Vascular Decline in the Oregon Pawpaw Regional Variety Trial

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Summary. A pawpaw (*Asimina triloba*) regional variety trial (PRVT) was established at the U.S. Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR), Corvallis, Ore., in Fall 1995. This orchard was a replicated planting of 28 commercially available varieties or advanced selections from the PawPaw Foundation (PPF; Frankfort, Ky.), with eight replicate trees of each selection grafted onto seedling rootstocks and planted in a randomized block design. Two years after planting, 32 trees had either failed to establish or had died after an initial healthy start. By July 1999, 25% of grafted trees had died due to a vascular wilt-like disease, and 2 years later mortality exceeded 50%. Grafted selections with the lowest symptom severity include 1-7-2, 2-54, 7-90, 8-58, 9-58, 'Mitchell', 'PA-Golden #1', 'Taylor' and 'Wilson'. Seedling guard trees were unaffected until July 2000, when six guard trees of 76 died and 10 more were declining. By July 2001, 14 guard trees were dead. No fungi were consistently isolated from declining trees. A number of bacteria were isolated from infected trees, but no specific pathogen has been confirmed as the causal agent. Polymerase chain reaction (PCR) tests for phytoplasmas and for the bacterium *Xylella fastidiosa* were also negative. Research is ongoing to determine if a bacterial pathogen was the cause of the pawpaw decline in the Oregon PRVT.

The pawpaw tree produces the largest edible fruit native to the United States (Darrow, 1975) and has great potential as a new high value crop (Layne, 1996; Pomper et al., 1999). Pawpaws are hardy to USDA growing zone 5 [–26.1 °C (–15 °F)] and grow wild in the mesic hardwood forests of 26 states in the eastern United States, ranging from northern Florida to southern Ontario (Canada) and as far west as eastern Nebraska (Kral 1960). In 1995, the PPF, embarked on a joint venture with the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) National Clonal Germplasm Repository in Corvallis, Ore., to test commercially available pawpaw varieties and PPF advanced selections in Oregon, a region outside the native range of the pawpaw (Layne, 1996; Pomper et al., 1999).

A PRVT was established at the NCGR, Corvallis, Ore. in autumn 1995. This orchard was a replicated planting of 10 commercially available pawpaw varieties and 18 PPF advanced selections, with 8 replicate trees of each variety or selection, grafted onto seedling rootstocks and planted in a randomized block design.

Named varieties in the trial included: 'Middletown' (selected in Ohio), 'Mitchell' (Illinois), NC-1 (Ontario, Canada), 'Overleese' (Indiana), 'PA-Golden #1' (New York), 'Sunflower' (Kansas), 'Taylor' (Michigan), 'Taytwo' (Michigan), 'Wells' (Indiana), and 'Wilson' (Kentucky).

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The other 18 clones to be evaluated were selected by R. Neal Peterson from PPF orchards at the University of Maryland Experiment Stations at Queenstown and Keedysville, Md. The PPF advanced selections were selected based on superior traits including fruit size and taste, high flesh-to-seed ratio, resistance to pests and diseases, and overall productivity on a year-to-year basis.

The perimeter of the Corvallis PRVT field was surrounded by a one tree wide border of pawpaw seedlings or guard trees. The planting was removed in October 2002 due to the decline and death of trees from an unknown disease that apparently spread through the orchard.

Development of disease symptoms in the trial

The initial PRVT planting consisted of 224 grafted trees and 76 seedling guard trees. Two years after planting, 32 grafted trees had either failed to establish or had died after an initial healthy start. Additional trees were observed to decline each succeeding year. Vascular-wilt-like symptoms became apparent each spring after trees leafed out. As transpiration demand increased with warmer and drier weather, severely affected trees collapsed and died. Moderately affected trees became chlorotic with stunted new growth. Blue and black vascular discoloration was observed beneath the bark of declining trees along the lower parts of the main stem, particularly at and above the graft unions. This symptom has been described as blue-stain. A canker-like bark splitting was also observed near the base of many declining trees (Fig. 1), with smaller cankers on upper scaffold branches. Several species of fungi in the genera *Ceratocystis* and *Leptographium* have been associated with blue-stain in conifer timber, and a few species are associated with tree diseases. In most cases, spread of these fungi has been attributed to assorted bark beetles or other insects (Jacobs et al., 2000; Jacobs and Wingfield, 2001; Sohlheim and Safranyik, 1997). Microscopic examination of wood and bark samples from stem cankers on declining pawpaw trees, including blue-stained wood from what appears to be an infection front found no evidence of fungal tissue. Blue discoloration beneath the bark of pawpaw trees seems to be a common response of this host to injury and may be associated with more than one disease or disorder.

By July 1999, 25% of grafted trees in the Corvallis PRVT had died, and 2 years later mortality exceeded 50% (Fig. 2). Initial poor establishment of selections 1-68 and 11-13 (three out of eight replicate trees were alive in Spring 1999) and 'Taytwo' (four out of eight replicate trees were alive in 1999) suggest that either scions of these selections may have been diseased when they were initially grafted, or these selections are more susceptible to this disease than the other genotypes in the planting. These selections had 100% survival in the Frankfort PRVT (Pomper et al., 2003). Disease symptoms were not restricted to grafted pawpaw trees. Seedling guard row trees in the trial were unaffected until July, 2000 when 6 of 76 guard trees died and 10 more were in decline. By July 2001 14 guard trees were dead. Trees in the PPF orchard at Queenstown have exhibited similar disease symptoms to those in Oregon, but only about 1% die annually (N. Peterson, personal communication).

