of the Linnaean Society 10:229-249.

Stakman EC and Fletcher DG. 1930. The common barberry and black stem rust. U.S. Department Agric. Farmers' Bulletin 1544. 28 pp.

Staub T and Williams PH. 1972. Factors influencing black rot lesion development in resistant and susceptible cabbage. Phytopathology 62:722-728.

Stein A, Kocks CG, Zadoks JC, Frinking HD, Ruissen MA and Myers DE. 1994. A geostatistical analysis of the spatio-temporal development of downy mildew epidemics in cabbage. Phytopathology 84:1227-1239.

Strandberg JO. 1973. Spatial distribution of cabbage black rot and the estimation of diseased plant populations. Phytopathology 63:998-1003.

Sutton JC and Williams PH. 1970. Relation of xylem plugging to black rot lesion development in cabbage. Canadian Journal of Botany 48:391-401.

Trangmar BB, Yost RS and Uehara G. 1985. Application of geostatistics to spatial studies of soil properties. Advances of Agronomics 38:45-94.

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.

Van de Lande HL. 1993. Spatio-temporal analysis of spear rot and 'marchitez sorpressiva' in African oil palm in Surinam. Netherlands Journal of Plant Pathology 99:129-139.

Van der Plank JE. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York. 349 pp.

Van der Plank JE. 1975. Principles of plant infection. Academic Press, New York. 216 pp.

Van der Zaag DE. 1956 Overwintering and epidemiology of *Phytophthora infestans*, and some new possibilities of control. Tijdschrift over Plantenziekten 72:89-156.

Van Hall CJJ. 1900. Twee bacteriënziekten. Tijdschrift over Plantenziekten 6:169-177.

Van de Ven, E. 1984. Zwartnervigheid slaat toe in spruitkool. Groenten en Fruit (40) 9:51.

Vos JGM 1994. Integrated crop management of hot pepper (*Capsicum* spp.) in tropical lowlands. PhD thesis. Agricultural University, Wageningen. The Netherlands. 188 pp.

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. 132 pp.

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

Walker JC. 1941. Origin of cabbage black rot epidemics. Plant Disease Reporter 25:91-94.

Walker JC. 1952. Diseases of crucifers. Pages 129-131 in: Diseases of vegetable crops. McGraw-Hill, New York. 529 pp.

Walker JC, Larson RH and Taylor AL. 1958. Diseases of cabbage and related plants. US Department of Agriculture. Handbook No. 144.

Walker JC and Tisdale WB. 1920. Observations on seed transmission of the cabbage black rot organism. Phytopathology 10:175-177.

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

Williams PH, Staub T and Sutton JC. 1982. Inheritance of black rot resistance in cabbage. Phytopathology 72:247-252.

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.

Woronin M. 1878. *Plasmodiophora brassicae*, Urheber der Kohlpflanze-Hernie. Jahrbuch Wissenschaftl. Bot. 11:548-574.

Xu X-M, 1996. On estimating non-linear response of fungal development under fluctuating temperatures. Plant Pathology 45:163-72.

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.

Young JM. 1969. An alternative weed host for *Xanthomonas campestris*. Plant Disease Reporter 53:820-821.

Yuan GY, Alvarez AM, Benedict AA and Trotter KJ. 1987. Use of monoclonal antibodies to monitor the dissemination of *Xanthomonas campestris* pv. *campestris*. Phytopathology 77:366-370.

Zadoks JC. 1961. Yellow rust on wheat, studies in epidemiology and physiological specialization. Tijdschrift over Planteziekten 67:69-256.

Zadoks JC. 1972. Reflections on disease resistance in annual crops. In: Biology of rust resistance in forest trees. Bingham RT, Hoff RJ and McDonald GI, eds. Proceedings NATO-IUFRO Advanced Study Institute, ESDA, Forest Service, Miscellaneous Publications 1221. pp. 43-63.

Zadoks JC. 1974. Methodology of epidemiological research. Annual Review of Phytopathology 10:253-276.

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. 427 pp.

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

Zawolek MW and Zadoks JC. 1992. Studies in focus development: An optimum theorem for the dual dispersal of plant pathogens. Phytopathology 82:1288-1297.

Zeven AC and Zhukovsky PM. 1975. Dictionary of cultivated plants and their centres of diversity: excluding ornamentals, forest tress and lower plants. Pudoc, Wageningen. 219 pp.

