HOST REACTIONS INVOLVED IN THE RECOVERY OF APRICOT TREES FROM *VERTICILLIUM* WILT

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Summary

Verticillium-infected wood of apricot trees became darkened and host ray cells and fungal hyphae in discoloured wood were darkened, internally disorganized, and apparently inactivated. *Verticillium* was rarely isolated from discoloured wood and hyphae were not observed in normal wood. Extracts of discoloured wood inhibited spore germination, germ tube elongation, hyphal respiration, and hyphal growth of *Verticillium*. The degree of inhibition was related to the amount of discoloration and to the phenolic content of the extracts. Gums and tyloses formed in infected wood and prevented transport of conidia along the vascular tissue. Trees recovered from infection when uninfected lateral shoots were produced or when new wood was laid down around infected, discoloured wood containing inactivated *Verticillium* hyphae.

I. INTRODUCTION

Wilt or "blackheart" of apricot trees caused by *Verticillium dahliae* Kleb. [*V. albo-atrum* Reinke & Berthold (microsclerotial strain)] is widely distributed in South Australia. In some orchards it causes marked reduction in quality and quantity of fruit, and losses appear to be increasing. Leaves of infected trees begin to wilt in early summer and by late summer most wilted leaves have fallen. After wilting has become apparent, a brown-black discoloration of wood can be seen in diseased parts of trees. The wilting is most severe on those branches containing the greatest volume of discoloured wood.

Even severely affected trees usually do not die completely but during the autumn of the year in which symptoms first appear they frequently produce vigorous lateral branches. At this time the pathogen can rarely be isolated from diseased trees although it could readily be isolated in the previous spring and summer (Taylor and Flentje 1968). Many trees recover entirely within 1–3 years and recurrence of the disease in trees which have recovered is infrequent.

The resistance of apricot trees to *Verticillium* wilt has been correlated with the intensity of discoloration in wood following infection (Dufrenoy 1929). Commercial varieties of apricots grown in South Australia are not completely resistant to *Verticillium* infection but it appears that phenolic substances in discoloured wood may be involved in preventing the spread of the pathogen in infected trees (Somers and Harrison 1967). This paper describes some reactions of apricot wood to infection by *Verticillium*, and the effects of extracts from infected wood on *Verticillium* spores and hyphae.

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II. Materials and Methods

(*a*) *Histology of Wood*

Longitudinal and transverse sections 10–20 μm thick of diseased and healthy wood were cut, using a sledge microtome. Sections were either mounted in 10% aqueous glycerol without further treatment, or were stained in safranin and picro-aniline blue (Cartwright 1929) and then mounted.

(*b*) *Isolation of* Verticillium *from Wood*

Diseased lateral branches were taken at random from 70 trees. Tissues surrounding the wood were removed using a rotating, circular, wire brush which caused very little apparent damage to the wood. The wood was flamed lightly and then cut into disks 2 mm thick using sterile secateurs. Each disk was placed flat on 1% water agar in a Petri dish and incubated at room temperature for 2 weeks. Disks from which *Verticillium* could not be isolated after 2 weeks incubation were transferred to fresh plates of water agar and incubated for a further 2 weeks.

(*c*) *Preparation and Bioassay of Wood Extracts*

Samples of ground shavings (10 g) from diseased or healthy wood were extracted at room temperature in either distilled water (600 ml, 4 hr), 95% aqueous ethanol (160 ml, 4 hr), redistilled methanol (twice in 40 ml, 30 min each), or 50% aqueous acetone (twice in 40 ml, 30 min each). Extracts were evaporated to dryness and the residues were dissolved in a weight of 10 mM phosphate buffer, pH 6·0, equal to that of the wood sample used. The resulting solutions were sterilized using Millipore filters.

The effects on spore germination were observed using the double Petri dish method (Somers and Harrison 1967). This technique was also used to determine the effect of extracts on hyphal extension in 7-day-old mycelial mats (Converse 1953) removed from the surface of a liquid medium of the following composition per litre: proteose peptone 2 g, glucose 10 g, yeast extract 0·25 g, $MgSO_4.7H_2O$ 0·5 g, KCl 0·5 g, 10 mM KH_2PO_4 10 ml, 11 mM Na_2HPO_4 10 ml. Mycelial mats were placed on this medium, which also contained 1% agar, after having been treated with extracts and rinsed in distilled water.

