Xylem Dysfunction in Peach Caused by Cytospora leucostoma

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ABSTRACT

Observations of the Valsa canker disease in New York peach orchards revealed wilting and defoliation of infected branches not girdled by cankers. A greenhouse study involving inoculation of potted peach trees with *Cytospora leucostoma*, the incitant of the disease, gave evidence of xylem dysfunction as an important cause of symptoms. Trees inoculated during active growth secreted gum within and outside of tissues at loci of infection and displayed symptoms of acute and chronic water stress in parts distal to sites of inoculation. Movement of eosin dye through xylem of segments cut from infected stems was interdicted at loci of infection. Foliage distal to nongirdling cankers showed symptoms of Ca deficiency and contained significantly lower concentrations of Al, B, Ca, Mg, Mn, P and Zn than comparable foliage from uninfected branches of the same trees. Transpiration rates of detached peach shoots standing in viscous solutions including culture fluids of *C. leucostoma* were 4 to 15% as great as those of comparable shoots standing in water. A 0.2% solution of gum from peach cankers was more viscous than the fluids tested on peach shoots. It was inferred from observations of gum in cambial and xylem tissues in and adjacent to cankers that gum is a major cause of the xylem dysfunction. It is hypothesized that gum may plug vessels directly or, when relatively dilute, impart viscosity to xylary fluid and thus impede cross transfer of the fluid among functional vessels.

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Additional key words: *Prunus persica*, *Leucostoma persoonii*, gummosis

Valsa canker as observed in New York peach orchards and described by others (8, 9, 11, 17) causes wilting or premature defoliation, then death of shoots and branches. We observed that infected branches show slow growth and sparse foliage with attenuated infrared reflectivity. Such foliage becomes prematurely senescent. Gum is secreted within and on the surface of infected tissues, and pycnidia of *Cytospora leucostoma* Sacc., the asexual stage of *Leucostoma persoonii* (Nits.) v. Höhnel [≡*Valsa-leucostoma* (Pers.) Fr.] develop most commonly in bark distal to sites of gum secretion on killed twigs. Foliar symptoms distal to nongirdling cankers indicate that stress is induced in tissues distant from loci of infection. This aspect of the disease was studied in young, artificially inoculated peach trees and detached shoots.

MATERIALS AND METHODS.—*Peach trees.*—One-year-old seedlings of Lovell peach and budlings of five varieties on seedling rootstocks were planted in containers of ca. 11-liters capacity in a mixture of clay-loam soil, peat moss, and sand (1:1:1) or perlite and peat moss (1:1). The potted trees were stored dormant at 3 C until used for experiments, which were done in March - October of two seasons in a greenhouse under natural daylight at temperatures ranging 13-27 C. The trees were initially irrigated with a dilute solution of soluble 20:20:20 fertilizer, then were watered every 2 days, and fertilized weekly with Hoagland's No. 2 solution (10). On removal from storage most trees were pruned to leave four lateral branches on mainstems 75 cm tall.

Inoculations.—A single isolate of *C. leucostoma*, typical of several for which pathogenicity was confirmed by inoculations on 'Baby Gold' budlings, was used for experiments. Inoculum consisted of plugs (5 mm diam) of mycelium plus malt extract agar (MA) from 14-day-old petri dish cultures, or blocks (10 X 5 X 2 mm) from cultures of the same age on previously dialysed, MA-saturated polyurethane (MAP). The method of Hildebrand (9) was modified for inoculations in wounds of several types: (a) oblique cuts ca. 2.5-cm long made to the wood; (b) bark flaps carefully cut to expose ca. 1.3 cm^2 of wood surface while minimizing injury to xylem; (c) girdles 1-cm wide from which bark was removed as above; (d) wounds made by removing bark discs with a No. 3 cork borer; (e) holes made by twisting a knife point through the bark and 2 mm into xylem. After inoculations, wounds were bound with masking tape or Parafilm. Control wounds received sterile plugs of MA or blocks of MAP.

Element analyses.—Samples consisting of three subterminal leaves each from one shoot bearing a non-girdling canker at its proximal end and from one uninfected shoot on each of five trees were analyzed by mass spectrography for P, Ca, K, Mg, Na, Zn, Mn, Fe, Cu, B and Al. The significance of mean differences in element concentrations between inoculated and uninoculated branches were determined by a t-test for paried values.

