SOUTHERN BACTERIAL WILT OF FIELD TOMATOES IN SOUTHWESTERN ONTARIO

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Abstract

Pseudomonas solanacearum E. F. Sm., the causal agent of southern bacterial wilt of tomato, was found in Kent County in six fields of processing tomatoes set with transplants imported from Georgia, USA. Infection ranged from 1 to 4%, and only limited secondary spread occurred in two of the six fields. This is the first report of bacterial wilt of tomato in Canada that could be traced to transplants of known origin and for which the identity of the pathogen was fully established. The overwintering potential of the pathogen in southwestern Ontario soils is being investigated. Until the possibility of overwintering is established, several precautionary measures have been recommended.

Southern bacterial wilt, caused by Pseudomonas solanacearum E. F. Sm., is an important disease of tomato, potato, tobacco, eggplant, pepper and peanut in the southern United States (2). The disease seldom occurs north of Maryland and Virginia or west of the Appalachian Mountains. Vaughan (11) proved experimentally that the causal bacterium overwintered in the soil as far north as central New Jersey. The disease has occurred on field tomatoes in the North Central States, where it was introduced from southern-grown transplants, but losses have been small and overwintering has not been established (10).

The only previous report of southern bacterial wilt in Canada appeared in 1949 (3). The causal bacterium was isolated in Ontario by Prof. E. H.. Gerrard from a field tomato plant of unknown origin that had symptoms of "brown rot." The diseased plant was found near Kitchener, Ontario. Identification was based on cultural characters of the bacteria and on disease symptoms **(4).** No confirmatory pathogenicity tests were made with the bacterial isolate.

In 1967, about three to four weeks after several tomato fields were set with imported transplants from Georgia, up to 4% of the plants in some fields showed symptoms sufficiently similar to those described for southern bacterial wilt (2, 5, 6) to suggest that this may have been the disease involved. Diseased plants were collected from several locations for isolation and identification of the pathogen **(s).** Several surveys of tomato fields in southwestern Ontario were made throughout the growing season to determine the incidence and extent of wilt, varieties affected, origin of transplants, and evidence of secondary spread.

Materials and Methods

Small pieces of internally discolored tissue from the xylem and pith were mounted in drops of sterile water and examined by phase-contrast microscopy (400X)

Crude sap expressed from systemically infected plants was strained through cheesecloth and applied to upper leaf surfaces by gently rubbing leaves from base to tip with pads of Cheesecloth soaked in inocula, as described by Layne ***(8)*** for inoculation with Corynebacterium michiganense (E.F. Sm.) Jensen.

Pieces of stem, root and petiole from diseased plants were surface-sterilized by immersion in 70% ethyl alcohol for **5-10** seconds and flamed to burn off surplus alcohol. These sections were split longitudinally and small pieces of discolored tissue were removed aseptically and placed in screw cap tubes containing 3 ml of sterile water The tubes with contents were thoroughly agitated with a Vortex Jr. Mixer. A loopful of the suspension was streaked on King's B media (7) in petri plates and incubated for several days at room temperature, about 25" C Observations of the resulting bacterial colonies for presence of pigment and fluorescence in ultraviolet light were made 3-5 days later. UV fluorescence was determined with a long-wave UV lamp. All bacterial isolates were transferred to slopes of mannitol agar and potato-dextrose-peptone agar (PDPA) and examined again for color, diffusible pigment and UV fluorescence. Transfers were also made to sterile steamed potato plugs, and the color of the bacterial growth and the change in color of the potato plugs were noted after incubation for **3-5** days.

Pure cultures of the bacterial isolates obtained were tested for pathogenicity on tomato 'Michigan-Ohio' and 'Ohio W-R 25', and on potato 'Irish Cobbler'. The tomato plants were at least **30** to 60 days old, were individually potted in a mixture of peat and sand, and were fertilized on a weekly schedule. Three methods were used to inoculate tomato plants:

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Leaf rub. Cheesecloth soaked in turbid suspensions of the bacterial isolates in steriIe water was rubbed over the leaf surface.

Stem inoculations. Tips of sterile needles were coated with bacteria from petri dishes and stems stabbed about midway between their bases and shoot apices.

