Survival of Xanthomonas campestris in Soil

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ABSTRACT

The survival of the black rot organism, *Xanthomonas campestris*, was determined in soil using an agar plate technique. When separated from host tissue, the pathogen was not recovered in field soil after 14 days in late summer or after 42 days in winter; half-lives of the pathogen during winter, late spring, early summer, and late summer were 2.6, 1.3, 1.2, and 0.4 days, respectively. When protected by cabbage stem tissue, the pathogen was recovered in large numbers from plant debris in soil for up to 244 days. More than one hundred thousand viable *X. campestris* cells per g tissue were recovered from the 244-day sample and the half-life of the pathogen was 13.7-days. Using a 13.7-day half-life, the theoretical survival time of *X. campestris* in host tissue in soil would be 615 days. The relationship between the survival of the pathogen in soil and recommended crop rotations for controlling black rot are discussed.

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Additional key words: black rot, cabbage, crop rotation.

Xanthomonas campestris, the causal agent of black rot, attacks many cruciferous plants (1, 9). The bacteria are present in host vascular bundles, and during fruit development may invade the xylem of the funiculus and siliques (4). The pathogen survives from season to season as seed-borne inoculum (3, 6, 7). Although infection can take place from infested soil (4, 7), the importance of soil in the ecology of *X. campestris* has not been clearly established. Clayton (2), using bait plants, first concluded that the bacterium did not survive in soil. However, 5 years later he stated "...the ability of the organism to live over one winter in the soil was thus definitely established" (3). When clean cabbage (*Brassica oleraceae* L.) seeds were sown in sterilized soil containing diseased cabbage leaves, seedlings became diseased (6). On the other hand, where healthy cabbages were set on land on which black rot of cauliflower was observed 2 years previously, no black rot was found (3). Chupp and Sherf (1) concluded that *X. campestris* cannot survive longer than 1 year in a field or seedbed and suggested a 2-year rotation.

Walker (9) concluded that epidemics were most often traced to seed or a seedbed which had become infested from refuse or seed, and recommended at least a 3-year rotation of the seedbed and of the transplant field. Most recently, Williams and Wade (10) recommended that seedbed ground should be selected so as not to have had crucifer crops on or near it for at least 5 years. Most of these control recommendations have been based not on quantitative data, but only on observations. It was the purpose of this study to determine quantitatively the survival of *X. campestris* in soil. The first part of this work dealing with the development of a selective medium (SX agar) for the quantitative recovery of the pathogen from soil has been published (8).

METHODS.—*Inoculum.*—For plant inoculations, early log phase cells were obtained by growing *X. campestris* strain BBS in 523 broth (5) on a rotary shaker at 27 C to a turbidity of 50 Klett units as described previously (8). Approximately 10^6 cells were injected into stems of 6-week-old, greenhouse-grown cabbage (cultivar Market Prize, Harris Seed Co.) with a sterile, disposable needle and syringe.

For soil infestations, bacteria were obtained from leaves of greenhouse-grown cabbage plants which had been inoculated 10 to 14 days previously. The black veins together with varying amounts of adjacent tissue were removed and weighed. Forty grams (fresh weight) of tissue were then ground in 200 ml sterile distilled water for 10-15 seconds at 5 C using a Waring Blendor. After incubation for 15.5 hours at 5 C, the suspension was filtered through a triple layer of cheesecloth to remove any visible black veins. The final volume was adjusted to 320 ml by washing the blender with sterile distilled water. One ml was removed and diluted to 10^{-7}, 10^{-8}, and 10^{-9} with sterile distilled water. The number of viable cells in the inoculum was determined by pipetting 0.1 ml from each dilution onto the surface of triplicate Difco nutrient agar plates as previously described (8). Finally the inoculum was placed in an ice bath and taken to the field.

Survival of X. campestris in soil.—Twelve 0.5-m-square sites approximately 90 m apart were selected in an open field of a Lloyd sandy clay loam soil. No cruciferous crop had been grown in this field and the pathogen was not recovered from several soil samples when assayed on SX agar (8). One hundred ml of inoculum was slowly poured onto the soil so as not to infest an area greater than 10 to 15 cm in diameter. Three sites were infested on 29 December 1971, 30 April 1972, 1 June 1972, and 1 September 1972.

