Variation in Horticultural Traits of ‘Bing’ Sweet Cherry Associated with Iarvirus Infection

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Abstract. Thirty-five ‘Bing’ sweet cherry (*Prunus avium* L.) clones were collected, primarily from old commercial orchards in central Washington; propagated on *P. mahaleb* L. rootstock; and their horticultural performance was evaluated. Nine of the 35 clones were not infected with the common pollen-borne ilarviruses prunus necrotic ringspot virus and prune dwarf virus—four of the clones after decades of exposure in commercial orchards. As a group, the nine virus-free clones produced larger trees with earlier fruit maturity and less rain cracking, but softer fruit, than did the 26 infected clones. These data challenge the general assumption that the presence of one or both of these ilarviruses is always detrimental. This assumption has driven development of many valuable virus certification programs and the adoption of virus-free trees as the standard for commercial fruit growing in most states.

‘Bing’ cherry has been grown commercially in the Pacific Northwest for more than a century. In the mid-1950s, the virus-free clone OB-260 was selected on the basis of limited horticultural evaluation as a typical ‘Bing’ that was free of known viruses. This clone has been used extensively by nurseries cooperating in the Washington, Oregon, and California nursery improvement programs. Since 1961, when the Washington State fruit tree virus certification program released the first virus-free ‘Bing’ cherry trees, most of the cherry orchards established in Washington and Oregon have been planted with that selection (Mink and Aichele, 1984).

Cherry trees grown in an orchard environment in Washington are exposed to viral infection, especially the pollen-transmitted ilarviruses prunus necrotic ring spot virus (PNRSV) and prune dwarf virus (PDV) (Mink, 1980; Mink and Aichele, 1984). Many trees in orchards established before the nursery certification program have been infected with PNRSV or PDV but express few, if any, symptoms (Mink and Aichele, 1984).

Sweet cherry propagation problems in the nursery caused by PNRSV and PDV include poor bud “take” and scion development. These viruses also can reduce growth of propagated trees (Gilmer et al., 1976; Nyland et al., 1976). Orchard problems also may occur with PNRSV-infected trees. Many show symptoms of some sort each year (Helton, 1955). Some PNRSV-infected trees annually express symptoms of rugose mosaic disease, typified by leaf distortion and delays of up to 2 weeks in fruit maturity (Howell and Mink, 1984). Fruit from trees with severely delayed maturity cannot be harvested with the rest of the crop and are a commercial loss. When the fruit is allowed to ripen, it is firmer and sweeter than fruit from virus-free trees (Howell and Mink, 1992). The number of trees infected with the cherry rugose mosaic disease strain of PNRSV rose dramatically in the 1970s (Mink, 1980).

PDV is best known as the causal agent for sour cherry yellows, a serious disease in ‘Montmorency’ cherry (*Prunus cerasus* L.). PDV causes the cherry tree to form flower buds at lateral nodes that would normally produce vegetative buds (Basak et al., 1962). Because cherry trees normally produce only a single simple bud at each node, a flower bud would preclude spur development at that node, ultimately reducing the bearing potential of the tree. This behavior is more serious in sour cherry than in sweet cherry. Yields of some sweet cherry cultivars may be seriously reduced (Posnette et al., 1968), while others are more tolerant of infection. Yield reduction is not believed to be serious on ‘Bing’ in Washington.

PNRSV and PDV exist as many distinct strains (Crosslin and Mink, 1992) whose effects range from severe to virtually symptomless. The use of symptomless strains to protect ‘Bing’ trees against the effects of disease-causing PNRSV strains (cross protection) is

feasible (Howell and Mink, 1984, 1988; Mink, 1986). In addition to cross protection, viruses sometimes can induce beneficial changes in horticultural attributes as reported in citrus (Duncan et al., 1980). The ideal cross-protectant virus for ‘Bing’ cherry would not only protect trees against disease but also improve the horticultural properties of the tree or fruit.

