This is to certify that the
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ROOTSTOCK AFFECTS FLOWER DISTRIBUTION AND
PATTERNING IN 'HEDELFINGER' (*Prunus avium* L.) SWEET
CHERRY AND 'MONTMORENCY' (*P. cerasus* L.) TART
CHERRY

presented by

KAREN MAGUYLO

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Major Professor's Signature

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ROOTSTOCK AFFECTS FLOWER DISTRIBUTION AND PATTERNING IN ‘HEDELFINGER’ (*Prunus avium* L.) SWEET CHERRY AND ‘MONTMORENCY’ (*P. cerasus* L.) TART CHERRY

By

Karen Maguylo

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ABSTRACT

ROOTSTOCK AFFECTS FLOWER DISTRIBUTION AND PATTERNING IN 'HEDELFINGER' (*Prunus avium* L.) SWEET CHERRY AND 'MONTMORENCY' (*P. cerasus* L.) TART CHERRY

By

Karen Maguylo

New precocious and highly productive cherry rootstocks have led to management challenges for balancing of crop levels with adequate leaf area to assure good fruit size and quality. To examine how different cropping potentials might be managed more strategically, the influence of individual rootstock genotypes on flower bud distribution and density was characterized using `Hedelfinger' sweet cherry (*Prunus avium* L.) and 'Montmorency' tart cherry (*Prunus cerasus* L.) scions. Flower bud formation occurs solitarily on one-year-old shoots, or on spurs of two-year and older shoot sections. In 2001 (trees planted spring 1998 as part of the NC-140 regional tree fruit rootstock project), the second- year shoot section of branches was used to characterize rootstock influence on the development of flowers, buds, spurs, blind nodes, lateral shoots, and ultimately crop load. Both rootstock and position within the second-year-shoot section affected flower number per bud. Rootstock genotype also influenced location of lateral shoots, spurs, and vegetative axillary buds. These spur locational and flowering characteristics provide helpful parameters for evaluating, and eventually managing, such rootstocks that can dramatically alter sweet and tart cherry scion precocity and/or vigor.

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Literature Review

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INTRODUCTION

Cherry Production

The United States (US) produced 369.3 million pounds and 230,100 tons of tart (*Prunus cerasus* L.) and sweet (*P. avium* L.) cherries, respectively, during 2001 (NASS, 2002). Michigan is the largest U.S. producer of tart cherries and one of the four largest sweet cherry – producing states (Westwood, 1993). 'Montmorency' is the main tart variety, and 'Hedelfinger' is one of the main sweet varieties grown in Michigan. Both varieties are also grown extensively worldwide (Westwood, 1993).

Many of the fruit crops in the US are becoming less profitable due, in part, to increased labor costs and increased foreign competition. However, fresh market sweet cherries are one of the few fruit crops in the US increasing in value (NASS, 2001). A short postharvest life and limited climatic adaptability foster a profitable niche in world markets and reduced competition. Although cherry production has increased worldwide due to current profitability, the US has a higher labor cost than many production areas. If production and labor costs can be reduced for sweet and tart cherries, the US has the potential to remain among the leading world cherry exporters. Growers, then, must explore ways to reduce input costs and increase productivity, thereby maintaining or increasing their profits. One strategy to both increase productivity and reduce input costs is to improve and utilize knowledge of flowering (and ultimately fruiting) habits, which can lead to the optimization of cultural practices such as tree training and pruning. This, in turn, can increase production efficiency.

Flowering and fruiting habits can be explored on a variety of levels. The whole plant as well as the individual bud is influenced by both exogenous and end

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endogenous factors. These factors can contribute to location of flowers and the density of flowers in a given area of the plant or shoot. This review will cover:

- Patterns in trees
- The concept of pattern and different levels of patterning within trees
- Flowering
- Juvenility, flower initiation, and factors affecting flower initiation
- The growth and fruiting habit of cherry

PATTERNS IN TREES

The concept of pattern

The three components in the study of living systems are theorized to be: (1) pattern, (2) structure, and (3) process (Maturana and Varela, 1987). The description of the pattern involves an abstract mapping of relationships within an organism, whereas the description of the structure involves describing the system's actual physical components (shape, chemical composition, etc.). The third component, process, is the link between pattern and structure. It is necessary to continually maintain patterns in living organisms and move matter and energy through the structure. In summary, patterns can be recognized only if they are embodied in a physical structure, and in living organisms, this embodiment is an ongoing process.

The study of trees can be divided into the study of pattern, structure, and process as well. Godin and Caraglio (1998) have divided the study of trees into the study of their different 'structures' such as spatial structure (the study of plant constituents in 3-

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dimensional space), mechanical structure (distribution of mechanical constraints), topological structure, etc. Topological structure is the breaking down of a plant into elementary constituents and then describing the connection between the constituents. Topological structure, by this definition, is the same as pattern as defined by Maturana and Varela (1987).

Levels of Pattern in Trees

Flower bud "quality" is a parameter often questioned by tree fruit growers, and Crabbé (1984) stated that better forecasting of flower location would provide a more accurate base from which to study this characteristic. Commitment of a meristem to one developmental pathway (flowering) stops that meristem from following another developmental pathway. The location of specific reproductive or vegetative meristems has been suggested to be a more accurate predictor of resource allocation in plants than fixed carbon or nutrients, and this resource allocation influences flowering and fruit quality. So far, it is hard to predict where and when flower buds are initiated. It is suggested that the rate of node production (plastochron) is related to the resultant meristem activity, such that shorter plastochrons result in floral meristems while longer plastochrons result in vegetative meristems (Crabbé, 1984).

The productive potential of a tree consists of three levels of growth: (1) general architecture, or the growth pattern of the main stem and its scaffolds, (2) type and arrangement pattern of the spurs on the branch, and (3) natural evolution, or aging, of the spurs (Lespinasse and Lauri, 1996). Clearly, then, pattern is a large part of the productive potential of the tree. Practical applications of studying pattern in fruit trees include: (1)

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the creation of new tree types through breeding due to the selection of specific growth and flowering habits, (2) development of new training systems that will have reduced or simplified pruning and thinning requirements, and natural regulation of fruit production, and (3) a more rational approach to cultivation, for example, pruning that would lead to an improvement in fruit quality characteristics and less labor costs (Lespinasse and Lauri, 1996). Another useful aspect of studying patterns is that the resulting training systems can be applied to other species with similar branch and flower patterns.

