Cabbage Refuse Piles as Sources of Inoculum for Black Rot Epidemics

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ABSTRACT

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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 month old) or fresh (2 week 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 3 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 to 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 0600 and 0900 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.

Black rot in cabbage crops, caused by the bacterium *Xanthomonas campestris* pv. *campestris*, is an important disease of crucifers worldwide (18). *X. c.* pv. *campestris* colonizes the vascular system (5). Characteristic black rot symptoms are V-shaped lesions at leaf margins with black veins, chlorosis, and necrosis (16).

In spite of intensive research, black rot still is not controlled sufficiently. Sanitation is an important component in the control of black rot (18). Sanitation is the action that reduces, excludes, or eliminates the initial inoculum from which epidemics start (17). Plant quarantine, crop rotation, seed disinfection, elimination of refuse piles, and eradication of alternative hosts are general sanitation practices (20). The importance of sanitation has been demonstrated for *Phytophthora infestans* (Mont.) de Bary (2), *Puccinia graminis* Pers.:Pers. (Lehmann, Kummer, & Pannenmann) (15), and *Puccinia striiformis* Westend. (Eriks. & Henn.) (19). For sanitation to be fully effective, the various primary inoculum sources must be identified.

The importance of seedborne inoculum (12), soilborne inoculum (10,13), and weed-borne inoculum (11) of *X. c.* pv. *campestris* has been demonstrated. Recently, Dzhalilov and Tiwari (6) 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 in 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 replenished 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.

MATERIALS AND METHODS

Plot establishment. Black rot epidemics in cabbage were studied in Emmen (coordinates 52.46 N, 6.58 E), the Netherlands, during 1992, 1993, and 1994. The white cabbage (*Brassica oleracea* L. var. *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 2 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 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 years, 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 at other locations in the field.

Each year, there were two treatments—fresh and old refuse piles—in two replicates. Plots comprised 14 × 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 year) were randomly placed in the vicinity of the plots (up to 70 m away from the plots) to check for *X. c.* pv. *campestris* infections outside the plots. Presence and absence of rain between 0600 and 0900 h were recorded daily.

Preparation of refuse piles. One isolate of *X. c.* pv. *campestris* (LUF 107) was used in all years. A culture of *X. c.* pv. *campestris* LUF 107 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 8-week-old cabbage plants were injected at the base of the leaf close to the petiole with about 0.03 ml of inoculum. Plants were placed in the greenhouse until symptoms developed. Plants with five symptomatic leaves were harvested (10-week-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 × 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 1). Fresh refuse piles were obtained by inocu-

Table 1. Dates of planting, plot infestation with old and fresh refuse piles, sampling pile infectivity, 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.

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lating plants in March and infesting plots in April (Table 1).

To check the infectivity of the refuse piles, they were sampled at planting time and in early summer (Table 1). An auger (1.6 cm diameter) was used to take four subsamples per pile (0 to 10 cm deep in refuse piles). Subsamples were mixed, and 25 g of the mixture was macerated in 50 ml of sterile water. Per refuse pile, one leaf on each of five test plants was inoculated with 0.03 ml of suspension. Test plants were scored for absence or presence of symptoms 3 weeks after inoculation. Koch's postulates were applied to symptomatic leaves.

Disease assessment. Black rot was assessed four or five times per summer until maturity of the crop. In the plots, symptom expression was recorded by visual assessment of disease incidence (absence or presence of disease) and severity (defined here as 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 severity. Year effects on black rot development, the latter expressed as $r \times K$ values (3), 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).

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 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*.

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 severity ($P \leq 0.05$) (Fig. 1). In plots with fresh refuse piles, disease incidence and severity were considerably higher than in plots with old refuse piles. Black rot foci appeared around fresh refuse piles (Fig. 2). Greatest distances between old refuse piles and diseased plants in the concerned plots were 0.5, 1.5, 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 progress curves for disease incidence for 1992, 1993, and 1994 are shown in Figure 3.

