Temperature, an Important Factor Determining Survival of Corynebacterium michiganense in Soil

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ABSTRACT

Corynebacterium michiganense, the tomato canker organism, survived in infected tomato leaflets and on infested cotton threads for at least 36 and 11 weeks, respectively, at −20 C, in sterile and unsterile soil at pH 7.7 ± 0.3, but did not survive more than 3 weeks at temp from 5-35 C. The organism was recovered from soil ranging in moisture content from 2.7 to 21%. Soil moisture, per se, did not limit its ability to survive. Phytopathology 60:825-827.

Corynebacterium michiganense (E. F. Sm.) H. L. Jens., the cause of tomato (*Lycopersicon esculentum* Mill.) canker survives in seed (6, 9, 11), soil (6, 9, 13), and alternate hosts (1, 2), but seed transmission of the disease was found to be less than 1% (9, 11), and infection was controlled by seed treatment (4, 5). The longevity of the organism in soil is variable, depending upon the region and locality (6, 7, 9, 10, 13). Grogan & Kendrick (9) reported that field carry-over of the organism was not important in California, but Strider (13) found that it was capable of overwintering in western North Carolina. Bryan (6) showed survival of the organism in sterile soil tubes for 2-2.5 years in Washington, D.C. Ciccarone & Carilli (7) recorded a case of possible survival of *C. michiganense* in soil for 4 years in Italy.

Several attempts at Ottawa, over a period of 3 years, to reisolate the organism from previously infested field plots failed, either because the pathogen was eliminated shortly after its incorporation into field soil or because its population was reduced to an undetectable level as determined by dilution plate and pathogenicity techniques (8, 13). Differences in the period of survival in soil could be due to the effects of temperature, moisture, pH, microbial antagonism, and possible other factors.

In the work presented here, the relative importance of temperature and soil moisture in relation to the survival of *C. michiganense* in the presence or absence of host tissue using both sterile and unsterile soil at a constant pH was studied.

MATERIALS AND METHODS.—A virulent strain of *C. michiganense,* hereafter referred to as *Cm7,* was isolated from infected tomato fruits showing typical disease symptoms (6) and identified by cultural characteristics (6) and pathogenicity tests (3, 13). Stock cultures of this organism were maintained on yeast-dextrose-carbonate agar slants at 5 C. An aqueous suspension containing 2×10^8 cells/ml was used for inoculations when required.

The soil mixture prepared for these studies was composed of garden loam, sand, and peat moss (2:1:1, v/v). It was passed through a 20-mesh sieve to ensure uniformity in particle size. The average moisture-holding capacity of the soil was 16.3% at 1/3 atm, as determined by the pressure plate method (14). The pH of the soil was adjusted to 7.7 ± 0.3 with $CaCO_3$. Its moisture content was determined by a Cenco moisture balance, and was adjusted to 20 ± 2% or to 10 ± 2% by moistening or drying as required. The soil was dispensed into test tubes (125 × 16 mm) and petri plates (100 × 15 mm). Each tube and plate contained 6 and 45 g soil, respectively. Half of the soil tubes and plates were sterilized by autoclaving for 1 hr at 20 psi on 4 successive days as a control to determine the microbial effect on the longevity of the organism. The pH and moisture levels of the soil showed little change following autoclaving.

To determine the longevity of the pathogen in soil in association with host tissue, artificially infected tomato leaflets (3), in groups of three, were introduced into the soil tubes. These were sealed with parafilm to avoid moisture loss and placed in two incubators maintained at −20 and 25 C. Every week during the first month and, subsequently, every 3 weeks for the next 7 months, the leaves were removed from four tubes of sterile or unsterile soil incubated at each temperature. Three leaflets from each tube were washed with sterile water to remove any soil particles, then ground with a mortar and pestle with 25 ml water. The resultant suspension, containing comminuted host tissue and cells of *Cm7,* was sprayed with an atomizer on 3- to 4-week-old John Baer tomato seedlings in the greenhouse and incubated for 5-7 days under high humidity (3). The production of typical blisterlike symptoms (3) on leaves indicated the presence of *Cm7* in the suspension as well as its pathogenicity and viability.To compare the infectivity of the incubated diseased leaf tissues, three kinds of control were used in the pathogenicity tests. Tomato seedlings were sprayed with (i) sterile water; (ii) a suspension of *Cm7* cells; or (iii) a suspension of bacterial cells and plant tissue prepared from fresh tomato leaves with lesions. For each test, 50 seedlings were inoculated and the percentage of infected leaflets was recorded.