The study of pattern in trees can be accomplished at many levels. Tree architecture organizes a tree in both space and time. The two main purposes for the study of tree architecture are: (1) plant growth modeling, and (2) analysis of architecture. The analysis of architecture can be used as a base to study the application of architecture in horticultural or forestry contexts. The application of architectural analyses, however, requires the quantification of architecture.

Hallé and Oldeman (1970) characterized the architecture of trees through the use of the architectural model. The parameters used to separate individual species into groups are based on patterns of meristematic development and branching behavior. The analysis of meristem development led Hallé and Oldeman (1970) to distinguish at least 25 architectural models. These models are stable and usually constant throughout the life of the plant. Architectural models allow the classification of trees into one of these models or the combination of two models via quantifying the architecture of the tree.

For a more specific analysis of pattern, a single tree can be divided into parts either artificially or naturally. Artificial division of a tree simply means that there is no biological reason for the division, such 10 cm of shoot length. Natural division uses mor

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morphological features to identify plant components, such as nodes on shoots. Plants can be divided into components with identical nature, called modules. Modules are the products of a single apical meristem, and so pattern is related to growth of each apical meristem (Godin and Caraglio, 1998; White, 1984).

Growth of angiosperm shoots can be described as a series of repeating units (metamers) that are formed sequentially by the apical meristem. In the most basic form, a metamer consists of a node with the associated leaf-like organ, lateral meristem in the axil of the leaf, and the internode (White, 1984). According to this concept, much, if not all, of the variation observed in shoot patterns can be accounted for by differences in the number of metameric units produced per shoot, their rotational orientation with respect to each other (phyllotaxy), and the metameric unit type (meristem fate). The developmental significance of the metameric unit concept in plants has not been established clearly (Rutishauser and Sattler, 1985), but it offers a logical and easy way to describe variation in shoot patterns (van Groenendael, 1985). Schultz and Haughn (1991) suggest that there is an ordered array of different metamers in a mature Arabidopsis thaliana (Arabidopsis) and that there must be mechanisms that specify the type of metamer to be produced at a particular time in development. This suggestion can also be applied to trees.

Pattern and arrangement of flowers have been noted across various fruit tree species. Perez-Gonzalez (1993) demonstrated that, of 50 different peach (*P. persica* L. Batsch) cultivars, there was a difference in flower bud distribution based on origin of the cultivar. Three broad classes were found: (1) genotypes with more than 60% of nodes having one bud, which were from Mediterranean climates, (2) cultivars with more than 30% of nodes having three buds, originating from colder climates, and (3) cultivars with a rel

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a relatively even distribution of nodes having one, two, or three buds. The proportion of blind nodes was correlated negatively with bud number per node in peach, and that proportion of nodes with one bud was correlated negatively with nodes that have 2 or 3 buds. Schaumberg and Gruppe (1985) reported that the effect of rootstock on the number of floral buds per spur was small on 'Hedelfinger' sweet cherry, but that there was a significant effect on the number of flowers per bud. They also noticed that the number of buds per spur was correlated strongly with the location of the spur within the branch. The number of buds per spur increased distally along two-, three-, and four-year old shoots. The greatest spur flowering and fruiting occurred on the two-year-old shoot sections, while flowering and fruiting of individual spurs declined with spur age (Schaumberg and Gruppe, 1985). In a similar rootstock trial in Germany, rootstock affected the distribution, as well as density, of spurs within the shoot (Franken-Bembenek and Gruppe, 1985).

FLOWERING

Juvenility

The growth of flowering plants is divided into two different phases. First, there is a juvenile vegetative phase, during which leaves and lateral shoots are produced, and a reproductive phase, during which (concomitant with leaves and lateral shoots) flowers are produced. Currently, there is no accurate way to distinguish the end of the juvenile phase from the non-flowering adult phase in trees, so the period during which the tree has the ability to flower, but is not currently flowering, is called the transition

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period (Zimmerman, 1973). The transition period is qualitatively the same as the adult phase, but cannot be distinguished accurately from the juvenile phase in trees (Hackett, 1985). The ability to distinquish between the juvenile and transition (adult vegetative) phases via molecular pathways may be possible.

The length of the juvenile phase is determined genetically, and once the juvenile phase has ended, flower initiation can occur. Many attempts have been made to shorten the juvenile phase (Zimmerman, 1972), since a long juvenile phase creates problems for both the tree fruit production industry (long time to first crop) and breeders (long time between breeding cycles) (Egea-Cortines and Weiss, 2001). Once a tree has reached the adult phase, each axillary meristem will either remain vegetative or make the transition to produce flowers (Pidkowich, 1999). Upon transition to flowering, the meristem takes on new characteristics. Most practices that result in early flowering of fruit trees probably do so by shortening the juvenile period. Treatments that speed the development of seedling trees probably shorten the transition period. Once a tree has reached the adult phase, it cannot make the transition back to the juvenile phase. However, cultural practices, such as the use of vigorous rootstocks, may influence a delay in flowering in adult scions by shifting them back into the transitional period, which is still part of the adult phase (Zimmerman, 1973).

Flower Initiation

Once the adult phase has been reached, an annual cycle of flowering occurs. The flowering process is composed of three parts: (1) flower initiation (or flower induction), (2) differentiation (or development) of the growing floral meristem, and (3) bloom (Kozlowski, 1971). Flower initiation is a qualitative change in which a meristem is programmed to form flowers (Bewley et al., 2000). In trees, the partially developed buds receive a signal for flower initiation to begin, although the nature of this signal is unknown (Faust, 1989). Flower bud differentiation is when visible morphological changes occur at the growing point. One idea is that hormones have a large influence over the early parts of bud development, and that carbohydrates and nitrogen availability are more of the limiting factor in later parts of bud development (Faust, 1989). Flower differentiation culminates in bloom (Bewley et al., 2000).

