A SWEET CHERRY PLANTING SYSTEM COMPARISON INVOLVING VIRUS EFFECTS WITH MULTIPLE GENOTYPES

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of JOSEPH BRETT ADAMS find it satisfactory and recommend that it be accepted.

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Abstract

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New sweet cherry (*Prunus avium* L.) orchards are planted at high tree densities using precocious size-controlling rootstocks to improve economic returns and labor efficiency. Growers are seeking alternative planting strategies to reduce high costs of establishing these systems. This research project evaluated the feasibility of a sleeping eye (dormant graft) planting system compared to the traditional nursery tree (two year grown) for multiple genotypes. Graft survival and growth data were collected in 2006 and 2007 for 'Bing', 'Chelan', and 'Tieton' grafted on Mazzard (*P. avium* L.), MxM® 60 (*P. avium* x *P. Mahaleb*), and Gisela®6 (*P. cerasus* x *P. canescens*) in 2006. Graft success was 24% greater for nursery trees vs. sleeping eyes. In 2006 the nursery trees were larger than the transplanted sleeping eye trees but in 2007 growth of sleeping eye trees exceeded that of the transplanted nursery trees. Tree growth was delayed in the first orchard year irrespective of planting system. Using virus-infected budwood reduced graft success significantly but did not affect tree vigor. Preliminary economic comparisons suggest that start up costs for a two acre sleeping eye block would be $1,865 to $1,092 less than costs for planting standard nursery trees.

In a separate experiment I investigated the role of the position of a bud within a budwood stick in graft success and tree growth. ‘Bing’ and ‘Tieton’ buds were alternately grafted to a rootstock Mazzard and Gisela®6 for survival and growth observations or analyzed by ELISA and PCR for virus titer. Bud position had a significant effect on tree survival. The basal three buds were 63% more likely to result in a tree loss - ca. 50% of the buds that didn’t produce trees were floral. There were significant difference in growth between rootstocks, seasons, scion varieties, and bud positions. Further, there was no clear relationship between the diameter of the bud stick at the point of bud removal and the growth of grafted trees in the subsequent year, irrespective of genotypes. Virus titer could not be assessed due to the timing of sample collection.

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1.1 – Introduction

Sweet Cherry Industry

Today sweet cherry (*Prunus avium* L.) production is in the midst of a modern revolution. Traditional methods of producing sweet cherries are being replaced with new approaches which include high density plantings, size-controlling rootstocks, and new cultivars. These factors have the potential to improve precocity, yield, and labor efficiency. With the demand and prices rising in the sweet cherry markets, more growers are entering the industry and current growers are expanding into new locations; creating more competition. Competition is one of the driving forces for the major change in the last decade and a half. Growers desire something different or cost efficiencies to give them the competitive edge to make their profits.

Since 1990 sweet cherry production world wide has been increasing (Figure 1.1). Sweet cherry production around the world in the 80's had been consistent at 290,000 hectares harvested or 1.5 million metric tons; but starting in 1991 a increase commenced that would reach 365,000 hectares harvested world wide by 2002. Between 1994-96 and 2006, the increases have ranged from 5% in Eastern Europe to 48.9% in the Near East (Turkey, Iran, etc.), 54.3% in North America and 66.1% in the rest of Asia. Production topped 2 million metric tons in 2006 being the largest on record harvested world wide. The trend in the four major producing countries, Turkey, the United States, Iran, and Italy has been upwards since 1994. The area harvested in the last decade has doubled in Turkey and the United States and tripled in Iran; Turkey in 2006 was the first country to produce over 300,000 metric tons.

Global trade in fresh sweet cherries grew in volume and value as exports exceeded 222,445 metric tons world wide in 2005. The value of the exported fruit exceeded $688 million in 2005. Europe is the single largest importer for fresh sweet cherries with Asia close ranked second. The United States is ranked number one in sweet cherry exports with 48,393 metric tons and Turkey close behind with 34,793 metric tons in 2005. Exported fresh sweet cherries from the United States are estimated to be worth $217,872,000 annually. Even with global production and acreage rising, cherries compared to other fruits remain a small part of the world market. In 2005, cherries accounted for only 0.4% of the world's fruit production; and only 1.6% of the total deciduous fruits, such as apples, grapes, oranges, and bananas.

The sweet cherry industry in North America has increased from 153,600 metric tons in 1985-87 to 275,200 metric tons in 2006 a 55.8% increase in production. The United States produce 250,219 metric tons annually, with the 48,393 metric tons exported and the rest distributed and consumed with in the nation. With the 2005 data from Food and Agriculture Organization of the United Nations trade flows were assessed; out of the 48,393 metric tons exported out of the US, 29,665 metric tons were shipped to North East Asia and 14,605 metric tons to Mexico and Canada. (O'Rourke, 2007).

In the United States, Washington State is the largest producer followed by California and Oregon. The acreage of sweet cherry in Washington was estimated at 10,100 hectares in 2005; estimates of current acreage are 14,500 hectares with around 15% not yet in production. The 2007 Northwest (Washington, Oregon, Idaho, Utah, and Montana) crop was 14.7 million 18 lb. boxes packed and sold, where as California packed 7.4 million boxes (BJ. Thurlby, Pers. Commun.). The Northwest industry mirrors

the worldwide trend of tremendous growth with change in many diverse areas such as labor expenses, labor shortages, new scions, new rootstocks, and new styles for planting systems. Many of these changes are due to the competition and the need to be more efficient.

As more area is being planted with new cultivars that lengthen the traditional cherry season, labor issues become an increasing point of expense and concern for the sweet cherry industry. Already cherries are the most labor intensive fruit because of the many hours of hand labor in picking, sorting, handling, and packing to maintain a high quality fresh product. The United States and parts of the world are experiencing an increase in labor prices. The United States federal wage and individual state minimum wages are increasing yearly so the expense of harvesting, packing, and selling is making it difficult to make a profitable return each year. In 2000 the minimum wage rate for Washington State was $6.50 a ca. 11% difference from 2005; and in 2008, it is $8.07, a 24% increase from 2000 (Washington State Department of Labor and Industries, 2008). Access to labor is also becoming a challenging problem for the U.S. growers. Much of the population is acquiring a higher education, and with that education, they do not want to be in a manual labor jobs. This leaves less willing to work in agriculture and a small work force to do the large task needed in the industry. Many growers are looking for other sources to help them with the large labor deficits but federal immigration laws are eliminating the use of fraudulent documents and tightening the border which is restricting a large source of needed labor for the agriculture industry.

New varieties are being introduced from breeding programs around the world. ‘Bing’ has been grown in the Pacific Northwest commercially for over a century. ‘Bing’

is the industry standard and the most familiar sweet cherry and still dominates the market in production today (Table 1.2). 'Bing' clearly is the market leader with 6 million boxes sold (BJ. Thurlby Pers. Commun.). Newer varieties such as 'Tieton' are increasing in production; in 2006 production of 'Tieton' was 25 thousand boxes and the following year this doubled (Table 1.2). New varieties have an appeal to growers for diversification. Large fruit size is a very desirable trait for sweet cherries destined to the fresh market (Long et al., 2005). Early- or late-season varieties are also in demand because of the decrease in returns for traditional cultivars such as 'Bing.' New varieties are needed to diversify the market and to commence the sweet cherry season earlier or lengthen it. 'Bing' is harvested late June to mid July; the cherry season is being extended to the start of June and to the end of August. 'Tieton' is an extraordinarily large cherry that ripens one week before 'Bing.' 'Chelan' is the earliest ripening sweet cherry currently available to the market, which ripens two weeks before 'Bing'. 'Sweetheart' is a Canadian-bred variety that is harvested about 18 to 22 days after 'Bing'. Consumers are willing to spend more on different tastes, sizes, colors, and times (earlier season or later season). These varieties bring profits to those who invest in them, but nurseries are struggling to keep up with the change.

Sweet cherry growers in the United States have been slow to adopt new high density orchard systems of 500+ trees per hectare (Long et al., 2005). The current trend for the sweet cherry industry is to grow and crop cherries with larger tree densities to maximize the fruit tonnage per hectare. In the Northeastern States growers are planting higher densities for more precocity, production, and to cover more easily with rain exclusion shelter (Andersen et al., 1999; Balmer, 2001; Lang and Perry, 2002; Weber,

2001). Smaller spacing results in increased labor efficiency, precocity and productivity (Webster, 1995; Lang, 2000) and reduced cropping risks (Santos, et al., 2007). The traditional method was to plant the tree as a multiple leader training system, grown on vigorous seedling rootstocks Mazzard (P. *avium* L.) or Mahaleb (P. *mahaleb* L.) at a density of 250 to 400 trees per ha (Whiting et al., 2005).

New rootstocks that reduce the tree size and improve fruiting sites are changing the traditional style of planting and training systems. Growers are currently planting sweet cherry orchards at a spacing of 1.5 to 3 m within the row and 3 to 5 m between rows (660 – 1000 trees per ha). The size-controlling rootstocks make this possible in that they fill much less space but come to production sooner and have many fruiting sites. Rootstocks like the Gisela® (*P. cerasus* x *P. canescens*) series from Justus Liebig University (Giessen, Germany) are size controlling rootstocks that reduce the size of the tree by up to 45% compared to Mazzard. This breeding program was started in 1965 and continued for about 25 years of intensive work in breeding and pre-selection (Gruppe, 1985). These rootstocks have economic potential to help solve the challenges that growers have been facing in reducing the unproductive time period during orchard establishment and the inefficiency of manual labor during harvest (Santos et al., 2005). Throughout the industry the consensus is that size controlling rootstocks are having a large impact on the future of commercial cherry production (Robinson et al., 2007; Sitarek et al., 2005; Santos et al., 2007) and are likely to be essential for commercial success (for example Trefois, 1981; Claverie et al., 1989; Edin, 1989, 1993; Bargioni, 1996). However, introduction of new size controlling rootstocks to production is often risky because some cultivars are not physiologically compatible with them (Sitarek and

Grzyb, 2007). This increases the challenge for nurseries to propagate new cultivars with new rootstocks.

New sweet cherry orchard systems are using these size controlling rootstocks at a higher than traditional tree density to improve economic returns and labor efficiency (Whiting et al., 2005). Higher densities increase the start up costs which drive growers to find alternate methods of planting a cherry orchard. In the apple industry, growers plant orchard blocks by different quality grades, and even alternate planting materials to start their orchards. A key goal in orchard establishment is to keep tree growth and development uniform so that production and harvest operations will be synchronized and have uniform yield. Buying nursery stock trees at one size grade provide this uniformity. Even buying multiple sizes but keeping them in blocks is still effective. Problems occur when smaller caliper nursery trees (or "number two" grades that are discounted) are used sporadically in the orchard. "Number two" grade is typically small and usually have scars and other defects make them a poor investment.

An alternate planting system being used more frequently in the apple industry is known as "sleeping eyes." These sleeping eyes are a dormant bud grafted on a single rootstock that is harvested in the spring at the nursery and sold to the orchard manager at planting time (Figure 1.5). Where a standard nursery tree is grafted in the fall over-winters in the field and then grown and trained into a tree during the next year; then harvested, stored and sold in the following spring. Recent research trials in apple have made comparisons between nursery standard trees, one year whips, and sleeping eyes (Robinson and Hoying, 2005). Planting a density of 850 trees per acre then feathered standard nursery trees are most profitable, but if planting 1,600 to 3,000 trees per acre,

the up-front investment for feathered trees may be too high, and the sleeping eyes are more profitable. Sleeping eyes require additional management effort and if not managed properly, they can be a poor investment (Robinson and Hoying, 2005; Warner, 2006). Sleeping eyes have potential for high risk and high success because of the nature of the product. When the orchard manager purchases a sleeping eye it is then his responsibility to grow the tree as if a nurseryman. Sleeping eyes can be one third to one half the price of a finished nursery tree. No research is available for using a sweet cherry sleeping eye planting system.

Nursery Tree Industry

Nurseries are the home of propagation in large numbers: their purpose is to grow a tender product through the difficult stages and then sell a hardy product to an orchard grower at a reasonable cost. Some of the first nurseries were rootstock nurseries, growing large numbers of roots available for buyers to plant and graft their own fruit varieties. As the size of orchards grew and the demand for trees increased, nurseries began to transform operations to produce finished grafted tree nurseries. Many growers found it difficult to grow their own nursery trees due to many different kinds of inputs and time to care for them; many factors go into the nursery tree process.

Through the 1980's typical sweet cherry tree production typically was around 30,000 for a large scale nursery. The world sweet cherry surge commenced around 1991; the Northwest nursery's tree sales began to increase by 1993. The following figures demonstrate the current trend in sweet cherry tree sales for all of the nurseries in the states of Washington and California (Figure 1.2 and Figure 1.3) respectively. By 2007 on average, one nursery in Washington State was producing 200,000 sweet cherry trees.

The rapidly changing demand for fruiting varieties and the increase in numbers for the sweet cherry industry strained nursery operations. Among the change, were the introduction of new precocious semi-size controlling and size controlling rootstocks. [Traditionally only a few rootstocks (Mazzard and Maheleb) were available for the orchard grower, and these rootstocks were grafted with one of only a couple of cultivars ('Bing' and Rainier')]. Gisela® series rootstocks have become the most requested sweet cherry rootstock series today but not the most produced (Figure 1.4). These rootstocks were developed in 1960 in Europe and didn't see American soil until 1980. These Gisela® rootstocks are very precocious and heavy bearing. For example Gisela® 5 can begin to flower and fruit in the second year and achieve full cropping in the 5th year (Franken-Bembenek, 2005). The heavy bearing helps eliminate the problem of cropping risks (Santos, et al., 2007) resulting from to frosts in the spring.

These precocious sweet cherry size controlling rootstocks are derived from species other than, or are hybrids with *Prunus avium.* These hybrids can be severely affected by infection with pollen-borne viruses such as prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV), where as the traditional *P. avium* and *P. mahaleb* rootstocks are tolerant (Lang and Howell, 2001). [Recent research (Lang *et al.,* 1997; Lang *et al*., 1998) revealed that trees on some these new dwarfing rootstock hybrids bred at Justus Liebig University (Giessen, Germany) exhibited detrimental reactions to infection of PDV.] Along with the promise of early success, these new precocious rootstocks bring uncertainty and frequent failure to the traditional nursery propagation systems, so new methods of handling and grafting them have to be developed.

The nursery process starts by selecting popular rootstocks. Propagation methods for commercial rootstock growers include tissue culture, seeds, and malling beds. Cherry rootstocks are either tissue cultured or grown from seed. If produced from seed, the seed crop is harvested from a seed source (Mazzard or Mahaleb), treated, and planted in the fall for spring germination. With a full year of watering, fertilizing, weed control and cultivating, a seedling crop can be harvested. Each rootstock is then dug, graded, and palletized for the winter storage. If the rootstock is tissue cultured then a complete year of time and labor is saved but the product is smaller. These rootstocks are then transported to the field for planting. All rootstocks are planted in the spring and that fall the nurseryman will graft a fruiting variety to the root by using a "T-bud" or more common today, a "chip-bud" (Hartmann and Kester, 1975). Buds are cut about 2 to 3 cm long from the bud stick (Figure 1.6) and an equal cut is made on the rootstock where the cambium layers are matched (Figure 1.7). Each bud is then sealed by a plastic strip wrapped around the bud holding it flush to the cut for duration of a month and a half (Figure 1.8). In October each plastic strip is removed (Figure 1.9). The rootstock with its new bud is then left out through the winter in the field to start growing next spring. The top of the rootstock is removed flush to the new grafted bud in March and the bud begins to grow into a finished nursery tree ready for harvest that fall. During that second year many hours of hand labor is spent training the tree to stakes for straightness and removing the sucker and low branches. The tree is then dug, sorted, palletized and stored through the winter and sold for planting in the spring, therefore this entire process is conducted over two years with a third year if the propagation of the rootstock is considered.

The economics of a nursery are complex, for a traditional seedling rootstock sweet cherry tree to be fully grown from seed to tree takes 4 years in the nursery. Many hours are needed as well as machinery and skills to handle all the different stages of the nursery process. In those four years the nursery is faced with all types of challenges; frost damage on the seed crop, poor germination in the spring, sudden death of rootstocks, wind breakage of the tender whips, etc. Propagation problems are increasing as new varieties and rootstocks are released commercially. Much research has been preformed on assessing bud-take and grafting success with *P. avium* in the nursery between various cultivars and rootstocks and external influences (for example Howard and Vasek, 1988; Feucht and Schmid, 1988; Schimmerlpfeng, 1988; Kappel et al., 2005; Santos et al., 2005; Sitarek and Grzyb, 2007; Santos et al., 2007). Nurseries over-propagate anticipating losses due to various reasons, and each loss represents wasted time and resources. Sweet cherry propagation problems in the nursery caused by PDV include poor bud "take" (Proebsting et al., 1995) and a reduction in tree growth (Gilmer et al., 1976; Nyland et al., 1976). With the cherry industry changing and moving to be more efficient and economical, nurseries need to make that transition also. Without the research, experience, and understanding of these problems such as virus infections, incompatibility, and the introduction of new cultivars and rootstocks it is difficult for nurseries to be efficient and to supply a cheaper product.

Prune Dwarf Virus

There is published evidence that virus infection of orchard trees can cause heavy losses (Deogratias et al., 1989; Proebsting et al., 1995; Lankes, 2007; Hansen, 2006). PDV exist as many distinct strains (Crosslin and Mink, 1992) whose effects range from

severe to virtually symptom-less (Proebsting et al., 1995). PDV is prevalent and widely distributed in sweet and sour cherries throughout North America. The initial symptoms in sweet cherry are the appearance of large chlorotic rings as well as stunting and leaf malformation. PDV is readily transmitted by budding, grafting, or through pollen and seeds of several stone fruits: mahaleb, sweet cherry, myrobalan, and sour cherry (Gilmer et al., 1976). In the nurseries, infected materials reduce grafting success at times as much as 40 to 50%. PDV causes the cherry tree to form flower buds at lateral nodes that would normally produce vegetative buds (Basak et al., 1962). Particles of PDV are isometric "spheres" about 22 mμ in diameter; it is one of the smallest plant viruses (Gilmer et al., 1976).

Several strategies are being investigated to reduce the impact of PDV. Continuous research is being conducted on rootstock tolerance testing (Lankes, 2007) as well as eradication and establishment of virus certification programs (Mink and Aichele, 1984). Natural cross protection has been researched (Howell and Mink, 1988) for protecting orchards from PDV and PNRSV pollen borne viruses. There are different methods for detecting and analyzing samples for PDV and PNRSV viruses. ELISA (Enzyme-Linked Immuno Sorbent Assay) and RT-PCR (Reverse Transcription-Polymerase Chain Reaction) are two popular methods used to detect viruses.

