Isolate Source and Daylight Intensity Effects on the Pathogenicity of *Verticillium dahliae* in Watermelon Seedlings

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ABSTRACT

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Watermelon (cv. Sugar Baby) seedlings, which were root-dip inoculated with *Verticillium dahliae* spore suspensions developed wilt symptoms usually starting with cotyledon wilt, continuing with leaf wilt, and ending in seedling death. These symptoms were observed in greenhouses with low daylight intensities of 60–320 μE m^{-2} · sec^{-1} and temperature of 19–30 C. More plants developed symptoms at the lower daylight intensities of 100 or 180 than at 320 μE m^{-2} · sec^{-1} at temperatures of 22 ± 2 C. As inoculum concentration increased the percentage of seedlings with disease symptoms also increased with both root-dipping and hypocotyl spore-injection techniques. Disease developed more quickly after root-dip than after hypocotyl inoculation. The influence of growth by the pathogen on three media and for different incubation times on pathogenicity to watermelon seedlings was compared. No differences were found for the three media after 4 days of incubation. Spores obtained after 14 and 21 days growth on yeast extract agar clearly had lost pathogenicity to watermelon seedlings. The longer incubation periods on potato dextrose agar or synthetic agar did not significantly influence pathogenicity, as compared with that of spores collected after 4 days. Loss in pathogenicity was due to virtually complete loss of spore viability. In pathogenicity tests, 15 isolates from seven hosts (cotton, melon, avocado, watermelon, peanut, potato, and tomato) caused typical disease symptoms in watermelon seedlings. One isolate from olive was nonpathogenic to watermelon.

Verticillium dahliae Kleb. is a prevalent soil fungus of Israel which causes serious diseases in fruit trees and many vegetable crops, including watermelon. To decrease the population of microsclerotia in soil, fungicide treatment with metham was found efficient (3). However, because this treatment is expensive, development of a watermelon cultivar tolerant of the fungus would be desirable.

Among several techniques of seedling inoculation with *V. dahliae*, the simplest and most useful is the dipping of seedling roots in a spore suspension. However, injection of spores into the stem was found to be more accurate and disease symptoms developed faster compared with root-dip inoculation of sunflower seedlings (6,10).

Verticillium isolates from other hosts were found (11) to be nonpathogenic to peppermint, and *Verticillium* from peppermint was nonpathogenic to other plant species. Kendrick and Middleton (9) compared 12 isolates of *V. albo-atrum* derived from 12 hosts and concluded that only the one from pepper caused severe stunting of pepper, two others induced mild stunting, and all the others were nonpathogenic. Other investigators (4,7) have reported that isolates from one host infected other hosts. Skotland (12) tested the pathogenicity (as expressed by disease symptoms) of four isolates of *V. dahliae* derived from three host species (mint, sweet cherry, and cantaloupe); eggplant and cantaloupe were sensitive to all isolates tested, but tomato and peppermint were sensitive to only one isolate each.

The effect of light duration on development of *Verticillium* and Fusarium wilt of tomato has been studied (2,8,14) and Jones et al (8) reported that photoperiod drastically affected the development of Verticillium wilt of tomato. However, the effect of daylight intensity on disease symptom development was not reported.

The purpose of this work was to determine the pathogenicity of several *V. dahliae* isolates and the effect of daylight intensities, growth medium, spore age and concentration, and inoculation procedure on the development of Verticillium wilt of watermelon seedlings.

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MATERIALS AND METHODS

***Verticillium dahliae* isolates.** Sixteen isolates of the fungus were used in this study, some of which were supplied by I. Dishon, R. Cohen, and A. Karib of the Division of Plant Pathology, ARO, The Volcani Center. Six tomato race 1 isolates, two isolates each from watermelon, potatoes, and avocado, and one isolate each from cotton, olive, melon, and peanut, were checked for pathogenicity to watermelon seedlings. An isolate ("A") from tomato was used for most of this work; the other isolates were used only to compare their pathogenicity to watermelon seedlings.

Yeast extract agar (YEA) (5) was used as growth medium for the fungus and 20 ml was poured into each petri dish. Incubation temperature and growth period were 20 C and 4 days, respectively.

