Black Rot of Cabbage in Hawaii: Inoculum Source and Disease Incidence

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ABSTRACT

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Commercial cabbage seed and soil of nine cabbage-growing areas in Kula, Maui, were assayed for the presence of *Xanthomonas campestris* to determine the relative importance of each in the annual recurrence of black rot. The pathogen was recovered from only 1 of 20,500 individually tested seeds from commercial lots. Disease-free seedlings planted in areas of high black rot incidence became severely diseased at maturity, whereas in areas with no history of crucifer production, they remained disease-free. The incidence of black rot was influenced by environmental conditions, soil properties, and rotation to nonsusceptible crops. Plants on farms located at low elevations (490-655 m) and in the Keahua soil series had a higher incidence of the disease than did those on the farms located at higher elevations (850-1,000 m) and in the Kula soil series. On farms at low elevations where 50-90% disease occurred, soils contained 1.1-2.3% organic carbon, 1.6-13.5 meq extractable Ca/100 g, and pH was 4.2-4.9. In farms at low and high elevations, where 0-5% disease occurred, soils contained 3.2-6.2% organic carbon, 15.4-42.2 meq extractable Ca/100 g, and pH was 5.5-6.8.

Cabbage production in Hawaii, which comprises 12-15% of the local vegetable industry in terms of total acres planted and yields 9,900,000 pounds of cabbage annually (3), is limited by black rot caused by *Xanthomonas campestris* (Pammel) Dowson. Black rot is of great concern to local cabbage growers, particularly those farming in the Kula district of Maui, where severe disease losses occur despite application of known disease control measures.

Populations of soil-borne bacterial pathogens may be reduced to insignificant levels in the temperate zones by low winter temperatures (9), but *X. campestris* probably is not affected by the mild Hawaiian climate and thus may increase to epidemic proportions with continuous cropping of cabbage. Seed certified to be pathogen-free is currently unavailable, and a low rate of contamination has been reported to initiate disease outbreaks under favorable weather conditions (1). Thus, in Hawaii research was needed to determine whether seed contamination or survival of *X. campestris* in the soil was the predominant factor in the annual recurrence of disease. In addition, the wide range of soil types which occur within small areas in Hawaii offered an opportunity to evaluate the role of edaphic factors on survival of the pathogen.

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MATERIALS AND METHODS

To determine whether commercial seed was a source of inoculum, 5,500 seeds of the most commonly used cultivars (C-G Cross and Golden Acre) were plated individually on a medium containing 250 mg/ml cycloheximide (8), and all yellow viscous colonies were transferred to a starch medium (SX) (6). Starch-hydrolyzing colonies were tested for pathogenicity to confirm the presence of *X. campestris*. Five hundredths ml inoculum (10^8 cells/ml) was placed on the notched leaf margin of each cabbage seedling in the greenhouse. Plants were left in the shade for 2 hr and then returned to the greenhouse bench. Characteristic V-shaped lesions were produced in 5-14 days by pathogenic isolates used as controls. In addition, 15,000 seeds were surface sterilized for 10 min in 1% NaOCl and plated directly on SX medium. An additional forty thousand seeds were tested by the enrichment method (5).

An indirect method also was used to assay seed and soil for the presence of the pathogen. Nontreated seed of the commercially used black rot-susceptible cabbage cultivar C-G and hot water-treated seed (pathogen incidence certified $< 0.01\%$) of two other susceptible varieties, Erin and Shamrock (Pieters-Wheeler Seed Co., Gilroy, CA 95020), were germinated in Jiffy Mix at the Maui Agricultural Research Center (MARC), Kula. One mo later, 72 seedlings of each cultivar were transplanted in marked rows within cabbage fields, with and without a previous history of black rot. Disease incidence was

recorded as a percentage of plants infected and *X. campestris* was isolated from infected leaves on yeast extract-dextrose-$CaCO_3$ (YDC) agar (13). Pathogenicity of suspected colonies was tested as previously described. Field trials were repeated during the rainy season (December-March 1976).

