Relationship of Incidence of Seedborne *Xanthomonas campestris* to Black Rot of Crucifers

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ABSTRACT

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The relation of amounts of seedborne *Xanthomonas campestris*, determined by laboratory assays, to the incidence of black rot in the field was determined. Seeds infested naturally with *X. campestris* were mixed with healthy seeds, assayed for *X. campestris*, and seeded in a field in South Carolina. The pathogen was detected in laboratory assays in two of four samples with 0.01% infestation, whereas black rot developed in the field in three of four such samples. In 1976, field plots that initially contained 0.03, 0.07, and 0.14% infected plants resulted in high incidences of black rot. In 1977, the incidences of black rot were high in plots that initially contained 0.05% infected plants but not in plots that initially contained 0.01% infected plants. We conclude that laboratory assays can be used to detect levels of seed infestation that may or may not result in a high incidence of black rot in the field.

Xanthomonas campestris is seedborne (3,5,7), and infested seed is an important source of inoculum (2,4,8,14,15). Several methods have been described for detecting the pathogen in or on seeds (1,9,11,13), but the number of infested seeds needed to initiate black rot in the field has not been determined. This information is essential to establish a minimum allowable percentage of seed infestation. This study was designed to determine the relationship of amounts of seed infestation to disease incidence in the field.

MATERIALS AND METHODS

Seed source and preparation. Danish Ballhead cabbage seeds were obtained from Alf Christianson Seed Co., Mount Vernon, WA. No infested seeds were found in a sample of 50,000 seeds assayed by the direct SX agar (10) plating technique (9). The germination was 92.0% and 10,000 seeds weighed 40 g. Green Comet broccoli seeds were obtained from seed plants inoculated with *X. campestris*. Ten percent of the broccoli seeds were infested with *X. campestris* as determined by the direct SX agar plating technique (9). The following amounts of infestation were prepared by mixing seeds in the following proportions: 1) 1,000 broccoli and 9,000 cabbage seeds (1.0% infestation), 2) 100 broccoli and 9,900 cabbage seeds (0.1% infestation), 3) 10 broccoli and 9,990 cabbage seeds (0.01% infestation), and 4) 10,000 cabbage seeds (0.0%). The seeds were mixed and then treated with a captan-ceresan mixture (about 1 g/40 g seed) to control seedborne fungi. For the 1976 test, four samples of each seed lot were assayed for *X. campestris* and four duplicate samples were sent to Charleston, SC, for planting. For the 1977 test, the samples with 1.0% infested seeds were eliminated and five samples of all other lots were assayed and duplicate samples sent to Charleston for planting.

Laboratory assays. Seeds were washed in Micro detergent (International Products Corp., Trenton, NJ), surface-disinfested in a 1.0% solution of sodium hypochlorite, washed twice in sterile distilled water, dried, and plated onto SX agar as previously described (9).

Field plots. A field of fine sandy loam was fertilized with 913 kg/ha of a granular 10-10-10 formulation and 1.8 m × 33 m beds were prepared with a Ferguson Til-O-Vator. Each plot contained one sample (10,000 seeds) and consisted of three beds with three rows per bed. Each plot was separated from every other plot by 15.2 m in 1976 and by 27.4 m in 1977. The experimental design was a randomized block. Seeds were sown on 15 March using a Stanhay precision planter. A preemergence treatment of Vegedex (CDEC) was applied at 3.5 kg/ha. When the first true leaf of the cabbage seedlings developed to about 15 mm in diameter, Tok (nitrogen) herbicide was broadcast at 6.8 kg/ha. Lannate (methomyl) and Dipel (*Bacillus thuringiensis*) at 0.62 kg/ha were used to control insects.

Disease incidence was determined by counting the number of plants with symptoms of black rot 8, 13, and 17 wk after planting.

RESULTS

Laboratory assays showed *X. campestris* in all samples with 1.0% and 0.1% infestation but not in all samples with 0.01% (Table 1). In the samples with 0.01% of infested seeds, black rot was observed in three of four samples of seed sown in the field (mean of 2.25 diseased plants per sample) and the pathogen was detected in two of four samples in the laboratory assays.

The first symptoms of black rot were not observed until 6 wk after sowing. Initially (8 wk), no infected cabbage seedlings were observed; all diseased seedlings were broccoli. During 1976, the amount of disease resulting from the different amounts of seed infestation was not significantly different after 13 wk (Table 2). All treatments resulted in a relatively high incidence of infected plants. In 1977, however, an initial seedling infection of one plant per 10,000 seeds resulted in significantly fewer infected plants than did an initial infection of five plants (Table 2).

DISCUSSION

Although investigators have concluded from field observations that epidemics of black rot can result from infested seed (6,14), no data are available that correlate amounts of seed infestation and subsequent incidence of black rot. By sowing seeds with known amounts of infestation, our data show that whereas five diseased seedlings per 10,000 seeds sown can result in a relatively high incidence of black rot, a single diseased seedling will not. Also, we have established that laboratory assays can be used to detect *X. campestris* infestation at percentages likely to result in severe black rot in the field.

The reason for the large number of diseased plants in our 1976 check plots after 17 wk is difficult to explain. Because no diseased plants were observed in the check plots initially at 8 wk and since the treatments were significantly different at 8 wk but not thereafter, the most likely reason is spread of inoculum from one

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plot to another. This explanation is further supported by the significantly different results of treatment when we increased the distance between plots by 80% in 1977. Results showing that infections of new plants with *X. campetris* depend on their proximity to infected plants (12) support this explanation.