Inoculum preparation. Spores and hyphal fragments of *V. dahliae* grown on slant cultures were removed with a bacteriological needle and seeded in a petri dish with potato dextrose agar (PDA) medium. After 4 days of incubation, the fungus was scraped from the agar layer, comminuted in a tissue homogenizer, and 0.1 ml of homogenate was spread on each petri dish. After the requisite incubation period, the agar layer in each petri dish was transferred into a 250-ml Erlenmeyer flask with 50 ml of distilled water. The Erlenmeyer flasks then were thoroughly shaken by hand for 20 sec and the spore suspensions were filtered through a coarse filter (100 μm) to separate agar particles. The spore suspensions were centrifuged at 10,000 *g* for 15 min. The supernatants were decanted, spore pellets were resuspended in sterile distilled water, and the number of spores was determined with a haemacytometer. To determine spore viability for each experiment, three replicates of 150 spores each were plated on PDA in petri dishes; after three days incubation, *V. dahliae* colonies were counted.

Seedling preparation and inoculation. Seeds of watermelon, cv. 'Sugar Baby', were seeded in a mixture of sand and peat (3:1, v/v) and 3–5 days after emergence the seedlings were removed and washed free of soil particles. Then, the water on the roots was adsorbed with filter paper, the roots were cut, and the seedlings were dipped into the various spore suspensions and immediately planted. Seven- to 9-day-old seedlings also were inoculated by injecting spores into the hypocotyl with a 10-μl syringe (Hamilton Reno Company, Nevada). After inoculation the watermelon seed-

lings were planted (30–40 per 54 × 23 × 12 cm nursery flat) in a mixture of loam and peat (3:1, v/v). Then seedlings were grown in greenhouses.

Effects of inoculum concentrations was tested at the following spore concentrations: 3×10^1, 3×10^2, 3×10^3, 3×10^4, 3×10^5, 3×10^6, and 3×10^7 spores per milliliter for both root-dip inoculation and hypocotyl injection. The inoculated seedlings were grown in greenhouses with daylight intensity of 60 μE $m^{-2} \cdot sec^{-1}$ at 20 ± 1 C.

V. dahliae was grown on YEA (5), PDA, and modified synthetic agar (SA) (1), without pentachloronitrobenzene, ethyl alcohol, or antibiotics, and incubated for 4, 7, 14, and 21 days at 20 C. Watermelon seedlings were root-dipped in 3×10^5 spores per milliliter, planted, and incubated in a greenhouse with daylight intensity of 80 μE $m^{-2} \cdot sec^{-1}$ and temperature of 25 ± 1 C.

V. dahliae (isolate A) from tomato was maintained in three different ways: subcultured on PDA every 10 days for 1 yr (designated A-1); stored 1 yr at 5 C on PDA (designated A-2); and stored 10 mos at 5 C, root-dip inoculated into watermelon seedlings, and reisolated from a wilted seedling (designated A-3). Cultures A-1, A-2, and A-3 were seeded on YEA and spore suspensions of 3×10^5 spores per ml were prepared from each. Then, watermelon seedlings were root-dip inoculated, planted in a loam peat mixture (3:1, v/v), and grown in greenhouses at a daylight intensity at 140 μE $m^{-2} \cdot sec^{-1}$ and a temperature of 23 ± 2 C.

Daylight and temperature conditions in greenhouses. Daylight intensities were measured with a Lambda Quantum-meter Model L1-185 and were expressed in μE $m^{-2} \cdot sec^{-1}$. Light intensity in each greenhouse was measured on several days during the growth period, which usually lasted for 24 days after seedling inoculation. A slight difference in the intensity of light between various days was observed. However, on any one day the light intensity increased until 1200 hours, and decreased toward evening. The values of daylight in this work represent the maximum daylight, which was recorded at 1200 hours. Inside the greenhouses, light intensity was about 1/15, 1/30, and 1/40 that of outside direct radiation (2,300 μE $m^{-2} \cdot sec^{-1}$. To achieve different daylight intensities in one greenhouse, screens which eliminated 70 and 30% of the incoming sunlight were used. Temperatures in each greenhouse were measured daily with a minimum-maximum thermometer, and the values reported in this work represent the means during the growth periods. Day length varied from 9–14 hr according to the season of the year.