Dye movement in stems.—Freshly severed inoculated and uninoculated peach stems were treated with aqueous solutions of eosin Y (1%) in two ways. 1) Trees in foliage were cut off 10 cm above soil (deradicated) and the butt ends of the stems were placed in solution. 2) Dye was drawn by a vacuum of ca. 30 Torr through stem segments 10-30 cm long. Treated stems and segments were split and/or cross-cut for examination of eosin patterns.

Uptake of fluids by peach shoots.—The butt ends of shoots cut from greenhouse trees were placed in sterile distilled water (SDW) for 1 hr, after which all but ten upper leaves per shoot were removed and the basal 10-cm of each shoot was immersed in 0.5% NaOCl for 2 min. The basal 2-cm of each shoot was next removed and discarded. Then the butt end was washed three times in SDW and inserted into a pre-plugged sterile flask which contained 20 ml of SDW or a test fluid. The viscosity of each fluid at 30 C was determined in a Series 200 Cannon-Fenske ASTM viscometer as: (time for test fluid to flow through viscometer ÷ time for water to flow through viscosimeter) X 100.

The relative influences of various fluids and SDW on transpiration of peach shoots were determined gravimetrically and symptoms induced by each liquid were noted. Each flask was weighed at intervals beginning immediately after insertion of the shoot. The rate of transpiration (RT) of each liquid relative to SDW was expressed as:

$$\frac{T_w \times DW_w}{T_f \times DW_f} \times 100$$

where T_w and T_f are times taken for loss of 10 g of SDW and test fluid, respectively; and DW_w and DW_f are weights of shoots from the SDW and test fluid after drying 48 hr at 30 C.

The test fluids were: (a) a culture medium, adjusted to pH 5.0, which contained 10.7 g maltose, 3.16 g KNO_3, 0.49 g $MgSO_4 \cdot 7H_2O$, 0.91 g KH_2PO_4, 5 μg biotin, 100 μg thiamin, and distilled water to 1 liter (12); (b) medium amended with polyethylene glycol (Carbowax 4000) to make 1% concentration; (c) medium after growth of *C. leucostoma* for 28 days; (d) medium filtered (0.2 μm pore size) after growth of *C. leucostoma* for 28 days; (e, f) fluids c and d, respectively, autoclaved. Cultures of *C. leucostoma* were started in 100 ml of medium in 300-ml flasks from five MAP culture blocks per flask and were incubated in diffuse light at 30 C. Culture fluids were obtained by decanting.

RESULTS.—*Inoculations.*—Gum was secreted and cankers formed at 50-100% of wounds of all types inoculated 0-70 days after removal of trees from storage. Control wounds remained free of gum. Neither gumming nor canker formation occurred on several inoculated trees that failed to develop foliage after removal from storage. Moreover, trees that foliated slowly secreted less gum at points of inoculation than did rapidly growing trees. The length of time from removal of trees from storage to inoculation did not affect the type and severity of symptoms.

Sequences and severity of symptoms were related to the type of wound. Wounds with relatively little

TABLE 1. Wilt at 4 days, and defoliation and canker lengths 112 days after inoculation of Early Elberta peach stems with *Cytospora leucostoma*[a]

	Inoculated trees		Control trees
	Mean	SD	Mean
Shoots per tree	10	1	10
Shoots wilted at 4 days (%)	52	17	0
Shoots defoliated at 112 days (%)	80	13	0
Canker lengths (mm) at A	54	26	0
B	42	11	0
C	32	18	0
D	23	15	0
E	23	23	0

[a]Five knife-point wounds 2 mm into xylem at 4-cm intervals in a helical arrangement on each of eight stems were inoculated with 5-mm mycelium-agar plugs of *C. leucostoma*. Two control trees received sterile plugs. Locations A – E are uppermost to lowest. Vertical bars indicate ranges of nonsignificance at 5% probability level.

damage to xylem; e.g., oblique bark cuts, showed gum secretion but limited canker development throughout observation periods of several weeks. Foliar symptoms distal to these wounds developed slowly, beginning with interveinal chlorotic mottling, then general chlorosis and abscission of leaves in acropetal progression. This syndrome is termed chronic.

Rapidly developing or acute symptoms occurred after the inoculation of more severe wounds in which recently differentiated xylem was severed. The acute syndrome was characterized by acropetally developing wilt and browning of leaves, which generally remained attached. If several shoots became symptomatic, this occurred in acropetal order. Cankers on or subjacent to wilting shoots extended within a few days to several times their original lengths and pycnidia formed in them. Sudden wilt and conversion from chronic to acute symptoms sometimes occurred on defoliating trees.