Soil samples for assay of *X. campestris* were taken immediately after the inoculum was added and every 7 days thereafter until the pathogen could no longer be recovered. Two, 2- to 3-g samples were removed from the top 5 cm of infested soil with a sterile spatula, placed into a sterile test tube, and brought to the laboratory. One gram of soil was removed from each sample, added to 9.0 ml sterile water, mixed with a Vortex mixer, and immediately serially diluted. One-tenth ml of the appropriate dilution was then pipetted onto the surface of triplicate SX agar plates and spread evenly using a turntable and an L-shaped glass rod. In Each case, the dilution giving the highest colony count < 500 per plate was recorded.

Survival of X. campestris in host tissue in soil.—Two cabbage stems, approximately 2×5 cm, showing numerous black veins were selected from several plants which had been inoculated with *X. campestris* 3 months previously. Using a sterile scalpel, two cross-sections

TABLE 1. Survival of *Xanthomonas campestris* in soil during different times of the year[a]

Days after infestation	*X. campestris* detected (mean no. cells per g wet weight soil[b])			
	Date of infestation			
	29 Dec 1971	30 April 1972	1 June 1972	1 Sept 1972
0	3.2×10^6	4.9×10^7	3.2×10^7	1.3×10^7
7	1.4×10^5	5.0×10^4	...	3.3×10^3
14	1.1×10^4	4.1×10^3	1.3×10^3	2.2×10^1
21	...	...	1.2×10^2	0.0
27	6.8×10^2	4.4×10^1	0.0	
42	4.9×10^1	0.0		
56	0.0			

[a]Soil infested by adding 100 ml of inoculum freshly extracted from diseased cabbage leaves.
[b]Figures represent average of two 1.0-g samples taken from each of three separate soil sites. Samples were diluted serially and 0.1 ml assayed on three SX agar plates.

approximately 1-mm thick were cut from each end of each stem and their weights recorded to the nearest mg. One section of each pair was dried at 40 C for 7 to 10 days to standardize moisture content. The other section was ground thoroughly in sterile water using a mortar and pestle, diluted serially, and assayed in triplicate on SX agar plates. The initial dilution of each sample of tissue varied from 1:20 to 1:100 depending upon dry weights. Each stem was wrapped tightly in a piece of nylon netting (3-mm-diameter holes) and buried 10-15 cm deep in natural, Lloyd sandy clay loam soil on 1 November 1973. Sixty days later and at 30-day intervals thereafter, the nylon nettings containing the stems were removed from the soil and the tissue assayed as stated above. The experiment was terminated after 244 days.

RESULTS.—*Soil survival independent of host tissue.*—Survival time of *X. campestris* was short when unprotected by host tissues, and depended upon the time of year. For example, the pathogen was recovered from soil infestations for a period of 42 days in winter, but only 14 days during late summer (Table 1). Half-lives (11) of the pathogen during January, May, June, and September were 2.6, 1.3, 1.2, and 0.4 days, respectively.

Soil survival in host tissue.—According to J. Ratcliffe *(personal communication),* cabbage plants with stems heavily infected with *X. campestris* are not uncommon in Georgia at harvest time. Since cabbage plants are disked into the soil after the heads are harvested, the infected, hardened stems could serve as an important source of inoculum. To determine whether such tissue might serve as an inoculum source from season to season, infected cabbage tissue was buried in soil and assayed as described.

The mean number of viable cells of *X. campestris* per g tissue declined very slowly (Table 2). From 1 January 1973, through 3 July 1973, the mean number of cells per gram tissue decreased from 2.2×10^9 to 1.7×10^5. Half-lives of the pathogen in stem tissue for these monthly intervals were 6.6, 12.6, 14.4, 18.7, 10.6 and 13.5 days, respectively. Apparently time of year had little or no influence on the survival of *X. campestris* when protected by host tissues.

No visible changes were evident in the physical condition of cabbage stems after being buried in soil for 63 days; the tissue was intact and no signs of rotting were observed. On 7 February, approximately one-half of the tissue was visibly decomposed and several black veins were visible. By 1 March, most tissue except the vascular bundles had decomposed. After 1 March, little or no change in decomposition was evident. The experiment was terminated after 244 days because assays had utilized all the available tissue.

