

## IN-SEASON VARIATIONS IN TRANSMISSION OF CHERRY X-PHYTOPLASMA AND IMPLICATION IN CERTIFICATION PROGRAMS

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### SUMMARY

Different aged shoots of X-disease cherry trees (*Prunus avium* cv Bing) collected in spring (June) or summer (August) were assayed using fruit spurs or bud chips from current, 1-, 2- and 3-year old shoots. Ten to 20 single t-grafts were made onto indicator trees of Bing scions on mahaleb (*P. mahaleb*) rootstocks. Spring collections, irrespective of shoot age, produced transmission rates of 10%. In contrast, over half of the indicators were infected with summer collections, which when t-bud propagated onto mazzard (*P. avium*) seedlings resulted in 90% diseased progeny trees. Results showed that biologic assays for cherry X-phytoplasma were optimized using summer collections. Besides cherry sources, index-transmissions and propagations of scion buds from X- and peach yellow leaf roll- (PYLR) peach trees produced high rates of transmission (both diseases) and high numbers of diseased progeny (PYLR).

*Key words:* cherry, phytoplasma, transmission rate, indexing.

### INTRODUCTION

In California, symptoms of chronic infections of X-phytoplasma in sweet cherry (*Prunus avium*) trees on mazzard (*P. avium*), Stockton Morello (*P. cerasus*), and Colt (*P. avium* x *P. pseudocerasus*) consists of sparse canopies, small leaves and small, pointed fruit borne on short, thick fruit stem or long, thin ones characteristics described, respectively, for Green Valley and Napa Valley strains of the pathogen. In contrast, X-infections of cherry trees on mahaleb (*P. mahaleb*) rootstock develop stem pits and grooves at the scion-rootstock junction and decline rapidly (Uyemoto 1989). In peach trees, leaf symptoms of X-infections consisted of chlorotic spots that turn necrotic and drop out leaving a shot-hole appearance. Chronically infected trees developed limb and scaffold branch dieback, poor fruit set, and tasteless flesh.

Although some strains of X-phytoplasma may be associated in trees symptomatic for PYLR, the biology of the predominant strain of PYLR-phytoplasma (= pear decline phytoplasma; Kirkpatrick 1993) is markedly different from X-phytoplasma in host reaction and insect vector species. In peach, symptoms consist of leaf chlorosis, downward leaf curl, and at mid-summer, a swelling of the midrib and lateral veins. Chronic infections cause tree decline and severely compromise fruit production. PYLR-phytoplasma does not infect sweet cherry.

We initiated a study to determine infective- and propagative-potentials of X-phytoplasma in cherry scion buds collected in spring and summer. This was done, in part, because in a large screening trial to determine the susceptibility of selected cherry clones and related species to X-phytoplasma, multiple bud-grafts in spring (June) from symptomatic sources oftentimes produced negative transmissions and completion of the trial required repeated bud-grafts the next year (Uyemoto *et al.*, 1991). Results of current investigations are being reported herein.

### MATERIALS AND METHODS

Orchard and nursery sites, located on the Department of Plant Pathology's Armstrong Tract, University of California in Davis CA (UC Davis), were planted one to 3 years in advance of utility with certified cherry trees of Bing scion (*Prunus avium*) on mahaleb (*P. mahaleb*) rootstock; peach trees of Fay Elberta scion on Lovell rootstock (*P. persica*); and seedlings of mazzard (*P. avium*) and nemaguard (*P. persica*) supplied by a commercial nursery. Diseased sources were pre-screened for presences of phytoplasmas via DNA (dot-blot) hybridization assays as described (Uyemoto *et al.*, 1989) and by ELISA for *Prunus necrotic ringspot virus* and *Prune dwarf virus* (Uyemoto *et al.*, 1989) and tree sources free of ilarviruses were sampled. The latter assays were done to reduce necrotic (shock) reaction associated with ilarvirus-infected buds grafted onto healthy test trees.

Other *Prunus* viruses are not known to affect bud-take. Healthy cherry and peach collections were from trees at Foundation Plant Services, University of California, Davis, CA.

Collections of X-disease scion wood were taken from three Bing cherry orchards in Stockton and Placerville, CA. Ten or 20 bud chips and spurs were removed from current, 1-, 2-, and 3-year old limbs collected in spring (June) and summer (August) and indexed on Bing/mahaleb trees.

Also, current season shoots of peach trees cv. Loadel symptomatic for X-disease or peach yellow leaf roll (PYLR), located at UC Davis, were collected in June to propagate on nemaguard seedlings (PYLR) or in August for indexing on Fay Elberta peach/nemaguard trees (both disease sources).

In index-transmission assays, individual trees were t-grafted with a single bud and overlaid with parafilm and held with budding rubber. With cherry, vegetative buds and fruit spurs, which bore symptomatic fruit, were used. Test trees were observed and symptoms recorded for up to three years post inoculations.

In progeny trials, two PYLR-Loadel peach trees (June collections) and four X-disease Bing cherry trees (August collections) were single t-bud grafted onto nursery rows of nemaguard and mazzard seedlings, respectively. Seasonal propagation times on host species coincided with commercial nursery practice.