The ELISA method is a biochemical technique using mainly immunology techniques to detect the presence of an antibody or an antigen in a sample. The process affixed a sample or antigen surface and then allowed a specific antibody to bind to the antigen. The antibody is linked to an enzyme, and in the final step a substance is added that the enzyme can convert to some detectable signal. However, ELISA has its own

limitations, lacking the sensitivity to reliably detect viruses when they occur at low titer (Borja and Ponz, 1992; Golino et al., 1992; Mathews et al., 1997; Rowhani et al., 1992).

RT-PCR has the potential to be an extremely sensitive alternative to ELISA (Rowhani et al., 1998). This method amplifies a defined piece of a RNA molecule. The first step is to make complementary DNA from messenger RNA through the process of reverse transcription. Then the second strand reaction occurs and the sample is taken through cycles of temperature treatments to multiply the sequences of DNA to a detectable level. The PCR product is then loaded onto an agarose gel for electrophoresis.

1.2 – Problem Statement

The sweet cherry industry is rapidly growing and changing. Competition and cost of production is forcing many forms of change on the sweet cherry industry. The cost and shortage of labor is increasing, and the profit margin and efficiency in production of common varieties are decreasing. The commercial release of new cultivars and rootstocks require research and experience to make their propagation proficient. Nurseries are the ones confronting the problems in their propagation methods. New varieties incompatible to new rootstocks is becoming a larger problem. Virus infected material only decreases propagation success and growth. New ideas for planting systems are changing the overall product sold from the nurseries. This is because of the increasing costs in producing sweet cherries with labor and start up costs. The concept of sleeping eyes for orchard establishment is being proposed in apples and has a possibility to be extended to cherries. To date, some research has addressed the role of common viruses in graft failure and tree growth in the nursery. There are no published reports

evaluating tree growth under a sleeping eye planting system. In addition, most published research was conducted on traditional varieties and rootstocks.

1.3 – Objectives

The goal of this research was to evaluate the potential for reducing orchard establishment costs from reducing tree losses in the nursery and utilizing a novel planting system. More specifically, the objectives of my research are to:

- assess the relationship between bud position/age and virus titer in correlation to growth and grafting success in the nursery
- assess tree survival and growth between the sleeping eye and standard planting systems
- analyze the role of Prune dwarf virus on grafting success and orchard growth, for nine sweet cherry scion-rootstock combinations

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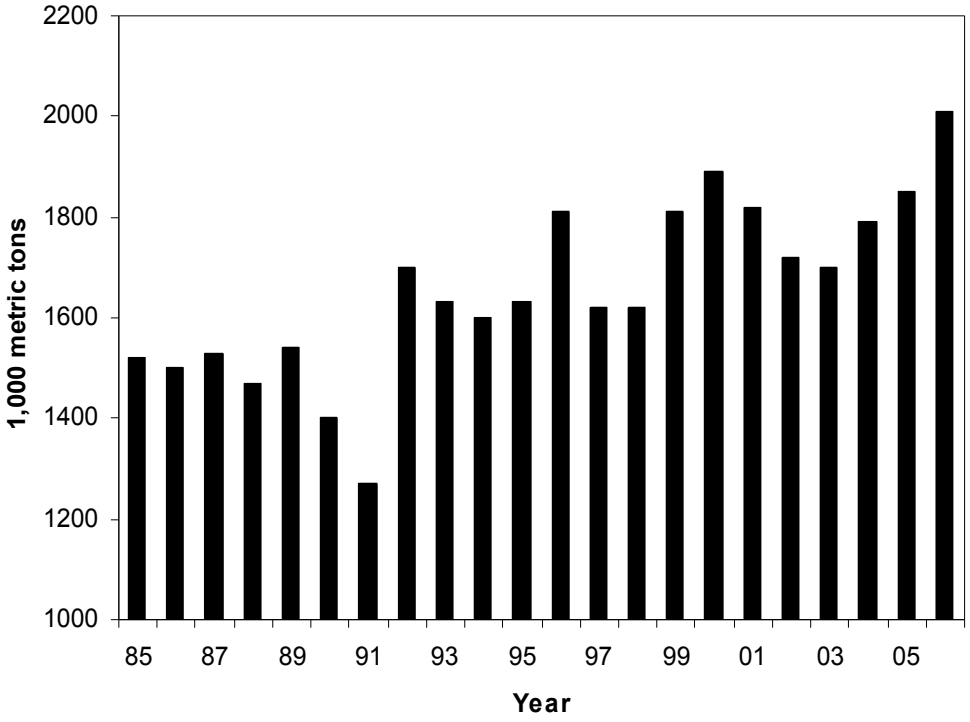

Firgure 1.1 – World sweet cherry production (O'Rourke, 2007).

Table 1.1 – Total boxes shipped by variety for the northwest in 2006 and 2007, 'Rainiers' reported in 15 pound equivalents and all others reported in 20 pound equivalents; n.a. is data not available (BJ. Thurlby, NWcherry).

Variety	2005 Boxes	2006 Boxes	2007 Boxes
Bing	6,526,074	7,358,475	6,571,859
Chelan	147,653	535,688	418,785
Lambert	162,964	187,981	248,444
Lapin	1,666,828	1,651,590	1,238,488
Rainier	1,265,893	1,526,364	1,687,429
Sweetheart	468,815	1,175,063	1,509,243
Vans	115,302	71,283	98,804
Staccato	n.a.	36,041	117,293
Tieton	n.a.	25,810	52,679
Skeena	n.a.	388,411	404,408
TOTAL	11,124,782	14,777,432	14,721,863

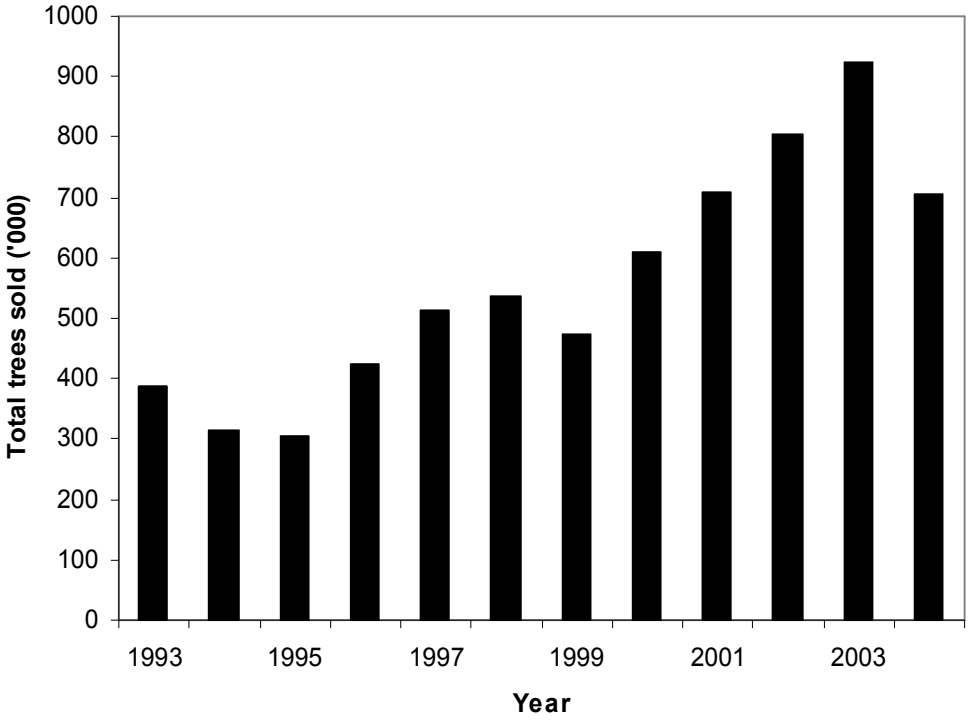

Figure 1.2 – Washington state sweet cherry tree propagation numbers for 1993-2004 (Fitch and Marshall, 2004).

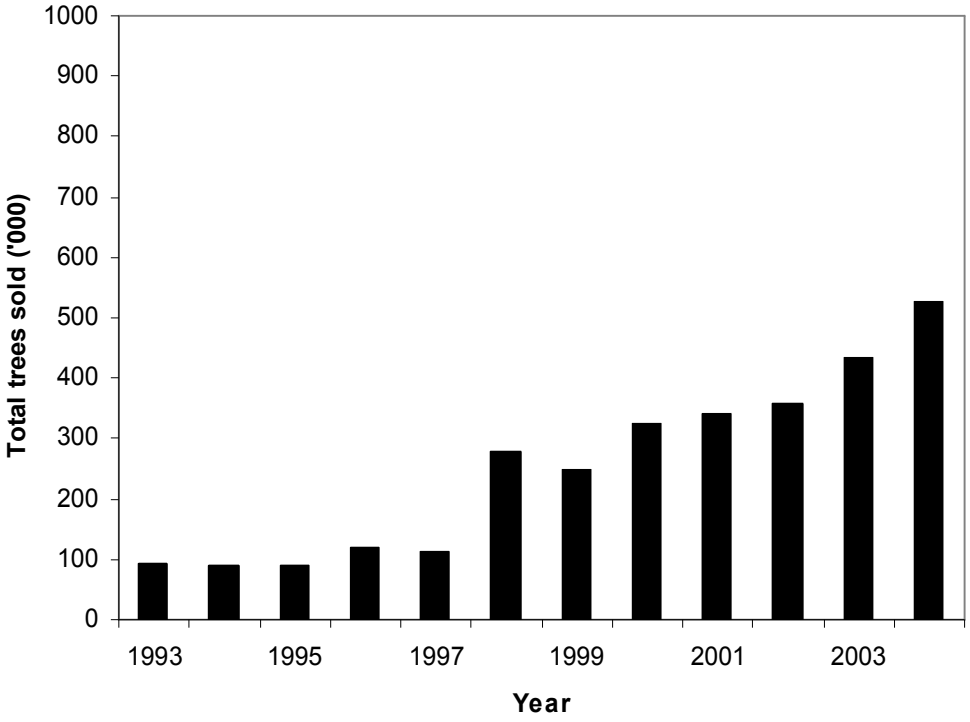

Figure 1.3 – California State sweet cherry tree propagation numbers for 1993-2004 (Fitch and Marshall, 2004).

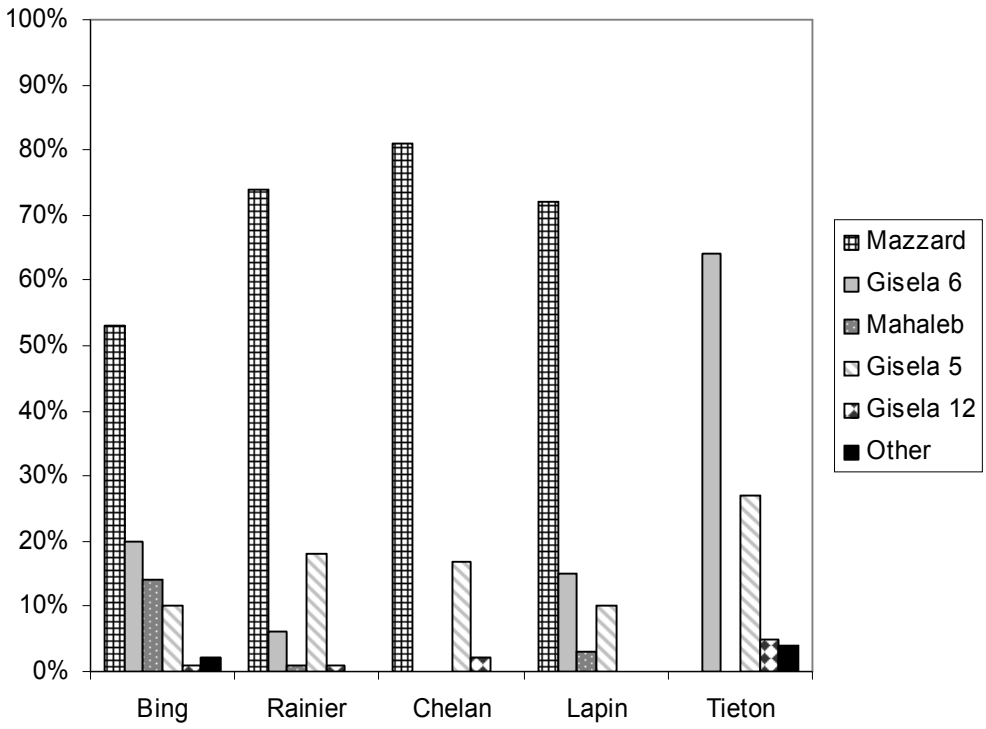

Figure 1.4 – Rootstocks used on trees sold by Washington nurseries in 2003 (Fitch and Marshall, 2004).

Figure 1.5 – An apple sleeping eye tree before harvest at a Willow Drive Nursery Ephrata, Washington.

Figure 1.6 – ‘Chip’ budding 2005 at Willow Drive Nursery Ephrata, Washington.

Figure 1.7 – Matching the cambium layers in the ‘chip’ budding method, 2005 at Willow Drive Nursery Ephrata, Washington.

Figure 1.8 – The use of polyethylene strips for securing and healing of the ‘chip’ buds, 2005 at Willow Drive Nursery Ephrata, Washington.

Figure 1.9 – Removal of the polyethylene strip once the ‘chip’ bud has healed in approximately 4 weeks after grafting in October 2005 at Willow Drive Nursery Ephrata, Washington.

CHAPTER 2 – THE ROLES OF BUD POSITION AND VIRUS TITER ON GRAFTING SUCCESS AND GROWTH

2.1 – Abstract

Poor graft success and weak tree growth in the nursery increase tree costs to growers. Current season shoots used as budwood for nurseries vary tremendously in length and caliper yet no published research has investigated the role of the position of buds on nursery budwood and the graft success and subsequent nursery growth. In the current trial, budwood sticks of varying lengths were collected from 'Bing' and 'Tieton' trees diagnosed with PDV in 2005 and 2006. In September of both years buds were alternately grafted to a rootstock (Mazzard (P. *avium* L.) or Gisela®6 (P. *cerasus* x P. *canescens*)) for survival and growth observations or analyzed by ELISA and PCR for virus titer. Graft success and tree growth were assessed in the season subsequent to budding by bud position and budwood caliper. Bud position had a significant effect on tree survival. The basal three buds were 63% more likely to result in a tree loss due to lack of growth. Approximately 50% of the buds that didn't produce trees were floral. There were significant differences in growth between rootstocks, seasons, scion varieties, and bud position. Trees grown on Gisela®6 were more uniform in growth than those on Mazzard. Bud position had a significant effect on trunk diameter after one year. A negative relationship was observed between bud stick length and its diameter. Analyses revealed no clear relationship between the diameter of the bud stick at the point of bud removal and the growth of grafted trees in the subsequent year, irrespective of genotypes. Virus titer and bud position could not be analyzed because ELISA values were too low for a reliable confidence threshold.

2.2 – Introduction

'Bing' cherry has been grown commercially in the Pacific Northwest for more than a century and many trees are infected with the pollen-transmitted virus Prune Dwarf Virus (PDV) (Proebsting et al., 1995). It was not until 1961 that the Washington State fruit tree virus certification program released the first virus-free 'Bing' cherry tree. Newly planted trees are highly susceptible to infection from transmitted via pollen from nearby PDV-infected orchards (Mink and Aichele, 1984). Washington State regulations require that all virus free scion blocks must be no less than 100 feet from any non-registered member of the rosaceae family. According to Mink and Aichele (1984), an 84% infection rate is probably a realistic representation of the older cherry orchards in Washington. PDV causes chlorotic spots and rings on the leaves in sweet cherry and is readily transmitted by budding and grafting with infected mother trees (Li et al. 1996) and through pollen and seeds of several stone fruits including sweet cherry (*Prunus avium* L.), Mahaleb (P. *mahaleb*), and sour cherry (P. *cerasus* L.). Severity of symptoms varies greatly among cultivars. Symptoms are particularly severe in sour cherry (P. *cerasus* L.) (Rampitsch et al., 1995). PDV is best known as the causal agent for sour cherry yellows, a serious disease in 'Montmorency' cherry (Proebsting et al., 1995).

These ilarviruses (PDV and PNRSV) are common in older trees and typically pose no problems for cherry trees on P. *avium* (Mazzard) or P. *mahaleb* (Mahaleb) rootstocks (Lang and Howell, 2001) However, recent research (Lang et al., 1997; Lang et al., 1998) revealed that trees on Gisela®6 rootstocks exhibited detrimental reactions to infection by PDV. Lang and Howell (2001) report that trees on some of these new rootstocks, particularly those Giessen hybrids which utilized P. *fruiticosa* as a parent

(e.g., Gisela 1, Gisela 10) exhibit rapid hypersensitive reactions including graft union gum exudation, premature abscission, and tree death within one to two growing seasons. Some genotypes utilizing *P. cerasus* or *P. canescens* in the parentage also exhibited hypersensitivity while others (e.g., Gisela 7, Gran Manier 79 [Camil]) exhibited an initially less severe "sensitive" reaction.

There is insufficient supply of virus-free propagation material to supply to demand of the rapidly growing sweet cherry industry, particularly for the newly-released cultivars. Many nurseries therefore are forced to propagate with budwood that is not certified virus free. Propagation with PDV-infected budwood can result in low graft success (Proebsting et al., 1995) and weak tree growth (Gilmer et al., 1976; Nyland et al., 1976). As well as poor growth in the tree, PDV can delay maturity in fruit development up to 2 weeks compared to virus-free trees (Howell and Mink, 1984). Fruit from the virus-infected trees therefore are not harvested with the rest of the crop, and are often lost. PDV also causes the formation of flower buds at lateral nodes on current season extension shoots that would normally bear vegetative buds (Basak et al., 1962). This effect reduces the fruiting and yield potential of trees because single flowering nodes induce by PDV become unfruitful blind wood in subsequent years (Proebsting et al., 1995). Further, obtaining budwood free of these viruses is very important to tree production because virus-free clones grow much more vigorously in the nursery than virus-infected tree (Gilmer et al., 1976; Nyland et al., 1976).

Virus-free budwood can be generated via high temperature followed by apical meristem culture (i.e., thermotherapy) (Deogratias et al., 1986; Deogratias et al., 1989; Mink et al., 1998). However, many woody species including sweet cherry are heat

sensitive and the production of virus-free plants by thermotherapy is difficult (Lenz et al. 1983). Micrografting *in vitro* and meristem tip culture are used with thermotherapy and chemotherapy to obtain virus-free fruit trees (Deogratias et al., 1989). Applying therapy procedures to plant material *in vitro* allows control of components of the nutrient media and other environmental factors that affect survival and regeneration of virus-free shoot tips (Spiegel et al., 1995). Using virus-free budwood, nurseries could improve graft success and tree growth and reduce costs. Further, fruit producers in turn receive a healthier tree that should reduce transplant stress and improve growth and orchard establishment.