To further test seed as a pontential carrier of inoculum, 1,500 seedlings of each cultivar, C-G, Erin, and Shamrock, were planted in subplots (with seven replications) at MARC's Pulehu substation, an area not previously cropped to crucifers and free of *X. campestris* on the basis of soil tests. Land preparation, fertilization, and other cultural practices were those recommended for optimal cabbage production in the Kula area. Plants were sprinkler-irrigated twice weekly for 1.5 hr and were examined regularly for disease incidence.

In an experiment designed to test the extent to which disease-free seedlings become infected under local conditions, a crucifer-free area at the Pulehu substation was infested. Naturally infected cabbage stumps with four to five wrapper leaves (6.8 kg net weight) were chopped, evenly distributed, and tilled into a 15 × 30 m area with a roto-tiller. Seedlings grown from hot water-treated (50 C for 30 min) seed (cultivars Erin and C-G) and nontreated seed (cultivar C-G) were planted in the three infested and three noninfested 3 × 6 m subplots with 40 cm spacing within rows and 70 cm between rows. Plants were sprinkler-irrigated to promote secondary spread of the bacterium. Disease incidence was recorded at maturity, as a percentage of heads infected; disease severity was recorded on a scale of 0-3 (0 = no lesions; 1 = 1-10 lesions; 2 = 11-25 lesions; 3 = > 26 lesions).

To assess the role of cultural and edaphic factors in perpetuation of disease, nine cabbage-growing areas at various elevations in Maui were selected and surveyed for black rot. Disease incidence, based on visible symptoms, was recorded at weekly intervals from transplanting through harvest. Cultural practices were recorded. On farms at elevations between 488-731 m, soils were silty clay loams and silty clays of Keahua series belonging to the Torroxic Haplustoll soil group. These are fine soils with an available water-holding capacity of less than 30% (11). On farms located at 853 m and above, soils were silt loams and cobbly loams belonging to the Typic Eutrandept soil group, with water-holding capacity greater than 50% (11). Additional soil properties were determined by standard methods (10). Calcium was extracted with ammonium acetate at pH 7.0 (10).

To determine whether *X. campestris* could be detected in soil, soils were assayed at 6 and 14 wk after harvest. Twenty 50-g samples were collected at random from fields, screened to remove plant debris, and mixed thoroughly. Three 10-g subsamples were then shaken in 100 ml of sterile, distilled water and diluted in a tenfold dilution series. Triplicate 0.1-ml aliquots were assayed for *X. campestris* with starch medium (6). To examine more thoroughly the survival capacity of *X. campestris* in infested soils, two farms in which disease incidence was high at harvest were assayed, as described previously, for the presence of *X. campestris* at 3, 4, 6, 9, 13, and 14 wk after harvest.

RESULTS

In seed assays of commercial cabbage cultivars only one seed per 20,500 seeds individually assayed was contaminated with *X. campestris*. A further assay of 40,000 seeds by the enrichment technique yielded no *X. campestris*. Disease-free seedlings planted in soils not previously cropped to crucifers did not develop black rot symptoms at maturity whereas 45% of seedlings from the same seed lots planted in soils with a previous history of black rot developed the disease at maturity (Table 2). The pathogen was recovered from soil samples of the latter farms 6 and 14 wk after harvest. At the Pulehu substation, where soils were infested to determine the significance of primary versus secondary infection, disease incidence recorded at maturity, was approximately twice as great in infested as compared to noninfested subplots, but no difference in disease severity was observed (Table 1). A higher incidence of symptoms occurred at borders between infested and noninfested plots in the direction of sprinkler irrigation.

Black rot occurred with greater frequency on farms located between 488-640 m than on those located between 731-1,006 m (Table 2). On farms A, B, and C disease incidence was 15-90%; soils contained 1.1-2.3% organic carbon, 1.6-13.5 meq extractable Ca/100 g, and pH 4.2-4.9. Farms F, G, H, I, and J had low disease incidence (< 5%). Soils contained 3.2-6.2% organic carbon, 15.4-42.2 meq extractable Ca/100 g, and pH 5.5-6.8. Disease incidence was lower in areas rotated to onions than in those continuously cropped to cabbage (Sites B-1 as compared to B-2 and C; Table 2).