Root inoculations. Turbid suspensions of the bacteria were poured into trenches made with sterile scalpels near the tap roots, thus ensuring that the secondary roots were severed.

Potato plants were stem-inoculated about 10 cm from the shoot apex. All plants were maintained in the greenhouse at 25-30°C. Plastic canopies were used to provide additional humidification for the leaf rub inoculations.

Cultural and pathogenic characteristics of nine isolates of P. solanacearum were compared with the three common bacterial pathogens of tomato: P. solanacearum (E. F. Smith) Altstatt, X. vesicatoria (Doidge) Dows., and Corynebacterium michiganense.

Fields of processing tomatoes known to be infected with the southern wilt pathogen were surveyed in June, July, August and September, 1967, to observe incidence of wilted plants and evidence of secondary spread. Soil samples were collected at the base of diseased plants on October 20 for subsequent studies of the overwintering potential of the pathogen.

Results

Symptoms—Naturally dtseased plants were stunted and wilted, and extensive breakdown of the pith occurred in the lower part of the stem and tap root (Fig. 1:A, C, D). In the stem, reddish-brown vascular discoloration and water-soaking of the pith extended well above and below the regions where internal breakdown was severe. Adventitious root development was extensive on stems of wilted plants. Spots were not present on the leaves, stems or fruit of wilted plants, but dark-brown to black streaks on the stems and petioles were sometimes observed (Fig 1:B).

Microscopy—Examination of water mounts of discolored stem tissues from affected plants revealed the presence of large numbers of highly motile, rod-shaped bacteria, occurring singly or in pairs.

Preliminary bioassay test—Three to five days after inoculation, small irregular lesions with tan centers and dark brown margins were observed and they were distinguishable from those associated with other bacterial pathogens of tomato (1, 6). The disease became systemic seven days after inoculation as indicated by wilting of leaflets and the appearance of dark-brown to black streaks on the petiole (Fig. 1:B). Wilting progressed until all leaves were affected.

Cultural characters in vitro—In the nine pathogenic isolates obtained from systemically infected tomato plants most cultural characters were similar. On PDPA media, they were at first white, but they became brown with age and produced a brown diffusible pigment that did not fluoresce in UV light. No diffusible pigment was produced on mannitol, but some isolates produced the brown pigment on King's B. No UV fluorescence was obtained with any of the isolates on King's B or mannitol. Bacterial growth on potato plugs was at first white but soon became brown. The potato plugs changed from white to gray to grayish brown after several days. Such characters have been described for P. solanacearum (5, 6).

Several important differences in color, pigment production, UV fluorescence, and other characters distinguished our isolates of P. solanacearum from other bacterial pathogens of tomato. P. tomato produced a diffusible green pigment on King's B and mannitol. The pigment gave a strong bluish green fluorescence in UV light. Neither X. vesicatoria nor C. michiganense produced a diffusible fluorescent pigment on any of the media tested. X. vesicatoria was yellow and distinctly mucoid on all media, whereas C. michiganense was cream on PDPA and mustard yellow on mannitol. P. tomato was gray to white on all media but P. solanacearum was white on mannitol and white to brown on King's B and PDPA. On steamed potato plugs, the color of bacterial growth and the color changes of the plug differed with each of the four bacterial pathogens. P. solanacearum isolates were brown and changed the color of the plugs from white to brown. P. tomato was white or gray and changed the plug color from white to gray. X. vesicatoria was yellow and mucoid, and changed the plug color from white to brown. C. michiganense was mustard yellow and changed the plug color from white to gray. C. michiganense was also gram-positive, whereas the other three pathogens were gram-negative. Other workers have obtained similar results with similar media and stains (5, 6, 9).

Pathogenicity tests—The pathogenicity tests on tomato were sufficiently definitive to distinguish our isolates of P. solanacearum from the other three bacterial pathogens of tomato. P. solanacearum and C. michiganense produced local as well as systemic symptoms on tomato, but their symptoms were distinctly different (2, 6, 8, 9). P. tomato and X. vesicatoria each produced distinctive local symptoms but no systemic symptoms (1, 5, 6).

