Plant Pathol. J. 17(4) : 222-226 (2001)

The Plant Pathology Journal

Identification of *Verticillium dahliae* and *V. albo-atrum* Causing Wilt of Tomato in Korea

Jong-Tae Kim*, In-Hee Park[1], Hyang-Burm Lee[2], Young-Il Hahm and Seung-Hun Yu[3]

National Alpine Agriculture Experiment Station, Rural Development Administration, Pyungchang 232-950, Korea
[1]Puyeo Tomato Experiment Station, Chungnam Agricultural Research Institute, Puyeo 414-810, Korea
[2]Genetic Resources Center, Korea Research Institute of Bioscience and Biotechnology, Daejon 305-600, Korea
[3]Department of Agricultural Biology, Chungnam National University, Daejon 305-764, Korea

(Received on May 3, 2001)

In 1997, 103 isolates of *Verticillium* were obtained from roots and stems of tomato plants showing wilt symptoms in greenhouses in eight areas of Korea. Out of these isolates, 75 were identified as *V. dahliae* while 28 were identified as *V. albo-atrum* based on their morphological and cultural characteristics. Both *Verticillium* species produced colonies with conidiophores, which were more or less erect, hyaline, with verticillate branches, and with 3-4 phialides at each node. *V. dahliae* produced microsclerotia, while *V. albo-atrum* produced resting dark mycelium. Optimum temperatures for mycelial growth of *V. dahliae* and *V. albo-atrum* on PDA were 22 and 26°C, respectively. Mycelial growth of *V. albo-atrum* was slower than that of *V. dahliae*. Pathogenicity tests revealed that tomato cvs. Zuikoh No. 102, Kyoryokubeiju No. 2, Zuiken, Kagimuza, and Momotaro were susceptible to *V. albo-atrum*, while cvs. Zuikoh No. 102 and Kyoryokubeiju No. 2 were susceptible to *V. dahliae*.

Keywords : pathogenicity, tomato wilt, *Verticillium dahliae*, *V. albo-atrum*.

Verticillium wilt of tomato (*Lycopersicon esculentum* Mill.) is a vascular wilt disease caused by *Verticillium dahliae* Kleb. and *V. albo-atrum* Reinke Berth (Bewley, 1922). Both pathogens infect many plant species, including trees, vegetables, field crops, ornamentals, and weeds (McCain et al., 1979). Verticillium wilt is a major limiting factor in tomato production in several areas in the United States (Alexander, 1962; Berkeley et al., 1931; Guthrie, 1960; Katazawa and Suzui, 1980). Yield losses in tomato cultivars susceptible to *Verticillium* spp. can reach as high as 30-70% (Ashworth et al., 1979; Hawksworth and Talboys, 1970). Symptoms of Verticillium wilt are almost identical to those of Fusarium wilt in tomato. The two diseases cannot be distinguished from each other in laboratory examination. In many hosts, however, *Verticillium* induces wilt at lower temperatures than *Fusarium*. The symptoms caused by *Verticillium* develop more slowly, and often appear only on the lower or outer parts of plants. In some hosts, Verticillium wilt develops primarily in seedlings, which usually die shortly after infection. Late infection of the disease causes epinasty of the upper leaves, followed by irregular chlorotic patches that become necrotic. Infected older plants are usually stunted in various degrees, and their vascular tissues show characteristic discoloration (Pegg, 1974).

Effective control of Verticillium wilt has been obtained by planting resistant cultivars (Jones and Crill, 1975). In 1951, Schaible et al. reported a high level of resistance to Verticillium wilt in small-fruited, wild Peruvian cherry tomato (*L. esculentum* var. *cerasiforme* Gray "Peru Wild"). This resistance conferred by a single dominant gene (*Ve*) has been incorporated into many current tomato cultivars. In 1957, an isolate of *V. dahliae* from tomato in California and an isolate of *V. albo-atrum* from potato (*Solanum tuberosum* L.) in Canada were reported to be pathogenic on "Loran Blood," a tomato cultivar with the *Ve* gene (Robinson et al., 1957). Since then, similar isolates of this new race (race 2) have been discovered in several countries (Alexander, 1962; Grogan et al., 1979).