Summary

Chapter 1

Black rot, caused by the bacterium *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson 1939 (*X.c.* pv. *campestris*), is an important disease in cabbage crops. *X.c.* pv. *campestris* overwinters in infected plant debris, overwintering cabbage, seed, or weeds. Survival of *X.c.* pv. *campestris* in soil or in plant refuse and debris is affected by climatic factors, crop rotation, microbial activity in the soil, chemical soil properties, and protection by the host. Seed is the major vehicle for transmission of *X.c.* pv. *campestris* from one area to another. Spread of *X.c.* pv. *campestris* in fields occurs by overhead irrigation (splash dispersal) and rain splash. Hydathodes are the most important port of entry.

Since crucifers are susceptible to black rot during the whole cropping cycle, all aspects of crucifer production must be considered to control black rot. Several guidelines are known to control black rot in cabbage. These guidelines emphasize seed treatment, sanitation, careful handling of seedlings during transplant, crop rotation, seedbed practices, and use of resistant cultivars. Notwithstanding these recommendations, black rot appeared to be very destructive during several years in The Netherlands. In the 1990s, the disease still was and is a problem.

Chapter 2

This chapter reports an exploratory survey of conventional cabbage production systems, with regard to black rot disease intensity. Survey data indicated that cropping aspects and farmers' practices may be important. In a few cases, some aggregation of disease was found at field entries and along the tractor tracks in the field made during the application of chemicals. Apart from this aggregation, black rot was randomly distributed. The black rot level may be related to cultivars, but other factors also may have played a (major) role in disease development. Plant refuse piles can be a source of inoculum for black rot infections. The existence of refuse piles corresponded often with presence of black rot in fields. Although destruction of refuse piles may prevent fields from black rot infection, the results also indicated that refuse piles were not the only infection source of black rot.

Obviously, none of the practised management methods is good enough to black

rot infection of cabbage. Black rot development at farm level is probably the outcome of a combination of different cropping aspects and farmers' practices.

Chapter 3

Refuse piles are common in Dutch growing areas and thus may be important sources of inoculum for black rot epidemics. Effects of cabbage refuse piles, infected with the bacterium *X.c.* pv. *campestris*, on black rot epidemics in cabbage were studied. Field plots of cabbage were infested by placing old (4-mo-old) or fresh (2-wk-old) refuse piles in the centre of each plot. Infection of the plots from seed and from unknown sources in or around the plots could be excluded by appropriate experiments, farm history analysis and visual observation.

Black rot development in the plots was far more intensive with fresh than with old refuse piles. Cabbage plots infested with old refuse piles had 1% diseased plants per plot and an average of 0.02 diseased leaves per plant. In contrast, fresh refuse piles resulted in 30-70% diseased plants and 1.0 to 3.5 diseased leaves per plant. Typical disease foci developed around the fresh refuse piles. Black rot development was positively correlated to the number of days with rainfall between 06.00-09.00 h during May and June. *X.c.* pv. *campestris* infected refuse piles may be serious sources of inoculum for black rot epidemics when they are fresh or when fresh cabbage debris is present on top of old refuse piles.

Chapter 4

During this study we focused on field resistance of a few cabbage cultivars, a susceptible, an intermediate and a more resistant cultivar. Field resistance is defined as any resistance which affects epidemics in the field but which is not immediately apparent in laboratory or glasshouse tests. Field resistance of the cabbage cultivars Bartolo, Erdeno, Perfect Ball, and Roxy were studied in The Netherlands. Plots were inoculated by single sources in the centre of each plot. Disease progress was described by the Gompertz model. The overall measure of absolute rate (disease progress rate r multiplied with maximum disease intensity K) was used to compare cultivar effects on disease progress. Disease gradients were described by the negative exponential model. The percentile distance (distance from the source at which disease intensity reached 1% of the empirical maximum disease intensity) was

used to compare cultivar effects on disease spread. Disease severity was a more sensitive measure than disease incidence to calculate the disease progress and spread of black rot. Disease progress and disease spread were about equally effective to screen cultivars for field resistance to black rot. Perfect Ball was the most susceptible, Erdeno and Bartolo were intermediate and Roxy was the most resistant with either measure.

Increased levels of field resistance reduced the development of black rot in time and in space. The field resistance of cabbage to black rot is thought to be composed of several mechanisms. Field resistance to black rot can be determined by one or more of the following mechanisms, a) passive resistance, b) active resistance, c) disease escape, d) tolerance, and e) mature plant resistance.