Better understanding of virus distribution could be beneficial for nursery production in that it would facilitate budwood selection by avoiding higher virus titers, which would increase bud survival and efficiency. Fridlund (1973) stated that apple chlorotic leaf spot virus (ACLSV) in apples does not totally invade the current-season growth, and that infected buds occur primarily toward the basal ends of current-season growth, but usually does not infect all buds on apple bud sticks. Snir and Stein (1985) suggested that fluctuations of virus titer are caused by an inherently irregular distribution of virus in the tree. Deogratias et al. (1989) stated also that virus distribution may also be influenced by the flexuous or isometric forms of the virus particles. However, detailed knowledge concerning distribution of deciduous fruit-tree viruses in their hosts is generally lacking. Moreover, the relationship between virus titer and bud position and graft success and nursery growth has not been documented.

The objective of this research was to understand better the effects of bud position in graft success and growth of nursery trees.

2.3 – Materials and Methods

Plant material - In early September of 2005 and 2006 current season shoots were collected from 20+ year old 'Bing' and 'Tieton' trees grown at the Washington State University Roza experimental farm. Trees had previously tested positive for PDV. Ten bud sticks were collected from random current season extension growth by pruning flush at the point of origin. Each shoot was defoliated and measured for length. The position of each axillary bud was measured in relation to the bud stick base. Immediately below each bud, shoot diameter was determined by digital caliper. Each bud was removed and alternately stored for subsequent virus analyses or utilized immediately for grafting. The measurements taken and the bud codes corresponding to the bud location on the stick are illustrated in Figure 2.1. Buds assigned for grafting were grafted using the standard chip budding method (Hartmann and Kester, 1975) in the field on September 7, 2005 and 2006 at Willow Drive Nursery, Ephrata, WA. Buds were cut about 2.5 to 3 cm long from the bud stick and matched on the rootstock 10 to 15 cm above the ground. With each graft the cambium layers of the scion and rootstock were aligned. All buds were grafted by the same person. Both cultivars were grafted onto both Mazzard (P. *avium* L.) and Gisela®6 (P. *cerasus* x P. *canescens*). All rootstocks were field-grown with 21 cm between plants within the row and 1.68 m between rows at Willow Drive Nursery, Ephrata, WA. Rootstock liners were raised following standard nursery practices with irrigation applied by T-tape (T-Systems International, Inc. San Diego, CA.). Rootstocks were planted in 2005 from either seeds (Mazzard) or tissue culture starts (Gisela®6). Mazzard rootstocks were nursery row seeds (from Willow Drive Nursery, WA.) grown in place, and all Gisela®6 (from Pro Tree Nursery, CA.) were tissue cultured, every root

remained in place for budding in fall of 2005. For the repeated trial in 2006, Mazzard rootstocks were used in place of seed starts and were planted at the same time as the Gisela®6 tissue culture starts.

Buds assigned to virus testing were refrigerated and transported to Prosser where every bud was halved longitudinally. One half was frozen in liquid nitrogen and stored at -80 ºC for future PCR analyses. The other halves were tested for virus presence by ELISA (Clark and Adams, 1977). Every ELISA sample was ground and mixed in a 5% BSA (Bovine serum albumin) (BSA, fraction V: Sigma Chemical Co.). The triple antibody-sandwich ELISA (TAS-ELISA) assay for PDV commenced with 96-well Maxisorp plates (NUNC) coated with 100 µl per well rabbit polyclonal antiserum (PDV-E: ATCC, Manassas, VA) diluted 1:500 in carbonate buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6) for 2 hr at 23 °C. The plates were washed three times with phosphate-buffered saline (PBS)(1.5 mM KH_2PO_4; 4 mM Na_2HPO_4; 137 mM NaCl; 0.2 mM KCl, pH 7.4) containing 0.05% polyoxyethylene sorbitan monolaurate (Tween 20)) and the excess solution was tapped out, and placed at 4 ºC overnight in a humidified chamber. Wells were blocked with 300 µl per well of 3% Carnation® skim milk powder in PBS-Tween 20 for 30 min and tapped out. Samples consisting of buds cut in two longitudinally were ground individually in porcelain mortars and pestles in 10 volumes of carbonate extraction buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.6; 0.5 mM polyvinylpyrrolidone molecular weight 40,000; 0.2 % powdered egg (chicken) albumin; 0.45% sodium diethyldithiocarbamate). Sample (100 µl) was added to each well and refrigerated overnight at 4 ºC. Plates were washed three times with PBS-Tween 20, the excess tapped out; then 100 µl of murine monoclonal antibody (PD A-3C) (Rampitsch et

al., 1995) diluted 1:3 in 3% Carnation® skim milk powder in PBS-Tween 20 was added and incubated at 37 ºC for 1 hour. Plates were washed again with PBS-Tween 20 three times and the excess solution was tapped out; and 100 µl of secondary antibody (alkaline phosphatase-linked goat anti-mouse serum (Gibco/BRL)) diluted 1:3000 in 3% Carnation® skim milk powder in PBS-Tween 20 was added and incubated for 2 hr at room temperature. The solution was tapped out and each well was filled with PBS-Tween 20 and allowed to sit for 3 min, tapped out and 100 µl per well substrate buffer (0.1% p-nitrophenyl phosphate (Sigma) in 9.7% diethanolamine, pH 9.6; 1 mM $MgCl_2$) and all plates were incubated at room temperature. Absorbance at 405 nm (A_{405}) was measured (Thermo-Labsystems Multiscan Asent plate reader with Labsystems assist robot Thermo electron Corporation: Waltham. MA).

A RT-PCR (reverse transcription polymerase chain reaction) test was performed to confirm the ELISA data and virus titer. The RT-PCR commenced with the extraction of RNA with the use of an RNeasy kit (Qiagen, Valencia, CA). A sample of approximately 0.1 grams was ground with 450 µl buffer RLT (lysis buffer containing guanidine thiocyanate) and β-Mercaptoethanol (β-ME) and the homogenate was then incubated at 56 ºC for 3 min. The sample was then transferred to a QIA shredder spin column and centrifuged for 2 min. The solution was then transferred to a 1.5 ml tube and mixed with a 0.5 volume ethanol (96-100%) and 650 µl of the solution was then placed in an RNeasy spin column and centrifuged for 15 s. The column was then removed and placed in a 2 ml tube and 700 µl of RW1 (Qiagen, Valencia, CA) buffer was added and centrifuged for 15 s. The column was then placed again in a 2 ml tube and 500 µl of RPE (Qiagen, Valencia, CA) was added and centrifuged for 15 s. The column was then placed

in a 2 ml tube and 500 µl of RPE was added and centrifuged for 2 min. The spin column was then placed in a final 2 ml tube and centrifuged for 1 min to dry. The RNeasy spin column was then placed in a 1.5 ml collection tube and 50 µl of RNase-free water was added and centrifuged for 1 min. The sample was then stored at -20ºC overnight. A duplex reaction was then conducted for PDV and PNRSV (Prunus necrotic ring spot virus) by adding 24 µl of the reaction mix to 1µl of sample. The reaction mix consisted of 9.5 µl of sterile double distilled water, 12.5 µl 2x Rxn mix, 0.25 µl of PNRSV 16 primer ((20 µM) 5'-ATA TTG GCA GGT ACA GAA GG-3'), 0.25 µl of PNRSV 17 ((20 µM) 5'-TTC GGA GAA ATT CGA GTG TGC-3') (Vaskova et al., 2000), 0.25 µl of PDV 3 primer ((20 µM) 5'-CCC TCC TGC TGG TTT TGT TA-3'), 0.25 µl of PDV 5 primer ((20 µM) 5'-CAC GGA CTT TCA TGG TGT AA-3') (Rampitsch et al., 1995), and 1µl SSIII/Platinum Taq (Invitrogen, Carlbad, CA). The BioRad thermocycler procedure consisted of 1 cycle of 55 ºC for 30 min and 94 ºC for 2 min, then 35 cycles of 94 ºC for 15 s. and 60 ºC for 2 min and 68 ºC for 90 s., and finally with 1 cycle of 68 ºC for 30 min and then held at 4 ºC and stored. A 2.4% agarose gel LE (low electroendosmosis) in tris-acetate-EDTA buffer (TAE) was produced and electrophoresis commenced at 90 volts for 100 min.

In the following spring, data were collected on graft success, indicated by growth and development of the bud. Between April and October, 2006, growth measurements were made every 30 days. These included trunk diameter ca. 8 cm above the graft union and tree height (graft union to upper most growing point). This study was conducted in a repeated measures design with 10 replicates for two different seasons for each combination (n=674). GLM procedure and a FREQ procedure of the Statistical Analysis

System computer package were used to analyze data (SAS Institute, 1982). Regression analysis also was used to assess relationships.

2.4 – Results and Discussion

Graft success – In April of 2006 and 2007, buds grafted in the previous fall began to break dormancy. No genotypic effect on the timing of the initiation of bud growth was observed. There was significant difference between growing seasons; graft success in 2007 was less than in the previous year (Figure 2.2). This is interesting because nursery practices were no different between the two seasons other than the region of the nursery field where the trees were grown (ca. 200 m apart). This suggests that environmental conditions may affect graft success. Overall, and irrespective of bud position, there were minor differences between 'Bing' and 'Tieton' in graft success, but nothing statistically significant. Between the rootstocks, buds grafted onto Mazzard had ca. 15% greater survival rate compared to Gisela®6 except for budding of 'Tieton' in 2006. The greatest incidence of graft failure of ca. 40% was with 'Tieton'/Gisela®6 from budding in 2006. The lowest incidence of graft failure was for 'Bing'/Mazzard from budding in 2005 when ca. 90% of the trees survived and grew. The difference in graft success between these two extreme, highlights the potential variability between years and among genotypes.

Bud position had a significant effect on graft success (FREQ Procedure). Figure 2.3 illustrates that the basal three buds were 63% more likely to result in a loss. Much of the tree loss from basal buds was due to their being reproductive. About ca. 50% of the buds that didn't grow into a tree were floral. Generally, the single, simple basal buds on current season extension growth of sweet cherry are reproductive and the number varies by cultivar. Further, PDV infection causes the cherry tree to form additional flower buds

at lateral nodes that would normally produce vegetative buds (Basak et al., 1962). Whether these floral buds were due to virus infection or inherent characteristic of the cultivar is not clear. Regardless, it seems prudent to avoid using the basal buds for grafting due to high potential for failure. Alternatively, the increased graft failure from non-reproductive basal buds could be due to their having high virus titer. Previous research has found that virus titer in apple increases with distance from the shoot apex (Fridlund, 1973; Fridlund, 1982). Interestingly, buds on the tip of the bud-sticks failed too (Figure 2.3). This is likely due to their being immature (Howard and Vasek, 1988).

Seasonal growth –There were significant overall effects of rootstock ($P < 0.0003$), year (with larger growth in 2007 ($P < 0.0001$)), cultivar ($P < 0.0121$), and bud positions based on trunk diameter ($P < 0.0001$). Trunk diameter at the end of each season was converted into standard nursery grading scale of 1", 3/4", 5/8", 1/2" and 7/16" caliper. Trees grown on Gisela®6 were more uniform in growth than those on Mazzard. Gisela®6-rooted trees were ca. 96% 3/4" and 5/8" size, where Mazzard grew 85% in the same range. 'Tieton' produced the largest trees with ca. 8% being 1" or larger in trunk diameter. I hypothesize that 'Tieton' is a more vigorous variety than 'Bing'. Differences among trees are not likely related to tree nutrition considering each plant received the same amount of nutrients and time to build up reserves in the fall.

For each cultivar/rootstock combination, bud position on the budwood had a significant effect on trunk diameter at digging (Figures 2.4, 2.5, 2.6, 2.7). Larger trees receive a small premium price and for the nursery, each tree has the same cost no matter what the size. 'Bing' had 84% death rate from grafting with buds from the basal position (n=19). In addition, 'Bing' buds from the budwood's apical end also had a high mortality

rate (Figure 2.5). Buds at the tip demonstrated weaker growth (Positions 11, 12, & 13). Figure 2.6 and 2.7 'Tieton'/Gisela®6 and 'Tieton'/Mazzard had grown the larger diameter trees from positions 5-12 (middle to 5 cm from the end of the sticks). Overall, across genotypes, it appears the best bud positions to use for grafting are those between ca. 5 cm from the tip to 15 cm from the base.

I recorded a negative relationship between bud stick length and its diameter for every cultivar/rootstock combination (data not shown). This is likely due to differences in the age of wood and greater secondary growth in older wood. The difference in branch diameter from the base to the terminal bud was greater than two-fold in most cases. The effect of bud stick caliper at each lateral node (i.e., bud position on the bud stick) on graft success and subsequent growth and vigor in the orchard was previously unreported. My analyses revealed no clear relationship between the diameter of the bud stick at the point of bud removal and the growth of grafted trees in the subsequent year for all genotypes assessed (Figure 2.8). For example, buds from 'Tieton' budsticks that had a diameter of ca. 6 mm yielded trees that ranged from 12 to 27 mm in caliper at the end of the nursery year (Figure 2.8D). It appears therefore that buds from small diameter bud sticks are not predisposed to creating smaller trees. However, bud position (i.e. age) on the bud stick did have an impact in grafting success and finished tree size.

Virus titer – Titer for each bud position could not be assessed because of the ELISA results were low for any reliable confidence threshold commonly used. Sutula et al. 1986 suggested the most commonly used methods for creating confidence thresholds for ELISA data were, 2x negative mean or negative mean + 4s; these thresholds give the lowest percentage of false negatives or false positives when analyzing the absorbance

levels. Figure 2.9 is a histogram of 'Tieton'/Gisela®6 samples for PDV and illustrating where the two thresholds are. The 'Tieton' histogram illustrates that all but two of the samples were below the negative threshold at the negative mean + 4s, and still the majority of the samples being below at 2x negative mean (all below the thresholds are virus free); data for 'Bing' were similar to 'Tieton'.

To verify that the low ELISA absorbance levels were not false negatives a RT-PCR was conducted to confirm the thresholds. This method (RT-PCR) is a more sensitive procedure for detecting viruses (Rowhani et al, 1998). PCR results confirmed that the lowest absorbance reading and highest were indeed positive infections (Figure 2.10). The ELISA data were therefore disregarded due to no confidence threshold being accurate after analyzing the same tissue sample with a RT-PCR test. I believe this was due to the timing of scion wood collection for budding. Samples for the current research were collected in early September, a timing of low natural virus titer (K. Eastwell, Pers. Commun.).

In summary, I report herein that it is important to consider bud position when grafting nursery trees because the extreme basal and apical buds have greater potential to fail. Prudent bud selection would avoid the buds on the basal 15 cm and the apical 5 cm of the budsticks. Further, buds from thicker budwood had no better graft success or growth in the nursery year (Figure 2.8).

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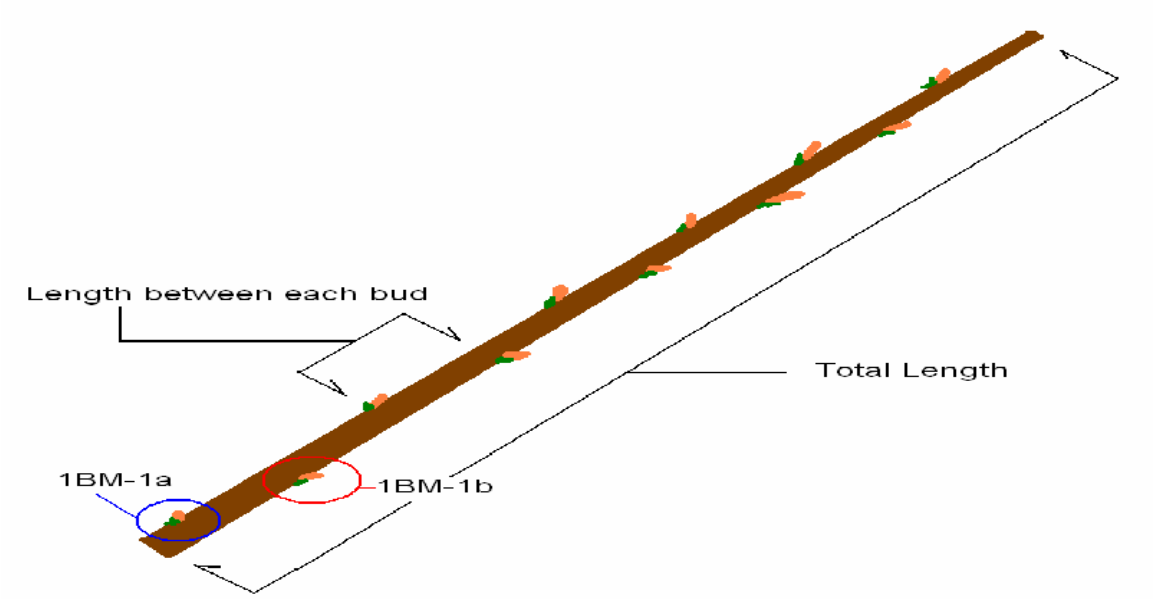

Figure 2.1 – The measuring and labeling approach for each bud for grafting or PDV analysis. (Example code: 1BM-1a translates to; Stick # 1, Bing grafted onto Mazzard, bud # 1, 'a' = lab analysis; where 'b' = budded in the field).