In both sweet and tart cherry trees, differentiation of the growing point has been observed using the scanning electron microscope by Guimond et al. (1998a) and Diaz et al. (1981), respectively. Changes in the sweet cherry meristem are evident 91-105 days after full bloom. Early in the visible transformation of a vegetative bud to a floral bud is a broadening and flattening of the rounded meristem. Then two to four small lateral protuberances (primordial), representing primordial bracts, which subtend each flower, develop. Individual flower primordia are then evident in the axil of each bract. Over the summer, sepal primordia differentiate first, then petals, stamens, and finally pistils. By leaf fall, all the floral parts are visible in an immature stage (Thompson, 1996).

Pathway of Flower Initiation

Progress has been made in understanding the pathway of flower initiation using molecular biology (Pidkowich et al., 1999). The shoot apical meristem (SAM) of higher plants is the site of floral initiation. The SAM consists of a small number of undifferentiated dividing cells that are laid out in an organized manner (Evans and

Barton, 1997). In the juvenile phase, the SAM is characterized by the production of primordia that develop into leaves, with a pattern of differentiation that is distinct from those produced in the adult phase (Simpson et al., 1999). The importance of the transition period to flower initiation is that, normally, only the adult vegetative meristem is competent to induce flowers (Telfer and Poethig, 1998; Weigel and Nilsson, 1995). Environmental signals that would normally induce flower initiation fail to during the juvenile phase, even though the transition period may appear phenotypically similar to the juvenile phase (Zimmerman, 1972).

Newly discovered pathways for flower initiation have shed some light on the question of whether a plant is in the adult or juvenile phase. Up until the discoveries of genes associated with flowering, the only consistent characteristic to assess the end of the juvenile period was the attainment and maintenance of the ability or potential to flower (Hackett, 1985). In Arabidopsis, an early-flowering mutant, *hasty*, is the result of a faster movement from the juvenile to adult vegetative phase (Telfer and Poethig, 1998). The vegetative-to-floral transition, however, is unchanged relative to wild-type. The gene, HASTY, promotes a juvenile pattern of vegetative development and inhibits flowering by reducing the competence of the SAM to respond to activity of the floral initiation genes (Telfer and Poethig, 1998).

The fate of a floral meristem is highly regulated (Ma, 1998). Floral initiation is accomplished and regulated by floral meristem identity genes (Weigel and Meyerowitz, 1993; Blazquez et al., 1997), which are also called FLIP (Floral Initiation Process) genes (Schultz and Haughn, 1993). Floral initiation is followed by pattern formation and organogenesis of the flower, which is carried out by floral homeotic genes (Weigel and

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Meyerowitz, 1993). Floral fate of a meristem is not due to a single switch, but is a condition that is acquired progressively. Interactions between at least five FLIP genes have been found to determine meristem fate in Arabidopsis. These genes include LEAFY (LFY), APETALA1 (AP1), CAULIFLOWER (CAL), APETALA2 (AP2), and UNUSUAL FLORAL ORGANS (UFO) (Pidkowich et al., 1999). Multiple pathways that regulate the timing of the floral transition act directly or indirectly on the FLIP genes, which are responsible for switching the fate of the meristem from vegetative to floral (Blazquez et al., 1997). Constitutive expression of some of these regulatory genes promotes precocious flowering (Ma, 1998).

All or some of these genes have been found to be well-conserved in species as diverse as *Pinus radiata* (Mellerowicz et al., 1998), *Populus trichocarpa* (Rottman et al., 2000), and *Eucalyptus globulus* (Southerton et al., 1998). The requirement for LFY to make the transition is well documented (Schultz and Haughn, 1991; Huala and Sussex, 1992; Shannon and Meeks-Wagner, 1991). Although experiments have shown that LFY is sufficient to determine floral fate in Arabidopsis, it also has been suggested that interactions between other genes are required (Liljegren et al., 1999). Evidence suggests that LFY, AP1, and AP2 gene products all have a role in switching meristems from a vegetative fate to a floral fate by suppressing the vegetative pathway and activating floral pathway. It has been shown that AP1 and LFY act together to specify floral meristem fate, even though constitutive expression of LFY alone can determine floral meristem fate, and cause precocious flowering in Arabidopsis. In an experiment by Weigel and Nilsson (1995), in which LFY was constitutively expressed via the 35-S promoter, both aspen (*Populus* spp.) and Arabidopsis formed solitary flowers instead of shoots in the axils of vegetative leaves. AP1 has been demonstrated to positively regulate LFY, and in turn, LFY can positively regulate AP1. They appear to work together to specify the floral meristem fate (Liljegren et al., 1999). Early expression of AP1 has been found to be the result of transcriptional activation by LFY, and was independent of protein synthesis (Wagner et al., 1999). It has been shown that TFL (TERMINAL FLOWER1) is a negative regulator of both LFY and AP1 in Arabidopsis (Liljegrin et al., 1999; Mandel and Yanofsky, 1995). Because constitutive expression of LFY results in a phenotype in Arabidopsis that is similar to mutations in TFL, and TFL is a spatial regulator of LFY, it has been suggested that LFY and TFL are antagonists (Shannon and Meeks-Wagner, 1991; Alvarez et al., 1992). UFO, another FLIP gene, requires LFY activity for its function, but not its expression, so UFO could lie downstream of LFY and only control a part of LFY function (Pidkowich et al., 1999). UFO appears to play a role in floral meristem identity, although it is known better for its function in defining the boundaries between floral organs in adjacent whorls of the flower as well as between floral organs of the same whorl (Levin and Meyerowitz, 1995). CAL is partially redundant with AP1. *cal* mutants have no significant phenotype alone, but *cal* mutations greatly enhance ap1 mutants, such that the resulting inflorescences of *cal/ap1* double mutants look like miniature cauliflowers, because there is a massive proliferation of floral meristematic tissue (Bowman et al., 1993).

Ectopic expression of LFY or AP1 only is not sufficient to direct floral development immediately after germination. A period of vegetative growth still occurs. As in wild-type plants, the duration of this vegetative growth is sensitive to environmental conditions such as photoperiod (Coupland, 1995). The connection

between the flowering time genes and LFY has been addressed directly (Blazquez et al., 1998; Nilsson et al., 1998). Indeed, the flowering time genes CO, GI, FCA, FVE, GA1, and GAI all play a role in activation of LFY and are required to some extent for the full expression of LFY function.