A comparison among years was made for plots with fresh refuse piles. Significant differences in $r \times K$ values for disease incidence and severity were observed among years (Table 3). The $r \times K$ product was largest in 1994. Mean incidence values for $r \times K$ were 2.61, 1.89, and 4.59 for 1992, 1993, and 1994, respectively. Mean severity values for $r \times K$ were 0.16, 0.08, and 0.30 for 1992, 1993, and 1994, respectively. Frequency of rainfall between 0600 and 0900 h during May and June was 14, 13, and 22 days for 1992, 1993, and 1994, respectively. Black rot development correlated positively with this rain frequency, with correlation coefficients of 0.96 and 0.94 for disease incidence and severity, respectively, ($n = 6$, $P \leq 0.05$).

Table 2. Infectivity of refuse piles. Number of diseased leaves observed 3 weeks after inoculation with 0.03 ml of *Xanthomonas campestris* pv. *campestris* suspension extracted from refuse piles

	First sampling[x]		Second sampling[x]	
Plot[w]	Old[y]	Fresh[y]	Old[y]	Fresh[y]
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 was inoculated per sample.
[z] Asymptomatic leaves with doubtful symptoms were not scored.

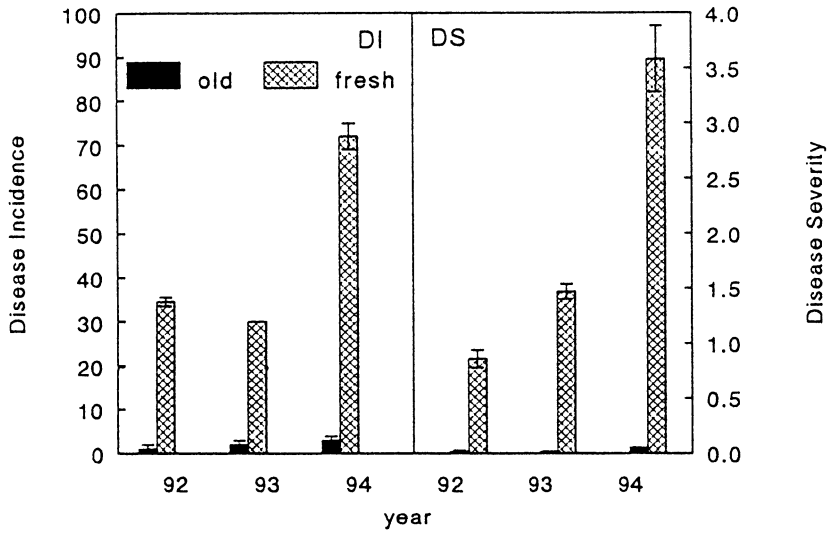

Fig. 1. Final black rot incidence (DI) and severity (DS) of cabbage grown around old and fresh refuse piles in 1992, 1993, and 1994. Bars and error bars represent average of replicates and deviation from the average, respectively.

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 surroundings 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 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 (21) 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 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 (8). Bain (1) and Hunter et al. (9) demonstrated that susceptibility to black rot decreased with plant age. Using $r \times K$ values, significant year effects in black rot development could be demonstrated. Gottwald et al. (7) 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 $r \times K$ values, in accordance with Cook et al. (5) and Williams (18), 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. (4), *Plasmodiophora brassicae* Woronin (14), and *Phoma lingam* (Tode: Fr.) Desmaz. (14). 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 in 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 auc-