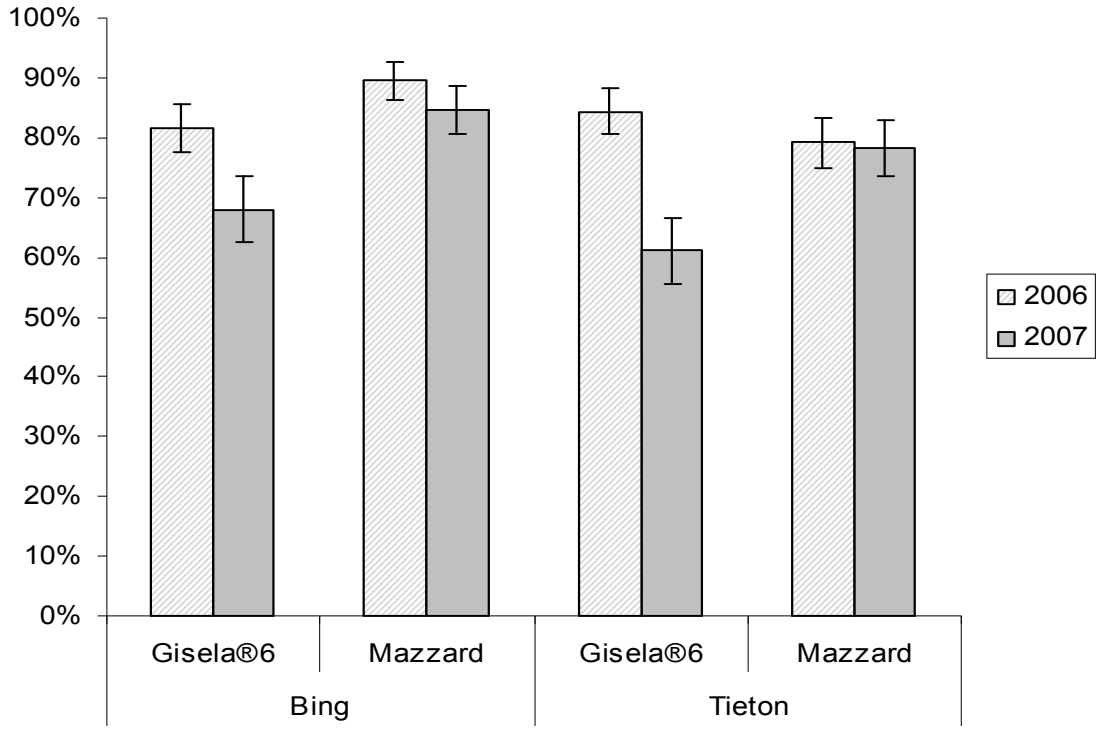

Figure 2.2 – Graft success for 'Bing' and 'Tieton' buds grafted on to Gisela®6 and Mazzard rootstocks in Ephrata, Washington in 2006 and 2007 (n=98).

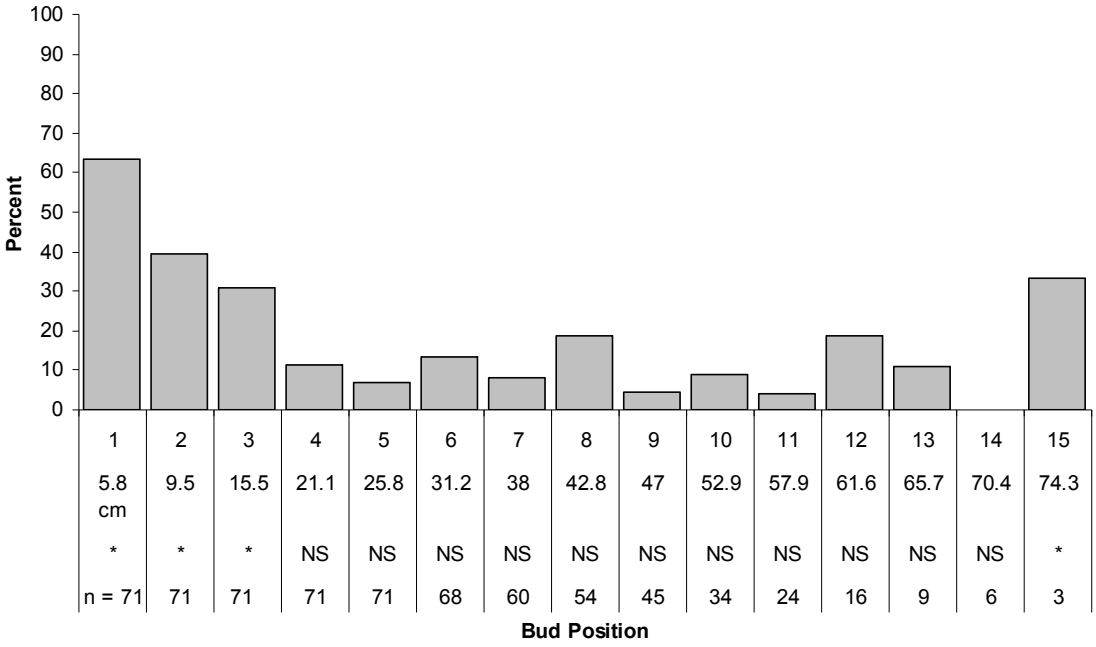

Figure 2.3 – Survival rate for chip budding in relation to the position of the scion-wood bud location (FREQ Procedure) accounting for all genotypes. Position #1 is the base of the scion-wood stick. N = the number of replicate bud sticks used in the study (not all the sticks used were the same length). * Significance at $P \leq 0.05$; NS = not significant.

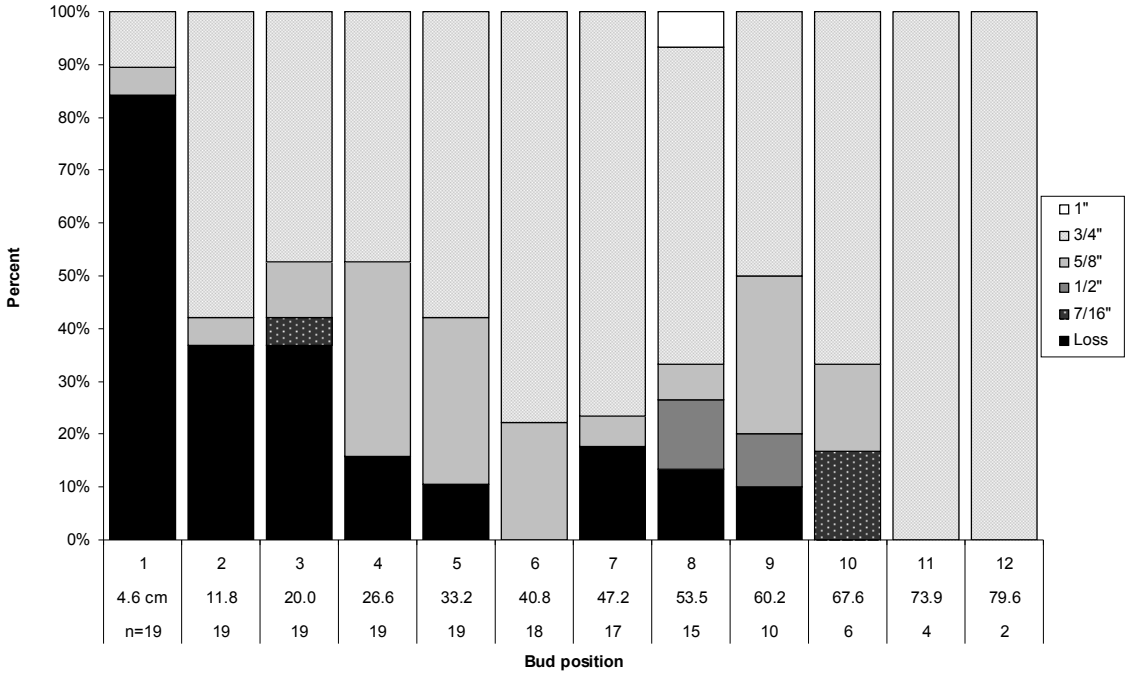

Figure 2.4 – Grade-out to nursery standard scale for bud position for 'Bing'/Gisela® 6 for both seasons (2006-2007). All buds are PDV infected and trees were propagated at Willow Drive Nursery Ephrata, Washington.

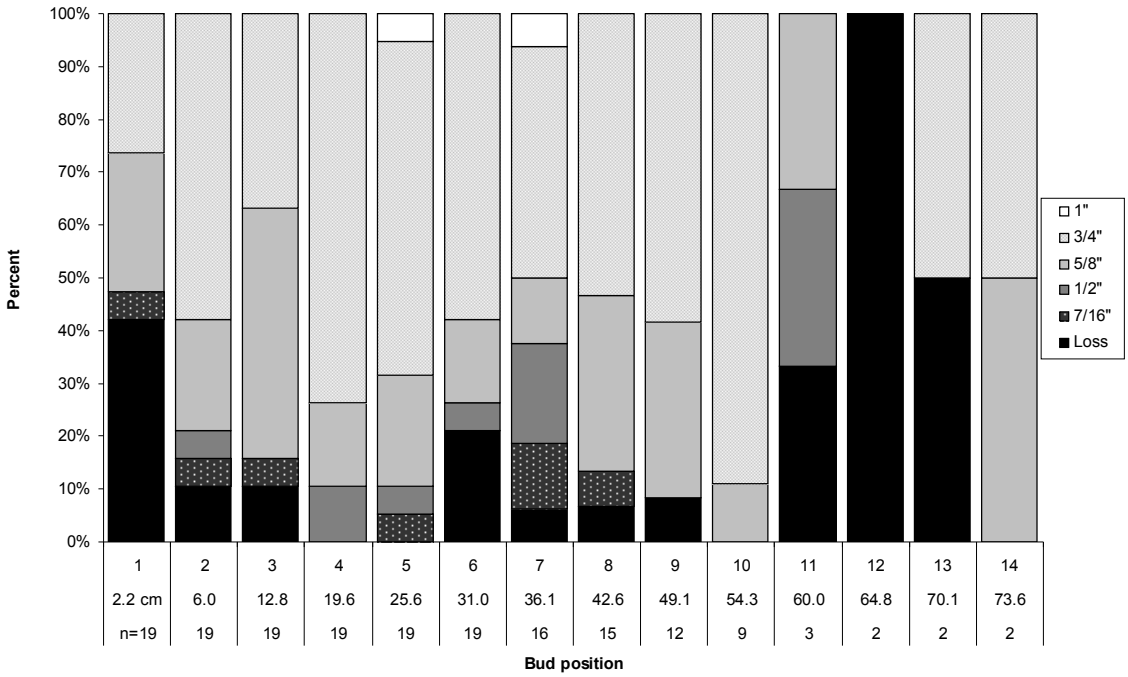

Figure 2.5 – Grade-out to nursery standard scale for 'Bing'/Mazzard for both seasons (2006-2007). All buds are PDV infected and trees were propagated at Willow Drive Nursery Ephrata, Washington.

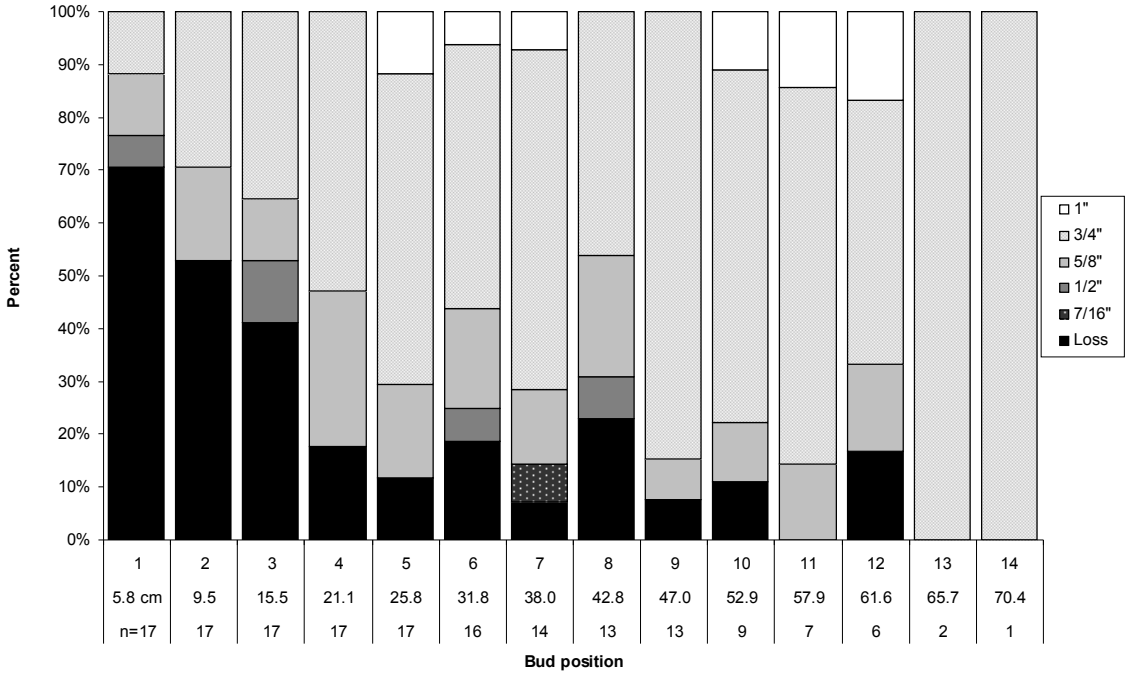

Figure 2.6 – Grade-out to nursery standard scale for 'Tieton'/Gisela® 6 for both seasons (2006-2007). All buds are PDV infected and trees were propagated at Willow Drive Nursery Ephrata, Washington.

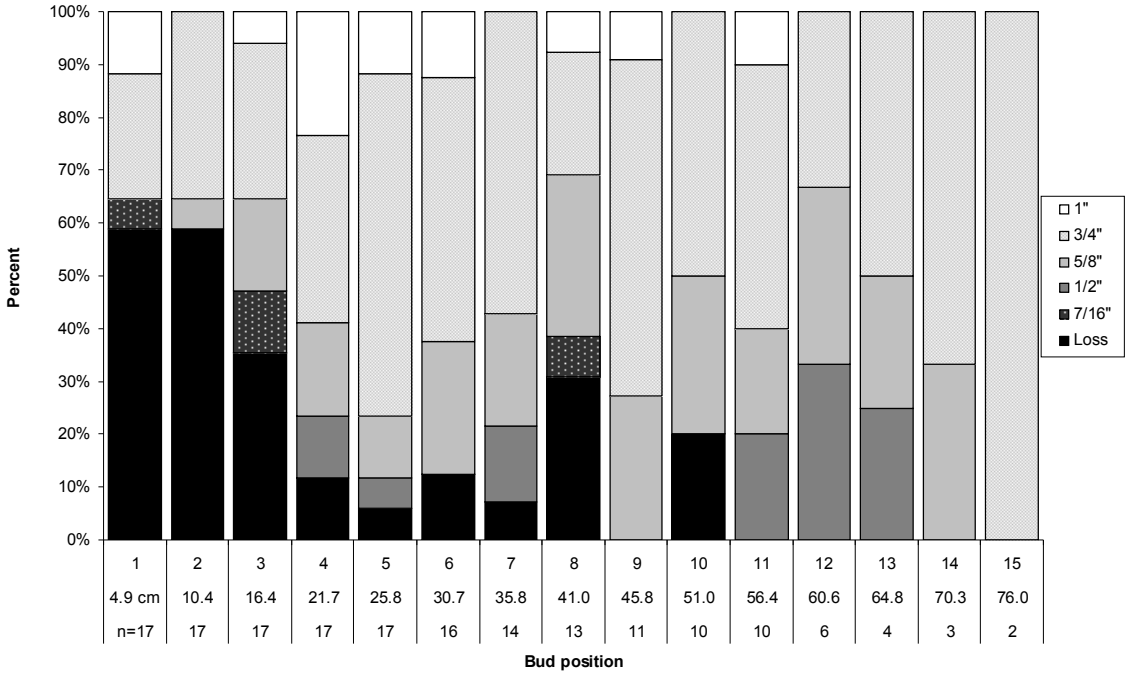

Figure 2.7 – Grade-out to nursery standard scale for 'Tieton'/Mazzard for both seasons (2006-2007). All buds are PDV infected and trees were propagated at Willow Drive Nursery Ephrata, Washington.

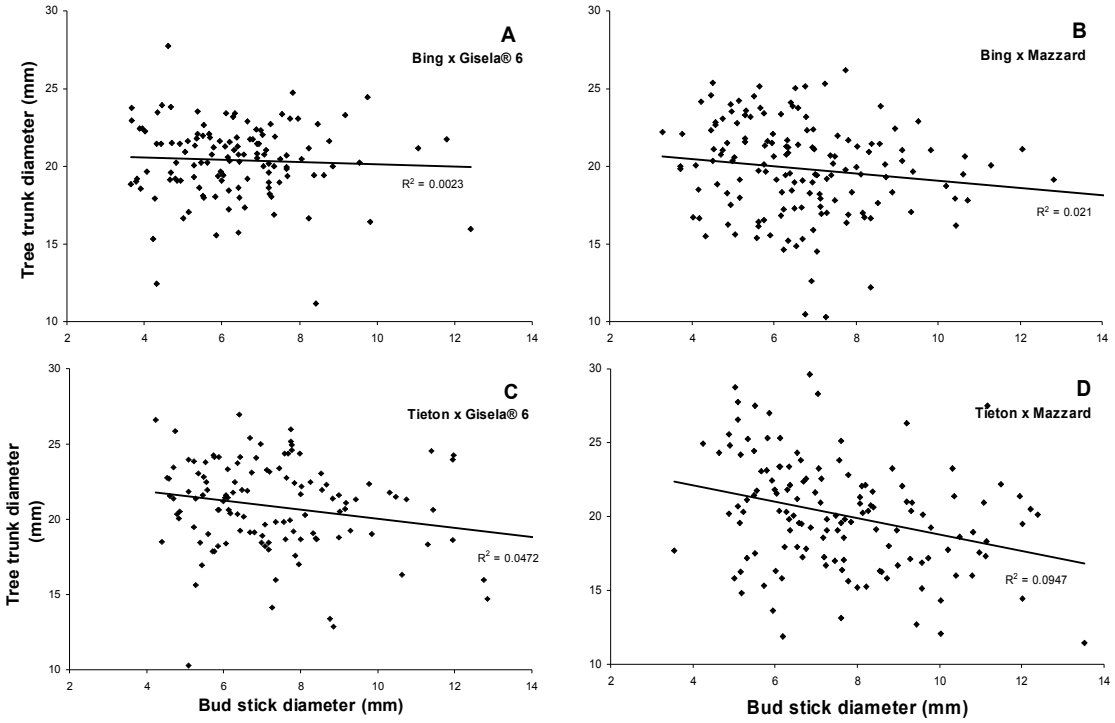

Figure 2.8 – Relationships between bud stick diameter at grafting in 2005 and 2006; and final tree diameter at the end of the subsequent growing season in 2006 and 2007 (n=165).