Chapter 5

Black rot on cabbage was evaluated in replicated field experiments to compare effects of various initial inoculum levels on spatio-temporal disease development from artificial sources, one per plot. The results support the hypothesis that black rot is a potentially polycyclic disease since secondary inoculum was found in harvested guttation droplets on plants outside the initial source. Furthermore, progress curves suggested the existence of two infection cycles during 1992, pointing at the possibility of polycyclic disease development.

Black rot development was inoculum-dependent because the progress rate of black rot epidemics and the spatial spread were both positively correlated with the strength of the source. Auto-infection and allo-infection were analyzed by evaluating incidence and severity data. The spread of black rot, associated with the primary gradient, was mainly due to allo-infection. Later in the season, increase of black rot near the foci was caused by auto-infection. The rate of disease development was related to the number of rain days.

Three-dimensional maps of disease severity showed the dominance of the primary focus. Maximum distance of black rot symptoms to the source of the focus was limited to a few meters so that damage of cabbage by focal inoculum was limited to the plants close to the source. Since black rot is considered a potentially polycyclic disease, we believe that the number of multiplication cycles was limited by unfavourable circumstances for bacterial growth and dispersal.

Spatio-temporal development and initial inoculum are related. High inoculum levels in point sources resulted in faster outward spread of black rot in cabbage, and

significant highest development was found for the high inoculum levels.

Under the conditions of the experiments, performed during three relatively dry seasons, a single source of infection measuring 0.5 x 0.5 m was not capable to cause disease in all plants in a plot of 6.5 x 6.5 m. The results imply that severe full-field disease, as observed regularly in The Netherlands, can only originate from a large number of foci per field.

Chapter 6

Disease progress of black rot in cabbage crops 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 (incidence and severity) was plotted over time, two and three dimensional maps were made, and disease aggregation was studied by means of geostatistics, black-black counts and Moran's *I* statistic.

The results show that black rot progress is primarily due to focus expansion. Secondary foci may appear at short distances from the initial focus but they merge with the expanding initial focus. The black rot disease of cabbage appeared to be more or less aggregated and sharply defined around the source of inoculum after uni-focal inoculation. The present paper shows that dispersal of black rot in cabbage under Dutch conditions is generally a short distance dispersal, resulting in increase of focus size, and that development of secondary foci by long distance dispersal seems to be of minor importance due to sharp decrease of inoculum density of *X.c.* pv. *campestris* with distance to the source.

The experiment of 1993 showed 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. This effect was attributed to the higher average daily temperature in the sunny plots on sunny days, by which black rot symptoms may have appeared faster and more bacteria may have been available for dispersal.

Disease proceeds faster in plots with multi-focal inoculation than in those with uni-focal inoculation. The results suggest that serious epidemics in Dutch cabbage fields should originate from large numbers of foci.

Chapter 7

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 a 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 the *X.c.* pv. *campestris* population 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, and over three years, infection foci of black rot disease were established. At harvest time, all plants were chopped and resulting plant debris was rotovated. The resulting soil infestation showed clear infestation foci of the soil 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. Comparisons are made between the polyetic situation in Russia and in The Netherlands and some practical conclusions are drawn.

Chapter 8

Geostatistics was used to analyze patterns of black rot in cabbage to optimize sampling plans in combination with observation methods, and to map the patterns based on samples of reduced sizes. For all plants in the plots, natural infection by *X.c.* pv. *campestris* was recorded by assessing disease incidence and severity during 1990 and 1991 on at least 6 days.

Spatial variability was analyzed by determining semivariograms. Semivariograms were influenced by the observation method and the sampling distance which gave changes in model types and parameter values. The dissimilarity of observation methods can result in different interpretations of spatial patterns. An increase of the sampling distance sometimes resulted in a shift from structured models of semivariograms into pure nugget models. Pure nugget semivariograms either indicate absence of spatial dependence or suggest that sampling distances

were too large to detect spatially dependent structures. A sampling distance up to 2.0m and to 3.0m for respectively disease incidence and severity was adequate to study small-scale patterns of black rot disease in small cabbage fields. In some cases semivariograms revealed anisotropy with preference for the northeast direction, in accordance with the prevalence of southwestern winds during rainfall.