In peach, t-budded nemaguard seedlings were pruned back to the insert bud after 45 days to stimulate scion shoot growth and trees remain in the nursery row.

With cherry, the tops of mazzard seedlings were pruned to the scion bud-inserts in February to promote scion shoot development. Later that summer, leaf petioles were sampled, extracted, and tested for X-phytoplasma via DNA (dot-blot) hybridization assays. Measurements of shoot lengths and diameters were done in October. During dormancy, the young Bing cherry/mazzard trees were uprooted and transplanted in an orchard setting for further observations. Orchard trees were re-assayed ca. mid-summer.

## RESULTS

With cherry, infectivity potential in spring collections averaged 10%, whereas summer collections from the same source trees averaged 64% (Table 1). Similar results were obtained in two other trials (data not shown). Symptomatic Bing cherry/mahaleb trees declined rapidly and were moribund by late summer.

In the progeny trial, four diseased Bing cherry sources successfully produced scion shoots of 70 to 80% on mazzard seedlings. Also, with the exception of four large Bing shoots derived from two disease sources, all other scion shoots were shorter in length and smaller in diameter compared to healthy Bing cherry shoots (Table 2). Then, under orchard conditions, all transplants with small scion shoots exhibited stunted growth, delayed bud-break, and downward curled, reddish leaves.

**Table 1.** In-season transmissions of X-phytoplasma in cherry.

Source tree	Age of shoots	Collections in:	
		June	August
Diseased	current season growth	1/10 <sup>1</sup>	6/10
	1-year old	2/20	6/10
	2-year old	2/20	6/9
	3-year old	1/10	7/10
	Totals:	6/60 (10%)	25/39 (64%)
Healthy	current season growth	0/10	0/10

<sup>1</sup> Number diseased/number Bing on mahaleb trees, each grafted with single bud.

Meanwhile, the four large individuals derived from disease sources and healthy controls grew vigorously.

While in the nursery, dot-blot hybridization assays revealed 18 (30%) Bing cherry trees were positive for X-phytoplasma (Table 3). However, among 41 surviving Bing cherry transplants in the orchard, 37 now tested positive (90%, Table 3). Nineteen transplants died (all derived from disease sources), which included 10 that tested positive the previous year. The aforementioned larger Bing cherry trees derived from two diseased sources and healthy controls were negative in both assays.

With diseased peach collections indexed on Fay Elberta peach trees, transmissions of both diseases were 55%, i.e., each with 11 diseased in 20 inoculations. Also, two progeny populations (June collections) of PYLR produced 14 diseased (58%) and 10 apparently healthy Loadel peach shoots (Table 4). In the same trial, 19 buds failed to grow, yet, 11 nemaguard trees (58%) developed symptoms of PYLR indicating infective scion buds had callused sufficiently to transmit the pathogen. The 21 healthy bud grafted controls produced 15 trees with Loadel peach shoots and six trees with failed buds; all units appeared healthy.

**Table 2.** Scion shoots of X-diseased and healthy-Bing cherry sources propagated on mazzard seedlings<sup>1</sup>

Source trees	Number of shoots	Avg. Shoot length (cm)	Range in shoot diameters (cm)
X-disease:			
Bing/mazzard T1	14	95.5	0.32 - 2.22
Bing/mazzard T2	16	67.0	0.31 - 0.95
Bing/Colt T3	14	66.7	0.32 - 1.59
Bing/Colt T4	16	57.4	0.32 - 1.27
Healthy Bing/mahaleb	20	127.5	0.32 - 3.18

<sup>1</sup> Twenty mazzard seedlings per collection t-budded in August; pruned to force scion growth in February; and shoots measured in October.

**Table 3.** Incidence of X-phytoplasma in Bing scions propagated on mazzard rootstocks<sup>1</sup>

Source trees	Dot-blot assays in:				
	Nursery row		Orchard trees		
	#positive	#negative	#positive	#negative	#dead
X-disease:					
Bing/mazzard T1	4	10	9	3	2 (0) <sup>2</sup>
Bing/mazzard T2	5	11	11	1	4 (3)
Bing/Colt T3	2	12	10	0	4 (2)
Bing/Colt T4	7	9	7	0	9 (5)
Totals:			37	4	19 (10)
Healthy Bing	0	5 <sup>3</sup>	0	20	0

<sup>1</sup> same trees in Table 2.

<sup>2</sup> Number in parenthesis were positive nursery trees that died in dormancy.

<sup>3</sup> Only 5 trees tested

## DISCUSSION

Our results showed that cherry X-phytoplasma transmissions were initially low (10%) and later, high (64%) in current and 1-3 year old shoots collected in spring or summer, respectively.

Thus, in assays for X-phytoplasma with spring, but not summer, collections of cherry shoots would require 10 grafts to effect a single transmission event. However, summer collections of cherry and peach, irrespective of collection times, would require, minimally, two bud grafts to detect X- and PYLR-phytoplasmas.