Populations of *X. campestris* declined at approximately the same rate in soils of the Pulehu

TABLE 1. Incidence of black rot of cabbage grown in artificially infested soil

Soil treatment	Cultivar/seed treatment	Avg. no. heads per replicate[w]	Diseased (%)	Severity[x]
Infested	Erin/hot water[y]	52.3	90.0 a[z]	1.2 a
	C-G/hot water	54.0	89.9 a	1.2 a
	C-G/nontreated	52.3	89.3 a	1.2 a
Noninfested	Erin/hot water	58.7	37.6 b	1.2 a
	C-G/hot water	55.3	47.9 b	1.1 a
	C-G/nontreated	56.0	44.9 b	1.1 a

[w]Three replicates.
[x]Disease severity based on 0 = no lesions, 1 = 1-10 lesions on leaf tips, 2 = 11-25 lesions, 3 = 26 or more lesions.
[y]Hot water treatment 50 C, 30 min.
[z]Column means not followed by the same letter are significantly different, $P = 0.01$ (LSD = 21.1).

substation (Farm E) as in naturally infested soils at farm site C-2 (Fig. 1). Low populations of *X. campestris* were recovered from all soils cropped to cabbage up to 14 wk after harvest, even when disease was not observed in the commercial plantings or test sites at several of these farms (Table 2).

DISCUSSION

Perpetuation of black rot may be related to survival of the pathogen in the soil, reintroduction of the pathogen on contaminated seed, or both (1, 4, 7, 9, 12). In Kula, Maui, seed used on commercial farms was found to carry very low levels of *X. campestris*, and seedlings planted from nontreated seed did not become infected if grown on disease-free farms or areas not previously cropped to crucifers. On the other hand, cabbage plants grown from hot water-treated seed became severely infected at maturity if transplanted in fields with a previous history of disease. Furthermore, by infesting a disease-free area with a small amount of infected plant debris at the Pulehu substation, 90% of the disease-free transplants contracted black rot before harvest. Thus, although the importance of seed transmission cannot be overlooked (1, 4, 12), survival of the pathogen in soil and/or plant debris must account for the high levels of disease that recur annually on Hawaiian farms.

Survival of pathogens in soil can be affected by climatic factors, physicochemical properties of the soil, crop rotation, and the microbial activity within the soil (9). In Maui, farms between 488 and 655 m were in a climatic

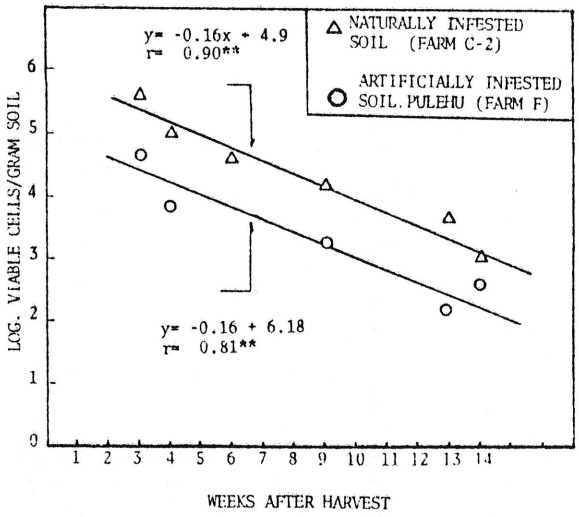

Fig. 1. Recovery of *Xanthomonas campestris* from a soil normally cultivated to cabbage (Farm C-2) and a crucifer-free soil infested with diseased cabbage stumps (Pulehu Substation). ** Indicates statistical significance, $P = 0.01$.