To determine the longevity of the organism in the absence of host tissue, Roizin's thread method (12) was employed. Washed and sterilized cotton threads (2 × 6 mm) were infested by soaking them in an aqueous suspension of *Cm7* containing 2×10^8 cells/ml. Four pieces of thread were placed, separately, in 6-7 mm-deep furrows in sterile or unsterile soil plates

and covered with soil. Plates were incubated in 5 temperature-controlled incubators at —20, 5, 20, 27, and 35 C, and in two Copenhagen chambers maintained at 27 and 35 C. These chambers were used to reduce moisture loss from the plates during incubation at the higher temperatures. At weekly intervals, threads recovered from four plates of both sterile and unsterile soil at each incubation temperature were shaken gently to dislodge adhering soil particles and plated on nutrient agar. After 4 to 5 days' incubation at room temperature (21-25 C), the organism, if viable, grew along the thread.

The pH and moisture content of soil from each plate and tube were determined after the removal of threads or leaf tissues.

RESULTS AND DISCUSSION.—The survival periods of *C. michiganense* within host tissue and on cotton threads buried in soil at various temperatures and moisture conditions are indicated by the heavier lines on the temp curves in Fig. 2, 3, and 4. The soil pH remained constant during the experimental period (Fig. 1).

Infected host tissues maintained at —20 C in both sterile and unsterile soil remained green, and the organism was viable and pathogenic for at least 36 weeks, but tissues incubated at 25 C deteriorated and the pathogen was not recovered after 3 weeks. The amount of infection produced on tomato seedlings sprayed with suspensions made from incubated diseased tissues ranged from 1 to 4.5% (Table 1). A similar range of infection was also obtained when the suspensions were prepared from fresh tomato leaves with lesions. Inoculation with pure bacterial (*Cm7*) suspensions, however, caused 37-50% infection of leaflets.

Sealed soil tubes containing infected leaflets held at —20 C retained the original moisture content (20 ± 2%), but those maintained at 25 C showed a gradual loss of moisture content to as low as 12.1% in 36 weeks. At the 9th week, however, the moisture content of soils held at —20 and 25 C was very similar (17-

TABLE 1. Infection of tomato leaflets produced by spraying seedlings with suspensions made from leaf tissues infected with *Corynebacterium michiganense* held in sterile and unsterile soil at —20 C and 25 C over a period of 36 weeks

Incubation period, weeks	% Infected leaflets[a]			
	—20 C		25 C	
	Sterile	Unsterile	Sterile	Unsterile
1	4.1	4.5	1.8	1.6
2	3.5	3.4	1.5	1.0
3	4.5	2.5	1.4	1.2
4	1.5	1.4	0.0	0.0
6	1.8	1.2	0.0	0.0
9	1.2	1.3	0.0	0.0
12	1.9	1.5	0.0	0.0
15	1.3	1.2	0.0	0.0
18	1.3	1.4	0.0	0.0
21	1.2	1.3	0.0	0.0
24	1.3	1.2	0.0	0.0
27	1.4	1.2	0.0	0.0
30	1.5	1.4	0.0	0.0
33	1.4	1.1	0.0	0.0
36	1.2	1.0	0.0	0.0

[a] Each based upon the number of leaflets showing blister symptoms out of 1,000-1,200 examined.