Factors that Influence Flower Initiation

Flower bud initiation is genetically, as well as biochemically, multifactorial, meaning that there are many genetic and biochemical factors that directly or indirectly affect flower initiation (Bewley et al., 2000). In reviews of the subject by Zimmerman (1972), Ryugo (1986), Buban and Faust (1982), Kozlowski (1971), Hackett (1985), Gur (1985), and Bernier (1988), the common factors included length of the juvenile period (and therefore, age of the tree), environmental considerations such as light and temperature, internal processes such as growth regulators and carbohydrate supply, and cultural practices.

Environmental Factors

Temperature has been shown to affect flower bud initiation in apricot 'Moorcot' (*Prunus armeniaca* L.). High temperatures in winter increased the number of 'Moorcot' flower buds per tree by increasing the number of flower buds per node (Jackson, 1970). It has also been demonstrated that leaf emergence and development are dependant upon temperature and are directly related to degree-day accumulations in tart cherry (Eisensmith et al., 1980). This is significant since a minimum leaf area is required for flower bud initiation.

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Light is a major factor in initiation of flower buds. Research has shown that under artificial conditions (shade houses) 'Montmorency' tart cherry will not flower below a light level of 21% full sun, and at 36% full sun, flower number per tree is greatly reduced, although flower number per bud is not different than 100% full sun conditions (Flore, 1980). Under field conditions, there was a significant decrease in flowering at light levels below 20% full sun (Flore, 1980). Lower light levels have also been known to decrease leaf dry weight, total leaf dry weight per spur, and specific leaf weight in 'Delicious' apple (*Malus domestica*) (Barritt et al., 1987). Although this is not a direct effect on flowering, exposure of spur leaves to light impacts flowering in the following season since a minimum spur leaf area is necessary for flower bud formation (Harley et al., 1942). In apricot trees, shading results in a very pronounced decrease in flower bud initiation (Jackson, 1970). The impact of light levels on flower bud initiation, then, influences placement of flower buds within the canopy of larger trees where within canopy shading is present at levels that inhibit flower bud initiation. In apple, spur leaf area and spur leaf size were positively related to long-term productivity of the spur (Rom and Ferree, 1984). It has been suggested that the greater yield efficiency of apple trees on a more dwarfing rootstock, M.9, relative to more vigorous rootstocks, was due to improved light distribution in the canopies of smaller trees (Schecter et al., 1991; Heinicke, 1964). This increase in yield efficiency is most likely due to an increase in fruit number, the result of an increase in flower initiation, since variation in fruit number rather then fruit weight account for most of the variation in yield in fruit trees (Browning, 1985).

Plant Hormones

Since flower initiation is multifactorial, the influence of a specific hormone on flower initiation is dependant on such things as time of the season, levels of other hormones, and availability of nutrients (Faust, 1989). Although many hormones and other molecules have been found to promote flower initiation, none have been found to be completely responsible for the control of this process (Bewley et al., 2000). It has been hypothesized that a universal flowering hormone, florigen, exists, although isolation and characterization of the hormone has been unsuccessful (Taiz and Zeiger, 1998). The floral initiation stimulus may involve the interaction of the hormones cytokinins and gibberellins (GAs) (Bernier, 1988). In fruit trees, GAs have been found to inhibit floral initiation (Westwood, 1993), while in conifers GAs promote flowering (Ekberg and Eriksson, 1985). High endogenous levels of GAs in angiosperms appear to be associated with the vegetative or juvenile condition (Hackett, 1985). Cytokinins have been implicated in cell division and enlargement, and in overcoming apical dominance by promoting growth of inactive lateral buds (Kozlowski and Pallardy, 1997). The interaction of cytokinins and gibberellins may be more important in floral initiation than the action of either hormone alone (Taiz and Zeiger, 1998). Another hormone implicated in flower initiation is ethylene. In pineapple (*Ananas comosus*), ethephon, an ethylene-releasing agent, increased flower initiation when an adequate amount of the ethephon was taken up by the plant (Turnbull et al., 1999).

The application of gibberellic acid (GA) accelerates precocious flowering of Arabidopsis (Langridge, 1957). GAs accelerate primordium initiation at the apex, and therefore, early manifestation of flower induction (Evans and Blundell, 1996). The role of GAs in activation of the LFY promoter has recently been analyzed (Blazquez et al., 1998). The level of LFY promoter activity is lower in GA-deficient, ga1, mutants. Therefore, GAs promote flowering in Arabidopsis at least in part by activating LFY expression (Blazquez et al., 1998).

Cultural Practices:

The time of floral initiation and the number and location of flower buds initiated are influenced by certain orchard management practices (Thompson, 1996). Since flowers tend to set when growth ceases, cultural practices that decrease vigorous shoot growth also enhance floral bud initiation (Forshey and Elving, 1989). Summer pruning was shown to encourage flower bud formation, while pruning during the dormant period encourages more vigorous growth and so reduces flower bud formation. If summer pruning is done before growth ceases, there is generally an early vigorous growth of new shoots. Pruning during early summer results in a delay of the maturation of seasonal tree growth, including fruit ripening (Chandler, 1923). Rootstocks, apart from their influence on vigor, can influence both precocity and the amount of flowering in consecutive years (Thompson, 1996). With very dwarfing cherry rootstocks, early senescence was observed in shoots and spurs of branches four years and older. Removal of 60 – 70% of the canopy in August rejuvenated shoot growth (Schaumberg and Gruppe, 1985).