The objectives of this study were to: 1) survey the distribution of *Verticillium* spp. at major tomato-growing areas of Korea; 2) determine the differences between *V. dahliae* and *V. albo-atrum* through morphological and cultural characteristics of the pathogens; and 3) determine the pathogenicity of these species to different tomato cultivars.

Materials and Methods

Isolation of soil-borne pathogens. In 1997, roots and stems of tomato were collected from wilted tomatoes in greenhouses in eight areas of Korea. A total of 103 *Verticillium* isolates were obtained. Stems and roots from diseased plants were washed with tap water. After removal of the outer stem cortex, small pieces of

*Corresponding author.
Phone) +82-33-330-7842, FAX) +82-33-330-7725
E-mail) teskjt@hanmail.net

vascular tissues were surface sterilized in 0.5% NaOCl for 30-60 seconds, then placed in petri plates containing 2% water agar or acidified potato-dextrose agar (APDA) containing 2 ml of 25% lactic acid per liter. The plates were incubated at 22°C for 5-7 days. The isolates were identified based on published descriptions (Smith, 1965; Hawksworth and Talboys, 1970) of morphological and cultural characteristics like resting mycelium, conidiophores, conidia and colony morphology, etc.

Effect of temperature on mycelial growth of pathogens. Petri dishes (8.7 cm diameter) containing 20 ml of PDA were inoculated centrally with 5 mm mycelial plugs taken from the periphery of young cultures of isolates TV-07 (*V. dahliae*), TV-19 (*V. albo-atrum*) with three replicates. The plates were incubated at 18, 22, 24, 26, 30, and 32°C for 2 weeks in the dark, and colony diameters were measured.

Pathogenicity test. Two isolates each of *V. dahliae* (TV-07, TV-11) and *V. albo-atrum* (TV-19, TV-29) were tested for their pathogenicity to tomato seedlings. All cultures were grown on potato dextrose agar (PDA) at 22°C prior to inoculation. Spore suspensions were prepared from 3-week-old cultures by adding 10 ml of sterile distilled water to each plate and scraping the cultures with a rubber spatula. Using a haemocytometer, the inoculum concentration was adjusted to 10^7 conidia per milliliter. Tomato seedlings of the susceptible (Zuikoh No. 102 and Kyoryokubeiju No.2) and resistant (Zuiken, Kagimuza and Momotaro) cultivars to *Verticillium*, produced by Japan companies, were inoculated at the 3rd-4th true leaf stage. These tomato seedlings were uprooted and inoculated by using the root-dip technique. Roots were washed with running water, and placed for 60 minutes in conidia suspension. The inoculated seedlings were transplanted to pots containing a mixture of peatmoss, vermiculate, and perlite (1:1:1, v/v/v). Three replications of five plants for each isolate were used. The plants were kept in a room bench at 21-23°C. Daylight was supplemented by fluorescent lights to provide a 12 hr day length. About 4 weeks after inoculation, disease incidence was determined according to disease index: 0 = health; 1 = slight vascular discoloration; 2 = slight wilting; 3 = severe wilting and death.

Results

Isolation rate of soil-borne fungi from wilted tomato roots. Samples of diseased stems and roots were collected from eight areas in Korea. From these samples, four genera of soil-borne fungi namely, *Fusarium*, *Verticillium*, *Colletotrichum*, and *Pyrenochaeta*, were isolated at average isolated rates of 80.5%, 11.3%, 2.0%, and 0.1%, respectively. In particular, average isolation rates for *V. dahliae* and *V. albo-atrum* were 9.7% and 1.6%, respectively. Out of 103 isolates, 75 were *V. dahliae* while 28 were *V. albo-atrum* (Table 1).