In one experiment the location of inoculated wounds on mainstems affected canker size and rapidity of symptom development. Five knifepoint wounds made 4 cm apart in a helical pattern in the mainstems of each of eight trees were inoculated with plugs from MA cultures when the leaves were 1-4 cm long. Two control trees were similarly wounded but were not inoculated. Some shoots began to wilt 96 hr after inoculation. Enlargement of cankers followed wilting. Cankers attained significantly greater length at the uppermost point of inoculation than at the three lowest points (Table 1). Defoliation of shoots on inoculated trees ranged from 63 to 100% at 112 days after inoculation Controls remained foliated and free of cankers. The pathogen could only be isolated from within cankers and radially subjacent xylem although wood below and above cankers was discolored brown. Sections examined under a stereoscopic microscope showed gum in cambial and recently differentiated xylem tissues 2-3 cm above canker margins.

Concentrations of nutrient elements in foliage showing chronic symptoms were studied in another experiment. In this case bark-flap wounds were made on four lateral branches on each of five trees after 2 months of tree growth. Two wounds per tree were inoculated with plugs from MA cultures. Gum appeared at the wounds within 10 days after inoculation. Interveinal chlorotic mottling appeared within 15 days in the terminal leaves on inoculated branches but not on wounded control branches on the same trees. Subterminal leaves for element analyses were collected 15 days after inoculation. Leaves of infected (I) branches had significantly less P, Ca, Mg, Zn, Mn, B and Al than leaves from uninoculated (U) branches on the same trees. Ca and B showed the greatest differences between I and U (Table 2).

TABLE 2. Content, determined by mass spectrographic analysis, of 11 elements in subterminal leaves of infected (I) and uninfected (U) shoots of Belle of Georgia peach trees, 21 days after inoculation with *Cytospora leucostoma*[a]

Type of	Percent dry weight					μg/g					
shoot	P	Ca	K	Mg	Na	Zn	Mn	Fe	Cu	B	Al
U	0.3	1.5	3.2	0.5	34	190	112	143	7	24	74
I	0.2	0.6	3.1	0.4	35	144	78	153	7	6	52
Difference	*	**	NS	**	NS	*	**	NS	NS	**	*
I/U	0.7	0.4	1.0	0.8	1.0	0.8	0.7	1.1	1.0	0.3	0.7

[a]Means of three-leaf samples from one I and one U shoot on each of five trees. One and two asterisks indicate mean differences significant at the 5% and 1% levels, respectively.

Dye movement.—The first of two experiments involved uptake of dye by transpiring trees which were deradicated 14 days after the following inoculation procedure. Twenty-five trees with fully expanded leaves were shorn of lateral branches along 30 cm of stem, and bark flaps were made bilaterally midway along each pruned length. Inoculated wounds received blocks from MAP cultures. Controls received similar blocks autoclaved or on which the pathogen had not grown, or no treatment. Some uninoculated trees were mechanically girdled. Inoculated trees gummed within 4 days and were girdled by cankers 3 days later. Leaves on shoots distal to infection sites wilted.

Eosin patterns showed that wounds infected by *C. leucostoma*, but not control wounds, were loci of obstruction of upward movement of water in the xylem. The dye was moved by transpirational pull to distal foliage of uninfected stems within the first 2 to 3 hr of a 12-hr test period. Dye movement showed only local diversion in the vicinity of uninoculated wounds. Dye did not enter xylem directly above cankers or where lateral shoots had wilted (Fig. 1).