Furthermore, our ability to distinguish broccoli and cabbage plants and the fact that no diseased cabbage plants were observed initially (at 8 wk) makes it unlikely that the diseased plants in our check plots resulted from seedborne inoculum.

Our results suggest that laboratory seed assays capable of detecting one diseased seed per 10,000 can successfully predict the field severity of black rot. Our results also suggest that a tolerance of one infested seed per 10,000 is acceptable for direct seeding of cabbage for head production but that a zero tolerance is necessary for transplant (seedbed) production.

However, establishment of tolerance levels will have to await further data on the effects of weather on the development of black rot.

Table 1. Comparison of laboratory assays of *Xanthomonas campestris* in seed and black rot incidence in the field in 1976

% Diseased seeds per sample[a]	Number of samples yielding *X. campestris* by:[b]	
	Laboratory assays	Disease incidence in the field
1.0	4	4 (57)
0.1	4	3 (26)
0.01	2	3 (12)
0.0	0	0 (0)

[a] Percentage calculated by adding 1,000, 100, 10, and 0 diseased seeds to 9,000, 9,900, 9,990, and 10,000 healthy cabbage seeds, respectively. The broccoli seeds were shown to be 10% infested by plating five 10,000-seed samples onto SX agar (9,10).
[b] Four replications of 10,000 seeds assayed in the laboratory and four replications of 10,000 seeds sown in the field. Field readings were taken on 8 May, 8 wk after sowing. Numbers in parentheses are total number of diseased plants.

Table 2. Development of black rot in the field from seeds with different percentages of black rot infestation, 1976 and 1977

% Diseased seeds per sample[a]	Expected	Mean number of infected plants[b]					
		1976			1977		
		Weeks after sowing					
		8	13	17	8	13	17
1.0	100	14 a	84 a	344 a	ND[c]	ND	ND
0.1	10	7 b	121 a	682 a	5 a	3 a	533 a
0.01	1	3 c	58 a	717 a	1 b	8 b	79 b
0.0	0	0 d	7 a	222 a	0 b	0 c	8 c

[a] Percentage calculated by adding 1,000, 100, 10, and 0 diseased broccoli seeds to 9,000, 9,900, 9,990, and 10,000 healthy cabbage seeds, respectively. Broccoli seeds were shown to be 10% infested by plating five 10,000-seed samples onto SX agar (9,10).
[b] Four replications in 1976 and five in 1977 each consisted of 10,000 seeds sown. Numbers in columns followed by the same letter are not significantly different ($P \geq 0.05$) according to Duncan's multiple range test.
[c] ND = not detected.

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LITERATURE CITED

1. ANDERSEN, H. 1973. A method for detection of Xanthomonas campestris (Pammel) Dowson in Brassica seeds. Statens Plantetilsyn 21:34-38.
2. CHUPP, C., and A. F. SHERF. 1960. Vegetable diseases and their control. The Ronald Press Company: New York. 693 pp.
3. CLAYTON, E. E. 1929. Studies of the black rot or blight disease of cauliflower. N.Y. Agric. Exp. Stn. Geneva Tech. Bull. 576. 44 pp.
4. CLAYTON, E. E. 1931. Vegetable seed treatment with special reference to the use of hot water and organic mercurials. N.Y. Agric. Exp. Stn. Geneva Tech. Bull. 183. 43 pp.
5. COOK, A. A., R. H. LARSON, and J. C. WALKER. 1952. Relation of the black rot pathogen to cabbage seed. Phytopathology 42:316-320.
6. HUNTER, J. E., G. S. ABAWI, and R. F. BECKER. 1975. Observations on the source and spread of Xanthomonas campestris in an epidemic of black rot in New York. Plant Dis. Rep. 59:384-387.
7. MONTEITH, J., JR. 1921. Seed transmission and overwintering of cabbage black rot. Phytopathology 11:53-54. (Abstr.).
8. RICHARDSON, J. K. 1945. Black rot of rutabagas. Sci. Agric. 25:415-425.
9. SCHAAD, N. W., and R. KENDRICK. 1975. A qualitative method of detecting Xanthomonas campestris in crucifer seed. Phytopathology 65:1034-1036.
10. SCHAAD, N. W., and W. C. WHITE. 1974. A selective medium for soil isolation and enumeration of Xanthomonas campestris. Phytopathology 64:876-880.
11. SHACKLETON, D. A. 1962. A method for the detection of Xanthomonas campestris (Pammel 1895) Dowson, 1939, in Brassica seed. Nature 193:78.
12. STRANDERG, J. 1973. Spatial distribution of cabbage black rot and the estimation of diseased plant population. Phytopathology 63:998-1003.
13. SRINIVASAN, M. C., P. NEERGAARD, and S. B. MATHUR. 1971. A technique for detection of Xanthomonas campestris in routine seed health testing of crucifers. Preprint No. 71. 16th Int. Seed Testing Assoc. Congr., Washington, DC. 10 pp.
14. WALKER, J. C. 1941. Origin of cabbage black rot epidemics. Plant Dis Rep. 25:91-94.
15. WALKER, J. C., and W. B. TISDALE. 1920. Observations on seed transmission of the cabbage black rot organism. Phytopathology 10:175-177.