Identification of the pathogen. Taxonomic differences between *V. dahliae* and *V. albo-atrum* were examined based on the size of conidia, formation of resting structures, and color of colonies. In case of *V. dahliae* on host materials, conidiophores were verticillately branched, hyaline, usually shorter (80-160 μm) than *V. albo-atrum*, and had one to five (usually three to four) phialides per whorl. Size of conidia was 2.5-8.8 × 2.0-3.0 μm. Infected tissues or artificial medium produced microsclerotium after 2 weeks of cultivation (Fig. 1B, D, and F). The color of colonies mainly changed from white to black on PDA media, but some isolates of the pathogen changed to yellow or partial yellow (Fig. 1E). On host materials, conidiophores of *V. albo-atrum* were verticillately branched, hyaline, and usually tighter (Fig. 1G). The size of conidia was 2.5-10.0 × 2.3-3.5 μm. Dark, thickened mycelia were produced as resting structures, but no microsclerotia were produced (Fig. 1I). Colonies were hyaline to white gray on PDA. These characteristics were compared with Hawksworth and Talboys description (Table 2). From these results, TV-11 and TV-29 were identified as *V. dahliae* and *V. albo-atrum*, respectively.

Table 1. Isolation frequency of soil-borne pathogens from wilted tomato plants in greenhouses of major producing areas in Korea

Area surveyed	*Fusarium oxysporum*	*Verticillium*		*Colletotrichum coccodes*	*Pyrenochaeta* sp.
		dahliae	*albo-atrum*		
Daegu Dalseong	86.7	32.8	0.0	7.8	1.1
Gyeongbuk Angang	75.6	5.1	0.0	0.0	0.0
Gwangju Gwangsan	67.9	3.8	0.0	0.9	0.0
Cheonnam Damyang	64.3	12.7	1.3	0.0	0.0
Cheonnam Boseong	78.0	2.5	2.5	0.0	0.0
Cheonbuk Iksan	80.7	12.7	3.8	0.0	0.0
Chungnam Buyeo	94.5	7.6	1.3	0.0	0.0
Chungbuk Cheongju	96.3	0.0	3.8	7.4	0.0
Average	80.5	9.7	1.6	2.0	0.1

Effect of temperature on mycelial growth. Optimum temperatures for mycelial growth of *V. dahliae* and *V. albo-atrum* on PDA were 22 and 26°C, respectively (Fig. 2).

Pathogenicity test. For the pathogenicity tests, 4-week-old tomato plants were inoculated with spore suspensions of the pathogens. After 4 weeks, inoculated plants exhibited stunting, chlorosis, and defoliation of lower leaves. In the optimal condition of disease development, white colonies appeared on the basal part of stems (Fig. 3A). *V. dahliae* and *V. albo-atrum* showed different pathogenicitiy to five tomato cultivars. Two isolates of *V. dahliae*, TV-07 and