In July 2001, after 6 years in the field, mortality at Corvallis was 75% or more for selections 1-7-1, 1-23, 1-68, 3-11, 3-21, 11-13, 'Overleese', and 'Taytwo' (Table 1). Selections with highest survival included 9-58 (seven surviving trees) and 'Wilson' (100% survival). Trees were rated for symptom severity each year since 1999 (data not shown). Selections with the lowest symptom severity include 1-7-2, 2-54, 7-90, 8-58, 9-58, 'Mitchell', 'PA-Golden #1', 'Taylor', and 'Wilson'.

Attempts to identify a pathogen

Efforts to isolate the pathogen as-

Fig. 1. Stem canker symptoms associated with vascular wilt of pawpaw (*Asimina triloba*) selections in the pawpaw regional variety trial, Corvallis, Ore. Blue discoloration is evident beneath the bark at the leading edge of cankers, becoming black immediately beneath the bark cracks illustrated.

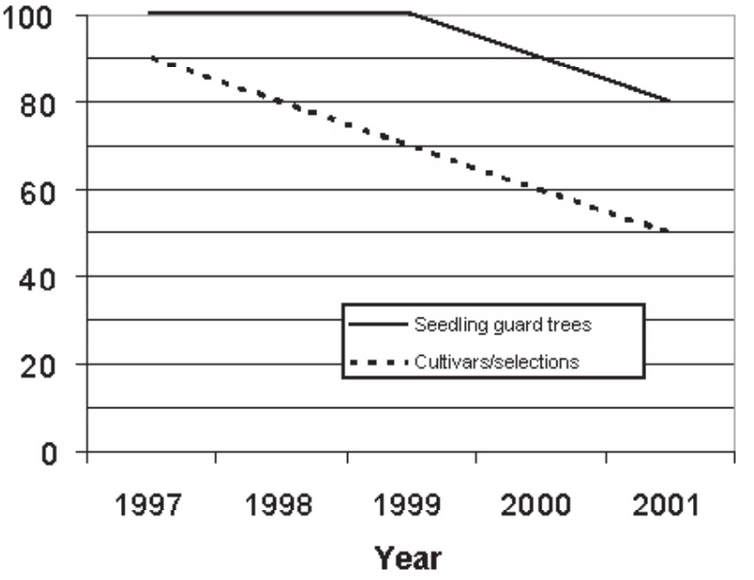

Fig. 2. Mean survival of pawpaw (*Asimina triloba*) trees in the pawpaw regional variety trial, Corvallis, Ore.; 224 grafted varieties and selections (8 trees each of 28 genotypes); 76 seedling perimeter guard trees; planted in 1995.

Table 1. Survival of pawpaw selections after 6 years in the Pawpaw Regional Variety Trial, Corvallis, Ore.; eight trees per selection.

Selection	Survival (%)
Wilson	100.0
9-58	87.5
1-7-2	75.0
2-10	75.0
2-54	75.0
Mitchell	75.0
Taylor	75.0
7-90	62.5
8-58	62.5
10-35	62.5
PA-Golden	62.5
4-2	50.0
5-5	50.0
9-47	50.0
11- 5	50.0
NC-1	50.0
Sunflower	50.0
8-20	37.5
Middletown	37.5
Wells	37.5
1-7-1	25.0
3-11	25.0
11-13	25.0
Overleese	25.0
Taytwo	25.0
1-68	12.5
3-21	12.5
1-23	0.0
Mean survival	49.1
Seedling guard trees[z]	81.6

[z]There were 76 seedling perimeter guard trees surrounding the planting.

sociated with decline symptoms have so far been unsuccessful. Samples were sent to the Oregon State University (OSU) Plant Disease Clinic in Corvallis in 1999. No fungi were consistently isolated from declining trees. In May 2000, the OSU clinic cultured several bacterial isolates from affected trees. Assays using Biolog culture plates (Biolog, Inc., Hayward, Calif.) were unable to identify any of the isolated bacteria as likely pathogens. In summer 2000, a number of bacteria were isolated by USDA–ARS collaborators in Corvallis, but again no likely pathogens were identified. PCR tests for phytoplasmas using universal primers, and for *Xylella fastidiosa* (causal agent of almond leaf scorch and Pierce's disease of grape) performed in 2001 by collaborators in Corvallis and Beltsville, Md., respectively, were also negative. During Winter 2001, bacteria cultures were isolated from surface-sterilized bark and wood at the leading edge of active stem cankers. Several of the cultures were fluorescent pseudomonads. Efforts are underway to test the pathogenicity of these isolates.

The future of the Oregon pawpaw trial

Dead and severely diseased pawpaw trees were removed from the Corvallis collection at the end of each growing season. Additional trees continued to decline as work proceeded to identify a pathogen. The planting was removed in October 2002, except for a row of guard trees that had been inoculated with bacteria isolates collected from stem cankers on declining trees. A new planting may be established on a nearby site to determine if disease symptoms are related to the present site of the trial. The Corvallis PRVT is the only site in this regional trial that is outside the native range of pawpaw. Despite the vascular wilt-like disease in this plot, healthy, producing pawpaw trees have been growing for more than 20 years at several gardens in Oregon's Willamette Valley (J. Postman, personal communication). The demise of the NCGR Corvallis PRVT may help to identify sources of resistance to this as yet unidentified disease. The NCGR will continue to research the cause of the disease and will replant early-ripening pawpaw varieties in another plot.

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