Rootstocks:

Rootstock use in commercial fruit production is common due to their beneficial effects on the growth and development of the scion. In tree fruits, rootstocks are known to affect tree size, precocity, flowering, yield efficiency, dry matter partitioning, and fruit quality (Autio et al., 1996; Anthony et al., 1938; Warrington et al., 1990; Westwood et al., 1976; Yadava and Doud, 1989). The tendency for trees on dwarfing rootstocks to produce more fruit relative to tree size has been well established (Hirst and Ferree, 1996). There is evidence, however, that tree size, as affected by rootstock, is not strongly related to spur density (number of spurs per unit shoot length) or flower density (number of flowers per unit shoot length) in 'Delicious' apple (Hirst and Ferree, 1996) or to yield in 'Montmorency' tart cherry (Westwood et al., 1976). In an experiment with 'Delicious' apple on nine rootstocks, there was a strong correlation between the number of spurs per limb circumference and limb yield efficiency (g fruit per cm limb circumference) (Schecter et al., 1991). Schecter et al. suggested that yield potential is influenced by rootstock, in part, by altering the spur density of the scion. In a study by Hirst and Ferree (1996), only half the variation observed in flower density could be explained by trunk cross-sectional area (TCSA) or shoot length, implying that rootstock has an effect on flowering independent of its affect on tree size. In this same study, rootstock effect on flower density was unrelated to its affect on spur density, so rootstocks did not influence flowering by producing a higher number of spurs. The number of leaves per spur, leaf area per spur, and leaf area per leaf of 'Delicious' apple were lowest on the dwarfing rootstocks, while specific leaf weight was higher. This means that the most dwarfing

rootstocks influenced both shoot growth as well as vigor of individual spurs, in terms of leaf area (Warrington et al., 1990).

Rootstock trials in Germany and the U.S. show an effect of rootstock on flowering in both sweet and tart cherry. Gruppe (1985a) noticed that rootstocks with at least one tetraploid species (*P. cerasus* and *P. fruticosa*) as a parent induced significant precocity on the scion. It is also interesting to note that the amount of dwarfing had no effect on precocity (Gruppe, 1985b). 'Montmorency' tart cherry on the precocious Gisela rootstocks produced a lot of blind wood, the result of a high percentage of flowering in one-year-old laterals (Perry, 1996). Sweet cherry trees on dwarfing rootstocks have an early senescence of the spurs, as well as decreasing amounts of vegetative growth each year (Gruppe, 1985a).

GROWTH AND FRUITING HABITS OF CHERRY TREES

Sweet and tart cherries produce simple buds, which contain either leaves or flowers. Floral buds are borne either in a lateral (axillary) position on one-year old shoots, or on spurs (shoots less than 4 cm) on two-year and older wood (Flore et al., 1996; Thompson, 1996). In sweet cherry, the most generative spurs are located on the two- and three-year old wood, so this is where the majority of flowers occur (Wustenberghs and Keulemans, 2000). Spurs differ between the tart and sweet cherries in general. In sweet cherries, spurs can remain active for up to 10 years, as long as sufficient environmental requirements are met, therefore can providing a sustainable form of flowering and fruiting. In tart cherry, spurs are most likely to be solely on the two- and three- year-old shoots, with only a few persistant spurs in four- and five- year old shoots

(Thompson, 1996). In tart cherry, a greater percentage of flowers are borne on axillary buds than on spurs. This is in contrast to sweet cherries, where most of the flowers are borne on spur buds. In sweet cherries, each floral bud is surrounded by several bud scales, and contains two to four flowers; from one to six or more buds may occur on each spur (Thompson, 1996). In tart cherries, there are also two to four flowers per bud. The percent of flowers borne on one-year-old laterals relative to spurs varies between cultivars, as well as within a cultivar, depending on cultural management. Thompson (1996) reported that 68% of 'Montmorency' flowers are on one-year-old shoots, whereas another study (not cited in Thompson, 1996) showed 'Montmorency' bore only 35-45% of its flowers on the one-year-old laterals.

Sweet cherry trees are characterized by their strong apical dominance. This apical dominance causes strong growth of terminal shoots and an inhibition of bud-break for long distances below the growing point. The tart cherry has less apical dominance and a greater tendency for production of blind wood (see below) and poor branch angles. Lateral shoots just below the leader of tart cherry trees have a tendency to become dominant over the leader (Flore et al., 1996)

If the axillary bud on a one-year-old shoot flowers, in the following year the node will be blind because it cannot initiate vegetative growth (Flore et al., 1996). Production of blind nodes is cultivar dependent (Wustenberghs and Keulemans, 2000), and decreases the productive area of the shoot. Because tart cherries have a significant percentage of flowers borne on axillary buds (e.g. 'Montmorency' produces only about 30 % of its flowers on spurs), there can be a large amount of blind nodes produced (Flore et al., 1996). Blind nodes can also be the result of buds that were vegetative in the first year and inactive in the second, or spurs that did not have vegetative buds and were thus rendered inactive in the following season (Wustenburghs and Keulemans, 2000). The latter production of blind nodes occurs because cherries initiate flowers only in buds that have attendant leaves opening early in the summer. If the leaves are not present, then the buds become inactive (Westwood, 1993).

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CHAPTER TWO

Rootstock Affects Floral Distribution and Patterning in 'Hedelfinger' Sweet Cherry

Rootstock Affects Floral Distribution and Patterning in 'Hedelfinger' Sweet Cherry.

ABSTRACT

The NC-140 regional tree fruit rootstock project established a trial of cherry rootstocks across North America in 1998 to evaluate their performance with the sweet cherry scions 'Bing' and 'Hedelfinger'. One 'Hedelfinger' trial was established near Traverse City, Michigan. This plot was used to characterize rootstock influence on the development of flowers, buds, spurs, blind nodes, lateral shoots, and ultimately crop load, on two-year-old shoots. Both rootstock genotype and node location on the shoot influenced bud number per spur and flower number per bud. Both bud number per spur and flowers per bud increased, in general, as tree vigor decreased. The exception was in the Gisela/Giessen series of rootstock, in which both bud number per spur and flower number per bud decreased with a decrease in tree vigor. Both parameters increased distally along the shoot. Rootstock genotype influenced location of lateral shoots, spurs, vegetative axillary buds, and blind nodes on two-year old shoots. Spurs dominated in the medial section, and lateral shoots in the distal section, of the less vigorous rootstocks, while vegetative axillary buds dominated in all sections of the more vigorous rootstocks. These spur locational and flowering characteristics provide helpful parameters for evaluating, and ultimately managing, such rootstocks that can dramatically alter sweet cherry scion precocity and/or vigor.