Kriging visualized the features of spatial heterogeneity and the main characteristics of the pattern, including higher disease intensities along wind-exposed directions and the location of foci, when sampling distances did not exceed a certain limit, the range. The range of influence was 2.5m for disease incidence and 3.5m for the disease severity. According to these results the sampling intensity could be reduced by 80% and 86% for disease incidence and disease severity, respectively.

Samenvatting

Hoofdstuk 1

Zwartnervigheid wordt veroorzaakt door de plantenpathogene bacterie *Xanthomonas campestris* pv. *campestris*. Het is een belangrijke ziekte in koolgewassen. *X.c.* pv. *campestris* overwintert in geïnfecteerde gewasresten, overwinterende koolgewassen, zaad of onkruiden. Overleving van *X.c.* pv. *campestris* in de bodem of in gewasresten wordt beïnvloed door klimatologische omstandigheden, gewasrotatie, microbiologische bodemactiviteit, chemische bodemtoestand en bescherming door de waardplant. Zaad is het belangrijkste middel voor verspreiding van *X.c.* pv. *campestris* van het ene gebied naar het andere. Verspreiding van *X.c.* pv. *campestris* binnen koolvelden geschiedt door spatverspreiding tijdens beregening, spatverspreiding tijdens regen en door middel van windverspreiding. De hydathoden vormen de belangrijkste invalspoort.

Omdat koolgewassen vatbaar zijn gedurende de gehele produktiecyclus moeten alle aspecten van de produktiecyclus beschouwd worden om beheersing van zwartnervigheid te realiseren. Aanbevelingen zijn inmiddels bekend. Deze aanbevelingen benadrukken zaadbehandeling, sanitatie, het voorzichtig behandelen van zaailingen tijdens overplanten, vruchtwisseling, behandeling van het zaadbed, en het gebruik van resistentie cultivars. Ondanks deze aanbevelingen was de zwartnervigheid vernietigend gedurende enkele jaren in Nederland. In de negentiger jaren was en is de ziekte nog steeds een probleem.

Hoofdstuk 2

Dit hoofdstuk beschrijft een exploratieve enquête over de gangbare koolteelt-systemen met betrekking tot de intensiteit van de ziekte zwartnervigheid. Enquête gegevens toonden aan dat teeltaspecten en handelwijzen van de kooltelers belangrijk kunnen zijn. In enkele situaties werd aggregatie van de ziekte gevonden rondom de toegang tot de percelen en in en direct naast het spuitspoor. Afgezien van deze aggregatie was de ziekte willekeurig verspreid door de percelen. De hoogte van de aantasting is mogelijk gerelateerd aan cultivareffecten, maar ook ander factoren kunnen een (belangrijke) rol in de ontwikkeling van de ziekte hebben gespeeld. Afvalhopen kunnen een inoculumbron zijn voor de ziekte. Het bestaan van

afvalhopen ging vaak gepaard met de aanwezigheid van de ziekte in het veld. Alhoewel vernietiging van afvalhopen kan voorkómen dat een veld besmet wordt, toonden de gegevens aan dat afvalhopen niet de enige besmettingsbron waren.

Blijkbaar was geen van de toegepaste beheersmethoden toereikend om tot een volledige preventie van de ziekte te komen. Zwartnervigheid op bedrijfsniveau is waarschijnlijk het resultaat van een combinatie van teeltaspecten en handelwijzen van de teler.

Hoofdstuk 3

Afvalhopen komen algemeen voor in de Nederlandse teeltgebieden en kunnen dus belangrijke inoculumbronnen zijn van zwartnervigheidepidemieën. De invloed van afvalhopen, geïnfecteerd met de bacterie *X.c.* pv. *campestris*, op zwartnervigheid-epidemieën werd bestudeerd. Proefveldjes met kool werden besmet door middel van het plaatsen van een oude (4 maanden oud) of een verse (2 weken oud) afvalhoop in het centrum van elke veldje. Infectie van de veldjes via zaad of door onbekende bronnen binnen of buiten de veldjes kon worden uitgesloten door passende experimenten, beoordeling van de bedrijfsgeschiedenis en via persoonlijke waarnemingen. De ontwikkeling van zwartnervigheid in de veldjes was veel intenser met de verse dan met de oude afvalhopen. Veldjes besmet met oude afvalhopen hadden 1% zieke planten per veldje en een gemiddelde aantasting van 0,02 zieke bladeren per plant. Daarentegen veroorzaakten verse afvalhopen in 30-70% zieke planten met 1,0 tot 3,5 zieke bladeren per plant. Karakteristieke ziekte-haarden ontwikkelden zich rondom de verse afvalhopen. De ontwikkeling van zwartnervigheid was positief gecorreleerd met het aantal dagen met regen tussen 06.00-09.00 uur gedurende mei en juni. Afvalhopen besmet met *X.c.* pv. *campestris* kunnen bronnen van inoculum zijn voor zwartnervigheidepidemieën als zij vers zijn of als vers plantmateriaal aanwezig is boven op de afvalhoop.

