

Occurrence and Incidence of Verticillium Wilt in Chilean Avocado Groves

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ABSTRACT

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Verticillium wilt was found in several new avocado groves in Chile. The disease has been particularly severe on the cultivar Hass grafted on Mexicola avocado rootstocks and planted after susceptible crops, primarily tomato, cabbage, squash, cauliflower, artichoke, or chrysanthemum. It has rarely occurred in orchards established after nonsusceptible crops. Incidence of the disease in seven groves varied from 1.3 to 10.4% of affected trees, with an overall incidence of 2.9%. Symptoms were characterized by sudden collapse of the foliage, death of leaves, and development of dark brown streaks in the sapwood. Trees usually recovered after the initial collapse. The economic loss due to Verticillium wilt was estimated at \$942/yr in an 8-yr-old Hass orchard where 10.4% of the trees were infected.

Verticillium wilt of avocado (*Persea americana* Mill.) was first described by Zentmyer (5) in California and it has

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apparently been observed in Chile for several years (6). Severe outbreaks have occurred in new plantation areas in the Quillota Province during the past 4-5 yr primarily affecting Hass avocados grafted on Mexicola rootstocks.

Symptoms were similar to those found in California or Florida (2,5), consisting of a sudden wilt of the leaves and shoots in actively growing trees. Symptoms initially appeared on one side of the infected tree, but later the entire tree showed symptoms. Leaves turned brown

and remained attached to the tree for several weeks. Fruits borne on diseased branches never reached maturity but became desiccated, turned brown, and remained on the tree (Fig. 1A,B). The sapwood of infected scaffold branches or trunks showed dark brown necrotic streaks similar to the vascular symptoms that develop in other woody plants (4).

After the initial collapse, trees usually recovered and new shoots appeared from adventitious buds. This vigorous new growth occasionally became infected but more often regenerated the tree. A complete dieback killing the entire tree has seldom been observed under Chilean conditions. We report the occurrence and economic significance of Verticillium wilt of avocado. This is the first confirmed report of this disease affecting avocados in Chile.

MATERIALS AND METHODS

Isolation and pathogenicity. Isolations were made on potato-dextrose agar (PDA) from small fragments (0.5-1 cm long) of discolored sapwood tissue taken from surface-sterilized trunks or scaffold

branches. Colonies of a fungus identified as *Verticillium* sp. consistently grew from about half of the sapwood fragments within 10 days of incubation. Mycelium from the edges of the colonies was subcultured on PDA for 7 days at 25 C, then kept at room temperature (20–22 C).

Pathogenicity tests were performed with four isolates obtained from Hass trees and one isolate from an Edranol avocado tree. The inoculum was prepared from actively growing colonies 4–7 days old on PDA. The fungus growth and the underlying agar medium was removed and blended with sterile distilled water. The resulting suspension was adjusted to a final conidial concentration of $1-2 \times 10^7$ conidia per milliliter, using a hemacytometer. Pathogenicity was established: 1) on detached immature

fruits of cultivar Bacon. Fruits were surface-sterilized with 95% ethanol for about 1 min, then transversely sectioned and inner surfaces flooded with an inoculum suspension. Both halves were tied together with adhesive tape and incubated at 25 C for 8 days in a moist chamber. Three fruits were used per isolate and three fruits flooded with sterile distilled water were left as controls; 2) on 1-yr-old stems about 20 cm long taken from Hass avocado trees and surface-sterilized with 95% ethanol for about 1 min. Stems were sectioned transversely with a sterile scalpel and the inner surfaces flooded with the inoculum suspension. Stems were incubated for 28 days in a moist chamber at room temperature. Three stems were used per isolate and three other stems treated with

sterile distilled water were left as controls; and 3) on 1-yr-old potted Hass trees on Mexicola rootstock inoculated through the roots or on the rootstock trunk. Root inoculation was achieved by digging out the first 8 or 9 cm of soil from each pot.