To test the hypothesis that there are differences in horticultural traits among cherry trees grown as ‘Bing’, some of which might be induced by the viruses they contain, we collected budwood from trees in several areas, primarily in Washington, and evaluated them at a single location.

Materials and Methods

During Summer 1984, budwood from 36 symptomless trees believed to be ‘Bing’ sweet cherry was collected from several locations in Washington, one location near The Dalles, Ore., and one location near Lewiston, Idaho. Trees were selected by people in each area who thought these trees represented a good or superior example of the cultivar. Trees were selected only if they had been planted before 1955. This assured that they had survived the severe freeze of that year and others in the 1960s (Bartram, 1969). It also meant that all had been propagated before the clone OB-260 had come into general use. In addition, all of these trees would have been planted before virus-free trees were available commercially. Included in the collection were five virus-free clones, including OB-260, obtained from the IR-2 repository of virus-free fruit trees (Fridlund, 1976).

The budwood was brought to Prosser, Wash., where 10 trees per clone were propagated on virus-tested *Mahaleb* rootstock. Survival of nursery trees ranged from 0 to 10, with five of the original clones being lost altogether. Surviving trees were planted in a randomized block design with a spacing of 6.1 × 6.1 m in Spring 1986 at the Washington State Univ. (WSU) orchard in Prosser. Single trees of all 36 surviving clones were planted in replication 1. Fewer clones appear in each successive replication. Only 20 of the 36 clones were planted in replicate 5.

The trees were grown as a commercial block of ‘Bing’ cherries using standard practice for cherry culture in the area, applied uniformly to all trees. They were trained to a modified central leader with minimal pruning until they began to bear fruit.

Samples of bud tissue from each tree were analyzed serologically by double-antigen sandwich (DAS) enzyme-linked immunosorbent assay (ELISA) for the presence of PNRSV or PDV (Mink, 1980). This test did not identify strains of these viruses. Samples for serology were collected in 1988, before the trees flowered, and thus likely represented the infection of the original source trees.

Subsequently, all the trees were indexed on Shirofugen flowering cherry for the presence of ilarviruses. The presence of PDV in one clone was still not certain after both tests so

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that clone was eliminated from the analysis.

Horticultural evaluation of the clones included yield and annual post-leaf-fall trunk circumference measurements. Samples of ≈ 200 cherries were collected when nearly all the fruit on the tree were darker colored than the No. 3 color comparator (Brearley et al., 1964). All the fruit on the selected branch were harvested to assure representation of the maturity range and defects present. Rain-crack data were the percentage of fruit affected following rain damage in the field. Fruit quality was measured with a standard series of tests (Proebsting and Mills, 1981). The firmness component was measured with an impact-type sensor with appropriate electronic circuitry (F. Younce, Dept. of Food Science and Human Nutrition, WSU, Pullman). This momentum transfer generator (MTG) measures firmness in dimensionless units derived from the slope of the response curve. The data correlate linearly ($r = 0.82$) with Instron values using the flat plate head.

Yield efficiencies were calculated in 1992 and 1993 from the total yield divided by the trunk cross-sectional area (TCSA).

The data were analyzed as a split-plot analysis of variance (ANOVA), with years as the main plots and virus infection as the subplots. Because all the clones had been grown as 'Bing', because no clonal differences were identified, and because the virus and clonal effects were confounded, we assumed for the purposes of the analysis that there were no genetic differences among the clones. The data were analyzed by the General Linear Models Procedure (SAS Inst., Cary, N.C.).

Results and Discussion

Nine clones were free of the pollen-borne viruses, five from the IR-2 collection of virus-free fruit trees. The other four came from commercial orchards and consequently were exposed to one or both viruses for many years without becoming infected. Importantly, while the majority of trees in the collection were infected with PNRSV, PDV, or both, they were selected because they were good or superior examples of commercial 'Bing'.