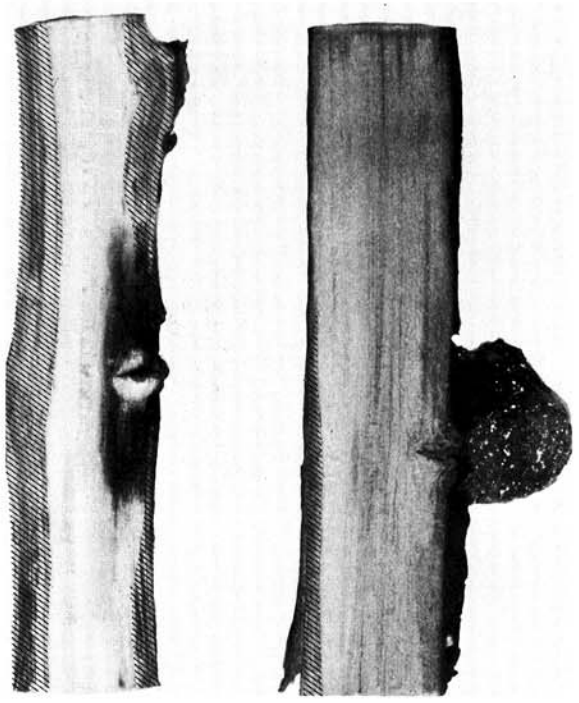

Fig. 1. Segments of 2-year-old peach stems showing patterns of movement of eosin dye solution (hatched areas) in the vicinity of wounds uninoculated (left) and inoculated with *Cytospora leucostoma*. Stems were severed from roots and allowed to take up dye solution by transpirational pull 14 days after wounding and inoculation.

In the second study eosin solution was drawn by vacuum through 30-cm segments from two infected and two control stems severed at weekly intervals after inoculation of one bark-flap wound per stem. Obstruction of eosin flow in infected stems was increasingly evident beginning 1 week after inoculation (Fig. 2). Transverse sections taken at random along infected stems distal to points of inoculation were checked microscopically. Never more than 20% of vessels were found occluded, even in a segment through which no dye passed during 12 hr of aspiration.

Uptake of viscous fluids by detached shoots.—The relative transpiration rates (RT) of shoots standing in untreated and autoclaved fluid from 28-day-old cultures of *C. leucostoma* were 4 and 6, respectively. Passage of these fluids through filters of 0.2 μm pore size caused increases in RT to 41 and 81, respectively (RT of SDW = 100). Viscosity values of the unfiltered fluids were 127 and 120; comparable values after filtration were ca. 100. The maltose-salts medium alone had RT and viscosity values of 62 and 100, respectively. When amended with polyethylene glycol (Carbowax 4000) to make 1% concentration, the medium had a viscosity value of 115 and a RT value of 14. A 0.2% (w/v) solution of gum collected from cankers had a viscosity value of 180. The influence of gum on RT, however, was not tested.

DISCUSSION.—Acute and chronic wilt of branches of peach trees infected by *C. leucostoma* seem attributable in large part to disruption of water conductivity of xylem at sites of infection. This disruption occurred rapidly when inoculation was done during the period of active growth. Inoculation of weak trees or those not yet growing elicited little or no response. Chronic symptoms, which are the rule in orchards, followed inoculation of wounds that did not penetrate xylem. Acute wilt was associated with infection courts made by wounding the living xylem as well as bark of transpiring stems. These observations at first seem contrary to the well-known association of Valsa canker with pruning wounds in orchards, but such wounds are often made during the dormant season when host responses to wounding and infection are slow.

All of the peach seedlings and budded varieties in the present study behaved similarly when inoculated with *C. leucostoma*. There was no indication that results were influenced by varietal differences.