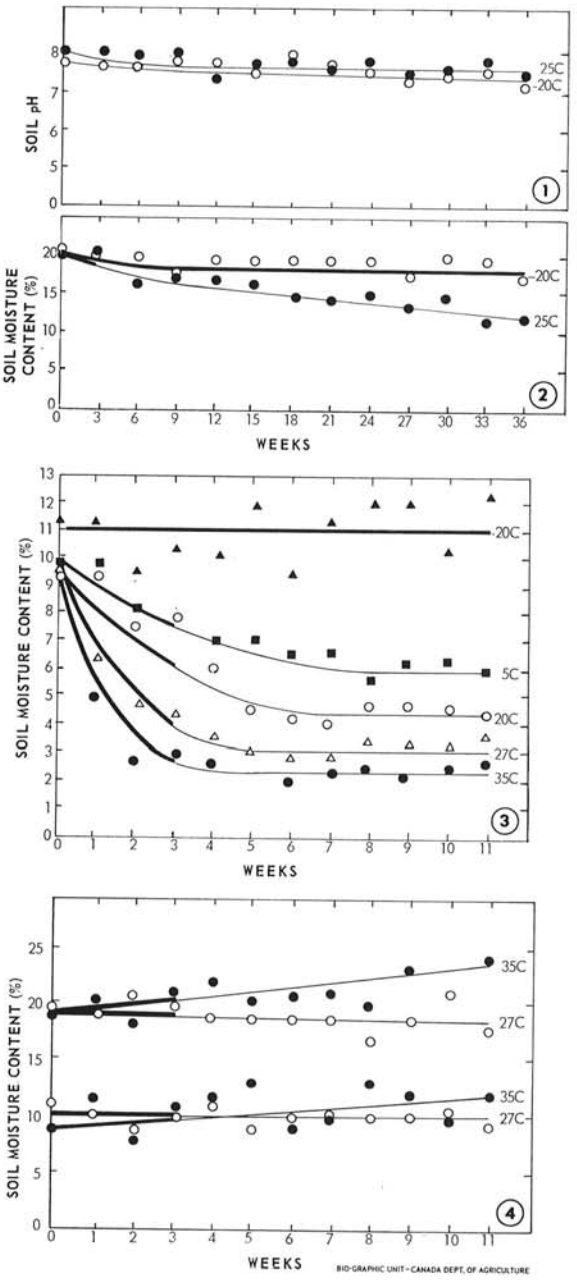

Fig. 1-4. **1)** pH of soil, containing infected leaf tissues, maintained at —20 C and 25 C in sealed tubes for 36 weeks. **2)** Period of survival of *Corynebacterium michiganense* (heavier lines) and moisture levels in sealed soil tubes, containing infected leaf tissues, maintained at —20 C in incubators for 36 weeks. **3)** Period of survival of *C. michiganense* (heavier lines) and trends of moisture levels in soil plates, containing inoculated threads, maintained at —20 C and 35 C in incubators for 11 weeks. **4)** Period of survival of *C. michiganense* (heavier lines) and trends of moisture levels in soil plates, containing inoculated threads, held in Copenhagen chambers 27 and 35 C for 11 weeks. Note two initial moisture levels, 10 ± 2% and 20 ± 2%.

18%), but the organism was viable only at the lower temperature (Fig. 2).

Since incubated tomato leaves were not sterile prior to inoculation, the possible effect of saprophytic microorganisms on the longevity of the pathogen at higher temp could not be ruled out in the above experiment. But this problem was resolved, to some degree, by determining the longevity of the organism on cotton threads instead of tomato leaflets, in both sterile and unsterile soil at different temp and moisture levels.

The longevity of *Cm7* on cotton threads exceeded 11 weeks or more only at −20 C in soil ranging in moisture content from 9.5 to 12.5%. At or above 5 C, the organism was recovered only during the first 3 weeks from soil containing 2.7 to 21% moisture, but after that period *Cm7* was not recovered from similar soil moisture ranges (Fig. 3, 4).