Surface colonies grown on this medium were also used for respiratory studies. They were removed from the medium, blotted on sterile filter paper, and placed with 2·25 ml of medium of the same composition in Warburg flasks. Aliquots (0·25 ml) of wood extracts were placed in the arms of the flasks. Oxygen uptake, at 25°C, was measured at 15-min intervals for either 45 or 60 min before the extracts were tipped from the side-arms into the flasks. The average rate of oxygen uptake over this initial period was taken as the initial rate. Oxygen uptake was measured at 15-min intervals for up to 2 hr after the extracts were added. The results were expressed as a percentage of the initial rate of oxygen uptake.

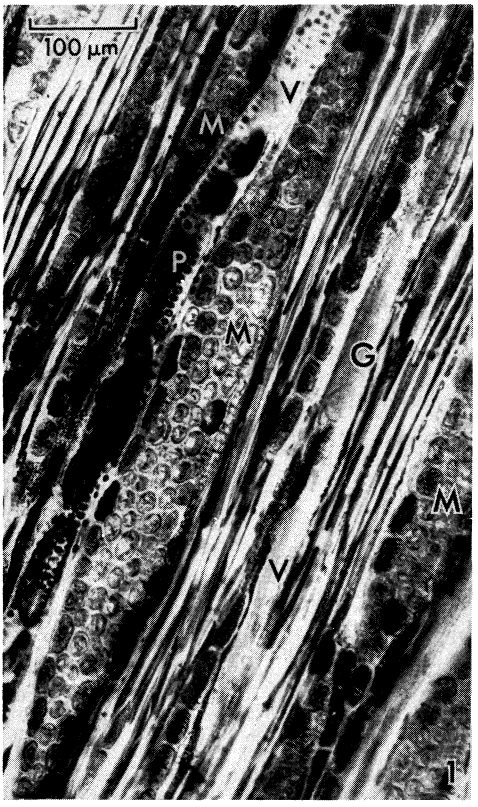
(*d*) *Total Phenols in Wood Extracts*

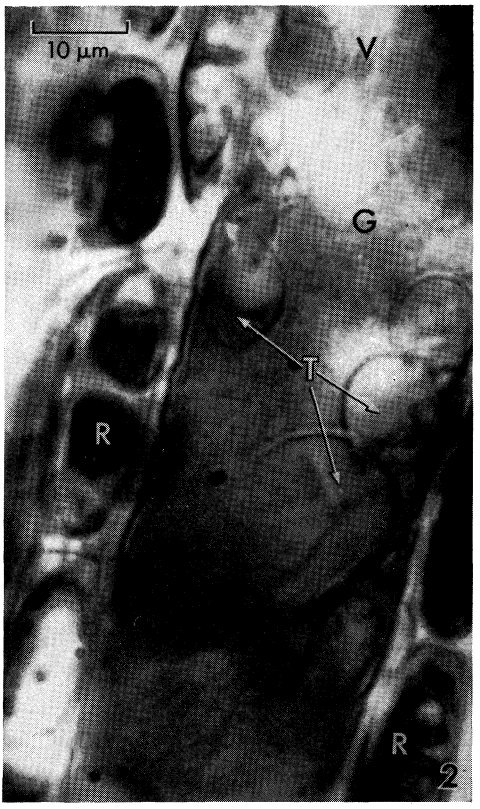
Extracts from wood with lesions of differing sizes were prepared using 50% aqueous methanol (Somers and Harrison 1967) and their phenolic contents determined using the Folin–Denis reagent (Swain and Hillis 1959).