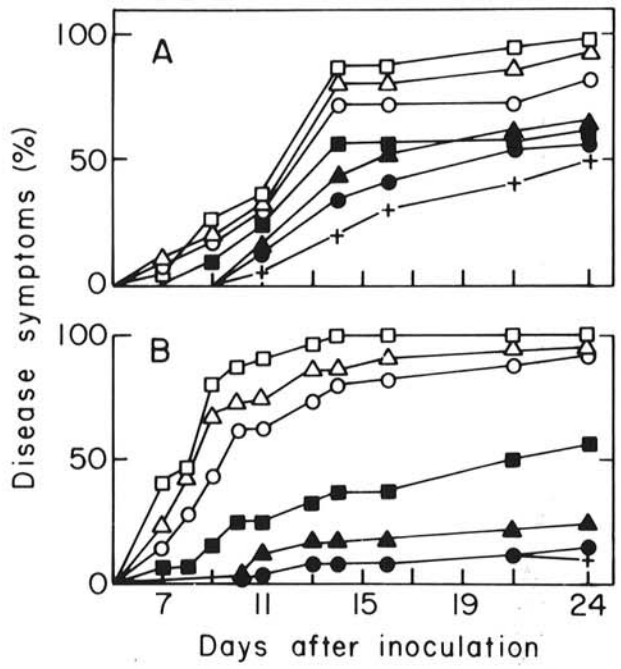

Fig. 1. Effect of inoculum concentration of *Verticillium dahliae* on the development of wilt symptoms on watermelon seedlings. **A**, Watermelon seedlings inoculated with spore suspensions injected into the hypocotyls. Spores per seedling: 3 (- + -), 10^1 (- ● -), 10^2 (- ▲ -), 10^3 (- ■ -), 10^4 (- ○ -), 10^5 (- △ -), and 10^6 (- □ -). **B**, Watermelon seedlings inoculated by root dipping in spore suspensions containing per milliliter: 3×10^1 (- + -), 3×10^2 (- ● -), 3×10^3 (- ▲ -), 3×10^4 (- ■ -), 3×10^5 (- ○ -), 3×10^6 (- △ -), and 3×10^7 (- □ -). The results represent the mean of three experiments, in each of which 40 seedlings were tested.

RESULTS

Effect of daylight intensities on the development of disease symptoms in watermelon seedlings. The following experiments were conducted to investigate whether *V. dahliae* could be present in a symptomless host. Roots of watermelon seedlings were dipped in a suspension of 3×10^7 spores per ml and the inoculated seedlings were divided in greenhouses with various combinations of light intensity and temperature. With light intensities of 140 μE $m^{-2} \cdot sec^{-1}$ and temperatures of 23 ± 2 C or 25 ± 5 C, disease was severe and resulted in 100% mortality of seedlings within 24 days after inoculation. Similar results were achieved in greenhouses with 80 and 60 μE $m^{-2} \cdot sec^{-1}$ light intensities and temperatures of 25 ± 1 C and 20 ± 1 C, respectively. With a light intensity of 280 μE $m^{-2} \cdot sec^{-1}$ and temperatures of 28 ± 5 C, disease symptoms consisted of yellow spots and wilt of lower leaves in 100% of inoculated seedlings. Stunting and mortality of inoculated seedlings were not observed. At the end of the experiment *V. dahliae* was reisolated from all seedlings which had been inoculated with the fungus.

To study more intensively effect of light on disease symptom appearance, watermelon seedlings were root-dip inoculated (3×10^5 spores per ml) and grown in a greenhouse at 22 ± 2 C. Daylight intensities were regulated within the greenhouse with screens to 100, 180, and 320 μE $m^{-2} \cdot sec^{-1}$. Disease symptoms appeared earlier and more plants wilted and died at 100 and 180 than at 320 μE $m^{-2} \cdot sec^{-1}$. Although symptom development was different, all treatments had the same percent plants colonized by the pathogen (Table 1).

