Endogenous Factors in Apple Bark Which Stimulate and Inhibit the Growth of Phytophthora cactorum

Z. Borecki, J. A. Ross, and D. F. Millikan

Visiting Professor, Research Organic Chemist, and Professor, respectively, Department of Plant Pathology, University of Missouri, Columbia 65201.

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Varietal differences in *Malus pumila* Mill. to invasion by *Phytophthora cactorum* (L. & C.) Schroet. suggest the presence or absence of biochemical substances influencing host susceptibility. The presence of such substances could function by preventing the establishment of the fungus or could regulate its growth after infection. Initial attempts to isolate substances with antifungal properties involved ether extraction of the entire bark tissue. When these extractions were fractionated into acidic, basic, and neutral fractions (1) and bioassayed, the growth of *P. cactorum* was stimulated with no correlation with the tolerance of the variety. Other investigations (2) indicated that the phloem-cambium tissue supported growth, whereas the unbroken periderm and xylem did not. Subsequent studies, therefore, were limited to this tissue. Alkaline extracts of this tissue also stimulated the growth, but this growth follows the varietal susceptibility if used to supplement mineral media (Table 1). Further evidence of difference is found in the growth on lima bean agar where inhibition occurred with all of the tolerant varieties except Jonared. No inhibition was found on the Grimes supplemented media, while the Canada Baldwin extract actually stimulated growth on lima bean agar.

The active substances are isolated by first removing the periderm and outer phloem, and then removing the exposed inner phloem-cambium tissue to the xylem, which was homogenized with 0.01 NaOH (1:3 w/v) in a Waring Blendor. The resulting extract was filtered by gel filtration, using a Sephadex G-25 column with 0.02 M phosphate buffer, pH 6.7, as the eluent. The effluent was continuously monitored at 254 nm with an ultraviolet light flow cell, and 20-ml fractions were collected. Four peak areas (Fig. 1) were combined and bioassayed, and the results shown in Table 2.

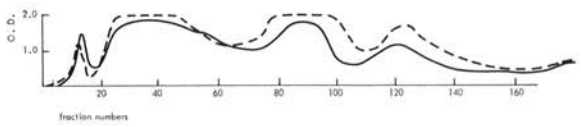

Fig. 1. Sephadex chromatography of NaOH extracts of phloem-cambium tissue from Grimes (—) and Starking (– –) apple. From left to right, peaks number 1-4.

Those fractions included in the first peak are stimulatory when bioassayed on plain or mineral agar media (3). On lima bean agar, only the fractions contained in the first part of the second peak show any stimulatory activity, and the activity is stronger with Starking than with Grimes. This same fraction showed the highest stimulation on the plain and mineral agar media. Those fractions beneath peaks 3 and 4 are strongly inhibitory when bioassayed on either agar or mineral media, but only the fractions found under the first part of peak 3 showed any inhibition when incorporated into lima bean agar. Greatest inhibition (35%) is found in the Starking fraction, including tubes 90-94.

The stimulatory fractions are found in the beginning of the second peak (Fig. 1), and therefore appear to be low molecular weight compounds of 500 or less. These fractions give a positive Molisch test for saccharides (5), and the stimulatory function is heat-labile and dializable. Further solvent studies indicate the presence of two active compounds, one of which is more

TABLE 1. Influence of alkaline bark extracts from some representative varieties of apple upon the growth of *Phytophthora cactorum* in vitro

Extract[a]	Disease Rating	Agar media[b]			
		Mineral		Lima bean	
		Total	Difference[c]	Total	Difference[d]
Control		7.0		29.5	
Starking	tolerant	10.8	3.6	25.8	3.7
Jonared	tolerant	11.1	4.1	28.6	0.9
McIntosh[e]	tolerant	29.5	22.5	26.6	2.9
Cortland[e]	tolerant	19.0	12.0	31.0	1.5
Wayne	unknown (new variety)	10.6	3.6	27.5	3.9
Grimes	susceptible	17.6	10.6	28.8	0.7
Canada Baldwin	susceptible	15.8	8.8	31.6	—2.2

[a] Extract prepared from bark of apple scions (1 yr growth) cut in December and stored at 6-8 C until used.
[b] Agar supplemented with 5% (v/v) extract. Each estimate is a mean of six diameter measurements after 2.5 days' growth at 27 C.
[c] Obtained by subtracting the measured growth on control media from supplemented mineral media.
[d] Obtained by subtracting measured growth on supplemented lima bean agar from control lima bean agar.
[e] McIntosh and Cortland possess field resistance, but this tolerance in stored excised stem tissue changes to susceptible in early April.

TABLE 2. Influence of bark extract fractions of Starking (tolerant) and Grimes Golden (susceptible) apple on the growth of *Phytophthora cactorum* in vitro[a]

Variety	Peak no.	Fraction no.	Agar media		
			Plain	Mineral	Lima bean
Starking	1	11-15	15.75	20.75	30.00
Grimes Golden	1	10-13	15.00	21.25	33.25
Starking	2	23-28	26.25	29.25	40.00
Grimes Golden	2	22-26	28.25	30.75	26.00
Starking	2	46-51	19.75	25.50	31.00
Grimes Golden	2	45-50	15.00	17.25	29.25
Starking	3	80-87			23.75
Grimes Golden	3	85-90			23.00
Starking	3	90-95			21.50
Grimes Golden	3	95-100			26.75
Starking	4	118-125			31.50
Grimes Golden	4	118-123			30.50
Starking Golden		177-187			32.50
Grimes Golden		183-187			32.50
Check				12.75	33.25

[a] Each estimate represents a mean of diameter growth of five colonies after 3 days at 27 C.

active than the other. Glucose, mannitol, and lactose have been identified by chromatography, but the stimulation of the rich lima bean agar indicates the presence of substances other than the common sugars. The inhibitory substances appearing in the third peak have ultraviolet light absorption maxima of 280 nm, typical of aromatic amino acids, and give a positive reaction with the Lowry reagents (4) specific for peptide linkages and tyrosin). Their appearance in the eluting stream suggests molecular weights of 4,000 to 5,000, and their activities are destroyed by heat and lost by dialysis. Further studies with Sephadex G-75 indicate the presence of at least three compounds, two of which appear to be active.

The role of these substances in host susceptibility has not been fully established, but quantitative correlations with observed varietal susceptibility make this relationship likely. In addition, changes in the proportion of these substances would explain seasonal changes in susceptibility within individual varieties (6).

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