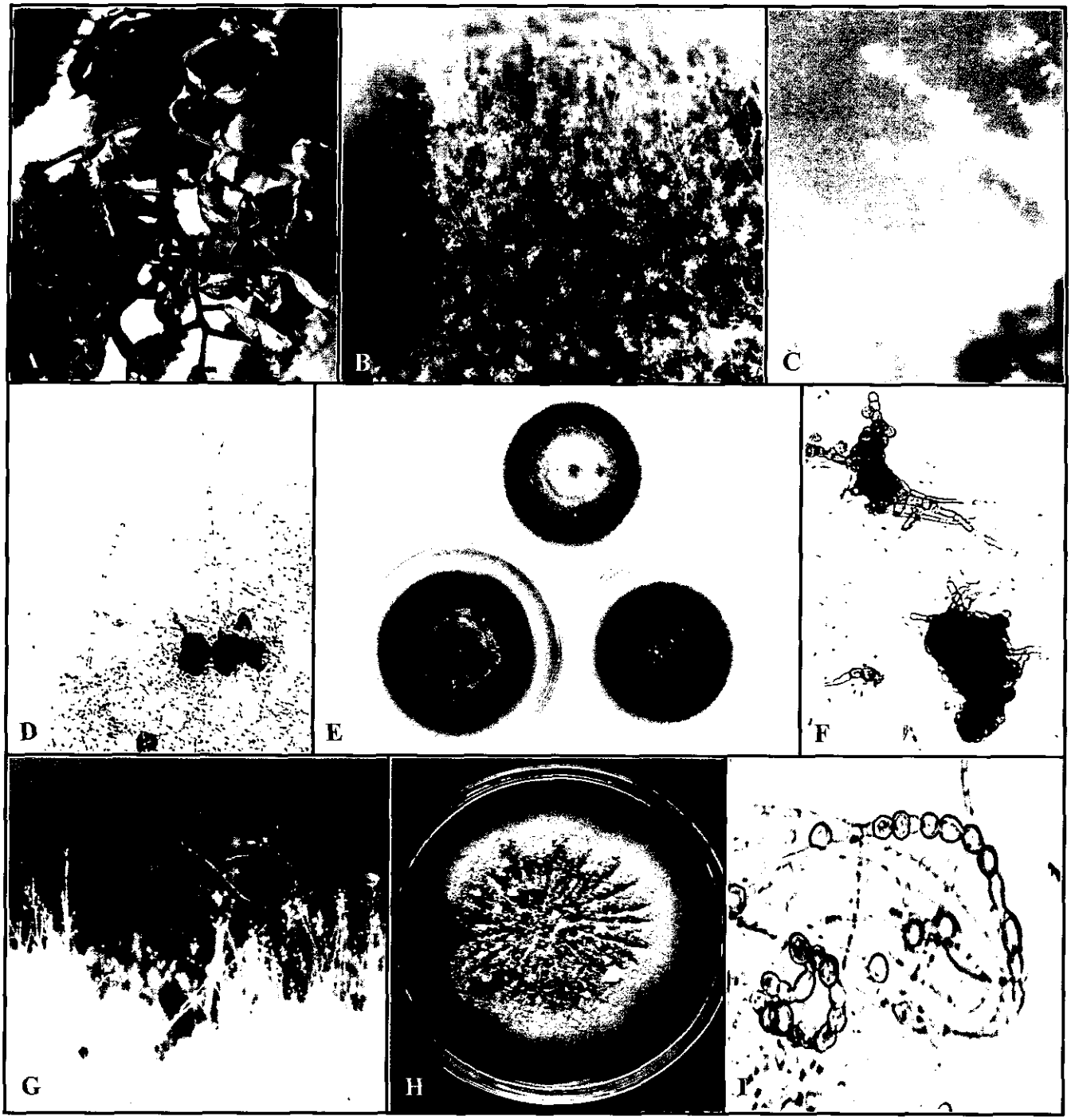

Fig. 1. Natural symptoms of tomato wilt in a greenhouse (**A**), morphological and cultural characteristics of *Verticillium dahliae* (**B-F**) and *V. albo-atrum* (**G-I**). (**B**) Conidiophores and microsclerotia on infected tomato tissue under stero microscope (40 x), (**C**) Magnified conidiophores as 80 x, (**D**) Magnified conidiophores and microsclerotia under compound microscope (200 x), (**E**) Colony types of *V. dahliae* on PDA, and (**F**) Microsclerotia on PDA medium 14 days after incubation at 22°C. (**G**) Conidiophores of *V. albo-atrum* on infected tissue, (**H**) Colony morphology on PDA medium, (**I**) Dark mycelum forming a "knot" in culture.

TV-11, were generally pathogenic to the susceptible cultivars (Zuikoh No. 102, Kyoryokubeiju No. 2). The disease severity ranged from 1.5 to 2.5 based on the 0-3 scale. Meanwhile, two isolates of *V. albo-atrum*, TV-19 and TV-29, were pathogenic not only to the susceptible cultivars but also to the resistant cultivars (Zuiken, Kagimuza, and Momotaro). The disease severity ranged from 0.5 to 3.0 based on the 0-3 scale (Table 3).