Hoofdstuk 4

Dit experiment was gericht op de veldresistentie van enkele koolcultivars, een vatbare, een middelmatig vatbare en een meer resistente cultivar. Veldresistentie wordt gedefinieerd als een resistentie die te velde de epidemie beïnvloedt, maar die niet direct zichtbaar is in laboratorium- of kasexperimenten. Veldresistentie van de

koolcultivars Bartolo, Erdeno, Perfect Ball, en Roxy zijn bestudeerd in Nederland. Veldjes werden geïnoculeerd door enkelvoudige bronnen in het centrum van elk veldje. De ziekteontwikkeling werd beschreven met het 'Gompertz'-model. De maat voor de totale absolute snelheid (snelheid van de ziekteontwikkeling r vermenigvuldigd met de maximum ziekte-intensiteit K) werd gebruikt om cultivar-effecten op de ontwikkeling van de ziekte te vergelijken. Ziektegradiënten werden beschreven met behulp van een negatief exponentieel model. De 1%-afstand (afstand tot de bron waar de ziekte-intensiteit 1% van de hoogste empirische ziekte-intensiteit bereikt) werd gebruikt om cultivareffecten te vergelijken op verspreiding van de ziekte. De aantastingsgraad van de ziekte was een gevoeliger maat dan de incidentie van de ziekte om de ontwikkeling van de ziekte en de verspreiding van zwartnervigheid te berekenen. Ziekte-ontwikkeling en ziekte-verspreiding waren vrijwel even effectief om cultivars te toetsen op veldresistentie tegen zwartnervigheid. Perfect Ball was het meest vatbaar, Erdeno en Bartolo waren tussenliggend en Roxy was het meest resistent bij beide maten.

Toename van de resistentie verminderde de ontwikkeling van zwartnervigheid in tijd en in ruimte. De veldresistentie tegen zwartnervigheid bestaat waarschijnlijk uit verschillende mechanismen, waaronder a) passieve resistentie, b) actieve resistentie, c) ontsnapping, d) tolerantie en e) oudersdomsresistie.

Hoofdstuk 5

Zwartnervigheid in kool werd onderzocht in herhaalde veldproeven om het effect van verscheidene startniveaus van inoculum te vergelijking op de ruimte-tijd-verbreiding van de ziekte vanuit kunstmatige bronnen (één bron per veld). De resultaten ondersteunen de hypothese dat zwartnervigheid een potentieel polycyclische ziekte is omdat secondair inoculum gevonden is in geoogste guttattie-druppels op planten buiten de oorspronkelijke bron. Bovendien suggereerden de ziektegroeicurven het bestaan van twee infectiecyclussen gedurende 1992, hetgeen duidt op de mogelijkheid van polycyclische ontwikkeling van de ziekte.

De ontwikkeling van zwartnervigheid was inoculum-afhankelijk omdat de groeisnelheid van de epidemieën en de ruimtelijke verspreiding beide positief gecorreleerd waren met de sterkte van de bron. Auto-infectie- en allo-infectie werden geanalyseerd met behulp van incidentie en aantastingsgraad. De verspreiding van zwartnervigheid, geassocieerd met de primaire infectie-gradiënt, was vooral het gevolg van allo-infectie. Later in het seizoen was de toename in aantastingsgraad

dicht bij de bron het gevolg van auto-infectie. De snelheid van de ziekte-ontwikkeling hing samen met het aantal regendagen.

Driedimensionale overzichtskaarten van de aantastingsgraad toonden de dominantie van de primaire haard. De maximale afstand tot de bron van planten met symptomen van zwartnervigheid bleef beperkt tot enkele meters zodat schade aan het koolgewas door middel van haard-inoculatie beperkt bleef tot planten dicht bij de bron. Omdat verondersteld is dat zwartnervigheid polycyclisch kan zijn, bleef het aantal vermenigvuldigingscyclussen beperkt bleef door ongunstige omstandigheden voor groei en verspreiding van de bacteriën.