INTRODUCTION

Cherry rootstocks have been selected for various reasons, including precocity, productivity, vigor control, disease tolerance, and adaptability to different soils. With some of these rootstocks, there is a potential problem of excessive cropping levels when grafted to very productive scion cultivars. Excessive cropping can result in poor fruit quality and stunted vegetative growth (Lang, 2000). One approach to study this phenomenon, and potentially develop strategies to manage it, is to more precisely characterize how rootstock genotype influences tree architecture and flower placement on the scion; that is, how the development of nodes is affected differentially within a shoot by different rootstocks. Determining growth and location patterns of different nodes can be used to assess how nodes are affected differentially within a shoot.

Pattern can be studied within a tree by dividing the tree either artificially, e.g., empirically dividing a shoot into equal units of length, or naturally, e.g., using biological growth parameters that are repeated throughout the tree or have an identical nature (Godin and Caraglio, 1998; White, 1984). Growth of angiosperm shoots can be described as a series of repeating units (metamers) that are formed sequentially by the apical meristem. In the most basic form, a metamer consists of a node with an associated leaf-like organ, a lateral meristem in the axil of the leaf, and the proximally located internode (White, 1984). It has been suggested that there is an ordered pattern of different metamers (Schultz and Haughn, 1991).

Distribution of different metamers within the plant plays a large part in the productive potential of tree fruit species (Lespinasse and Lauri, 1996). Metamer types in sweet cherry include blind nodes, lateral shoots, single vegetative buds, and spurs.

Lespinasse and Lauri suggested that studying the patterns and distribution of different metamer types in fruit trees would result in several practical applications including the development of new tree growth habits through breeding, new training systems that reduce pruning and thinning, and a more rational approach to cultivation that could lead to an improvement in fruit quality characteristics. A better ability to predict flower location would also provide a more definitive base for the study of flower bud 'quality' (Crabbé, 1984).

Rootstocks are known to affect spur characteristics. Franken-Bembenek and Gruppe (1985) found that yield potential in sweet cherry was mainly dependent on rootstock. One of the main ways the rootstock altered yield potential was by altering location of spur insertions. Schaumberg and Gruppe (1985) tested 'Hedelfinger' sweet cherry on some of the Giessen series rootstocks, and observed that the number of buds per spur was not altered, but that the number of flowers per bud was greatly affected by rootstock. The number of floral buds per spur depended greatly on the position of the spurs within the shoot. More distally located spurs produced a greater number of buds per spur. Other correlations have been made between location of the spur and the number of floral buds per spur in sweet cherry cultivars, indicating that position within the shoot affects spur flowering characteristics (Wustenberghs and Keulemans, 2000).

The limitation of the above trials is that they were done on a limited number of rootstocks, and some rootstocks not in the trial may be more ideal for conditions in Michigan. A regional tree fruit rootstock (NC-140) trial has been established in the US to evaluate rootstocks for suitability in the US. Rootstock can significantly influence flower density, as well as vigor and yield efficiency. Vigor and yield efficiency are not enough, however, to predict where the flowers will be borne within the canopy, which is important for predicting and managing the productive potential of the tree.

Sweet cherries initiate flowers in the summer before flowering occurs, after active growth has ceased (Guimond et al., 1998). Spurs are first produced in the two-year-old shoot sections in sweet cherry. Flowering and fruiting of individual spurs declines with spur age, therefore the two-year old shoot section is a good predictor of the predominant distribution of spur flowering and different metamer types.

The greatest spur flowering in 'Hedelfinger' sweet cherry occurs in the two-year old shoot section. Schaumberg and Gruppe (1985) reported that the effect of rootstock on the number of floral buds per spur was small on 'Hedelfinger' sweet cherry, but that there was a significant effect on the number of flowers per bud. They also noticed that the number of buds per spur was correlated strongly with the location of the spur within the branch. The number of buds per spur increased distally along two-, three-, and four- year old shoots.

Schaumberg and Gruppe's (1985) trials were in Germany, and so may not have accurately assessed rootstocks examined in trials in the US. The objectives for this experiment were (1) to assess the effect of rootstock and node location within two-year-old wood on spur flowering characteristics (flower number per bud and bud number per spur) and (2) to assess the effect of rootstock on location of the different metamer types (blind nodes, single vegetative buds, lateral shoots, and spurs) in 'Hedelfinger' sweet cherry in Michigan.

MATERIALS AND METHODS

Plant Materials

As part of the NC-140 regional tree fruit rootstock project, a plot of 'Hedelfinger' sweet cherry (*Prunus avium* L.) trees on 21 rootstocks was established in spring 1998 at Michigan State University's Northwest Horticultural Research Station near Traverse City, Michigan. The trees were planted in an Emmet-Leelenau sandy loam in a randomized complete block design with 8 replications; however, a completely randomized design was used for this study since the 5 most uniform replications of 16 selected rootstocks (Table 2-1) were used. The trees were trained to a modified central leader and drip irrigated. Fertilization and protective sprays were applied as by local standard recommendations.

Five trees per rootstock were selected for uniformity in early spring 2001. Three shoots of comparable size and including second-year wood were randomly selected from each tree's central leader. Markings were made using paint so that the second-year section of each shoot was divided empirically into three equal sections (proximal, medial, and distal) (Figure 2-1).

Data Collection

In 2001, data for each node along the two-year-old section of each tagged shoot were collected throughout the season. Each node was characterized according to its placement within the shoot section and its type. Node types used for this study were: (1)

Table 2-1. Rootstocks, listed in order of increasing vigor, characterized in the 1998 NC-140 'Hedelfinger' sweet cherry trial at the NWHRS near Traverse City, Michigan. Vigor values are based on 2001 TCSA. Genotypes are clonal selections unless otherwise noted.