The secretion of gum in xylem subjacent to

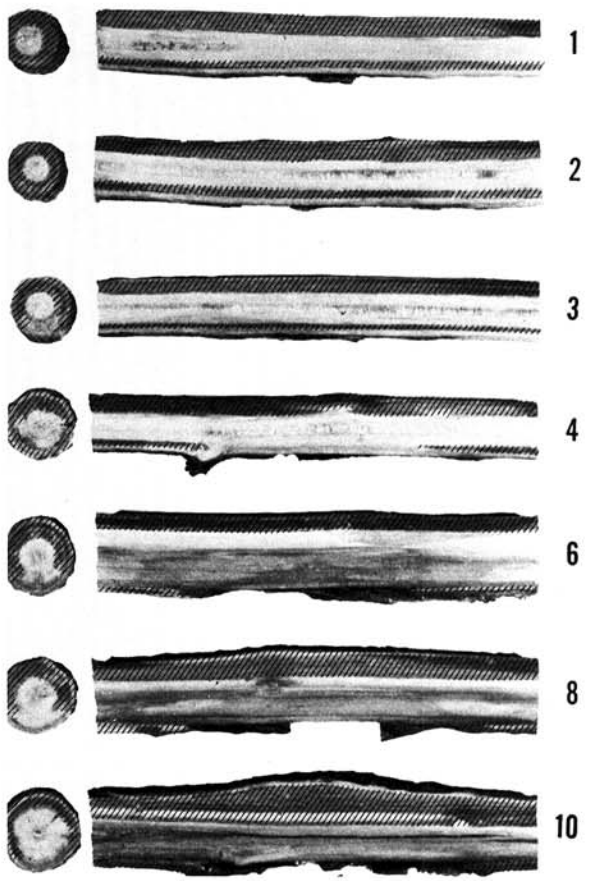

Fig. 2. Eosin dye patterns (hatched areas) in the vicinity of bark-flap wounds (lower center on each segment) 1, 2, 3, 4, 6, 8 and 10 weeks after inoculation of the fresh wounds with *Cytospora leucostoma*. Dye solution was drawn by vacuum through the stem segments.

cankers is probably important in the impairment of xylem conductivity. *C. leucostoma* was isolated from this tissue, and occlusions thought to be gum were observed in xylem as much 2-3 cm above cankers. Moreover, the flow of eosin was interdicted at sites of cankers but not at control wounds. Mechanical removal of bark caused no effect on distal foliage comparable to that associated with cankers.

Microscopic inspection of transverse sections taken at random along infected and healthy peach stems showed occlusion of less than 20% of vessels and tracheids in the conducting zone in all cases. This observation, however, does not negate the thesis of xylem dysfunction as a cause of distal symptoms. Vessels may presumably become plugged at various distances from points of inoculation, and a section taken at a given point would thus show only a minority of vessels occluded. Moreover, it is likely that the occlusions within vessels are short in relation to vessel length since movement of occluding substances within a vessel would cease as soon as a plug is formed. Thus, the observation of a low proportion of occluded vessels would refute the hypothesis of obstructed xylem only if the same vessels were found occluded in a long series of sections.

The recent literature on wilt diseases also suggests that important impedance to water flow may be caused by reduction of cross transfer between vessels (3, 4, 5, 20). Substances that could impart viscosity to xylary fluid, e.g., gum or possible products of the metabolism of *C. leucostoma*, might interfere with cross-transfer channels in peach wood. This would impede bypasses around plugged vessels (6, 7, 13, 15) and thus intensify the dysfunction of the xylem tissue.

The observation of low concentrations of elements, especially Ca and B, in leaves distal to cankers may also be interpreted in part on the basis of impairment of solute transport through xylem. Ca in particular is apparently immobile in phloem tissue and moves to permanent sites by xylem translocation (16).

It is also possible that Ca and other minerals are removed from xylem sap at canker sites. This hypothesis is supported by information that radioactive Ca accumulates at lesions of several types in wheat leaves (18) and at lesions caused by *Rhizoctonia solani* in bean hypocotyls (1). In any case, the reduced element concentrations in distal leaves of infected shoots as compared with healthy may contribute to the senescence and early loss of foliage in the chronic syndrome.

The experimental practice of pruning trees to assure active vegetative growth at the time of inoculation probably also promoted the expression of mineral deficiency symptoms in leaves distal to sites of xylem occlusion. Such symptoms might not occur on shoots inoculated after completion of a flush of vegetative growth.

The greater extension of cankers at upper than at lower sites of inoculation on stems bearing multiple sites is probably attributable to induction of water stress in tissues above the lowermost inoculated sites. Pathogenesis in several canker diseases is more rapid in tissues under water stress (2).

The fruiting behavior of *C. leucostoma* was typical of canker pathogens in that pycnidia formed after shoots showing wilt or advanced chronic symptoms became moribund and rapid extension of cankers occurred. Cankers remained small as long as growth continued or tissue surrounding the cankers remained well hydrated. In these respects Valsa canker on peach is typical of cankers caused by other facultative parasites (2). In the induction of foliar symptoms, especially acute wilt, however, this disease has much in common with the vascular wilts. In Dutch elm disease, for example, greatest susceptibility occurs during the period of rapid vegetative growth within ca. 3 to 8 weeks after budbreak (14, 19). In the present inoculation studies, gum production and canker extension were both minimal when trees were inoculated before the onset of active growth or when foliage did not develop, when tissue was dead or moribund, or shoots were shorn of leaves. Gum production and xylem occlusion were greatest when trees were inoculated in full foliage and during vegetative growth. This suggested that active xylem translocation was a necessary internal factor for disease development.

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