Ruimte-tijd-ontwikkeling en startinoculum zijn gekoppeld. Een hoger inoculumniveau in puntbronnen leidde tot een snellere ruimtelijke verspreiding van zwartnervigheid.

Onder de omstandigheden van de experimenten, uitgevoerd gedurende drie relatief droge seizoenen was een enkele infectiebron met een oppervlakte van 0,5m x 0,5m niet in staat om alle planten in een veldje van 6,5m x 6,5m ziek te maken. De resultaten betekenen dat zware volveldse ziekte, zoals regelmatig waargenomen in Nederland, alleen kan ontstaan vanuit een groot aantal bronnen per veld.

Hoofdstuk 6

De ziekteontwikkeling van zwartnervigheid in koolgewassen werd gedurende drie jaren in proefveldjes bestudeerd om het effect te vergelijken van 'unifocale' inoculatie (één bron per veld) en 'multifocale' inoculatie (meerdere bronnen per veld) bij het gebruik van dezelfde hoeveelheid inoculum per veldje. De ziektegroei (incidentie en aantastingsgraad) werd uitgezet tegen de tijd, twee- en drie-dimensionale overzichtskaarten werden gemaakt en de aggregatie van de ziekte werd beoordeeld door middel van geostatistiek, 'black-black counts' en 'Moran's *I* getal'.

De resultaten toonden aan dat de groei van zwartnervigheid allereerst het gevolg is van uitbreiding van de haard. Secondaire haarden kunnen ontstaan op korte afstand van de begin-haard, maar zij versmelten met de uitbreidende begin-haard. Na 'unifocale' inoculatie bleek zwartnervigheid in kool min of meer geclusterd en scherp begrensd te zijn rondom de inoculumbron. Dit hoofdstuk toont aan dat verspreiding van zwartnervigheid in kool onder Nederlandse omstandigheden voornamelijk een korte-afstand-verspreiding is, die leidt tot toename van de omvang van de haard, en dat de ontwikkeling van secundaire haarden door middel van lange-afstand-verspreiding van minder belang is door de snelle afname van de

inoculumdichtheid van *X.c.* pv. *campestris* met de afstand tot de bron.

Het experiment van 1993 toonde aan dat beschaduwing de ontwikkeling van de ziekte beïnvloedde. Veldjes beschaduwd tot 09.30-10.00 uur hadden een vertraagde ziektegroei in vergelijking tot veldjes beschaduwd tot 08.00-08.30 uur. Dit effect werd toegeschreven aan de hogere gemiddelde dagtemperatuur in de zonnige veldjes op zonnige dagen, waardoor de symptomen van zwartnervigheid sneller konden ontstaan en meer bacteriën beschikbaar waren voor verspreiding.

De ziektegroei ging sneller in veldjes met 'multifocale' inoculatie dan in de veldjes met 'unifocale' inoculatie. De resultaten suggereren dat ernstige epidemieën in koolvelden ontstaan vanuit een groot aantal bronnen.

Hoofdstuk 7

Overdracht van inoculum van *X.c.* pv. *campestris* in de bodem van het ene seizoen naar het volgende werd bestudeerd in veldproeven gedurende drie jaren. Deze studies werden ondersteund door laboratorium- en kasproeven over de schatting van het aantal bacteriën door middel van een biotoets en de 'Most Probable Number' methode en betreffende de teruggevonden bacteriën vanuit de bodem.

De gemiddelde hoeveelheid bacteriën teruggevonden uit kunstmatig besmette grond was 58%. Uitdoving van *X.c.* pv. *campestris* in grond besmet met geïnfecteerde plantenresten was exponentieel. De snelheid van de afbraak van plantenresten en van de uitdoving van de bacteriepopulatie waren afhankelijk van temperatuur. In herhaalde veldjes, en gedurende drie jaren, werden infectiehaarden van zwartnervigheid vastgesteld. Op het moment van de oogst werden alle planten met de frees in stukken geslagen en ondergewerkt. De aldus veroorzaakte bodembesmetting is bemonsterd en toonde een duidelijke haard van besmetting van de bodem aan, ter plaatse van de oorspronkelijke infectiehaard van het gewas. Deze besmettingshaarden namen af met de tijd en verdwenen na de winter. Volggewassen bleven vrijwel geheel vrij van infectie.