Our seasonal variations in X-phytoplasma transmissions compared similarly to insect vector acquisition-transmissions reported by Suslow and Purcell (1982). They provided the leafhopper vector, *Colladonus montanus*, access feeds on diseased or healthy cherry trees at monthly intervals (April to September) and transmission tested individual leafhoppers on celery and cherry seedlings.

**Table 4.** Incidence of peach yellow leaf roll (PYLR) with propagations on nemaguard<sup>1</sup>

Source Trees	No. diseased Loadel/total	No. diseased nemaguard/total
PYLR Loadel:		
Tree 1	6/12 <sup>2</sup>	8/10 <sup>3</sup>
Tree 2	8/12	3/9
Totals:	14/24 (58%)	11/19 (58%)
Healthy Loadel:	0/15	0/6

<sup>1</sup> Nemaguard seedlings t-budded with June collections; pruned to force bud growth 60 days later; and symptoms recorded after 15 months incubation.

<sup>2</sup> Number diseased/number trees budded.

<sup>3</sup> Number diseased nemaguard trees where diseased scion buds callused, yet failed to develop.

The relative percentages of infective leafhoppers were: zero in April; under 5% in May and June; and 24 or 20%, respectively, in August-September. Leafhoppers on healthy cherry trees were non-infective.

Also, the seasonal colonization pattern of European stone fruit yellows phytoplasmas (ESFY-P) was studied in different plum species by Jarausch *et al.* (1999). Irrespective of the season, they detected ESFY-P routinely in roots. However, in the aerial portions of the same trees, the pathogen was more reliably detected at mid-summer to early fall and declined significantly thereafter. For large scale assays, they suggested tissue collections of aerial plant parts be made from July to September.

Previously in California, assays for X-phytoplasma involved grafting four buds per accession onto two plants per indicator species of Bing cherry and Fay Elberta peach. Grafts were done during spring season with dormant or spring collections. After informal discussions of our research findings, summer grafts with fresh collections were adopted.

Previous work on seasonal distribution of X-phytoplasma in trees was first reported by Rawlins and Horne (1931). They observed both symptomatic and asymptomatic leaves and fruit on the same trees and variability in graft-transmission rates. Later, Stoddard (1947) found that transmissions from peach to peach can be 100% with more successes taking place at the early stages of disease expression and a gradual decline thereafter. Hildebrand (1952) reported some terminal peach buds on otherwise symptomatic limbs were not infective. In his study, transmission rates were highest in June-early July, lower in August, and zero in October. Reeves *et al.* (1951) also confirmed high transmissions in early season collections and low to none thereafter.

In the above reports, caution must be exercised in interpreting absence of X-phytoplasma transmissions in field grafts done in fall season. This is based on observations made while indexing for grapevine viruses (un-

published information). In a trial comprised of Cabernet Sauvignon indicators, seven leaf roll collections from one commercial vineyard were inoculated 10 days apart using six bud grafts per plant across a panel of nine indicator plants. Four collections were grafted on October 6, 2002 and three others, October 16. After two years, symptoms of leaf roll developed on 32 (89%) and 1 (4%) test plants, respectively, for the early- vs mid-October grafts. A comparable level of diseased test plants was expected from all seven collections.

An explanation for the discrepancy in leaf roll virus transmissions was gleaned from the CIMIS daily weather report, a database maintained by the University of California Statewide Integrated Pest Management Program. For the period October 6 to 14, day and night temperatures averaged 28°C and 8°C. For October 16 to 22, it averaged 21°C and 6°C. Hence, cooler ambient temperatures affected the callusing process. This hypothesis was subsequently confirmed.

In another series of late season grafts, we found green tissues in inoculum bud chips after 30 days post-inoculations. However, a closer examination revealed a lack of bud-chip to test plant connection. Also, the exposed cambium of the host stem cuts and bud chips showed both surfaces were necrotic. It was apparent that hydration of bud chips under late season ambient conditions was maintained up to 30 days, albeit undercover of parafilm wrap held in place with budding rubber, and thereby provided a false reading of bud viability.

To circumvent expected seasonal temperature changes, Rosenberger and Jones (1977) performed their graft-inoculations in greenhouses. For each collection period, actively growing indicators of peach or chokecherry (*P. virginiana*) were used by removing dormant seedlings from a cooler into warm greenhouses in advance of utility. Diseased shoots of field peach trees were collected from August 1972 to June 1974 and wild chokecherry, February to June 1974.

They reported peak disease transmissions of peach for the months of June-August (82-100%) and chokecherry in May and June (100%). Transmissions for peach collections made from mid-September to mid-December ranged from 32% down to 10%. Also, collections in February-May for peach or February-April for chokecherry produced infections ranging from zero to 21% (peach) and 20 or 40% (chokecherry).

In a study on ESFY-P, Seemüller *et al.* (1998) reported on phytoplasma titers in dormant season among various *Prunus* species. Bud grafts were made from January to March for three years and test plants held in a greenhouse at 18-25°C. Irrespective of year, all grafting dates from all donor taxa transmitted ESFY-P, confirming in part results of Rosenberger and Jones (1977).

Thus, and even though inoculum potentials varied widely, winter collections remain infectious provided tissue callusing conditions were met.

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