Roots were injured with a sterile scalpel over which 50 ml/tree of inoculum suspension was poured. Trunk inoculation was performed by placing the inoculum suspension into small V-shaped cuts made on the rootstock trunk. Cuts were wrapped with polyethylene bands to avoid contamination and rapid dehydration. Thirteen trees were root-inoculated and 18 other trees were inoculated on the trunk (Table 1). Trees were held under greenhouse conditions for 1 yr. Five trees uninoculated but wounded and treated with sterile distilled water were left as

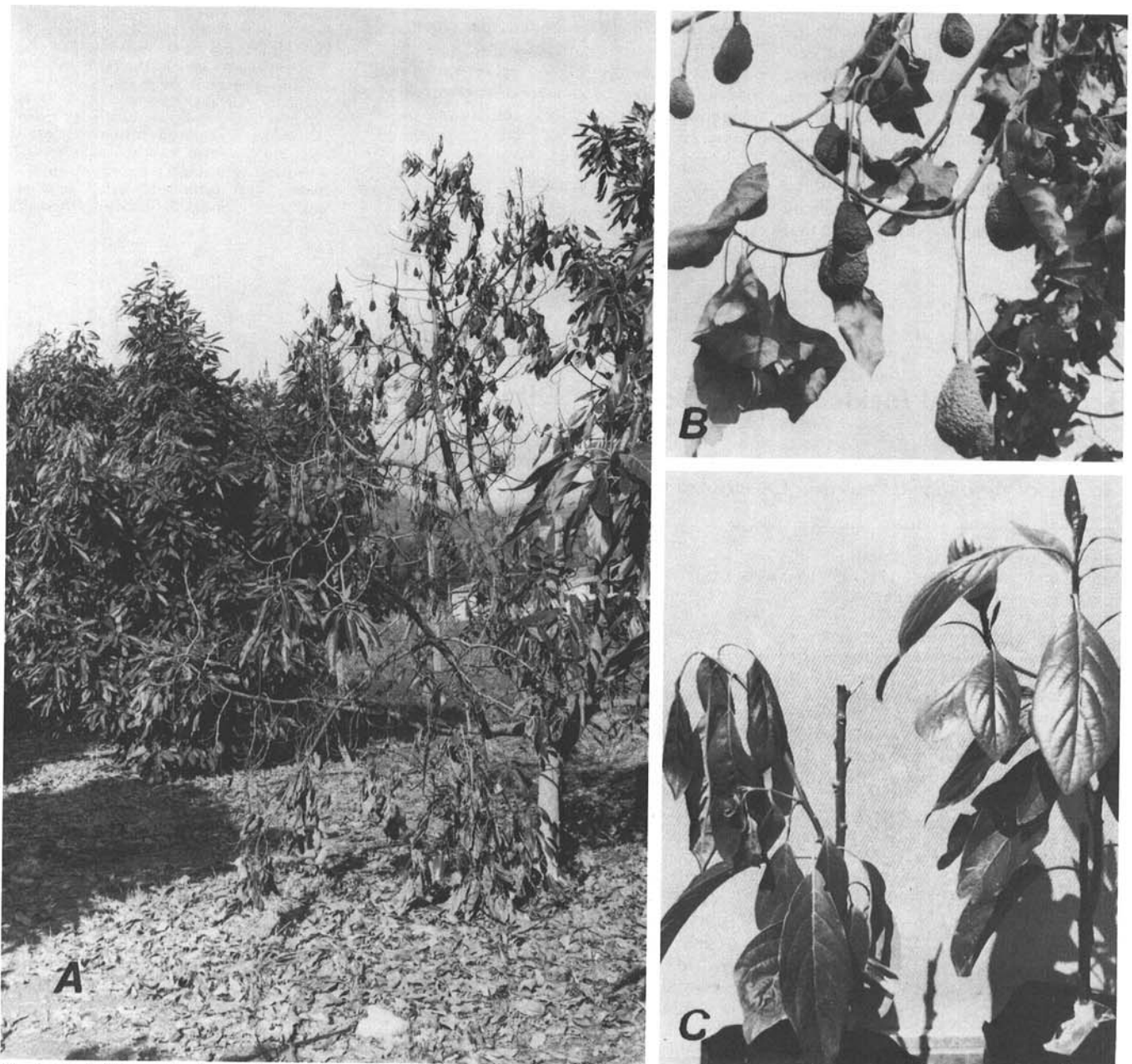


Fig. 1. *Verticillium* wilt of avocado. (A) Naturally infected Hass avocado in contrast with a healthy tree in the background. (B) Wilting, partial defoliation, and dryness of the fruits observed several weeks after the initial collapse. (C) Wilting of the leaves observed about 25 days after the inoculation on (left) 1-yr-old avocado and (right) healthy check.