Het resultaat toont aan dat in Nederland goed management van gewas en grond overleving van inoculum van het ene jaar naar het volgende bemoeilijkt, zodat kool onafgebroken kan worden geteeld. Meerjarige overdracht van inoculum door middel van planteresten in de bodem kan worden voorkomen in Nederland. Vergelijkingen zijn gemaakt tussen meerjarige situaties in Rusland en Nederland en enkele praktische conclusies werden getrokken.

Hoofdstuk 8

Geostatistiek werd benut om ruimtelijke patronen van zwartnervigheid in kool te analyseren om zo bemonsteringsschema's te optimaliseren in combinatie met verschillende waarnemingsmethoden, en de patronen in kaart te brengen op basis van een beperkte hoeveelheid gegevens. Voor alle planten in de veldjes werd de natuurlijke infectie door *X.c.* pv. *campestris* beschreven door de incidentie en de aantastingsgraad van de ziekte te bepalen op tenminste 6 dagen gedurende 1990 en 1991. De ruimtelijke variabiliteit werd geanalyseerd door middel van semivariogrammen. Semivariogrammen werden beïnvloed door de waarnemingsmethode en de bemonsteringsafstand waardoor modeltypen en parameterwaarden wijzigden. De ongelijkheid van waarnemingsmethoden kan zelfs leiden tot verschillende interpretaties van de ruimtelijke patronen. Een toename van de bemonsteringsafstand resulteerde soms in een verandering van gestructureerde semivariogrammodellen naar 'pure nugget'-modellen. Een 'pure nugget'-semivariogram geeft aan dat er geen ruimtelijke afhankelijkheid bestaat of suggereert dat de bemonsteringsafstand te groot is om ruimtelijk afhankelijke structuren te ontdekken. Een bemonsteringsafstand tot 2,0 m en tot 3,0 m voor respectievelijk incidentie en aantastingsgraad was adequaat om kleine-schaal-patronen van zwartnervigheid te bestuderen in kleine koolveljes. In sommige situaties toonden de semivariogrammen anisotropie met een voorkeur voor de zuidwestelijke windrichting tijdens regen.

'Kriging' liet de kenmerken zien van de ruimtelijke heterogeniteit en de belangrijkste kenmerken van het ruimtelijk patroon met inbegrip van de hogere ziektegraad in de aan de wind blootgestelde richtingen en toonde de plaats van haarden, mits de bemonsteringsafstand niet een bepaalde grens (de 'range') overschreed. De 'range' was 2,5 m voor de incidentie en 3,5 m voor de aantastingsgraad. Naar aanleiding van deze resultaten kon de bemonsteringsintensiteit teruggebracht worden met 80% en 86% voor de incidentie en aantastingsgraad van de ziekte.

Acknowledgements

This book is the result of four years' research work, but could not have been prepared without the cooperation and support of many colleagues, friends, and family.

I thank Prof. J.C. Zadoks for being my principal supervisor. His guidance and interest in the research subject was very important. I am very grateful to my advisor Dr. ir. M.A. Ruissen, for his practical support and the many discussions.

I thank Prof. A. Stein (Dept. of Soil Science and Geology of the Wageningen Agricultural University) and Prof. M. Jeger (Dept. of Phytopathology of the Wageningen Agricultural University) for fruitful discussions and valuable comments on the first draft of several chapters of the present thesis.

I am grateful to Prof. A.H.C. 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 one of the experiments.

I thank ir. H. Schneider and ir. B.M. Schober for the critical reading of several manuscripts.

I thank M. van den Bogert, W. van Noort, M.A.J. van de Scheur, C.E. Winterswijk, F.G.H.H. van Kesteren, and R.Th.M. van der Vossen for technical assistance, field maintenance and/or collecting data.

I am grateful to Unifarm from the Wageningen Agricultural University for growing disease free seedlings.