Effect of inoculum concentration on the development of disease symptoms in watermelon following root dipping or hypocotyl injection. Seedlings inoculated either by root-dipping or by hypocotyl injection usually first developed wilt of the cotyledons, followed by leaf wilt and finally seedling mortality. Sometimes disease symptoms started with leaf wilt, followed by cotyledon wilt. Increasing inoculum concentrations with both methods of inoculation resulted in increasing percentage of disease symptoms 24 days after inoculation. Three hypocotyl-injected spores per seedling caused disease symptoms in 50% of plants whereas 3×10^4 spores per milliliter were required to cause the same effect with root-dip inoculation. However, the range of percent diseased seedlings was greater with root-dip inoculation (10–100%) than with hypocotyl injection (50–100%). Disease symptoms appeared faster with root-dip inoculation than with hypocotyl injection (Fig. 1). Seedlings which did not show disease symptoms 24 days after inoculation were cut, and two segments from the hypocotyl and stem of each seedling were

TABLE 1. Effect of daylight intensities on symptom development on watermelon seedlings root-dip inoculated with *Verticillium dahliae*

Daylight intensity (μE $m^{-2} \cdot sec^{-1}$)	Plants with symptoms(%)[a]		
	Wilted and dead	Dead	Hypocotyl-colonized plants[b]
100	73 L	49 l	74 N
180	74 L	48 l	74 N
320	57 M	34 m	72 N
Control[c]	0	0	0

[a]Mean of three replicates (36 seedlings tested per each replicate) rated after 24 days incubation. Growth temperature was 22 ± 2 C.

[b]Pathogen reisolated (%).

[c]Noninoculated seedlings. Values followed by a different letter, in each column, are significantly different, $\alpha = 0.01$, as determined by Duncan's multiple range test.

seeded on synthetic medium. Almost all symptomless seedlings that were root-dip inoculated were free of the fungus, whereas the fungus was isolated from symptomless plants that had been injected with spores.

Effect of growth medium and length of incubation on the pathogenicity of *V. dahliae* to watermelon seedlings. Spores collected after 4 days of incubation in any of the three media tested caused disease symptoms in 100% of the 40 watermelon seedlings tested, within 24 days after inoculation. Four- and 7-day incubation periods on PDA or SA resulted in spores slightly more pathogenic than those collected after 14 and 21 days. On YEA, spores from 7-day-old cultures caused disease symptoms only in 50% of seedlings, no visible symptoms or infections were caused by inoculation with spores from 14- and 21-day-old cultures. The experiment was repeated three times with the same results. Spore viability was only slightly affected by extended (14- and 21-day) incubation periods on PDA or SA. Identical conditions for spores on YEA resulted in a complete loss of spore germinability. Germinability of spores from 7-day-old cultures was only 50% of that for spores from 4-day-old cultures.

In YEA and PDA, maximal sporulation of 2×10^9 and 10^9, respectively, spores per petri dish was observed after 4 days of incubation. On SA, sporulation was about 3×10^6 spores per petri dish after 4 days of incubation and about 10^7 spores per petri dish after 21 days of incubation.

Effect of culture storage methods on the pathogenicity of *V. dahliae* to watermelon seedlings. Watermelon seedlings were inoculated with culture A-1, A-2, and A-3. Based on the mean of three experiments each of which comprised 40 seedlings, the disease symptom percentages 24 days after inoculation were 83, 80, and 70 for culture A-1, A-2, and A-3, respectively. No significant difference between the pathogenicity of the three cultures was detected (Duncan's multiple range test, $\alpha = 0.01$).

Pathogenicity to watermelon seedlings of *V. dahliae* isolates from various hosts. *Verticillium dahliae* isolates from various field crop and fruit tree hosts (tomato, watermelon, melon, potato, peanut, cotton, avocado, and olive) were seeded on YEA. Spore concentrations were adjusted to 3×10^5 spores per milliliter for each isolate and then watermelon seedlings were root-dipped and planted. Fifteen of 16 isolates caused disease symptoms in watermelon seedlings. However, significant differences were observed in the pathogenicity of the isolates from different hosts or within one host. The isolate from olive, although isolated from 3% of the inoculated watermelon seedlings, did not lead to visible disease symptoms (Table 2).