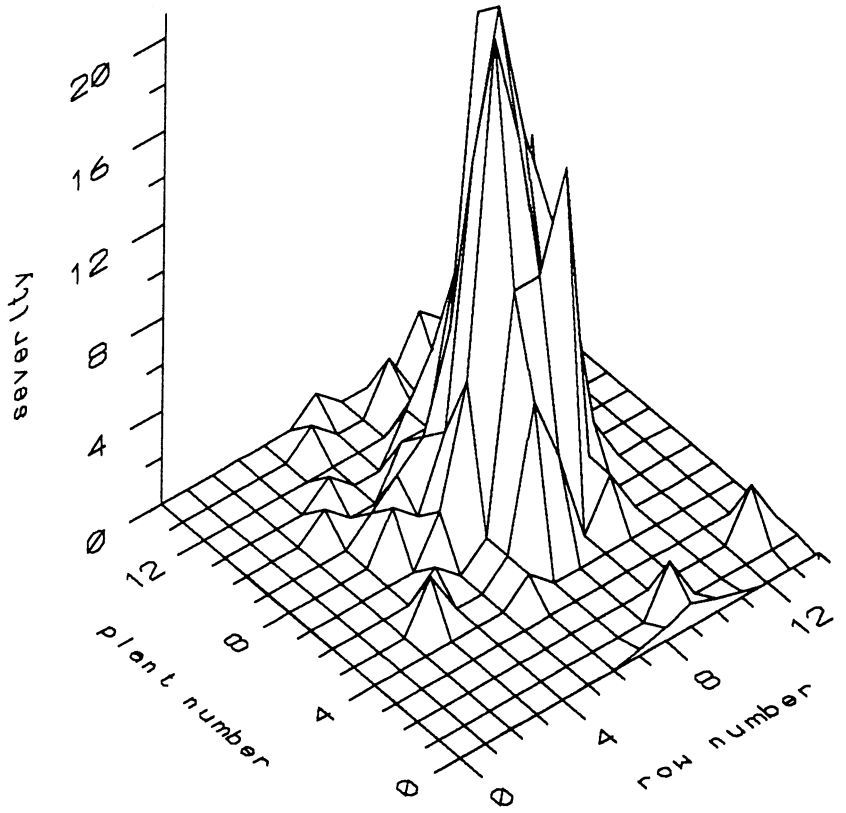

Fig. 2. Typical black rot focus in cabbage plot. The graph shows disease severity data on day 251, 1993, for a plot infested by a fresh refuse pile.

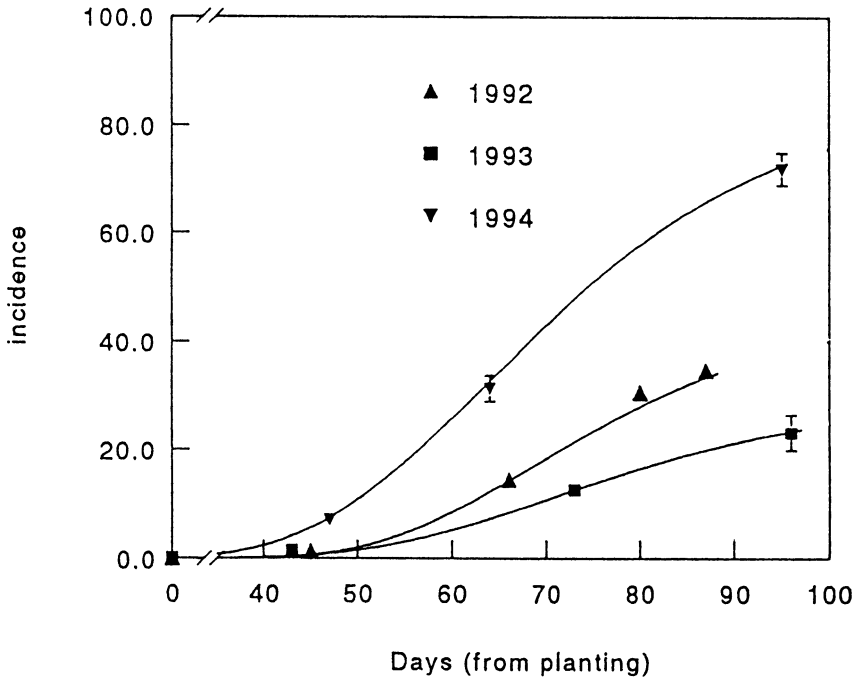

Fig. 3. Gompertz progress curves for black rot disease incidence in plots with a fresh refuse pile (means of two replicates with standard error bar) for disease incidence in 1992, 1993, and 1994. Where markers do not have an error bar, the error bars are too small to plot.

Table 3. Seasonal effects on black rot development in cabbage. Estimated Gompertz rate parameters (r), estimated final disease intensities (K), and $r \times K$ values in the cabbage cultivar Perfect Ball grown around fresh refuse piles during 1992, 1993, and 1994

	Disease incidence			Disease severity		
Plot[y]	r	K	$r \times K$	r	K	$r \times K$
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] $r \times K$ 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.

tions every day. Therefore, farmers prepare cabbage heads by removing diseased leaves from the heads so that the piles are replenished 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 to 1993 (data not shown) and found refuse piles on 40% of the visited cabbage-growing farms. Piles were found in 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 United States, the relative importance of refuse piles may be far less than in the Netherlands and some other parts of Europe.

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