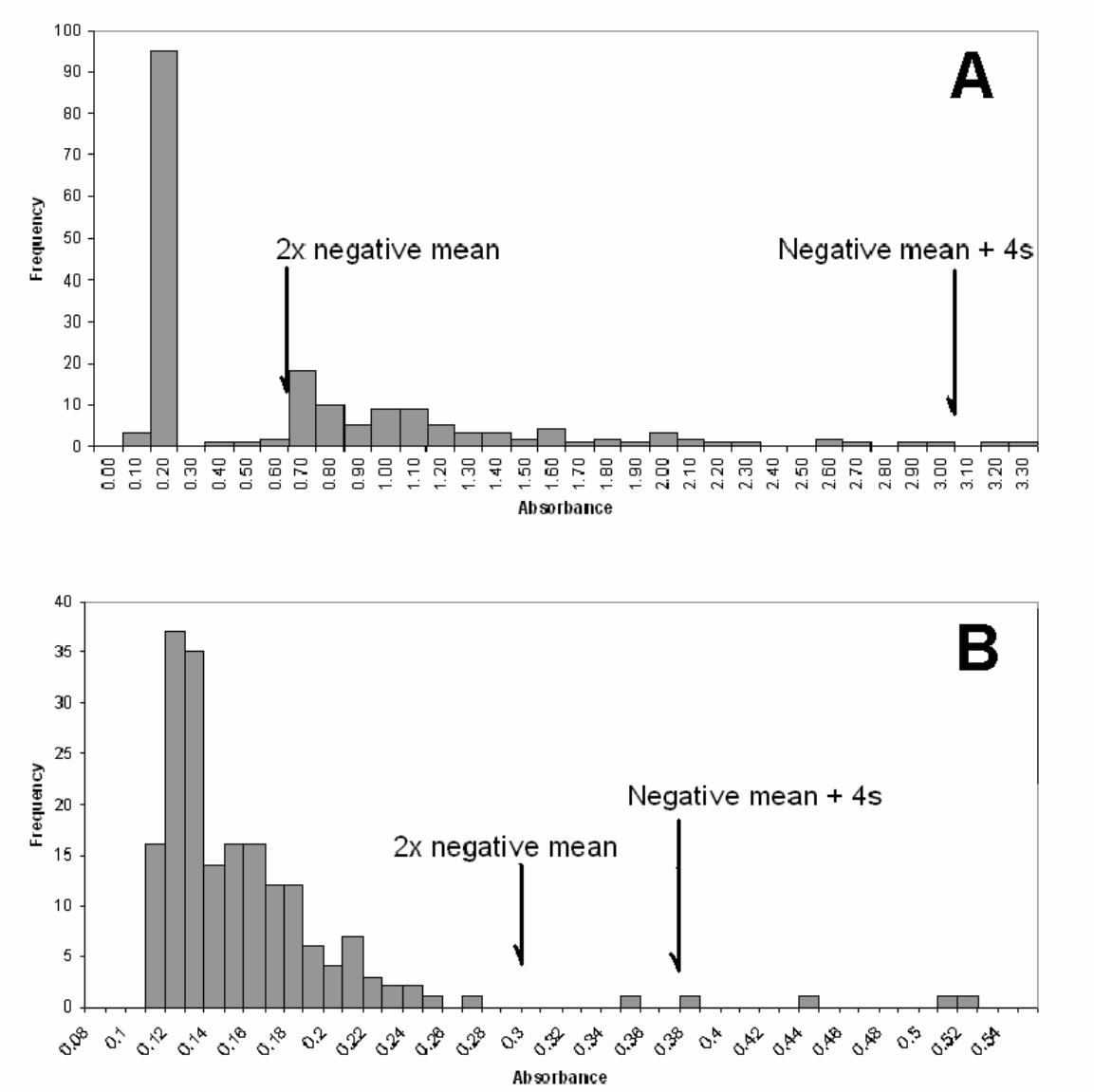

Figure 2.9 –ELISA results and the confidence thresholds for prune dwarf virus in 188 'Tieton' buds (A) and 189 Bing buds (B).

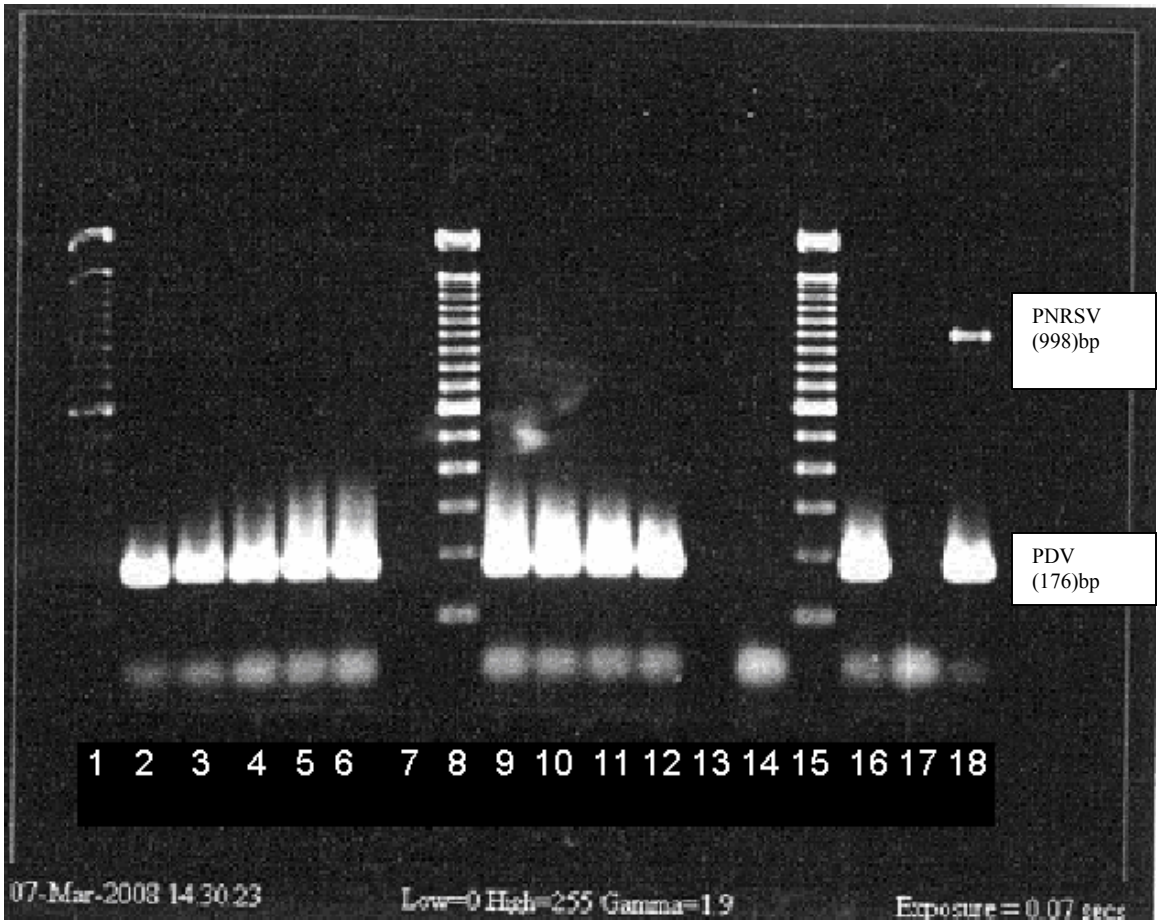

Figure 2.10 – Ethidium bromide-stained gel of PCR products: of 'Tieton' samples for PDV. Lanes 1, 8, and 15 are markers (marks are in 100 bp intervals). Lanes 2-6 and 9-12 are 'Tieton' bud samples. Lane 14 is the water sample. Lane 16 is the same source virus-infected sample. Lane 17 is the virus free sample and Lane 18 is the positive check for the PDV and PNRSV duplex.

CHAPTER 3 – A SWEET CHERRY PLANTING SYSTEM COMPARISON INVOLVING VIRUS EFFECTS WITH MULTIPLE GENOTYPES

3.1 - Abstract

New sweet cherry orchard systems use size-controlling rootstocks at a higher than traditional tree density to improve economic returns and labor efficiency. Greater tree densities increase orchard establishment costs and growers seek alternative planting methods. This research evaluated the feasibility and profitability of a sleeping eye planting system compared to the traditional 2 year nursery tree for multiple genotypes. Graft survival and growth were collected in 2006 and 2007 for 'Bing', 'Chelan', and 'Tieton' grafted on Mazzard, MxM® 60, and Gislea®6 in 2006. One hundred buds were placed for each combination, resulting in 1,800 trees in total.

In 2007 the nursery trees were larger than the transplanted sleeping eye trees but in 2007 growth of sleeping eye trees exceeded that of the transplanted nursery trees. This was due in part to transplant shock. Trees went through a delay in growth in their first orchard year irrespective of planting system. Rootstock and cultivar combination clearly affected growth habit within each planting system which follows previously reported research. Virus infection reduced graft success significantly but did not affect tree vigor. This is inconsequential for orchard growers because failed grafts in the nursery will be culled. For sleeping eyes it is important to utilize virus free budwood to minimize death and costs of replacement trees.

Preliminary economic comparisons suggest that start up costs for a two acre sleeping eye block would incur $1,865 - $1,092 less than the costs for planting standard nursery trees.

3.2 - Introduction

Traditional sweet cherry (*Prunus avium* L.) production systems utilized vigorous seedling rootstocks and large, open-center canopies at low tree densities. These systems can be productive at maturity and bear good quality fruit. However, these systems are being replaced with new cultivars and rootstocks planted at higher density and managed for higher initial yields, and improved labor efficiency. During the late 1990s, sweet cherry production soared due to high returns and depressed apple prices. The resulting competition is the driving force for change, and growers are looking for different strategies to save money and make money.

Orchard make-ups are changing from traditional cultivars (e.g., 'Bing', 'Van') to new diversified types such as 'Chelan' and 'Tieton'. 'Bing' cherry has been grown in the Pacific Northwest commercially for over a century. 'Tieton' is well known for its tree vigor and large fruit. 'Chelan' is the first sweet cherry to be harvested for the season and is a heavy cropper. Both are dark cherries like 'Bing', but their primary advantage is that their fruit ripen 1 to 2 weeks earlier. New varieties have an appeal to growers for diversification, and large fruit size is a very desirable trait for sweet cherries destined to the fresh market (Long et al., 2005).

Sweet cherry growers in the United States have been slow to adopt new high density orchard systems of 500+ trees per hectare (Long et al., 2005). The traditional method was to plant the tree as a multiple leader training system grown on vigorous seedling rootstocks Mazzard (P. *avium* L.) or Mahaleb (P. *mahaleb* L.) and planting at 250 to 400 trees per ha (Whiting et al., 2005). The consensus is that size controlling rootstocks will have a large impact on the future of commercial cherry production

(Sitarek et al., 2005; Robinson et al., 2007; and Santos et al., 2007). In the Northeastern states growers are planting higher densities with these rootstocks for more precocity, production, and to easy management (Andersen et al., 1999; Balmer, 2001; Weber, 2001; Lang and Perry, 2002). Smaller spacing results in increased labor efficiency and productivity per hectare (Webster, 1995; Lang, 2000). More fruiting sites also reduce cropping risks (Santos, et al., 2007). These rootstocks have potential to help solve the economic challenges that growers have been facing by reducing the unproductive length of orchard establishment and the inefficiency of manual labor during harvest (Santos et al., 2005). The size controlling rootstocks make this possible in that they fill much less space thereby reducing the need for ladders, and come to production sooner with more fruiting sites than on a vigorous rootstock. These precocious sweet cherry size controlling rootstocks are derived from species other than, or are hybrids with *Prunus avium.* Varieties like the Gisela® (*P. cerasus x P. canescens*) series from Justus Liebig University (Giessen, Germany) are size controlling rootstocks that reduce the size of the tree by up to 45 percent compared to Mazzard. These hybrids have a low tolerance to pollen-borne viruses (PDV), where typically the traditional *P. avium* and *P. mahaleb* rootstocks are tolerant (Lang and Howell, 2001). Recent research (Lang *et al.,* 1997; Lang *et al*., 1998) revealed that trees on some of these new size controlling rootstock hybrids exhibited detrimental reactions to infection of PDV and PNRSV.

New sweet cherry orchard systems are using size controlling rootstocks at a higher than traditional tree density to improve economic returns and labor efficiency (Whiting et al., 2005). Higher densities increase the start up costs; and drive growers to find alternate methods of planting a cherry orchard. Alternatives such as “sleeping eyes”

are being used more and more in apple production but there are no known plots where sleeping eyes were used in a sweet cherry orchard. Dr. Terence Robinson, (Cornell University) conducted trials with apple trees in a comparison between nursery standard trees, one year whips, and sleeping eyes (T. Robinson, Pers. Commun.) His results found if planting at a density of 850 trees per acre then feathered standard nursery trees are most profitable, but if planting 1,600 to 3,000 trees per acre, the up-front investment for feathered trees may be too high, and the sleeping eyes are more profitable. He did caution that unless the sleeping eyes are managed properly, it can be a poor investment (Warner, 2006). Sleeping eyes have high risk or high potential for success because when the orchard manager purchases a sleeping eye it is then his responsibility to grow the tree as if a nurseryman. Sleeping eyes can be one third to half the price of a finished nursery tree. It seems to be the consensus in the industry that although sleeping eyes are cheaper they are more hassle and riskier than a standard nursery tree (Warner, 1995; Witney, 1998).

Along with the promise of early success, these new precocious rootstocks bring uncertainty to the traditional nursery propagation systems, so new methods of handling and grafting them have to be evaluated. Much research has been formed on assessing bud-take and grafting success with *P. avium* in the nursery between various cultivars and rootstocks and external influences (for example Howard and Vasek, 1988; Feucht and Schmid, 1988; Schimmerlpfeng, 1988; Kappel et al., 2005; Santos et al., 2005; Sitarek and Grzyb, 2007; Santos et al., 2007). Nurseries over-propagate anticipating tree losses due to various causes. Sweet cherry propagation problems in the nursery caused by prune dwarf virus (PDV) include poor bud "take" and scion development (Proebsting et

al., 1995) and a reduction in growth (Gilmer et al., 1976; Nyland et al., 1976). With the cherry industry changing and moving to be more efficient and economical, nurseries need to make that transition also. Without the research, experience, and understanding of these problems such as virus infections, incompatibility, and the introduction of new cultivars and rootstocks it makes it difficult for nurseries to be efficient and to supply an economically competitive product.

A sweet cherry planting system comparison was conducted to evaluate the feasibility and profitability between the traditional 2 year nursery tree and the sleeping eye. Also included in this study is the comparison of nine different genotypes and two different virus statuses and their effects on propagation and growth.

3.3 – Materials and Methods

Three rootstock varieties were utilized: Mazzard (*P. avium L.*), the traditional industry standard vigorous rootstock; MxM® 60 (*P. avium* x *P. mahaleb*), a vigorous hybrid clonal rootstock, and Gisela®6 (*P. cerasus* x *P. canescens*), a slightly size controlling and precocious rootstock. All rootstocks were field-grown at 21 cm between plants within the row and 1.68 m between rows at Willow Drive Nursery, Ephrata, WA. Rootstock liners were raised following standard nursery practices with irrigation applied by T-tape (T-Systems International, Inc. San Diego, CA.). Rootstocks were planted in 2005 from either seeds or tissue cultures. Mazzard rootstocks were nursery row seeds (Willow Drive Nursery, WA.) grown in place and all Gisela®6 (Pro Tree Nursery, CA.) and MxM® 60 plants (from North American Plant, OR.) were tissue cultured, each rootstock remained in place for budding in fall of 2005.

All rootstocks were grafted on the same day in the first week of September, 2005 by chip budding at 15 to 20 cm above the ground. Briefly, a ca. 2.5 to 3 cm long 'chip' (i.e., vegetative auxiliary bud plus surrounding tissue) was removed from the bud stick and grafted into a similar sized cavity cut into the rootstocks. To secure each bud they were wrapped with a polythene strip. Strips were removed after one month. Each cultivar ('Bing', 'Tieton', and 'Chelan') were grafted on to three different rootstock liners (Mazzard, Gisela®6, and MxM® 60) to yield one hundred propagated plants of each combination. Among the cultivars, virus status was also included (virus-free or infected): 1,800 trees in total. In early March, all upper portions of the rootstock were pruned to just above the dormant bud 20 cm above the ground. During the second week of March, 2006, one half of each combination of trees were harvested by hand shovel and stored for shipment to the Washington State University Roza experimental orchard north of Prosser, WA. These sleeping eye trees were therefore comprised of a rootstock shank and a dormant bud. In Prosser, each sleeping eye combo was planted in north to south rows at 1.75 m spacing and 5 m between rows in a completely randomized design. We planted ten 5-tree replications of each cultivar/rootstock/virus status combination. The other set of trees remained in the nursery row to complete the standard two year growth cycle of a nursery tree. In each location the trees were handled as they would be commercially. Trees were trained relatively the same until the trees at the orchard site (Prosser, WA) reached a height of 117 cm where they were pruned to encourage lateral branching. Nursery trees were not pruned until harvest at which time they were pruned 143 cm for the convenience in storage. Nursery trees were dug the first week of November, 2006

and stored until April 14, 2007 when they were planted in 10 5-tree replicates adjacent to the sleeping eye trees planted the previous spring in Prosser.

Once bud break commenced, buds were counted and assessed for survival for each combination in 2006. Between April and October, 2006, growth measurements were made every 30 days apart at each location (Ephrata and Prosser). These included trunk diameter ca. 8 cm above the graft union and tree height (graft union to the most vertical growing point). At the end of the season (November 2006) trees were harvested from each location. The total lengths of all the branches were measured and also each tree was dried for its respective weights of roots and scions shoots. In the spring of 2007, a second set of samples were selected (one from each replicate of five) and measurement of caliper was performed the same way as the year before with a digital caliper (8 cm from the union and every 30 days). At this time all plant material are now planted in Prosser, WA. On April 15, 2007 all trees were pruned back to 85 cm, leaving single unbranched uprights. This was done to facilitate growth comparisons between planting systems in 2007 but may have obviated a potential advantage of planting sleeping eye trees – the ability to impose training system treatments in the first growing season (i.e., nursery year for standard trees). Dry weights of scion shoots and roots were collected at harvest as well as the total length of lateral branches. No height measurements were taken in 2007.

Data were analyzed with SAS (Statistical Analysis System) for significance. A mixed procedure of the Statistical Analysis System computer package was used to analyze data (SAS Institute, 1982). Multiple comparisons were made to analyze two and three way interactions. Each comparison was made within each planting system for the

first season. In the second growing season (2007) comparison were made between each planting system in Prosser WA. Real data was used in every case other than those specified where transformed data was used to improve normality.