The virus-free trees, as a group, had larger TCSA, an indication of overall tree size, than the infected but symptomless trees (Table 1). While both viruses reduced TCSA, the PNRSV effect was significant at $P \leq 0.01$, while the PDV was significant only at $P \leq 0.05$ (Table 2). The demonstration that virus-free trees were larger than virus-infected trees is not surprising. The virus-free certification programs now widely used were established, in part, because virus-free clones grew much more vigorously in the nursery than did virus-infected trees (Gilmer et al., 1976; Nyland et al., 1976). Since smaller trees are now favored for use in higher density growing systems, tree size reduction resulting from virus infection would be a horticultural advantage.

Yields were not affected significantly by virus infection (Tables 1 and 3). If all else is equal, bigger trees have higher yields per tree. Virus-positive and virus-negative trees had

Table 1. Effect of prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV) on trunk cross-sectional area (TCSA) in 1993; yield and yield efficiency in 1992 and 1993; and harvest date, fruit weight, firmness, and rain cracking in 1990–93.^a

Characteristic	Virus infection			
	None	PNRSV	PDV	Both
<i>1993</i>				
TCSA (cm ²)	373	263	305	254
n = ^y	40	37	4	65
<i>1992–93</i>				
Yield (kg/tree per year)	23.8	17.5	23.4	20
Yield efficiency (kg·cm ⁻² TCSA)	0.07	0.07	0.09	0.08
n =	72	71	8	128
<i>1990–93</i>				
Harvest date (June)	21.4	22.8	20.6	22.5
Fruit weight (g)	8.5	8.8	8.8	8.6
Firmness (MTG) ^x	660	718	702	714
Rain crack (%)	26	34	36	33
n =	156	142	15	233

^aAnalysis of variance, using the GLM procedure of SAS (SAS Inst., Cary, N.C.), is presented in Tables 2–4.

^yn = number of trees in each category of virus infection.

^xMeasured with a momentum transfer generator (MTG).

Table 2. Analysis of variance for trunk cross-sectional area (TCSA) (cm²) of 'Bing' cherry measured in 1993, as affected by prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV).

Source of variation	df	TCSA	
		MS	F ratio
Replication	4	3,759.0	0.88
PNRSV	1	82,782.8	19.35**
PDV	1	17,176.1	4.02*
PNRSV × PDV	1	10,637.8	2.49
Error	138	4,277.6	

*,**Significant at $P \leq 0.05$ or 0.01, respectively.

Table 3. Analysis of variance of yield (kg) of 'Bing' cherry as affected by prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV) averaged over 2 years, with yield efficiency (kg·cm⁻²) calculated as the average from yields in those 2 years divided by the trunk cross-sectional area.

Source of variation	df	Yield	Yield efficiency		
		MS	F ratio	MS	F ratio
Replication (Rep)	4	289.54	1.78	0.0043166	1.80
Year	1	4381.16	26.95**	0.0185115	7.70**
Rep × Year	4	212.89	1.31	0.0029572	1.23
PNRSV	1	579.89	3.57	0.0000017	0.00
PDV	1	20.34	0.13	0.0029069	1.21
PNRSV × PDV	1	59.36	0.37	0.0000027	0.00
Error	266	162.57		0.0024036	

**Significant at $P \leq 0.01$.

similar yield efficiencies (Tables 1 and 3).

There were no differences in fruit size (Tables 1 and 4). Fruit size was not related to yield or yield efficiency in this test.

Symptomless PNRSV infection increased the firmness of ripe fruit (Tables 1 and 4). This response is similar to the increased firmness found in fruit from trees infected with the rugose mosaic strain of PNRSV (Howell and Mink, 1992).

Soft fruit is a serious constraint in shipping sweet cherries. Much of the crop requires mass handling of the fruit from picking to sorting to packing and shipping. Soft fruit do not withstand this handling as well as firmer fruit. A virus-free cherry clone does not appear to be automatically a better clone in this respect.