Curriculum vitae

Corné Kocks werd geboren op 9 april 1964 te Emmen. Na het behalen van het HAVO-diploma aan de Gemeentelijke Scholen Gemeenschap te Emmen begon hij in 1982 met de studie Landbouw aan de toenmalige Hogere Landbouwschool (later Prof. H.C. van Hall Instituut) te Groningen, alwaar hij in 1987 afstudeerde in de richting Akkerbouw. Eind 1987 begon hij, als een van de eerste "doorstromers" de studies Planteziektenkunde en Landbouwplantenteelt aan de Landbouwuniversiteit te Wageningen. In maart 1991 behaalde hij het ingenieursdiploma voor beide studierichtingen met afstudeervakken Fytopathologie en Theoretische Produktie-ecologie. In mei 1991 werd hij aangesteld als Assistent in Opleiding bij de vakgroep Fytopathologie van de Landbouwuniversiteit. Het onderzoek uitgevoerd tijdens dit AIO-schap staat beschreven in dit proefschrift. In 1994 was hij secretaris van het organisatiecommissie voor de "summer school" "Quantitative ecology of pests and diseases: sampling, spatial statistics, spatial dynamics & molecular identification", 5-8 september 1994, van de C.T. de Wit Onderzoekschool voor Produktie Ecologie. Vanaf december 1991 tot september 1994 was hij bestuurslid van het WAIOO (Wagenings AIO Overleg) en het LAIOO (Landelijk AIO Overleg). Per 21 augustus 1995 is hij consultant voor de Stichting Agrotransfer (dienstencentrum van de Christelijke Agrarische School) en docent Plantenteelt / Gewasbescherming aan de Christelijke Agrarische School te Dronten. Sinds april 1996 is hij bestuurslid van de KNPV. Vanaf 1 februari 1998 is hij accountmanager van de produktgroep "Onderzoek & Advies" van de Stichting Agrotransfer.

List of publications

Publications included in this thesis

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

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

Kocks CG, Zadoks JC, and Ruissen MA. Spatio-temporal response of black rot in cabbage to initial inoculum levels. Submitted

Kocks CG, Ruissen MA, and Zadoks JC. Response of black rot in cabbage to spatial distribution of inoculum. Submitted

Kocks CG, Ruissen MA, and Zadoks JC. Survival and dwindling away of *Xanthomonas campestris* pv. *campestris* in soil. Submitted

Other publications

Ende E van de, Folkers M and Kocks C. 1992. *Mycosphaerella*: winterteelt speelt schimmel in de kaart. Groenten en Fruit: weekblad voor de voedingstuinbouw. Vollegrondsgroenten 2(19):6-7.

Kocks CG and Ruissen MA. 1994. Spatial pattern analysis of black rot epidemics in cabbage using geostatistics. Summerschool "Quantitative ecology of pests and diseases: sampling, spatial statistics, spatial dynamics & molecular identification", 5-8 september 1994. Publication in the reader.

Ruissen MA, Vossen RTM van der and Kocks CG. 1993. Growth of *Xanthomonas campestris* pv. *campestris* populations at constant and variable temperatures. Netherlands Journal of Plant Pathology 99:173-181.

Schneider JHM and Kocks CG. Sampling efficiency of rhizotonia-infected tulip bulbs in experimental fields determined by means of geostatistics. Submitted

Stein A, Kocks CG, Zadoks JC, Frinking HD, Ruissen MA and Myers DE. 1994. A geostatistical analysis of the spatio-temporal development of downy mildew epidemics in cabbage. Phytopathology 84:1227-1239.

Werf W van der, Been T and Kocks CG. 1995. Analysis of spatial variability in pest management. In: Griffin CT, Gwynn, RL and Masson JP (eds). Ecology and transmission strategies of entomopathogenic nematodes. pp. 14-35.

'Ze hebben ogen en zien niet', zei Jezus.
Geldt dat ook voor de moderne mens?

Met zijn microscoop ontleedt hij het kleinste stofdeeltje.
Met de telescoop kijkt hij naar de sterren.
Met fijne technieken peilt hij naar wat er omgaat op Mars.
Met de verrekijker brengt hij de hoogste bergtoppen aan zijn voeten.
Van in de onderzeeër overziet hij het leven onder water.
En zelfs de dichtste mist doorklieft hij met zijn radar.

Niettemin blijft de mens vaak blind voor wat er omgaat
in hemzelf
in de meest nabije mens
in zijn familie
en voor de nabijheid van God blijven we stekeblind.
Moet men dan een bijzonder oog hebben
om de diepste werkelijkheden te zien?

Vrij naar Bisschop E.J. De Schmedt, zondagsmis 23 oktober 1994.