3.4 – Results and Discussion

Survival

Poor graft success in the nursery leads to increased nursery costs which can lead to increased tree costs to the grower. A study by Sitarek and Grzyb (2007) reported that budding effectiveness (i.e., trees survival) among six sweet cherry varieties on four different rootstocks varied from 74 to 97%. One of the greatest potential risks to growers adopting a sleeping eye planting strategy is that they assume all costs associated with graft failure. In the nursery, buds that do not heal successfully are culled and not delivered. Significant variability in tree survival in the first season following grafting (and transplanting of sleeping eyes) was documented. Tree survival of nursery trees was 11% significantly ($P < 0.0001$) greater than for sleeping eyes although both were reasonably high overall – 87% and 76% for nursery trees and sleeping eyes, respectively across all scion/rootstock combinations and virus status. In practical terms however, the 13% of failed nursery trees would have been culled in the nursery and not delivered. The tree loss to the orchard grower associated with sleeping eye planting was therefore 24%. The true difference between survival of sleeping eye and nursery trees depends on how many nursery trees that grew in the nursery do not survive the digging, storing, and transplanting in the subsequent season. In the current study, no nursery tree deaths were noted after transplanting in 2007, the year the nursery trees were planted adjacent to the sleeping eye orchard in Prosser. To date, very little research has assessed graft success of

sleeping eye trees in general, and none in sweet cherry. It is possible that mechanical damage at harvest (i.e., when the budded rootstocks were dug) and transplant stress contributed to part of the increased tree death with sleeping eyes. The dormant chip buds grafted to the rootstock shank are particularly fragile and exposed (Figure 3.1) and special care must be taken when handling sweet cherry sleeping eye trees to avoid breaking off the bud. It would seem prudent to protect the dormant buds by not pruning the tops of the rootstocks off until after planting or even using a protective material such as parafilm to prevent the bud from being rubbed. However the practicality of individually wrapping each rootstock would increase the price dramatically and void any savings which were intended by purchasing a sleeping eye tree. Nurseries use the practice of double budding when it seems prudent to increase chances of survivability with known difficult combinations. This concept of double budding would be an easy way to improve the potential of success for each sleeping eye rootstock without great additional cost. With the new idea of sleeping eyes for sweet cherry trees, the development of specific grafting and handling protocols for this type of planting is an area that deserves further investigation. Transplant stress also can reduce tree growth and survivability. Research conducted with hard wood ornamentals and apples showed that if newly planted trees are stressed by root pruning, high planting densities, herbicide damage and replant disease that bud-take can be reduced significantly (Howard and Vasek 1988). Sleeping eye trees are likely more susceptible to such stress.

Trees made from virus free budwood had a significantly ($P < 0.0001$) higher (+10%) survival rate in both planting systems. Earlier research reported that viruses (PNRSV and PDV) have a negative effect on trees survival (Gilmer et al., 1976, Nyland

et al., 1976). I found survivability with virus-infected budwood was highest for 'Chelan' (83.3%) whereas 'Tieton' and 'Bing' were similar at 76% and 74%, respectively. Within a cultivar, virus-infected budwood reduced survivability for 'Tieton'/Gisela®6 nursery grown trees the most with a ca. 18% difference in survival rates, where 96% and 78% were for virus-free and virus-infected, respectively; and 'Bing'/Gisela®6 virus-free sleeping eye with a 88% survival rate and virus-infected sleeping eye 64%, ca. 24% difference. Among rootstocks, the greatest differences in survival between virus-infected and virus-free budwood were found with Gisela®6. Previous research has documented sensitivity of many of the Giessen rootstocks to common viruses (Lang et al., 1997; Lang et al., 1998), but Gisela®6 was classified as tolerant (Lang and Howell, 2001). Differences between my research and that reported may be related to methodology. The current research investigates survival of rootstocks grafted with infected budwood whereas the above-mentioned research studied tree survival following artificial inoculations with virus. When assessing all Gisela® combinations it was found to have a 12% greater in survivability with virus-free material. 'Bing', 'Chelan', and 'Tieton' trees made with virus free budwood had ca. 90%, 89%, and 82% survival, respectively. 'Bing' appeared to be the most sensitive to virus status of the budwood since the difference in grafting success between virus-free and virus-infected budwood was ca. 16%, 6%, and 6% for 'Bing', 'Tieton', and 'Chelan', respectively.

Among rootstocks, MxM® 60 exhibited a significant difference ($P < 0.0022$) in lower bud-take for all combinations (78%) when compared to Gisela®6 (83%) and Mazzard (85%). The primary contributor to this lower average for MxM® 60 is the combination of 'Tieton'/MxM® 60. 'Tieton' virus-free in the nursery had a 73%

survival and when virus-infected was 75%. Sleeping eye 'Tieton' virus-free was 76% and virus-infected being 68% (Table 3.1). Virus infected scions showed an 82% survival on Mazzard, 77% on Gisela®6, and 74% on MxM® 60. Among all possible combinations, I recorded a range in graft success from 'Chelan'/Gisela®6 virus free nursery stock having the highest survivability (98%) and 'Bing'/MxM® 60 virus-infected sleeping eye with the lowest (60%). When planning sleeping eye material, my data suggest that one should anticipate an 11-20% loss, and if propagation is conducted with virus-infected material then a 21-40% loss should be expected.

2006 growth

In 2006, nursery trees grew in place with 21 cm between trees whereas the sleeping eye trees were transported to the WSU-Roza experimental farm and planted at 1.75 m between trees in the row. These environments are not dissimilar; mean growing season high temperature from April 1st to November 1st was 25.26 °C at Willow Drive Nursery in Ephrata, Washington and 25.17 °C for the Roza farm in Prosser, Washington. Mean lows for the same time period were 9.04 °C at Willow Drive Nursery and 7.17 °C at the Roza farm. Soil at the Roza farm is a sandy loam and 1.25 to 2 m deep, in Ephrata, soil type is a coarse sandy loam and 1 to 1.5 m deep. Despite similarities between sites, I will restrict growth comparisons between planting systems to the second year, when nursery trees were transplanted adjacent to the sleeping eye orchard.

Tree Height – For nursery stock, final tree height is insignificant because every tree is pruned to ca. 158 cm for the ease of storage and handling. However, for sleeping eye trees tree height is important because rapidly filling orchard space is fundamental towards precocity and productivity. I did however record a slower rate of growth in the

first two months for virus-infected trees, but by mid summer all combinations were similar. Finished tree height in the nursery was ca. 10% greater with virus-free material. Results in Prosser with the sleeping eye trees showed virus-free material only grew 3% taller. Neither difference was statistically significant. Through the trial, rootstock selection had no effect on first season vertical growth. Among the cultivars 'Bing' grew the shortest, with 'Chelan' and 'Tieton' having similar growth.

Trunk diameter – From the nursery's perspective, trunk diameter is critically important because larger diameter trees are more valuable. Larger trees receive a small premium price for the nursery, where each tree has the same cost to produce no matter what the size. Research has shown that in apple, larger caliper trees are more efficient in filling their space sooner in new plantings system and in turn lead to earlier production (Robinson and Hoying, 2005).

Comparing all treatment combinations, it is apparent that nursery trees had greater radial caliper in 2006 than sleeping eye trees (Figure 3.2 graph A). Overall, I recorded a significant difference in ($P < 0.0001$) of ca. 15% greater trunk diameter for trees grown in the nursery. This is likely related to transplant shock for sleeping eye trees which had to re-establish rooting before scion growth commenced. Root growth generally occurs in the early spring and late fall. With the sleeping eye roots pruned back new growth to re-establish roots for nutrient absorption had to be first before all reserves were depleted. Other research conducted with similar genotypes of *P. avium* has seen a delay in growth due to transplanting late in the spring (Santos et al., 2007). I recorded significantly greater growth with virus-free vs. virus-infected budwood for only 'Bing'/Mazzard sleeping eye trees (+24%). Interestingly, virus-infected 'Chelan'/MxM® 60 nursery trees

exhibited 14% greater growth than that of the same genotype made with virus-free budwood. Across all treatment combinations, the greatest final tree caliper was from 'Chelan'/MxM® 60 virus-infected nursery trees (30.5 mm) and the lowest caliper was from 'Bing'/Mazzard virus-infected sleeping eye trees (15.6 mm).

Among cultivars I recorded only minor differences in trunk diameter and few were statistically significant. On Mazzard, 'Bing' virus-free had 14% and 18% greater growth than 'Tieton' virus-free for sleeping eye system and nursery trees, respectively, only in the sleeping eyes was it significant. Infected 'Chelan' vs. infected 'Tieton' on Mazzard had 'Tieton' 19% greater growth in the sleeping eyes ($P < 0.0418$) however in the nursery it was not significant that 'Chelan'/Mazzard was 12% greater growth. 'Chelan' virus-free vs. 'Tieton' virus-free on Gisela®6 had 'Tieton' 21% greater growth in the sleeping eyes ($P < 0.0004$) and again it was not significant in the nursery for 'Chelan' being 8% greater in diameter. Data leads me to believe that these three cultivars have no real distinct difference in radial growth in the first season and that these differences are either related to environmental factors, stress (transplant shock), or rootstocks.

Among rootstocks, MxM® 60-rooted trees had the largest tree diameter compared to Mazzard and Gisela®6. In the nursery, scions grafted to MxM® 60 were significantly larger ($P < 0.001$) in trunk diameter than those grafted to Gisela®6 and Mazzard, which were similar. Mazzard and Gisela®6 produced the same caliper of trees with all cultivars except for 'Tieton'/Gisela®6 virus-free (20.2 mm) in the sleeping eye plot being significantly different ($P < 0.0109$) from 'Tieton'/Mazzard (17.6 mm)) but not significantly different ($P < 0.12$) from 'Tieton'/MxM® 60 (22 mm). 'Bing'/Gisela®6

virus-free (23 mm) in the nursery being significantly different from 'Bing'/Mazzard ((P < 0.0413) 20.1 mm)) and Gisela®6 in both cases were larger than Mazzard trees.

Total lateral growth – All branch lengths were added together for analyzing total lateral growth (some trees may have had only one branch but equal in growth to another with ten branches). Many commercial orchardists would prefer planting nursery trees with lateral branching, believing this branching will improve precocity. However, assessing lateral growth is a good way to observe growth habits of scion cultivars. Similar to the assessment of trunk diameter, I observed no clear trends in total lateral growth evaluated by the main effects of planting systems, virus titer, cultivar, or rootstock. There was no difference between planting systems, both sleeping eye trees and nursery trees had similar lateral growth. This is interesting because nursery trees had significantly greater radial growth in 2006 compared to sleeping eye trees. This may be because secondary growth is a weak sink for photoassimilates compared to apical meristems of lateral branches (Whiting and Lang, 2004).

Within each planting system there were few differences among the various combinations of virus status and genotype. Among sleeping eye trees, only 'Tieton'/MxM® 60 from virus-free material had significantly greater lateral growth (+26%, P < 0.0028) than the same genotype from virus-infected bud wood. Among nursery trees, both 'Tieton'/Mazzard and 'Bing'/MxM® 60 virus-free materials had greater lateral growth +66% and +43%, respectively compared to virus-infected trees. In all other cultivar/rootstock combinations, virus status was unimportant for lateral growth.

Comparing lateral growth of cultivars within planting systems revealed that 'Tieton' is significantly more vigorous than 'Chelan' and 'Bing'. 'Tieton' trees had ca.

21% and 6% more lateral growth compared to ‘Chelan’ and ‘Bing’, respectively. For specific comparisons within the sleeping eye system, ‘Tieton’/Gisela®6 virus-free had 34% and 59% more lateral growth than ‘Bing’/Gisela®6 virus-free and ‘Chelan’/Gisela®6 virus-free, respectively. In addition ‘Tieton’/Mazzard virus-infected had 46% more lateral growth compared to ‘Chelan’/Mazzard virus-infected. Among the nursery trees there were more significant differences in lateral growth than for sleeping eyes. Virus-free ‘Chelan’/MxM® 60 and Gisela®6 rootstocks had significantly less lateral growth compared to ‘Bing’ on the same rootstocks ($P < 0.0056$ and $P < 0.0301$). In addition, virus-free ‘Tieton’/Mazzard had greater lateral growth compared to ‘Bing’ on the same rootstock but ‘Bing’ had nearly twice the amount of lateral growth on MxM® 60 compared to ‘Tieton’. Further, virus-free ‘Tieton’/Mazzard and Gisela®6 had significantly greater growth than ‘Chelan’ on the same rootstocks (Table 3.2). ‘Tieton’ producing on average more lateral growth is only significant in the sleeping eye comparison; in the nursery, the results illustrated that ‘Bing’ produced on average the most lateral growth.

Rootstock selection affected total lateral growth in the first year of growth. Among the differences in rootstocks, MxM® 60-rooted trees had ca. 37% greater lateral growth in the sleeping eye site compared to the other two rootstocks. This growth advantage was 43% for MxM® 60-rooted trees in the nursery in 2006. All comparisons showed that there was a significant difference between MxM® 60 and Gisela®6 or Mazzard rootstocks when compared within each planting system respectively. However no significant differences were assessed in a comparison of Gisela®6 and Mazzard in both planting systems.

Above- and below-ground tree dry weight - Many root systems were damaged in the harvest process. Data were transformed in SAS to analyze in a more normal distribution. Each root zone of 66 cm wide by 46 cm deep was the soil volume harvested, and any roots inside this were intact for dry weights. Nursery trees compared to the sleeping eye trees demonstrated a ca. 15% increase in root mass. Sleeping eye trees exhibited a significant difference in root dry weight in relation to virus infection (virus-free root systems showed a 3% increase in mass ($P < 0.0136$)). It was also confirmed that each rootstock was different in structure and mass ($P < 0.0001$). Nursery trees demonstrated the same results in that virus infection did reduce rootstock mass by 10% ($P < 0.0051$). Also it was the same with significant differences in structure and mass between each rootstock ($P < 0.0001$).

Above-ground dry weight data was also transformed to improve normality and homogeneity of variances for SAS analysis. Nursery trees had ca. 42% greater scion dry weight than the sleeping eyes. This is likely due to greater secondary growth of nursery trees vs. sleeping eye trees because total lateral growth was not different between the planting systems. Creating trees with virus-infected or virus-free budwood did not appear to affect scion dry weight, irrespective of planting system. Among cultivars in the sleeping eye orchard, virus-free 'Tieton'/MxM® 60 trees had 34% greater scion dry weight vs. 'Bing' on the same rootstocks ($P < 0.0048$). Virus-infected 'Tieton'/Mazzard trees had 54% more above-ground dry weight compared to 'Chelan'/Mazzard ($P < 0.0096$). 'Tieton'/Gisela®6 virus-free weighed 43% more to 'Chelan' ($P < 0.0055$). Among the nursery trees, 'Tieton' was consistently greater dry weight than 'Chelan'. Rootstocks were significantly different from each other with scion dry weight which

supports other research reported that rootstocks demonstrate a physical characteristic on the budded scion variety (Seif and Gruppe, 1985; Autio and Southwick, 1986; Yadava and Doud, 1989; Schecter et al., 1991; and Usenik et al., 2005). Mazzard and Gisela®6 were similar in scion dry weights, but each was significantly different when compared to MxM® 60 in each respective planting system.

2007 growth

Survival – In 2007, the second year in place for sleeping eyes and the transplant year for nursery trees, there were no tree deaths recorded.

Tree trunk diameter – Trunk diameter at 8 cm above the graft union was assessed every 30 days throughout the season to record secondary growth. Rate of secondary growth is a good indicator of tree vigor and source-sink relations because it is a weak sink (Whiting and Lang, 2004). In general, larger trunk diameter is related to greater canopy volume though no published research has reported a benefit to larger caliper trees in the orchard. In the current trial, the seasonal increase in trunk diameter of sleeping eye trees was significantly greater than that for standard nursery trees (overall ca. 27% more growth). For some genotypes this radial expansion of sleeping eye trees was nearly double that of nursery trees (Figures 3.3, 3.4, 3.5). This is likely due to the nursery material going through transplant shock in its first orchard year. Secondary growth of nursery trees was particularly low during the early spring and rates improved later in the summer.

Virus presence appeared to have only a subtle effect on growth in the second year. I recorded significantly improved growth on virus-free 'Bing'/Mazzard ($P < 0.0447$), 'Chelan'/Mazzard ($P < 0.0057$), 'Chelan'/MxM® 60 ($P < 0.0001$), and 'Bing'/Gisela® 6

($P < 0.0071$) in the sleeping eye stock, with these combinations having ca. 19% greater growth when virus-free vs. virus-infected are compared. For all other genotypes, there were no differences in growth between trees made with virus-free or virus-infected bud wood in the second season.

There was no consistent trend among scion cultivars on trunk diameter in the second season compared to the first season. Among the cultivars in 2007, minor discrepancies again were observed with differences only statistically significant when comparing genotypes in the sleeping eye stock. 'Bing'/Mazzard virus-infected were smaller compared to 'Chelan' and 'Tieton' on Mazzard. 'Bing'/MxM® 60 virus-free had 18% less trunk cross sectional area than 'Chelan'/MxM® 60. In addition, 'Bing'/MxM® 60 virus-infected wood had 19% less radial expansion than 'Tieton'/MxM® 60 trees in the sleeping eye orchard. In the previous season there were no differences in radial growth among the genotypes. Among the nursery trees, only 'Chelan'/MxM® 60 trees had 18% more growth than 'Bing'/MxM® 60.

Among rootstocks, MxM® 60 produced larger caliper trees than Mazzard and Gisela® 6. Compared to the first season's growth, fewer genotypes had a significant difference; 10 of the possible 36 combinations were statistically different and 80% of those were for sleeping eye trees. Nine of these significant comparisons were between Mazzard/MxM® 60 and MxM® 60/Gisela® 6 (in both cases MxM® 60 being larger caliper trees). I believe that the transplant effect eliminated any of the nursery trees from being significantly different from each other when analyzing rootstock effects.

Total lateral growth – Statistical analysis was conducted with the natural log transformed data. After being pruned in the spring to a stock in the ground, all new

growth in 2007 was comprised of lateral branches. By this time it is relevant for orchard growers to introduce such branching to create an open center, multiple leader training system. Total lateral growth of sleeping eye trees was about 74% greater than nursery trees. This was likely due to transplant shock reducing growth of the nursery trees. Trees made with virus-infected bud wood had similar lateral growth to those made with virus free budwood. Virus-free sleeping eye 'Chelan'/MxM® 60 had 10% more growth than infected trees. Virus-free 'Bing'/Gisela® 6 nursery trees and sleeping eye trees showed a difference in relation to infection (virus free had 42% more lateral growth).

No significant differences in lateral growth were observed among cultivars. Rootstocks however affected total lateral growth in 2007. Among rootstocks 13 of the 36 combinations were significantly different. The majority of these differences were observed in the nursery-grown trees (9 of the 13). Among these significant comparisons 5 of the 13 are Mazzard/MxM® 60 and 4 of 13 between Gisela®6/MxM® 60. Four combinations only in the nursery trees were found significant between Mazzard/Gisela®6; 'Bing' virus-free, 'Chelan' virus-free, 'Tieton' virus-free, and 'Tieton' virus-infected. Trees grown on Mazzard produced more lateral growth for 'Tieton' virus-free and 'Chelan', the others were greater on Gisela®6. I have no explanation for these four to be different here and not anywhere else. Overall, MxM® 60-rooted trees produced 44% greater lateral growth than Mazzard and Gisela® 6 (Table 3.3).