Fruit from trees infected with PDV was significantly more susceptible to rain cracking than that from virus-free trees, whereas the effect of PNRSV was nonsignificant (Tables 1 and 4). Crack resistance is desirable but is less important to the Pacific Coast cherry shipping

industry than firmness, as shown by the dominant position of 'Bing', a relatively crack-susceptible cultivar, as the cultivar of choice.

A highly significant delay in fruit maturity, as expressed by date of harvest, was associated with PNRSV infection (Tables 1 and 4). As noted above for firmness, this result is consistent with the effect of rugose mosaic disease strains of PNRSV, which can delay fruit maturity by as much as 2 weeks (Howell and Mink, 1984, 1992). Delaying maturity can be advantageous in the later-ripening districts as they often are harvested during a strengthening market.

This planting, propagated primarily with budwood collected from selected trees being grown as 'Bing' around the state, revealed significant differences in important characteristics among the accessions. The genetic effect is confounded with the virus effect since each clone brought its own virus infection. Virus inoculation was not a controlled treatment. Clones infected with PNRSV, PDV, both, or

Table 4. Analysis of variance for fruit weight (g), firmness (MTG units), rain crack (percent), and date harvested (date in June) in 'Bing' cherry as affected by prunus necrotic ringspot virus (PNRSV) and prune dwarf virus (PDV) averaged over 4 years.

Source	df	Fruit wt		Firmness		Rain crack		Date harvested	
		MS	F ratio	MS	F ratio	MS	F ratio	MS	F ratio
Replication (Rep)	4	0.395	0.37	10,753.5	1.26	468.38	2.09	14.655	1.11
Year	3	213.799	202.19**	264,533.5	31.09**	66,201.95	294.90**	6,797.026	514.86**
Rep × year	12	0.556	0.53	8,056.0	0.95	134.98	0.60	21.004	1.59
PNRSV	1	0.428	0.40	45,905.1	5.40*	384.70	1.71	173.292	13.13**
PDV	1	0.079	0.07	13,587.7	1.60	1,199.32	5.34*	29.081	2.20
PNRSV × PDV	1	1.710	1.62	20,388.0	2.4	1,133.85	5.05*	3.623	0.27
Error	522	1.057		8,508.1		224.49		13.202	

*,**Significant at $P \leq 0.05$ or 0.01 , respectively.

neither were identified, and the virus affected certain horticultural characteristics significantly. It was not possible to quantify genetic differences within the collection of clones so that variability appears in the error term of the ANOVA. Therefore, at this stage of the research, we conclude that there are important favorable horticultural effects of the viruses at a statistically significant level. The possibility remains that genetic differences within the collection may be identified in the future.

If we assume that data presented here represent how these clones, with their virus infection, would perform under commercial orchard conditions, there seems to be potential to improve production characteristics of commercial 'Bing' plantings. The potential is to produce later-maturing and firmer fruit on smaller trees. Much of this potential theoretically could be tapped immediately by using one or more of the clones in this collection with their existing virus complement. However, the virus strains, which are apparently benign in 'Bing', may cause disease in other cherry cultivars or in other crop species.

Out of 31 clones collected from commercial orchards, 26 were infected with PNRSV or PDV or both. The assay for virus produced indeterminate results in one case. The 84% infection rate is probably representative of the older cherry orchards in Washington (Mink and Aichele, 1984).

Despite their infections, these clones came from orchards that have been productive for at least 38 years and were selected because they were good or superior 'Bing' trees.

The spread of rugose mosaic can be limited by inoculating trees with symptomless virus isolates (Howell and Mink, 1988). If certain virus isolates improve 'Bing', it might seem

logical to inoculate OB-260 with isolates selected for their beneficial effects beyond being merely symptomless. Whether this collection includes a strain of PNRSV that would confer both protection against disease-causing isolates and, at the same time, improve tree performance or fruit characteristics needs to be determined.

The beneficial characteristics of the 'Bing'-induced virus combinations in this experiment are large enough to improve the variety significantly. This potential warrants continued research to answer the many questions that arise if one extrapolates these results to commercial application.

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