Our isolates of P. solanacearum produced brown water-soaked streaks, sometimes with brown

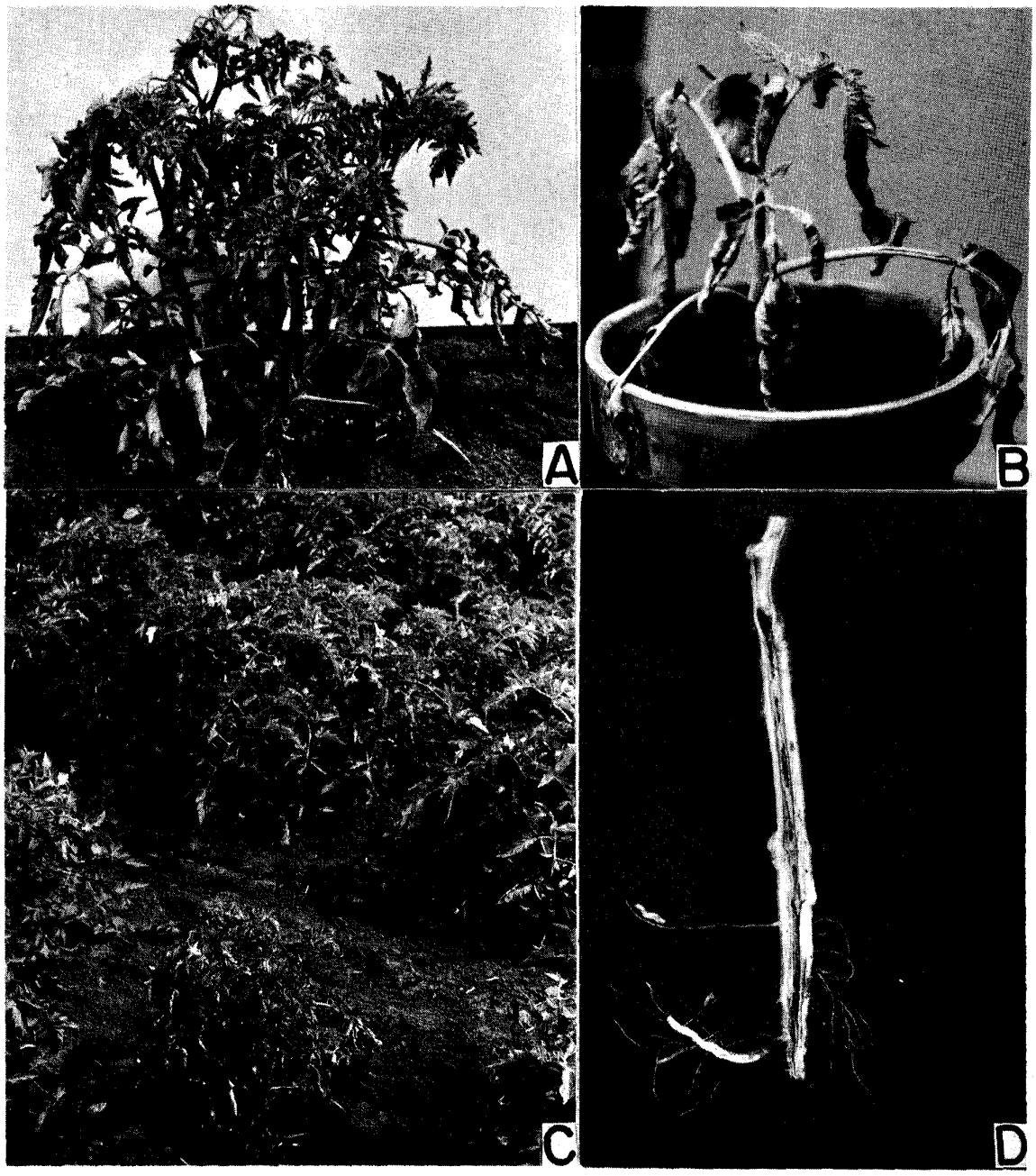

Figure 1. Tomato plants infected with Pseudomonos solanaceorum. A and C) Naturally infected plants at mid-season. Note severe stunting and wilt compared with healthy neighboring plants, B) Typical symptoms obtained from stem or root inoculation. Note severe wilting of leaves and black streaks on petiole and leaflet on 2nd lowest leaf. Photographed 11 days after inoculation. D) Longitudinal section of the lower portion of a stem from a naturally infected plant showing vascular discoloration and typical internal breakdown with cavities in the pith.

bacterial ooze, on inoculated stems of potato. Epinasty and wilt of the upper leaves occurred in plants inoculated with the most virulent isolates. The three other bacterial pathogens of tomato were nonpathogenic on potato.