DISCUSSION.—We have shown that *X. campestris* obtained directly from host tissues can survive in soil independent of its host for at least 42 days. Taking into account the 10% efficiency of recovery of *X. campestris* actually present in soil of SX agar (8), nearly 500 viable cells per gram of soil were present after 42 days. The inference from these data is that the pathogen has the potential of surviving in soil for nearly 60 days as free bacteria. Furthermore, survival of the pathogen was six times longer during the cool, wet, cabbage-growing season than during the hot, dry summer. It would appear that whereas soil might be an important source of secondary inoculum, it is doubtful that free bacteria in soil could serve as a primary source of inoculum after 2 months.

TABLE 2. Survival of *Xanthomonas campestris* in cabbage stem tissue buried in field soil

Days after burial	Date (1973)	Colonies per plate[a] mean no.	Cells per g tissue[b] mean no.
63	3 Jan	306.7±81.3[c]	2.2×10^9
98	7 Feb	27.8±9.6	5.7×10^7
120	1 March	24.2±9.9	9.6×10^7
152	2 April	35.5±7.1	2.8×10^7
182	2 May	39.6±14.1	2.7×10^7
215	4 June	21.3±9.9	1.1×10^5
244	3 July	1.9±1.3	1.7×10^5

[a]Counts are from four stem tissue samples; each sample was weighed, ground in a known volume of sterile water, diluted serially, and 0.1 ml assayed on three SX agar plates.
[b]Dry weight basis, 40 C for 7 to 10 days.
[c]Confidence limits, $P = 0.05$.

Whereas survival of free *X. campestris* cells in soil was limited to a relatively short time, the pathogen survived in host debris for a relatively long time. Extrapolation of our data indicated that *X. campestris* was capable of surviving in soil for at least 615 days when protected by host tissues. Although survival of the bacteria in host tissue did not appear to be affected by the time of year, survival in absence of host tissue in soil was greater in winter than in summer. While others (7, 9, 10) have suggested a 3- to 5-year-rotation for controlling black rot, our data indicated that a 2-year rotation would be sufficient.

We have shown under conditions in Georgia that *X. campestris* would most likely survive for less than 2 years in host debris. A rotation longer than 2 years would therefore seem to be an unnecessary and expensive practice for controlling black rot of crucifers, under the weather conditions of the southeastern United States. Whether or not similar results would occur in other crucifer-growing areas must await experimental evidence.

LITERATURE CITED

1. CHUPP, C., and A. F. SHERF. 1960. Crucifer diseases, p. 237-240. *In* C. Chupp, and A. F. Sherf, Vegetable diseases and their control. The Ronald Press Co., New York.
2. CLAYTON, E. E. 1924. A progress report on black-rot investigations, with special reference to cauliflower on Long Island. Phytopathology 14:24 (Abstr.).
3. CLAYTON, E. E. 1929. Studies of the black-rot or blight disease of cauliflower. N. Y. Agric. Exp. Stn. (Geneva) Bull. 576. 44 p.
4. COOK, A. A., R. H. LARSON, and J. C. WALKER. 1952. Relation of the black rot pathogen to cabbage seed. Phytopathology 42:316-320.
5. KADO, C. I., and M. G. HESKETT. 1970. Selective media for isolation of Agrobacterium, Corynebacterium, Erwinia, Pseudomonas, and Xanthomonas. Phytopathology 60:969-976.
6. MONTEITH, JOHN, JR. 1921. Seed transmission and overwintering of cabbage black rot. Phytopathology 11:53-54.
7. RICHARDSON, J. K. 1945. Black rot of rutabagas. Sci. Agric. 25:415-425.
8. SCHAAD, N. W., and W. C. WHITE. 1974. A selective medium for soil isolation and enumeration of Xanthomonas campestris. Phytopathology 64:876-880.
9. WALKER, J. C. 1952. Diseases of crucifers. Pages 128-131 *in* J. C. Walker, Diseases of vegetable crops. McGraw-Hill, New York.
10. WILLIAMS, P. H., and E. K. WADE. 1973. Recommendations for minimizing the threat of blackleg and black rot of cabbage. Control Plant Diseases #78, Cooperative Extensive Programs, Univ. of Wisconsin, Madison.
11. YARWOOD, C. E., and E. S. SYLVESTER. 1959. The half-life concept of longevity of plant pathogens. Plant Dis. Rep. 43:125-128.