TABLE 2. Soil factors associated with the incidence of black rot of cabbage in farms at varying elevations in Kula, Maui

Farm and site no.	Elevation (m)	Soil properties: Type[u]	pH	Extractable bases (Ca meq/100 g)	Water holding capacity at 1/3 bar (%)	Organic carbon (%)	Diseased plants at maturity[v]: Commercial field (%)	Diseased plants at maturity[v]: Test plot (%)	Recovery of *X. campestris* from soil after harvest (cells/g soil × 100): 6 wk	Recovery of *X. campestris* from soil after harvest (cells/g soil × 100): 14 wk
A	488	Kn C	4.7	5.46	< 30	1.46	40	30	66	t
B-1	640	Kn C	4.3	1.60	< 30	1.45	15-40	2[w]	t[z]	t
B-2	640	Kn C	4.2	2.62	< 30	1.09	60	9[x]	610	t
C	640	Knh C	4.9	13.53	< 30	2.32	40-90	30-45[x]	310	65
D-1	640	Kn C	6.0	24.14	< 30	4.80	-[y]	0[y]	0	0
D-2	640	Kn C	6.6	25.08	< 30	4.43	-[y]	0[y]	0	0
E-1	655	Knh C	6.4	15.57	< 30	1.68	-[y]	0[y]	0	0
E-2	655	Knh C	6.3	17.64	< 30	2.09	-[y]	0[y]	0	0
E-3	655	Knh C	6.4	17.49	< 30	1.59	-[y]	0[y]	0	0
F-1	731	Kns C	6.8	29.91	< 30	3.15	< 5	...	t	t
F-2	731	Kns C	6.2	17.73	< 30	3.15	< 5	...	t	t
G-1	853	Kx C	6.4	35.26	> 50	4.80	0	...	t	t
G-2	853	Kx C	6.5	42.20	> 50	5.07	0	...	t	t
H	914	Kx D	5.5	15.35	> 50	3.45	< 1	...	t	t
I-1	975	Kxa D	6.4	20.54	> 50	3.67	< 5	0	t	t
I-2	975	Kxa D	6.4	15.88	> 50	3.04	< 5	0	t	t
J	1,006	Kxa D	5.5	15.98	> 50	6.20	< 1	0	t	t

[u]Soils designated as Kn C, Knh C, and Kns C, are Keahua silty clay loams, cobbly silty clay, and stony silty clay, respectively (all Torroxic Haplustoll) with water holding capacities less than 30%. Soil designated as Kx D, Kxa D, and Kx C are Kobo silt loams, Kula cobbly loams, and Kula loams, respectively (all Typic Eutrandept) with water holding capacities less than 50%.

[v]Percentage of plants showing symptoms at leaf margins and/or discoloration of vessels in cabbage stalks. Average of summer and winter trials.

[w]Land rotated to onions leaving 1 yr between cabbage plantings.

[x]No rotation; land fallowed 4-6 wk between cabbage plantings.

[y]Areas not previously cropped to crucifers. Data represent first trial only. In the second commercial planting Farm D had pockets of affected plants, presumably from seed contamination. Farm E (Pulehu substation) was purposely infested after first crop.

[z]t = 1-50 cells/g soil × 100.

zone of low annual rainfall (381-635 mm) but high mean annual soil and air temperature (23 C), while farms at 853 m and above had more rainfall (635-1,016 mm) with lower soil and air temperatures (19 C). Although the higher temperature would favor disease development at low elevations (2), farms at high elevations had similar temperatures (22-24 C) during the summer months, yet disease levels remained low. In addition, some farms at low elevations had higher year around incidence of disease than adjacent farms. These observations indicate that differences in disease incidence cannot be explained totally by climatic conditions. On the other hand, edaphic characteristics may contribute to differences in disease incidence on these farms. In this respect, soils of low organic matter content, low water holding capacity, low clay content, low Ca content, and/or low pH were associated with higher disease incidence. These factors may affect plant susceptibility to disease and/or survival of the pathogen.

When the survival of *X. campestris* in two Keahua series soils, one of pH 4.9 and the other of pH 6.5, was compared, populations declined at approximately the same rate, indicating that high disease incidence in soils of low pH must be related to factors other than survival of the pathogen alone. Further experiments are underway to determine what soil properties directly influence population levels of *X. campestris* and its capacity to incite black rot.

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