Rootstock	Abbreviation	Origin	Genotype	Vigor (% Mazzard)
Ahrensburg 209/1	Gi. 209/1	Giessen and Ahrensburg, Germany	*P. cerasus* 'Schattenmorelle' X *P. canescens*	12
Edabriz	Edabriz	France (INRA)	*P. cerasus*	30
Gisela 5	Gi. 5	Giessen, Germany	*P. cerasus* X *P. canescens*	34
Gisela 7	Gi. 7	Giessen, Germany	*P. cerasus* X *P. canescens*	41
Weiroot 53	W. 53	Weihenstephan, Germany	*P. cerasus*	41
Weiroot 72	W. 72	Weihenstephan, Germany	*P. cerasus*	45
Giessen 195/20	Gi. 195/20	Giessen, Germany	*P. canescens* X *P. cerasus*	51
Gisela 6	Gi. 6	Giessen, Germany	*P. cerasus* X *P. canescens*	64
Weiroot 158	W. 158	Weihenstephan, Germany	*P. cerasus*	76
Weiroot 13	W. 13	Weihenstephan, Germany	*P. cerasus*	84
Weiroot 10	W. 10	Weihenstephan, Germany	*P. cerasus*	84
CT 500	CT 500	Hungary	seedling selection of *P. mahaleb*	100
Mazzard	Mazzard	south and central Europe and Asia Minor	*P. avium* L. seedling	100
Mahaleb	Mahaleb	south and central Europe and Asia Minor	*P. mahaleb* seedling	110
MxM 60	MxM 60	Oregon, USA	*P. mahaleb* hybrid	115
MxM 2	MxM 2	Oregon, USA	*P. mahaleb* hybrid	118

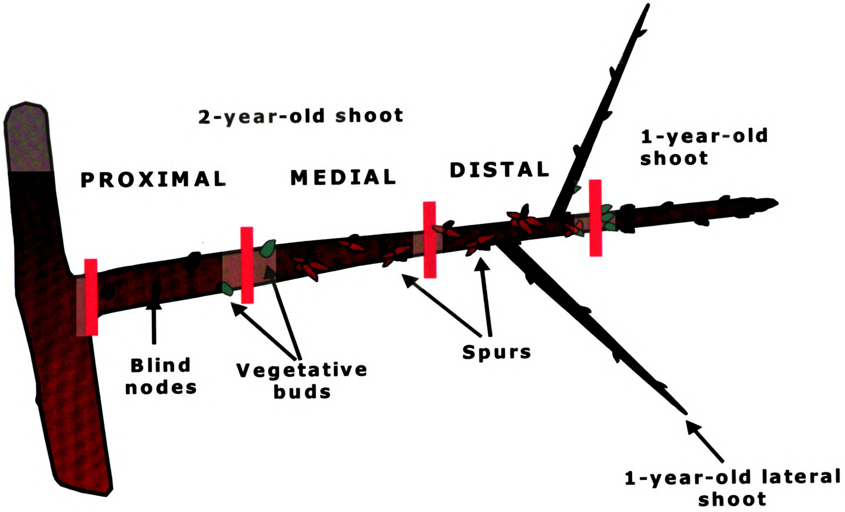

Figure 3-1: Illustration of a two-year-old (and associated one-year-old) cherry shoot. Red lines indicate the different sections by which the shoot was analyzed.

vegetative ("axillary") bud; (2) spur; (3) lateral (axillary) shoot ("lateral"); and (4) blind node.

Three spur parameters were measured. Bud number in each spur was counted in April 2001 and the number of floral buds was determined by subtracting one (for the vegetative bud) from the total buds within the spur. Flowers within each spur were counted on 3 May 2001. Flower number per bud was estimated by dividing the flower number per spur by the floral bud number per spur.

The only parameters measured on nodes with lateral shoots were length of growth that occurred during 2000, and length of growth that occurred during 2001 (made in November 2001, after active growth had ceased and the leaves had dropped). Metamer lengths were estimated for each section (proximal, medial, distal). Since shoots were split into three equal sections, the estimate was done using the following equation:

$$\text{Metamer length (section)} \equiv \frac{\text{total section length}}{\text{number of nodes within the section}}$$

Blind nodes were considered to be those that did not exhibit any active growth, whether vegetative or reproductive. Although there are different reasons for blind node development (Wustenberghs and Keulemans, 2001), these were not ascertained in this study. Most blind nodes of sweet cherry are due to the formation of solitary flower buds at the base of one-year-old shoots; blind nodes in this study are most likely due to such flowering during 2000.

Trunk cross-sectional area (TCSA) is an indicator of tree size (Westwood and Roberts, 1970). Trunk cross-sectional area increase (TCSAI), then, indicates vigor of the scion. TCSAI measurements used in this study were taken as part of the NC-140 regional tree research project (Cowgill and Clements, 2002).

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The only parameters measured on nodes with lateral shoots were length of growth that occurred during 2000, and length of growth that occurred during 2001 (made in November 2001, after active growth had ceased and the leaves had dropped). Metamer lengths were estimated for each section (proximal, medial, distal). Since shoots were split into three equal sections, the estimate was done using the following equation:

$$\text{Metamer length (section)} \equiv \frac{\text{total section length}}{\text{number of nodes within the section}}$$

Blind nodes were considered to be those that did not exhibit any active growth, whether vegetative or reproductive. Although there are different reasons for blind node development (Wustenberghs and Keulemans, 2001), these were not ascertained in this study. Most blind nodes of sweet cherry are due to the formation of solitary flower buds at the base of one-year-old shoots; blind nodes in this study are most likely due to such flowering during 2000.

Trunk cross-sectional area (TCSA) is an indicator of tree size (Westwood and Roberts, 1970). Trunk cross-sectional area increase (TCSAI), then, indicates vigor of the scion. TCSAI measurements used in this study were taken as part of the NC-140 regional tree research project (Cowgill and Clements, 2002).

Statistical Analysis

Statistical analyses were performed using the SAS proc glm program (SAS Institute, 1989). In all studies, pairwise comparisons and the analysis of variance were used in the comparisons among rootstocks. When comparing effects of rootstock on metamer location and flowering characteristics, all values were expressed as mean ± standard error. Statistical significance was calculated using Student's t-test. For the regression analyses, highest R^2 was used to determine the best fitting regression curve.

RESULTS

Flower Bud Number per Spur

In general, the more vigorous rootstocks had fewer buds per spur (Table 2-2). Trees more vigorous than those on W. 10 had no flowering spurs in the proximal section (Table 2-3) and very few flowering spurs, if any, in the medial section (Table 2-4). Spur location within the shoot section also affected flower bud number per spur. The distal section had significantly more buds per spur than the proximal section for all rootstocks (Table 2-5).