Table 1. Pathogenicity of five isolates of *Verticillium dahliae* to avocado

Isolate	Origin	Inoculation results obtained on			
		Fruits	Stems (1 yr old)	1-yr-old Hass avocados ^a	
				Roots	Trunks
IV	Hass	3/3 ^b	3/3 ^b	1/3 ^b	3/3 ^b
VI	Hass	3/3	3/3	1/2	2/3
XIII	Hass	3/3	3/3	1/2	3/3
B	Hass	3/3	3/3	1/2	3/3
D	Edranol	3/3	3/3	1/2	3/3
Checks	...	0/3	0/3	0/2	0/3

^aTrees were grafted on Mexicola avocado rootstock. Inoculation was performed on the rootstock, either roots or trunk.

^bNumber of fruits, stems, or trees developing symptoms/total number of fruits, stems, or trees inoculated.

controls (Table 1).

Disease incidence and estimated losses.

The incidence and significance of *Verticillium* wilt was estimated in seven groves totaling 3,589 trees 5–8 yr old budded on Mexicola rootstock. Economic losses were estimated from 250 8-yr-old Hass avocado trees in the same grove. Trees were rated as 0 = healthy, 0.5 = less than one half of the tree canopy showing symptoms, and 1 = more than one half of the tree canopy showing wilting symptoms.

RESULTS AND DISCUSSION

Isolation and pathogenicity.

A microsclerotium-forming fungus was consistently isolated from eight of 16 affected avocado trees assayed. Colonies were initially white and turned dark brown or black as microsclerotia formed on PDA. Abundant hyaline and verticillate conidiophores with unicellular, ovoid, and hyaline conidia borne on short phialides were produced on PDA. We identified this fungus as *V. dahliae* Kleb. (1). The initial description of *Verticillium* wilt of avocado was attributed to *V. albo-atrum*; however, we suspect that *V. dahliae* was indeed the causal agent because microsclerotia were produced by the fungus described (5).

The pathogenicity of each of five isolates was first demonstrated in fruit and stem inoculation tests (Table 1). Dark brown necrotic lesions developed in the vascular tissues below the inoculation sites on the fruits and stems. These tests

were consistent and correlated well with results obtained later when inoculations were made on 1-yr-old trees. Therefore, they provide a reliable and rapid technique for initial pathogenicity screenings. The 1-yr-old inoculated trees developed symptoms about 25 days after inoculation, consisting of a sudden wilt of the leaves followed by a dieback from the tips of the shoots (Fig. 1C). The leaves died, turned brown, and remained attached to the trees. Dark brown necrotic streaks developed in the vascular tissue on stems of the infected plants. No differences were observed in symptom expression among the five isolates tested. All the diseased plants except one recovered after 60 days of the initial collapse, developing vigorous new shoots from adventitious buds. No recurrent infection was observed within a year after the appearance of the first symptoms, suggesting that the fungus may not invade the new xylem tissue (3–5). The fungus was reisolated from all five infected trees about 45 days after first symptoms appeared, but a year later, *V. dahliae* was recovered from only two of 14 artificially inoculated plants.

Disease incidence and estimated losses.

The presence of *Verticillium* wilt was only found in avocado groves planted on soils intensively cultivated with susceptible crops, primarily artichoke (*Cynara scolymus* L.), cauliflower (*Brassica oleracea* var. *botrytis* L.), cabbage (*B. oleracea* var. *capitata* L.), chrysanthemum (*Chrysanthemum hortorum* L.), squash

(*Cucurbita maxima* Dche.), or tomato (*Lycopersicon esculentum* Mill.).

No evidence of the disease was found in three orchards totaling 2,100 trees from the same nursery but established after nonsusceptible crops, primarily cereals, in the same region. The incidence of *Verticillium* wilt in seven different groves varied from 1.3 to 10.4% affected trees, and 105 trees were found infected of 3,589 trees observed (2.9%). Diseased trees were found randomly distributed in the orchard.

The economic significance of *Verticillium* wilt was determined in a 250-tree grove where we observed the highest disease incidence. In this grove, nine trees were completely affected and 17 showed symptoms in about one half of the canopy. The latter 17 trees were considered as 8.5 entirely infected trees. Consequently, an equivalent to 17.5 of 250 trees was considered for yield-loss estimation from which no crop was harvested for at least two consecutive years. If we assume that trees should have yielded 40 kg/tree per year (average yield obtained on healthy trees), the actual yield loss per hectare (278 trees per hectare) was estimated as 778.4 kg/yr. Growers received an average of \$1.21/kg when this study was conducted. Consequently, the annual economic loss was \$941.86/ha per year without considering the interest rate and other costs relative to normal farming needed to keep the grove in good condition. Therefore, *Verticillium* wilt should be regarded as a major disease affecting avocados in Chile.

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