Table 2. Comparison of morphological and cultural characteristics between *Verticillium dahliae* and *V. albo-atrum*

Verticillium isolate	Color of colony	Resting structure	Size (μm)	
			Phialides	Conidia
TV-11 (*V. dahliae*)	Hyaline to black	Microsclerotium	17.5~35.0 × 1.0~2.5	2.5~8.8 × 2.0~3.0
TV-29 (*V. albo-atrum*)	Hyaline to white grey	Dark mycelium	17.5~27.5 × 1.0~2.5	2.5~10.0 × 2.3~3.5
V. dahliae[a]	Hyaline to black	Microsclerotium	16.0~35.0 × 1.0~2.5	2.5~8.0 × 1.4~3.2
V. albo-atrum[a]	Hyaline to white grey	Dark mycelium	14.0~26.0 × 1.0~2.5	3.5~10.5 × 2.0~4.0

[a]Hawksworth and Talboys (1970)

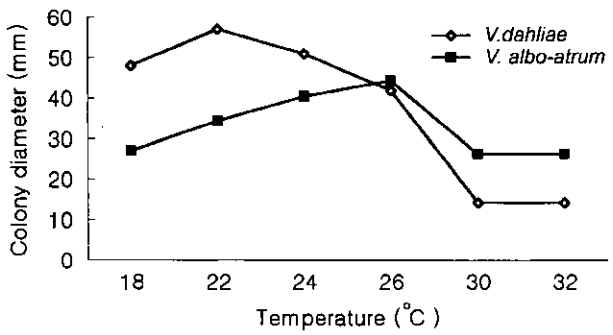

Fig. 2. Mycelium growth of *Verticillium dahliae* and *V. albo-atrum* on PDA media at different temperatures. Colony diameter was measured 2 weeks after inoculation.

Discussion

The occurrence of tomato caused by *Fusarium* and *Verticillium* was 81% and 11%, respectively. *Verticillium* wilt has not been investigated in Korea except for the first description of Park et al. (1995) on tomato wilt caused by *V. dahliae*. The occurrence of *V. albo-atrum* in Korea is first recorded in this study. Klebahn (1913) first described *V. dahliae* which formed black microsclerotia from budding of hyphae (see ref. Hawksworth and Talboys, 1970). The original description of *V. albo-atrum* was made by Reinke and Berthold (1879) from wilted potato plants in Germany.

Fig. 3. Verticillium wilt of tomato seedlings (cv. Kyoryokubeiju No. 2) caused by artificial inoculation with spore suspensions. Formation of white colonies on basement of a stem, 30 days after inoculation at 23°C (**A**). Tomato seedlings wilted by *V. dahliae* (**B**), and *V. alboatrum* (**C**), respectively.

Table 3. Pathogenicity of *Verticillium dahliae* and *V. albo-atrum* against five different tomato cultivars

Tomato cultivar	Disease index (0-3)[a]				
	V. dahliae		*V. albo-atrum*		Control
	TV-07	TV-11	TV-19	TV-29	
Zuikoh No. 102	2.0	1.5	1.3	3.0	0.3
Kyoryoku-beiju No.2	2.3	2.5	1.0	3.0	0.1
Zuiken	0.0	1.0	0.9	1.0	0.0
Kagimuza	0.0	0.0	1.8	0.5	0.0
Momotaro	0.5	0.0	2.0	2.0	0.1

[a]Disease index: 0 = health; 1 = slight vascular discoloration; 2 = slight wilting; 3 = severe wilting and death.

They described the mycellium of this fungus. Some differences between *V. dahliae* and *V. albo-atrum* have been clearly described by Isaac (1952), Van der Meer (1925), Berkeley et al. (1931), Ludbrook (1933), Williams (1946), and Isaac (1949). All of these authors have stressed the significance of the morphological differences of the resting mycelium, i.e., microsclerotia in *V. dahliae* and dark thickened hyphae in *V. albo-atrum*. Results of this study were consistent with these descriptions.

The differentiation of these two species can also be attributed to the differences in temperature requirements. The optimal temperatures for mycelial growth of *V. dahliae* and *V. albo-atrum* were 22 and 26°C, respectively. These results were consistent with Katazawa and Suzui (1980) and Smith's (1965) studies, where they reported that *V. albo-atrum* failed to grow at 30°C in culture, while *V. dahliae* showed some growth (Ludbrook, 1933).