Above- and below-ground tree dry weight – Under given environmental conditions the best estimation of the vigor of a tree is its annual accumulation of dry matter (Franken-Bembenek and Gruppe, 1984). At the conclusion of the 2007 season,

one tree from each 5-tree replication was dug and dried to constant temperature. Trees were separated into above-ground and below ground segments and weighed. It was apparent at tree removal that root systems of the sleeping eye trees were much more developed and healed in compared to nursery trees which had been planted earlier that year. Weights were not 100% accurate due to damage at time of harvest. Observations illustrate that MxM® 60 had the largest root structure with long large anchor roots (Figure 3.6). Mazzard was similar in structure but smaller in overall size (Figure 3.7). Gisela®6 roots were very long and slim in comparison to the other two (Figure 3.8). Across all cultivar/rootstock combinations, sleeping eye trees had ca. 55% greater root dry weight compared to nursery trees. The main effects of virus status of the budwood and cultivar had no effect on root weight.

Overall, differences in scion dry weight mirrored those I reported for lateral growth. Sleeping eye trees had about 68% greater above-ground scion dry weight due to more branching and longer shoots than compared to nursery trees after the 2007 growing season (Figure 3.9). Again, this is likely due to the sleeping eye trees growing in place for a second season and transplant stress for nursery trees. Trees made from virus-free budwood had a significant increase by 27% dry weight than trees made from virus-infected wood ($P < 0.0001$). Among the cultivars, 'Bing' had significantly less scion dry weight than both 'Chelan' ($P < 0.0334$) and 'Tieton' ($P < 0.0006$) which were similar. Among rootstocks, MxM® 60-rooted trees had significantly greater scion dry weight ($P < 0.0001$) by ca. 33% compared to Mazzard- and Gisela®6-rooted trees which were similar.

Conclusion

The first year nursery trees grew the best with the larger sized trees but in the second year all sleeping eyes surpassed the nursery trees in growth. I believe this is due in part to transplant shock. The poor growth of sleeping eyes in 2006 supports this contention. Trees will go through a delay in their first orchard year irrespective of planting system. Rootstocks and scion cultivars clearly affected growth habits with in each planting system which fallows previously reported research (Seif and Gruppe, 1985; Yadava and Doud, 1989; Usenik et al. 2005.). The virus status of bud wood had a significant effect on graft survival but not on growth or tree vigor. This is inconsequential for orchard growers purchasing material from a nursery because nursery trees that fail will be culled in the nursery, however if they use sleeping eyes it becomes very consequential due to 24% chance for losses. For sleeping eyes it is important to utilize virus free budwood to minimize death and costs of replacement trees.

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Figure 3.1 – Sleeping eye bud grafted on a Mazzard rootstock upon delivery to WSU Roza farm in Prosser, Washington 2006.

Table 3.1 – Final tree height, trunk diameter, and survival in 2006 for each of the 36 rootstock/cultivar combinations. Data are reported as means of 10 replications. Data are grouped by planting system and the virus status of the bud wood. Trees were budded in fall, 2005 at Willow Drive Nursery in Ephrata, Washington.

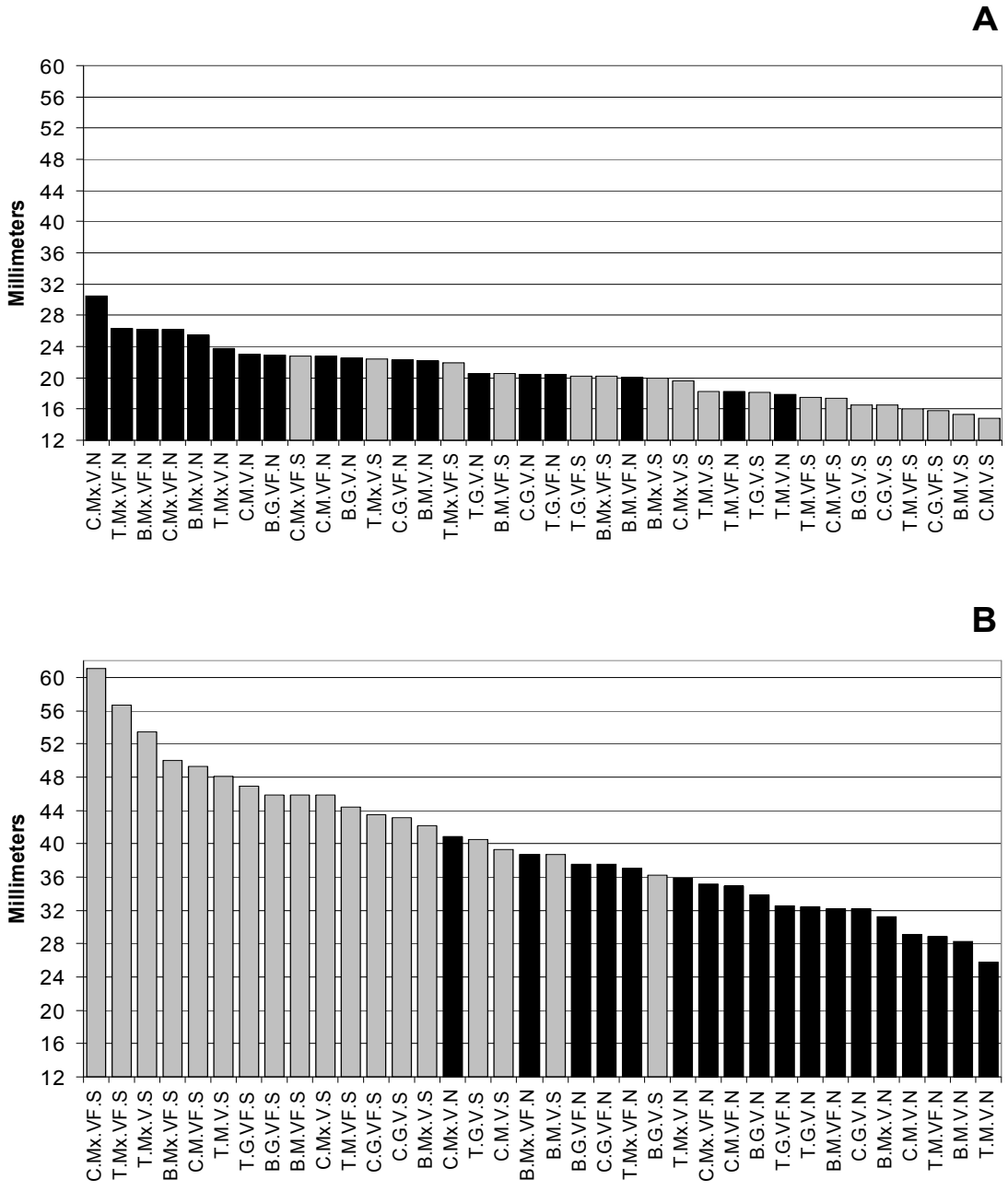
Nursery Trees (V.F.)		Height (cm)	Standard error	Tree trunk diameter (mm)	Standard error	Survival (%)
Gisela® 6	Bing	128.6	3.0	23.0	0.7	94%
	Tieton	139.2	4.4	20.4	0.6	96%
	Chelan	123.4	3.2	22.1	0.6	98%
MxM 60	Bing	111.6	3.2	26.2	0.7	90%
	Tieton	139.9	10.1	23.4	3.1	73%
	Chelan	138.0	1.5	26.2	1.0	90%
Mazzard	Bing	117.2	2.6	20.1	0.6	97%
	Tieton	123.5	14.0	16.4	1.9	84%
	Chelan	140.6	5.3	22.8	1.8	96%
Nursery Trees (Virus infected)						
Gisela® 6	Bing	111.4	3.1	22.5	0.8	88%
	Tieton	133.2	4.0	20.6	0.7	78%
	Chelan	124.7	6.8	20.5	1.0	86%
MxM® 60	Bing	90.2	12.5	22.6	3.4	78%
	Tieton	106.8	15.0	21.1	2.8	75%
	Chelan	139.1	2.9	30.5	0.9	86%
Mazzard	Bing	109.7	6.9	22.2	1.0	81%
	Tieton	103.1	3.6	17.9	1.2	83%
	Chelan	127.1	14.5	20.5	2.7	94%
Sleeping eye (V.F.)						
Gisela® 6	Bing	109.6	3.4	17.5	1.5	88%
	Tieton	126.2	7.7	20.2	1.1	78%
	Chelan	102.1	6.4	15.8	1.3	82%
MxM® 60	Bing	104.1	3.8	20.2	1.1	80%
	Tieton	115.5	11.5	22.0	2.0	76%
	Chelan	111.6	9.6	22.8	1.3	86%
Mazzard	Bing	110.1	2.8	20.6	0.8	88%
	Tieton	112.5	8.7	17.6	1.3	86%
	Chelan	110.0	11.4	17.4	1.6	80%
Sleeping eye (Virus infected)						
Gisela® 6	Bing	101.1	2.3	16.5	0.6	64%
	Tieton	113.2	2.8	18.1	0.5	76%
	Chelan	108.5	5.4	16.5	0.9	72%
MxM® 60	Bing	101.9	6.9	19.9	0.6	60%
	Tieton	128.9	5.5	22.4	1.6	68%
	Chelan	122.9	2.1	19.6	1.8	76%
Mazzard	Bing	98.0	6.7	15.6	0.9	70%
	Tieton	110.6	4.5	18.3	1.3	75%
	Chelan	89.7	9.8	14.8	1.0	86%

Table 3.2 – Final tree root dry weight, scion dry weight, and total lateral extension growth in 2006 for each of the 36 rootstock/cultivar combinations. Data are reported as means of 10 replications. Data are grouped by planting system and the virus status of the bud wood. Trees were budded in fall, 2005 at Willow Drive Nursery in Ephrata, Washington.

Nursery Trees (V.F.)		Root dry weight (g)	Standard error	Scion dry weight (g)	Standard error	Total lateral growth (cm)	Standard error
Gisela® 6	Bing	234.9	10.3	231.0	17.6	193.3	35.8
	Tieton	167.6	20.6	170.2	35.6	223.8	32.2
	Chelan	149.5	11.1	90.6	25.7	87.0	27.6
MxM 60	Bing	368.1	17.0	312.5	16.5	402.9	45.1
	Tieton	389.6	24.0	329.1	34.2	227.8	35.0
	Chelan	352.2	20.5	286.9	23.1	267.3	33.9
Mazzard	Bing	207.0	21.7	147.3	10.3	95.5	28.8
	Tieton	158.2	17.1	166.3	14.4	246.1	33.6
	Chelan	217.5	34.3	212.0	39.0	109.8	32.8
Nursery Trees (Virus infected)							
Gisela® 6	Bing	187.7	18.4	224.5	19.8	123.6	31.3
	Tieton	149.3	11.8	226.0	19.6	168.6	35.4
	Chelan	164.2	21.8	177.3	15.5	107.7	33.0
MxM® 60	Bing	296.0	27.6	263.5	39.3	227.8	48.4
	Tieton	275.8	18.7	247.3	22.6	212.8	36.9
	Chelan	430.0	28.1	364.8	23.1	260.4	23.0
Mazzard	Bing	169.0	12.1	164.5	19.3	49.8	31.4
	Tieton	121.8	19.5	122.8	20.5	83.3	27.8
	Chelan	205.7	14.3	189.0	15.7	113.4	24.6
Sleeping eye (V.F.)							
Gisela® 6	Bing	145.0	13.9	109.0	23.0	143.3	29.9
	Tieton	165.6	16.9	160.6	30.0	217.8	28.6
	Chelan	145.7	8.1	90.1	19.1	87.9	20.3
MxM® 60	Bing	289.7	27.8	146.6	24.2	240.9	32.9
	Tieton	289.2	56.9	222.0	48.8	260.2	51.1
	Chelan	356.2	43.6	195.3	38.8	244.2	40.5
Mazzard	Bing	213.1	20.0	137.6	22.1	188.0	21.8
	Tieton	161.7	11.0	85.2	8.3	117.3	14.2
	Chelan	157.4	25.3	107.5	21.0	134.9	23.9
Sleeping eye (Virus infected)							
Gisela® 6	Bing	139.6	6.7	88.1	5.7	128.0	10.3
	Tieton	129.3	10.6	108.2	6.3	154.5	9.9
	Chelan	139.6	7.5	92.9	9.7	109.1	14.8
MxM® 60	Bing	260.8	28.2	138.9	20.1	226.0	22.9
	Tieton	242.5	23.7	160.7	27.5	191.7	23.9
	Chelan	272.2	28.8	148.7	26.7	188.4	29.7
Mazzard	Bing	168.4	23.0	80.3	9.5	132.3	17.9
	Tieton	185.5	21.7	130.1	23.3	176.1	23.0
	Chelan	152.5	10.2	59.8	8.1	94.3	13.7

Table 3.3 – Final tree trunk diameter, root dry weight, scion dry weight, and total lateral extension growth in 2007 for each of the 36 rootstock/cultivar combinations. Data are reported as means of 10 replications. Data are grouped by planting system and the virus status of the bud wood. Trees were budded in fall, 2005 at Willow Drive Nursery in Ephrata, Washington.

Nursery Trees (V.F.)		Tree trunk diameter (mm)	Std. error	Root dry weight (g)	Std. error	Scion dry weight (g)	Std. Err.	Total lateral growth (cm)	Std. error
Gisela® 6	Bing	37.49	1.00	484.71	35.06	643.57	67.15	515.71	65.24
	Tieton	32.58	1.01	317.63	16.24	549.00	49.75	343.75	33.01
	Chelan	37.48	1.86	409.22	35.86	645.78	96.07	401.11	56.58
MxM® 60	Bing	32.22	2.03	430.57	57.95	359.57	86.44	301.43	77.60
	Tieton	28.83	2.72	349.17	60.9	369.83	93.02	248.33	78.55
	Chelan	34.88	1.85	440.78	62.33	383.78	77.10	243.33	71.02
Mazzard	Bing	38.78	2.30	771.14	145.49	636.29	133.49	572.86	144.50
	Tieton	37.04	2.90	770.17	120.81	770.83	152.29	601.67	133.78
	Chelan	35.14	1.97	607.67	82.97	490.17	89.92	351.67	86.08
Nursery Trees (Virus infected)									
Gisela® 6	Bing	33.91	0.91	296.25	18.44	458.00	33.73	288.75	25.53
	Tieton	32.42	1.71	332.83	55.02	548.00	121.05	351.67	67.41
	Chelan	32.20	0.80	270.20	25.85	401.00	27.03	238.00	22.23
MxM® 60	Bing	28.28	2.35	244.86	50.26	223.57	45.84	214.29	66.88
	Tieton	25.83	1.76	250.00	45.14	189.00	26.07	104.29	15.87
	Chelan	29.07	0.43	325.89	26.78	242.00	32.48	157.78	35.39
Mazzard	Bing	33.50	2.67	531.25	120.46	409.25	121.66	340.00	117.36
	Tieton	35.86	2.16	636.14	101.71	549.71	123.43	390.00	86.91
	Chelan	40.91	2.00	846.71	77.46	728.00	115.90	507.14	85.57
Sleeping eye (V.F.)									
Gisela® 6	Bing	45.88	2.16	688.89	109.85	1400.00	165.83	1283.33	152.69
	Tieton	46.91	2.92	811.11	104.67	1788.89	284.04	1200.44	173.34
	Chelan	43.48	3.38	650.00	98.86	1291.67	213.37	862.50	116.28
MxM® 60	Bing	45.83	1.95	1150.00	140.84	1280.00	160.43	1378.00	136.29
	Tieton	44.46	3.36	1288.89	280.60	1533.33	314.02	1165.56	213.17
	Chelan	49.24	5.11	1580.00	247.14	1870.00	349.01	1222.00	207.06
Mazzard	Bing	49.69	1.65	1242.86	160.57	1885.71	181.95	1844.29	200.65
	Tieton	56.65	5.40	1428.57	252.31	2500.00	507.98	1962.86	383.16
	Chelan	61.06	2.13	1955.56	158.21	2677.78	275.27	2153.33	217.44
Sleeping eye (Virus infected)									
Gisela® 6	Bing	36.26	2.66	460.00	42.69	840.00	123.12	742.00	70.76
	Tieton	40.53	1.38	580.00	51.21	1060.00	97.99	763.00	76.09
	Chelan	43.12	2.07	544.00	29.40	1155.56	119.15	770.00	67.14
MxM® 60	Bing	38.68	2.74	766.67	113.04	900.00	144.34	964.44	142.41
	Tieton	48.11	3.14	1028.57	139.23	1628.57	240.72	1130.00	175.90
	Chelan	39.35	3.42	766.67	175.59	955.56	218.65	890.00	150.85
Mazzard	Bing	42.18	3.54	814.29	138.74	1171.43	233.70	1364.29	240.20
	Tieton	53.40	0.72	1380.00	226.72	2300.00	104.90	1840.00	170.33
	Chelan	45.80	3.30	950.00	206.17	1490.00	283.83	1917.00	679.76



1st letter = Scion cultivar (C = Chelan, B = Bing, T = Tieton)
2nd letter = Root cultivar (M = Mazzard, Mx = MxM® 60, G = Gisela® 6)
3rd letter = Virus titer (V = Virus infected, VF = Virus Free)
4th letter = Planting system (N = Nursery, S = Sleeping eye)

Figure 3.2 – Graphs (A) 2006 and (B) 2007; trunk diameter data for each individual level rootstock/cultivar combination (36) for nursery grown trees (Black) and the sleeping eyes (Gray) (n=10).