Soil plates held at 5, 20, 27, and 35 C, where no attempt was made to prevent moisture escape, rapidly lost moisture for the first 4-5 weeks, after which the moisture values leveled off (Fig. 3). Soil plates incubated in Copenhagen chambers at 27 C retained the original moisture levels, but the soil absorbed a small amount of moisture in the high humidity of the chamber maintained at 35 C (Fig. 4).

These results indicate that the longevity of the organism in soil was dependent on temperature rather than on soil moisture. Furthermore, since the survival periods of the organism on cotton threads in sterile and unsterile soil were equal, it was apparent that microbial antagonism did not affect survival time. The results show that *C. michiganense* is capable of overwintering in frozen host tissue buried in soil. It would be necessary to study the longevity of the organism in soil within a temperature range from −20 to 5 C to determine the critical temperatures which just begin to affect survival of the organism. From a practical standpoint, it seems reasonable that, under normal greenhouse conditions for growing tomatoes, the pathogen is not likely to survive in soil for more than 3-4 weeks. Therefore, to avoid the possibility of primary infection from contaminated soil, the soil to be used for seed bed or potting tomato seedlings should be maintained in a warm temperature for at least 4 weeks prior to seeding or planting. Frozen soil, usually maintained near greenhouses, should not be used until it has reached the greenhouse temp and maintained at that temp for at least a month.

LITERATURE CITED

1. Ark, P. A., & J. P. Thompson. 1960. Additional hosts for tomato canker organism, *Corynebacterium michiganense*. Plant Dis. Reptr. 44:98-99.
2. Baines, R. C. 1947. Perennial nightshade, a host for *Corynebacterium michiganense*. Phytopathology 37: 359 (Abstr.).
3. Basu, P. K. 1966. Conditions for symptomatological differentiation of bacterial canker, spot, and speck on tomato seedlings. Can. J. Plant Sci. 46:525-530.
4. Blood, H. L. 1933. The control of tomato bacterial canker (*Aplanobacter michiganense* E. F. S.) by fruit-pulp fermentation in the seed-extraction process. Utah Acad. Sci., Arts and Letters Proc. 10:19-23.
5. Blood, H. L. 1937. A possible acid seed soak for the control of bacterial canker of the tomato. Science 86:199-200.
6. Bryan, M. K. 1930. Studies on bacterial canker of tomato. J. Agr. Res. 41:825-851.
7. Ciccarone, A., & A. Carilli. 1948. Osservazion di campo su *Corynebacterium michiganense* (Smith) Jensen e considerazioni su un possible caso di sua supravvivenza nel terreno. [English summary]. Bollettino della Stazione di Patologia Vegetale 6:177-179.
8. Diachun, S., & W. D. Valleau. 1946. Growth and overwintering of *Xanthomonas vesicatoria* in association with wheat roots. Phytopathology 36:277-280.
9. Grogan, R. G., & J. B. Kendrick. 1953. Seed transmission, mode of overwintering and spread of bacterial canker of tomato caused by *Corynebacterium michiganense*. Phytopathology 43:473 (Abstr.).
10. Loest, F. C. 1949. Bacterial canker of the tomato. Farming in South Africa 24:393-394 & 412.
11. Patino-Méndez, G. 1964. Studies on the pathogenicity of *Corynebacterium michiganense* (E. F. Sm.) Jensen and its transmission into tomato seed. Ph.D. Thesis, Univ. of Calif., Davis. 57 p.
12. Roizin, M. B. 1964. A thread method for assaying microbial survival in soil. Microbiology 33:950-952.
13. Strider, D. L. 1967. Survival studies with the tomato bacterial canker organism. Phytopathology 57:1067-1071.
14. US Salinity Laboratory Staff. 1954. Diagnosis and improvement of saline and alkali soils, p. 109. L. A. Richards [ed.] USDA Agriculture Handbook No. 60.