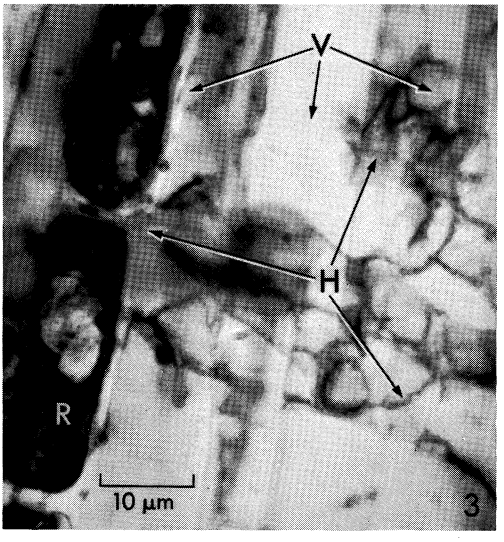
III. Results

In unstained sections of diseased wood the walls of most vessels and tracheids were discoloured brown to black or encrusted with black deposits or both, while medullary ray cells were disorganized or blackened internally (Figs. 1–3). The first ray cells to react in this way were immediately adjacent to vessels or tracheids. Vessels were often completely occluded by yellow-orange to brown gum deposits (Figs. 1 and 2) or tyloses (Fig. 2) or both. In stained sections, hyphae were detected within vessels, passing through vascular pits and within ray cells which were either internally discoloured or disorganized (Fig. 3). Hyphae were not observed outside areas of discoloured wood.

Verticillium was initially isolated from 18 of a total of 1225 diseased disks taken from 73 trees and placed on water agar. Isolates were made from an additional 23 of







Figs. 1–3.—Longitudinal–tangential sections of apricot wood infected by *Verticillium*: *1*, section not artificially stained. Vessels (*V*) contain gums (*G*) and black deposits on the walls and in pits (*P*). Medullary rays (*M*) contain blackened cells. *2*, section not artificially stained. Gums (*G*) and tyloses (*T*) are present in the vessel (*V*). Adjacent uniseriate ray cells (*R*) are discoloured. *3*, section stained with safranin and picro-aniline blue. *Verticillium* hyphae (*H*) are present in vessels (*V*) and discoloured uniseriate ray cells (*R*).

the disks when disks, from which *Verticillium* had not been isolated initially, were replated. Colonies grew sparsely from disks on to the agar. While most conidiophores

were triverticillate, some were unbranched and stunted. Agar under the disks became discoloured brown and the intensity of discoloration was greatest under disks with the largest amount of discoloration. Although hyphal growth was poorest on darkly

TABLE 1

EFFECTS OF APRICOT WOOD EXTRACTS ON THE GERMINATION AND GERM-TUBE GROWTH OF CONIDIA OF *VERTICILLIUM*

Lesion Size (%)*	Total Length of Germ-Tubes per Conidium† (μm)	Conidia Germinated (%)	Lesion Size (%)*	Total Length of Germ-Tubes per Conidium† (μm)	Conidia Germinated (%)
	Aqueous extracts‡			Ethanolic extracts§	
0	59	96	0 (6)	99	94
5	16	100	5 (9)	95	88
15	15	84	15 (14)	52	90
20	5	68	30 (19)	14	61
30	0	0	85 (31)	0	0

* As percentage of wood sample discoloured.

† Average from 100 conidia.

‡ Conidia incubated in extracts at 22°C for 18 hr.

§ Conidia incubated in extracts (diluted to one-sixteenth of original concentration) at 22°C for 24 hr. Values in parenthesis indicate the polyphenol content (mg/g wood) of the samples of wood from which extracts were prepared.

TABLE 2

EFFECT OF AQUEOUS SOLUTIONS OF METHANOLIC EXTRACTS OF APRICOT WOOD ON THE GROWTH OF *VERTICILLIUM* COLONIES

Period of Treatment with Extract (hr)	Increase in Colony Diameter (mm) after Growth on Nutrient Agar for following Periods (hr):				
	24	48	72	96	120
	Extract from uninfected wood				
0	3	7	11	15	19
15	2	6	8	12	16
36	2	5	9	13	17
60	2	5	9	12	16
84	3	6	10	13	18
108	2	6	10	14	18
	Extract from diseased wood				
0	3	7	11	15	19
15	0	*	*	*	0·5
36	0	*	*	*	0·5
60	0	0	*	*	*
84	0	0	0	*	*
108	0	0	0	0	*

* Weak growth.

discoloured agar, there was no correlation between the frequency of isolation from disks and the intensity of discoloration disks caused in the agar. Germination of *Verticillium* spores placed on discoloured agar was not inhibited. *Alternaria, Fusarium,*

Mucor, *Penicillium*, *Pullularia*, and two unidentified fungi were also isolated from disks but were rare.