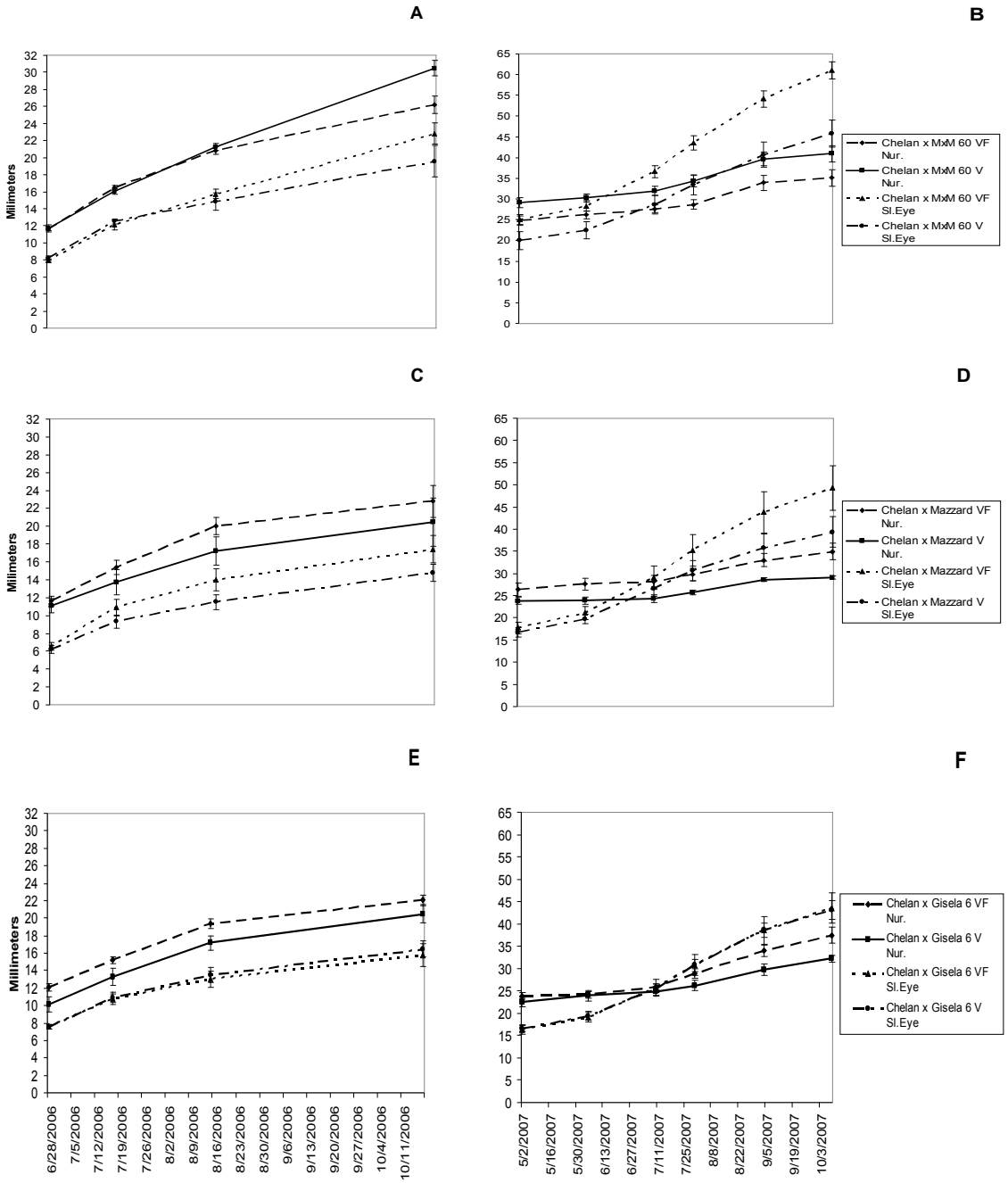


Figure 3.3 – Tree trunk diameter for 'Chelan' with different virus infections, planting systems, and rootstocks data for 2006 (A, C, E) and 2007 (B, D, F) seasons (n=10). Each data point is the mean ± SAM.

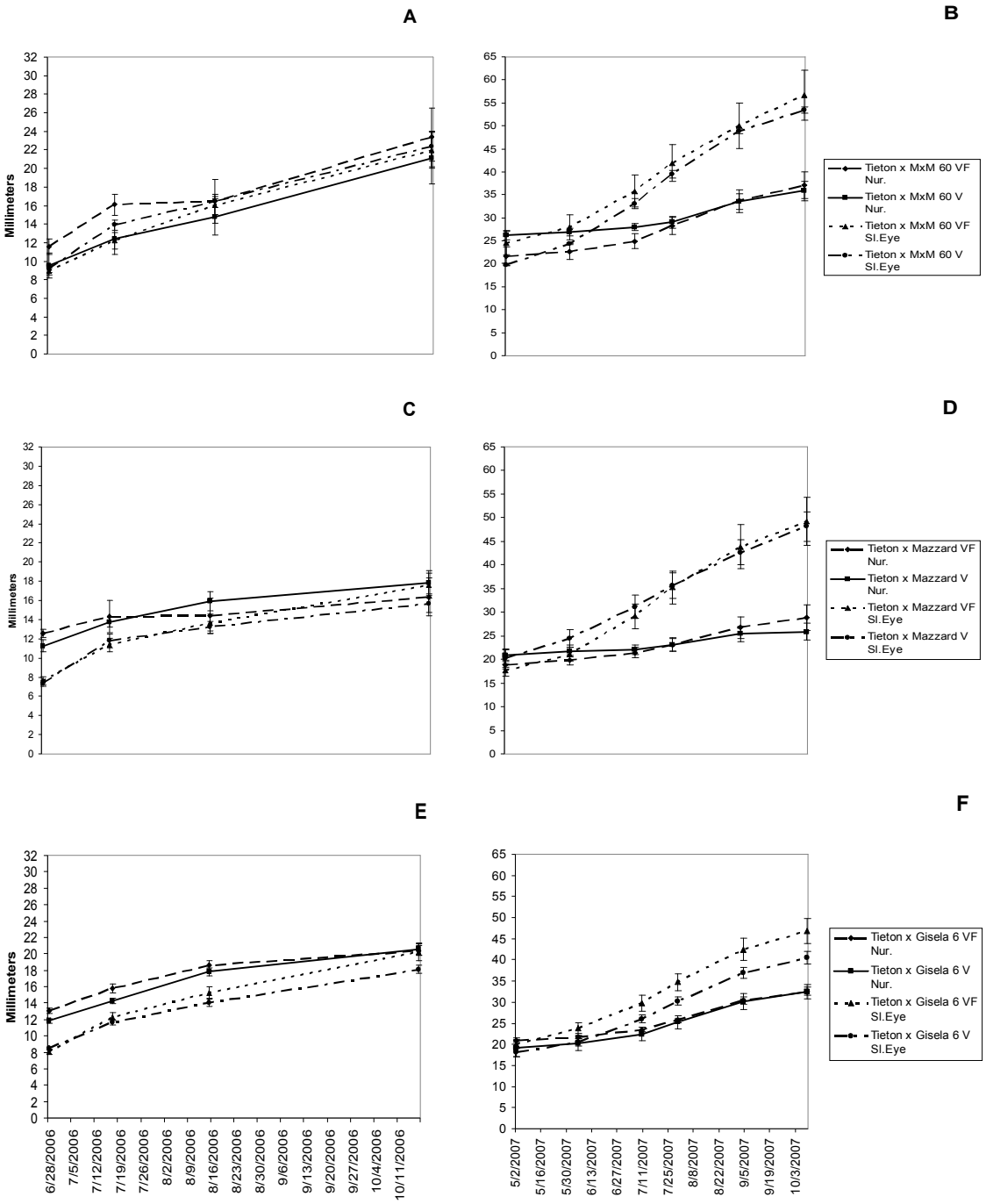


Figure 3.4 – Tree trunk diameter for 'Tieton' with different virus infections, planting systems, and rootstocks data for 2006 (A, C, E) and 2007 (B, D, F) seasons (n=10). Each data point is the mean ± SAM.

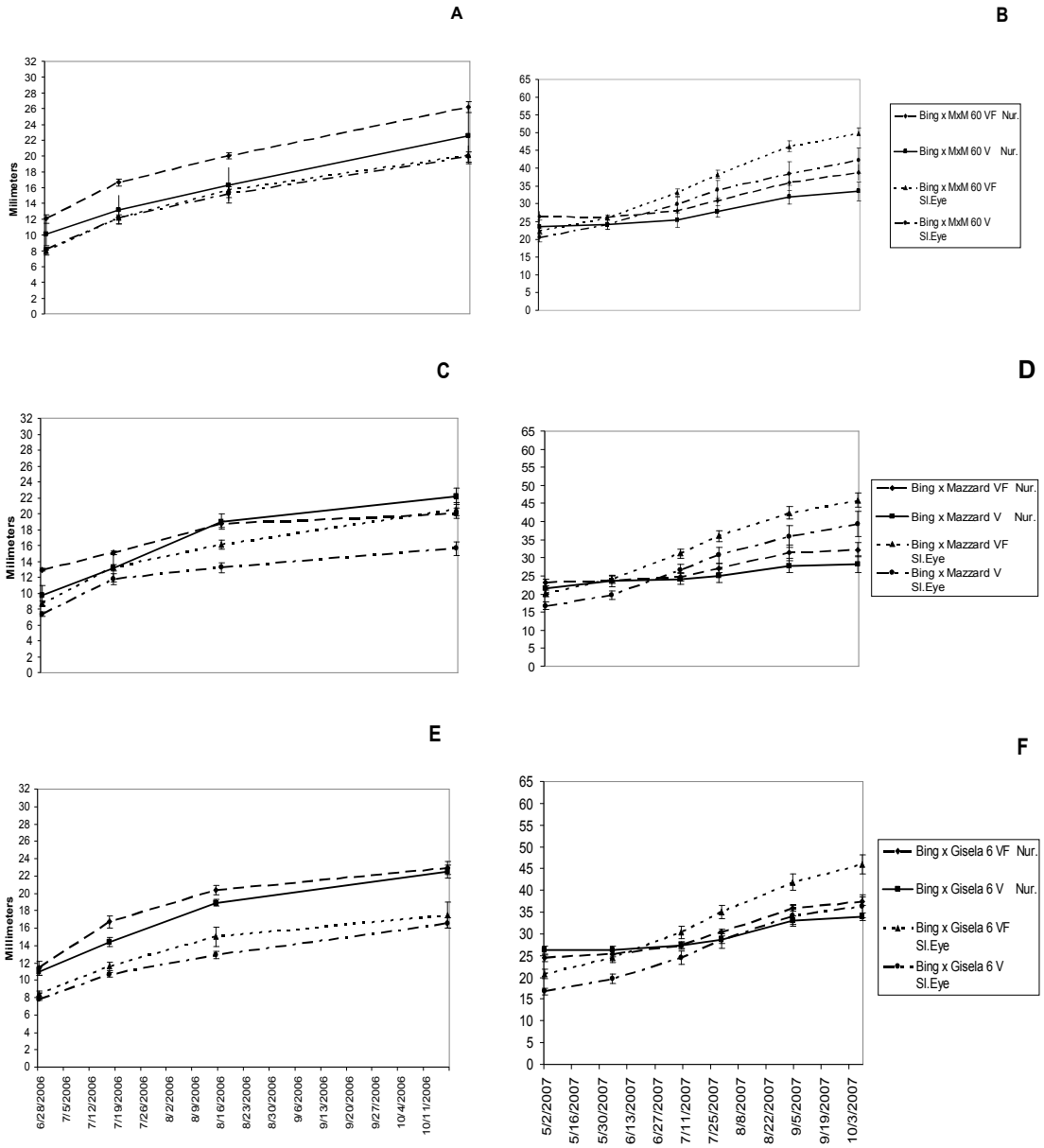


Figure 3.5 – Tree trunk diameter for 'Bing' with different virus infections, planting systems, and rootstocks data for 2006 (A, C, E) and 2007 (B, D, F) seasons (n=10). Each data point is the mean ± SAM.

Figure 3.6 – ‘Chelan’/MxM® 60 virus-free sleeping eye root section for dry weights at the WSU Roza farm in Prosser, Washington.

Figure 3.7 – 'Chelan'/Mazzard virus-free sleeping eye root section for dry weights at the WSU Roza farm in Prosser, Washington.

Figure 3.8 – Left to Right; ‘Chelan’/Mazzard virus-infected, ‘Chelan’/Gisela®6 virus-infected, and ‘Chelan’/MxM® 60 virus-infected sleeping eye trees for dry weights at the WSU Roza farm in Prosser, Washington.

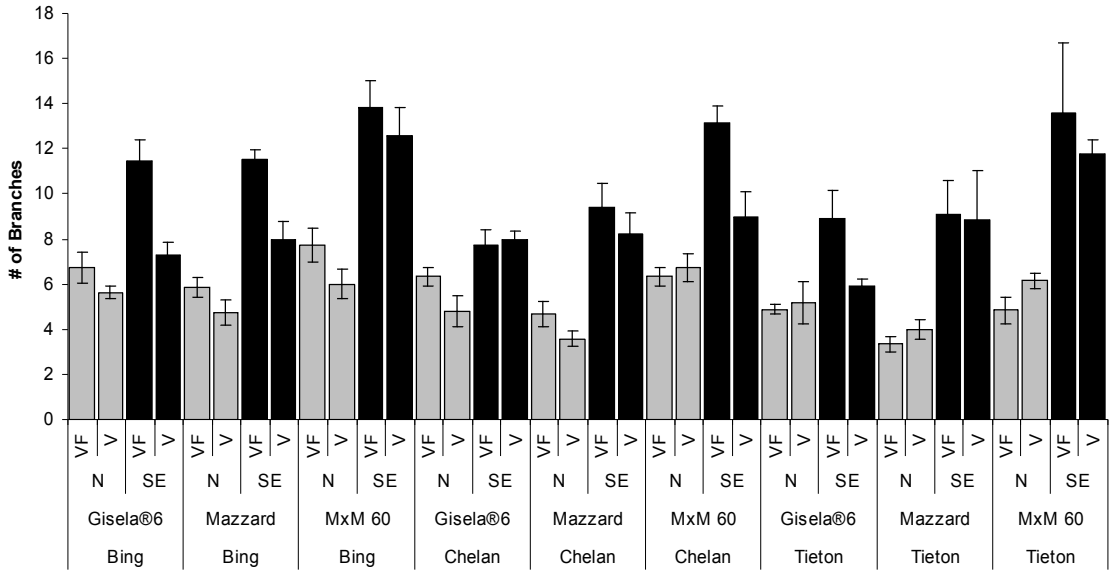


Figure 3.9 – Total number of branches in 2007 for each of the 36 rootstock/cultivar combinations. Data are reported as means of 10 replications. Data are grouped by cultivar, rootstock, and planting system (Black=sleeping eye, Gray=Nursery tree). Trees were budded in fall, 2005 at Willow Drive Nursery in Ephrata, Washington.

APPENDIX

Preliminary economic comparison

This trial consisted of 1800 trees on 2 acres of which half were dormant grafts (sleeping eyes) and the others nursery trees. Table A.1 and A.2 breaks each combination down to its price by variety, root, and tree price for each type of material; prices are estimated figures and do not represent actual purchases. For establishing this 900 tree block with 2 year nursery trees it would cost $7,431; for 900 sleeping eyes it would cost $3,825. When accounting for a 25% tree loss and need to purchase replacement trees the latter cost increases to $4,819. Training costs for young trees include labor, tree stakes, growth tubes, tape, and tools, each are included in the time and materials needed to produce a finished tree from a sleeping eye. Approximately 60 labor hours are needed in training 900 trees through the season (i.e., 4 min per tree). The cost for establishing this sleeping eye block with a 100% success rate would be $746. Table A.3 shows the cost break down for each item and makes a final comparison in price for establishing this 1800 tree block. 900 Nursery trees for this trial came to $7,431 and 900 sleeping eyes with a 25% allowance for replacement trees is $5,566; an $1,866 savings for this trial or $933 an acre. By purchasing replants in the holes of the dead grafts the savings would be $1,093 or $547 an acre. Critical to the ultimate economic analyses are data on tree precocity and productivity. The trees remaining from my research will be used for this purpose.

Table A.1 – Sleeping eye pricing table.

	Variety Charge	Root Charge	Tree price	Sale price	# of Trees	Finished price
Chelan x Mazz	$ 0.75	$ -	$ 2.50	$ 3.25	50	**$ 162.50**
Chelan x MxM®60	$ 0.75	$ 0.50	$ 2.50	$ 3.75	50	**$ 187.50**
Chelan x Gisela®6	$ 0.75	$ 3.00	$ 2.50	$ 6.25	50	**$ 312.50**
Bing x Mazz	$ -	$ -	$ 2.50	$ 2.50	50	**$ 125.00**
Bing x MxM®60	$ -	$ 0.50	$ 2.50	$ 3.00	50	**$ 150.00**
Bing x Gisela®6	$ -	$ 3.00	$ 2.50	$ 5.50	50	**$ 275.00**
Tieton x Mazz	$ 1.00	$ -	$ 2.50	$ 3.50	50	**$ 175.00**
Tieton x MxM®60	$ 1.00	$ 0.50	$ 2.50	$ 4.00	50	**$ 200.00**
Tieton x Gisela®6	$ 1.00	$ 3.00	$ 2.50	$ 6.50	50	**$ 325.00**
				Virus Free	450	**$ 1,912.50**
				Infected	450	**$ 1,912.50**
				Total	900	**$ 3,825.00**

Table A.2 – Nursery tree pricing table.

	Variety Charge	Root Charge	Tree price	Sale price	Trees	Price	
Chelan x Mazz	$ 0.75	$ -	$ 6.34	$ 7.09	50	**$**	**354.50**
Chelan x MxM®60	$ 0.75	$ 0.50	$ 6.34	$ 7.59	50	**$**	**379.50**
Chelan x Gisela®6	$ 0.75	$ 3.00	$ 6.84	$ 10.59	50	**$**	**529.50**
Bing x Mazz	$ -	$ -	$ 6.34	$ 6.34	50	**$**	**317.00**
Bing x MxM®60	$ -	$ 0.50	$ 6.34	$ 6.84	50	**$**	**342.00**
Bing x Gisela®6	$ -	$ 3.00	$ 6.84	$ 9.84	50	**$**	**492.00**
Tieton x Mazz	$ 1.00	$ -	$ 6.34	$ 7.34	50	**$**	**367.00**
Tieton x MxM®60	$ 1.00	$ 0.50	$ 6.34	$ 7.84	50	**$**	**392.00**
Tieton x Gisela®6	$ 1.00	$ 3.00	$ 6.84	$ 10.84	50	**$**	**542.00**
				Virus Free	450	**$**	**3,715.50**
				Infected	450	**$**	**3,715.50**
				Total	900	**$**	**7,431.00**

Table A.3 – Pricing table for orchard establishment between nursery trees and sleeping eyes for 900 trees each.

Sleeping eye	$	3,825.00
Training cost	$	486.00
Materials	$	260.00
25% extra sleeping eyes	$	994.50
Re-plant (std. nursery tree) 24%	$	1,767.51
Total with extra sleeping eyes	$	5,565.50
Total with re-plant	$	6,338.51
Nursery tree	$	7,431.00
Difference with extra sleeping eye	$	1,865.50
Difference with re-plant trees	$	1,092.49