Confirmatory identification—Our limited cultural and pathogenicity tests showed that all nine of our isolates were P. solanacearum. Dr. Arthur Kelman, Department of Plant Pathology, University of Wisconsin, confirmed our identification after he examined four of our isolates.

Survey of tomato fields in Kent County—On six Kent County farms where southern bacterial wilt was present, transplants imported from Georgia were being grown (Table 1). Infection ranged from 1 to 4%, and there was evidence of secondary spread within the row on *two* farms. Very few plants adjacent to diseased ones became infected later in the season at one farm. At another farm, where secondary spread was considered to be moderate, the disease was mainly confined to a low area, which was flooded several times in June and July. Vaughan (11), too, found that the disease spread more readily in wet, low-lying areas. The diseased plants were removed in July and no further evidence of spread was observed in August and September. Losses caused by the disease were quite small on all farms.

Discussion

With only a few cultural and pathogenicity tests we were able to identify P. solanacearum and to distinguish it from P. tomato, X. vesicatoria and C. michiganense. The tests were easy to perform and gave positive results in 2-10 days.

The sudden collapse of the foliage and the extensive decay of the pith were the symptoms that distinguished southern bacterial wilt from fusarium or verticillium wilts. In addition, it has been shown that the exudate obtained by squeezing the base of diseased tomato stems infected with P. solanacearum is not obtained from plants infected with the fungus-induced wilts (10).

During the past five years, about 80% of the field tomato plants grown in Essex and Kent counties in southwestern Ontario have been imported transplants from Georgia. The disease is of common occurrence in Georgia (2). It has probably been introduced into Ontario on infected transplants in previous years but it was only detected once (4). In 1967, exceptionally warm, wet weather prevailed during June, when the transplants were quite small. Rapid disease development is common under these conditions (2, 6, 11). Conspicuous stunting and wilt occurred during this period, so that by July diseased plants were easily recognized (Fig. 1:A, C).

Table 1. Occurrence of southern bacterial wilt in processing tomatoes imported as transplants from Georgia and grown in fields in Kent County, Ontario, 1967

Location of fields	Infection (%)	Varieties affected	Secondary spread
Lot 2, Conc. 4, Tilbury E.*	4	Campbell 17 and 19	trace
Lot 3, Conc. 3, Tilbury E.	2	Campbell 17 and 19	none
Lot 15, Conc. 4, Dover+	2	Campbell 19 and 24	moderate
Lot 9, 10, Conc. 2, 3, Chatham	1	Campbell 24	none
Lot 4, Conc. 1, Howard	1	Campbell 17 19, 24, 135	none
Lot 14, Conc. 8, Raleigh	1	Campbell 19	none

* P. solanacearum was isolated and identified as the pathogen causing the wilt and internal breakdown symptoms on plants sampled.

Later in the season, diseased plants were not readily detectable because they were overgrown by healthy plants.

Southern bacterial wilt is repeatedly introduced into the northern United States from southern-grown transplants but it has failed to become established and has caused only minor damage to the field tomato crop (10). It has overwintered in field soils as far north as New Jersey (11), but overwintering in Ohio, Michigan, Illinois or Wisconsin has not been demonstrated. We are presently investigating the overwintering potential of the pathogen in southwestern Ontario.

Until proof or disproof of overwintering in southwestern Ontario is obtained, certain precautions should be followed with subsequent crops. No solanaceous crop, especially potatoes, peppers, eggplants, or tobacco, should be grown in tomato fields that had plants infected with southern bacterial wilt the previous season. Diseased plants should be removed and burned when they are first observed. Replants should not be made where diseased plants were located.

Acknowledgments

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