In the pathogenicity reaction, three tomato cultivars (cvs. Zuiken, Kagimuza, and Momotaro) showed resistance to *V. dahliae* but were susceptible to *V. albo-atrum*. Guthrie (1960), who worked with both species, also recorded differences in resistance between the two pathogens. However, cvs. "Loran Blood" and "Gem" resistant to microsclerotial isolates of *V. dahliae* were also resistant to *V. albo-atrum* (Blackhurst and Wood, 1963).

References

Alexander, L. J. 1962. Susceptibility of certain *Verticillium*-resistant tomato varieties to an Ohio isolate of the pathogen. *Phytopathology* 52:998-1000.

Ashworth, L. J., Jr., Huisman, O. C., Harper, D. M., Stromberg, K. L. and Bassett, D. M. 1979. *Verticillium* wilt disease of cotton: influence of inoculum density in the field. *Phytopathology* 69:483-489.

Bewley, W. F. 1922. Sleepy disease of the tomato. *Ann. Appl. Biol.* 9:116-133.

Berkeley, G. H., Madden, G. O. and Willison, R. S. 1931. *Verticillium* wilts in Ontario. *Phytopathology* 11:739-759.

Blackhurst, F. M. and Wood, R. K. S. 1963. Resistance of tomato plants to *Verticillium albo-atrum*. *Trans. Brit. Mycol. Soc.* 46:385-392.

Guthrie, J. W. 1960. Early dying (Verticillium wilt) of potatoes in Idaho. *Idaho Agric. Exp. Sta. Res. Bull.* 45, 24 p.

Hawksworth, D. L. and Talboys, P. W. 1970. C.M.I descriptions of pathogenic fungi and bacteria. No. 255.*Verticillium albo-atrum*, No. 256. *V. dahliae*. CAB, Kew, England.

Isaac, I. 1949. A comparative study of pathogenic isolates of *Verticillium*. *Trans. Brit. Mycol. Soc.* 32:137-157.

Isaac, I. 1952. A further comparative study of pathogenic isolates of *Verticillium*: *V. nubilum* Pethybr., and *V. tricorpus* sp. nov. *Trans. Brit. Mycol. Soc.* 36:80-195.

Jones, J. P. and Crill, P. 1975. Reaction of resistant, tolerant and susceptible tomato varieties to Verticillium wilt. *Plant Dis. Rep.* 59:3-6.

Katazawa, K. and Suzui, T. 1980. *Verticillium* wilt disease of various crops caused by *Verticillium dahliae* Klebahn. *Ann. Phytopath. Soc. Japan* 46:267-270.

Ludbrook, W. L. 1933. Pathogenicity and environmental studies on *Verticillium* hadromyosis. *Phytopathology* 23:117-154.

McCain, A. H., Raabe, R. D. and Wilhelm, S. 1979. Plants resistant or susceptible to Verticillium wilt. *Univ. Calif. Coop. Ext. Leafl.* 2,703, 10 p.

Park, Y. S., Kim, J. S., Kwak, Y. H., Cho, M. R. and Kim. W. G. 1995. Tomato wilt caused by *Verticillium dahliae*. *RDA. J. Agri. Sci.* 37:303-307.

Pegg, G. F. 1974. Vertivillium disease. Rev. Plant Pathol. 53:157-182.

Robinson, D. B., Larson, R. H. and Walker, J. C. 1957. Verticillium wilt of potato in relation to symptoms, epidemiology and variability of the pathogen. *Wis. Agric. Exp. Stn. Res. Bull.* 202, 49 p.

Schaible, L., Cannon, O. S. and Waddoups, V. 1951. Inheritance of resistance to Verticillium wilt in a tomato cross. *Phytopathology* 41:986-990.

Smith, H. C. 1965. The morphology of *Verticillium albo-atrum*, *V. dahliae*, and *V. tricorpus*. *New Zealand J. Agric. Res.* 8:450-478.

Van der Meer, J. H. H. 1925. *Verticillium* wilt of herbaceous and woody plants. *Meded. Landbouwhogeschool Wageningen* 28:1-82.

Williams, D. H. 1946. *Verticillium* wilt. *Rep Exp. Res. Sta. Cheshunt.* 1945, pp. 25-65.