DISCUSSION

My results demonstrated that reduction of daylight intensities in greenhouses to 60–320 μE $m^{-2} \cdot sec^{-1}$ (at 20 ± 1 to 25 ± 5 C) enabled development of visible disease symptoms in watermelon seedlings after inoculation with *V. dahliae* spores. However, with an intensity of 320 μE $m^{-2} \cdot sec^{-1}$, significantly fewer plants wilted and died than with 100 or 180 μE $m^{-2} \cdot sec^{-1}$. Thus, it appears that light intensities below 320 μE $m^{-2} \cdot sec^{-1}$ are most likely to enhance appearance of disease symptoms. Although the same percentage of diseased plants was detected at temperatures of 25 ± 5 C or 23 ± 2 C in a greenhouse with light intensity of 140 μE $m^{-2} \cdot sec^{-1}$ further studies are required to determine the effect of different light intensity-temperature combinations on disease symptom expression. Daylight intensities of 60–140 μE $m^{-2} \cdot sec^{-1}$ also were found to be suitable for development of disease symptoms in tomato seedlings (Ben-Yephet, *unpublished*).

Hoes (6) and Moser and Sackston (10) compared stem injection with root dip inoculation in sunflower seedlings, and concluded that the former method was more accurate and disease symptoms developed faster than with root-dip inoculation. A positive correlation was observed between inoculum concentration and disease symptom development. I also found a positive correlation between inoculum concentration and disease symptom development. Also, hypocotyl-inoculated plants responded to lower inoculum concentration. However, symptom development was more rapid after root-dip inoculation than after hypocotyl injection.

Whereas incubation time of *V. dahliae* had a slight effect on the pathogenicity of spores to watermelon seedlings, growing the fungus inocula on different media did not affect its pathogenicity. Apparently loss of pathogenicity of the spores which grow on YEA was due to loss of viability.

Tolmsoff (13) demonstrated variation of *V. albo-atrum* (MS and DM types) on a culture medium. He also observed that pathogenicity of the variants to cotton seedlings was different from that of the parental wild types. In the present study the pathogenicity of *V. dahliae* isolate A was the same whether it was consecutively transferred or stored for 1 yr before passage through the host.

Skotland (12) tested the pathogenicity of four *V. dahliae* isolates from three different hosts to eggplant, tomato, peppermint, and cantaloupe seedlings. He found eggplant and cantaloupe to be sensitive to all isolates which were tested, and that tomato and peppermint each were sensitive to only one isolate. Kendrick and Middleton (9) tested the pathogenicity of 12 isolates of *V. albo-atrum* from 12 hosts to pepper. They observed that only the isolate from pepper caused severe stunting, that two other isolates induced mild stunting, and that all the others were nonpathogenic to pepper. In the present study, except for one isolate from olive, 15 isolates from seven hosts (watermelon, melon, cotton, potato, peanut, tomato, and avocado) were pathogenic to watermelon seedlings. Significant differences in pathogenicity to watermelon were found between *V. dahliae* isolates from single hosts including tomato, watermelon, potato, and avocado. In addition, there are indications that isolates from different hosts also differ significantly in pathogenicity, but further work is required to fully substantiate this point. Since all the isolates (except from olive) were found to be pathogenic to watermelon, watermelon grown after any of the above mentioned crops may become infected by the fungus in the soil.

TABLE 2. Pathogenicity of *Verticillium dahliae* from various hosts on watermelon seedlings[a]

Source of isolate	Seedlings with wilt symptoms (%)[b]					
	Isolate no.					
	1	2	3	4	5	6
Tomato	90 B	91 AB	94 A	89 B	74 CD	74 CD
Watermelon	90 B	80 C				
Potato	90 B	61 E				
Avocado	68 D	61 E				
Peanut	70 D					
Melon	53 F					
Cotton	53 F					
Olive	0					
Control	0					

[a]Watermelon seedlings were grown in greenhouses with daylight intensity of 80 μE $m^{-2} \cdot sec^{-1}$ and temperature of 25 ± 1 C. The results represent the mean of three experiments, in each of which 30 seedlings were tested. Disease ratings made after 24 days of incubation.

[b]Spore concentration for root-dip inoculation was 3×10^5 spores per ml. Values followed by different letters are significantly different, $\alpha = 0.01$, as determined by Duncan's multiple range test.

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