All extracts from infected, discoloured wood inhibited germ-tube elongation, hyphal growth, and hyphal respiration: typical results are given in Tables 1 and 2 and in Figure 4. Germ-tube initiation was less affected than germ-tube elongation

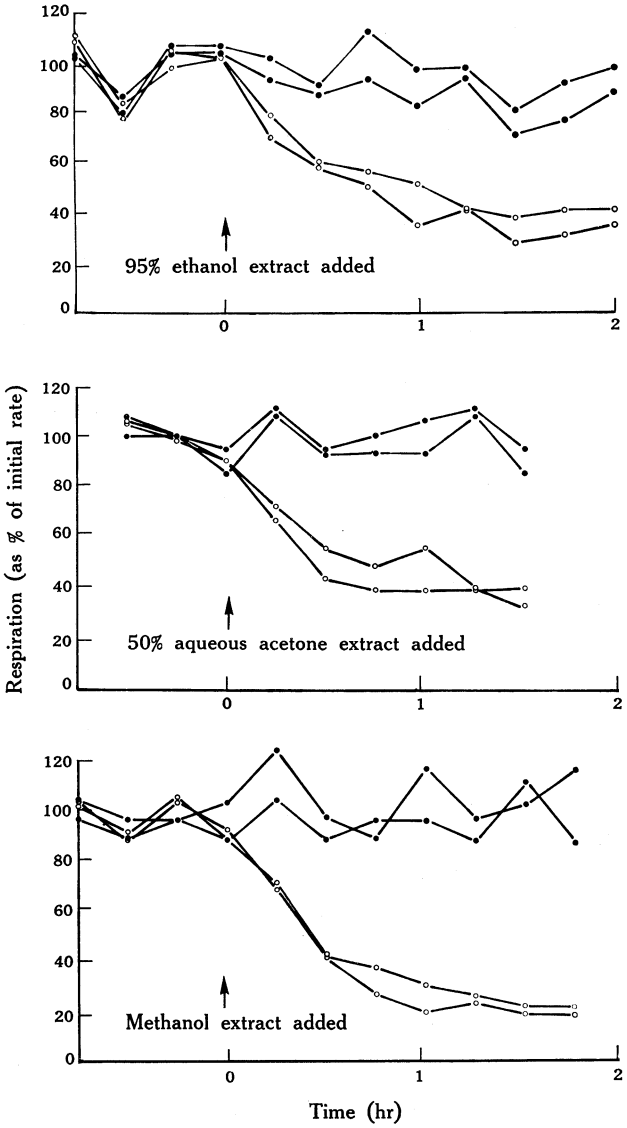


Fig. 4.—Respiratory rates of *Verticillium* hyphae in aqueous solutions of apricot wood extracts prepared with different solvents. ● Wood not discoloured. ○ Wood discoloured.

(Table 1). In each case, the degree of inhibition induced by wood extracts was proportional to the amount of discoloured wood in the samples extracted (Tables 1 and 2).

Total phenolic content of wood extracts increased as the amount of discoloured wood in the samples increased (Table 1).

IV. Discussion

These results indicate that recovery of apricot trees from *Verticillium* wilt is the result of physiological changes which occur in infected wood. They also suggest that these reactions to infection may be responsible, in part, for wilting of leaves on infected branches.

Gums and tyloses form in infected vascular elements and, by occluding them, prevent the upward movement of conidia from infected roots and stems. Transpiration would be reduced and wilting increased by these occluding materials. Infected ray cells and vascular elements become darkly pigmented and hyphae in these cells are discoloured internally, disorganized, and are apparently inactivated since the fungus was rarely isolated from discoloured wood. Spore germination, germ-tube elongation, hyphal respiration, and hyphal growth are inhibited by extracts of discoloured infected wood and the degree of inhibition is related to the degree of discoloration and to the phenolic content of the extracts. It would appear that phenolic substances, produced as a result of infection, are responsible for inactivating *Verticillium* in infected wood. It has been demonstrated that polymeric phenolics, fractionated from extracts of infected apricot wood, inhibit spore germination, probably by reacting with fungal proteins (Somers and Harrison 1967). It is possible that phenolics may be translocated from infected wood to leaves and contribute to wilting by reacting similarly with leaf proteins (Goldstein and Swain 1965). Since hyphae were not detected outside areas of discoloured wood, and since hyphal growth from disks of discoloured wood is extremely limited, it appears that lateral spread of the pathogen from discoloured areas is negligible. Trees are thus able to recover from infection when new lateral branches are produced and when new wood is produced enveloping discoloured, infected wood. Recurrence of the disease in a tree would result, almost entirely, from reinfection from the soil through roots as suggested by Taylor and Flentje (1968).

V. Acknowledgments

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VI. References

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