Flower Number per Bud

The number of flowers per bud followed the same trends as the number of buds per spur. Rootstock affected flower number per bud, with the more vigorous rootstocks having fewer flowers per bud (Table 2-2). No spurs were present in the two-year-old shoot section of trees more vigorous than those on W. 10. There was a difference in the number of flowers per bud between the proximal and distal sections for all rootstocks

Table 2-3. The effect of rootstock, listed in order of increasing vigor, on the overall number of flowers per bud and floral buds per spur (± standard error) in the proximal part of two-year-old shoot section of 'Hedelfinger' sweet cherry. Counts were taken in the 2001 growing season.

	Buds per spur	Flowers per bud
Gi. 209/1	0.9 ± 0.5	1.2 ± 0.7
Edabriz	0.0 ± 0.0	0.0 ± 0.0
W. 53	0.8 ± 0.4	1.6 ± 0.6
W. 72	0.6 ± 0.2	1.7 ± 0.7
Gi. 5	1.7 ± 0.3	2.9 ± 0.4
Gi. 7	2.2 ± 0.6	2.4 ± 0.7
Gi. 195/20	3.2 ± 0.3	3.3 ± 0.1
Gi. 6	1.8 ± 0.6	2.6 ± 0.9
W. 158	0.1 ± 0.1	0.4 ± 0.4
W. 10	0.3 ± 0.2	0.8 ± 0.6
W. 13	0.0 ± 0.0	0.0 ± 0.0
Mazzard	0.0 ± 0.0	0.0 ± 0.0
Mahaleb	0.0 ± 0.0	0.0 ± 0.0
MxM 2	0.0 ± 0.0	0.0 ± 0.0
MxM 60	0.0 ± 0.0	0.0 ± 0.0

Table 2-4. The effect of rootstock, listed in order of increasing vigor, on the overall number of flowers per bud and floral buds per spur (± standard error) in the medial part of two-year-old shoot section of 'Hedelfinger' sweet cherry. Counts were taken in the 2001 growing season.

	Buds per spur	Flowers per bud
Gi. 209/1	3.2 ± 0.4	2.5 ± 0.5
Edabriz	1.7 ± 0.1	3.3 ± 0.2
W. 53	2.2 ± 0.2	3.4 ± 0.2
W. 72	1.8 ± 0.2	2.8 ± 0.2
Gi. 5	3.0 ± 0.3	3.6 ± 0.1
Gi. 7	3.9 ± 0.6	3.5 ± 0.1
Gi. 195/20	4.5 ± 0.2	3.4 ± 0.1
Gi. 6	2.8 ± 0.4	3.5 ± 0.2
W. 158	1.5 ± 0.2	3.0 ± 0.4
W. 10	1.6 ± 0.5	2.1 ± 0.6
W. 13	0.4 ± 0.3	0.9 ± 0.6
Mazzard	0.1 ± 0.1	0.1 ± 0.1
Mahaleb	0.3 ± 0.2	0.7 ± 0.5
MxM 2	0.0 ± 0.0	0.0 ± 0.0
MxM 60	0.1 ± 0.1	0.2 ± 0.2

Table 2-5. The effect of rootstock, listed in order of increasing vigor, on the overall number of flowers per bud and floral buds per spur (± standard error) in the distal part of two-year-old shoot section of 'Hedelfinger' sweet cherry. Counts were taken in the 2001 growing season.

	Buds per spur	Flowers per bud
Gi. 209/1	4.1 ± 0.5	2.6 ± 0.4
Edabriz	3.5 ± 0.2	3.6 ± 0.3
W. 53	4.0 ± 0.3	3.1 ± 0.2
W. 72	4.1 ± 0.3	2.7 ± 0.2
Gi. 5	4.1 ± 0.5	3.1 ± 0.2
Gi. 7	5.1 ± 0.3	3.3 ± 0.2
Gi. 195/20	5.1 ± 0.2	3.2 ± 0.2
Gi. 6	4.2 ± 0.3	3.2 ± 0.2
W. 158	3.2 ± 0.5	3.1 ± 0.1
W. 10	3.4 ± 0.6	2.4 ± 0.3
W. 13	1.7 ± 0.3	2.6 ± 0.4
Mazzard	0.4 ± 0.2	0.8 ± 0.4
Mahaleb	1.9 ± 0.3	2.0 ± 0.2
MxM 2	1.3 ± 0.8	1.5 ± 0.4
MxM 60	0.7 ± 0.4	0.9 ± 0.5

(Tables 2-3, 2-5). Proximal sections had fewer flowers per bud than distal sections, and medial sections (Table 2-4) were intermediate between the proximal and distal sections.

Metamer Lengths

Metamer lengths did not differ significantly between rootstocks, but did by location within the shoot (Table 2-6). Average metamer lengths were: 4.44 ± 0.11 for the proximal, 2.62 ± 0.04 for the medial, and 2.13 ± 0.03 for the distal sections. Proximal section metamer lengths ranged from 3.8 cm (Gi. 209/1) to 5.0 cm (Mahaleb). Distal section metamer lengths ranged from 2.0 cm (Gi. 209/1) to 2.4 cm (Gi. 7). Medial metamer lengths ranged from 2.4 cm (CT500) to 3.0 cm (Gi. 7). All proximal section metamer lengths were significantly different from those of the distal section.

Distribution of Metamer Types

The distributions of four different metamer types (blind node, vegetative axillary bud, lateral shoot, and spurs) were analyzed as a percentage of the total metamer number within each section (proximal, medial, and distal) (Figure 2-2). For all four types, there were significant differences both among rootstocks as well as among shoot sections (Table 2-7). The proximal section (Table 2-8) had a relatively large amount of blind nodes, ranging from 12 % (MxM 60) to 72 % (Gi. 209/1). A large number of vegetative buds also occurred in the proximal section; however, no lateral shoots occurred in this area. Laterals were mostly in the distal section (Table 2-10). In fact, most of the metamers in the distal section were either lateral shoots or spurs, particularly in the less vigorous rootstocks. In the more vigorous rootstocks, with few spurs and lateral shoots, the single vegetative axillary buds were the most common metamer type. Relatively few blind nodes occurred in the distal section. A broader distribution of metamer types was

Table 2-6. Analysis of variances for the number of buds per spur, flowers per bud, and metamer lengths from different rootstock treatments and from different sections within the two-year-old shoots of those treatments. Analyzed data were collected during the 2001 growing season.

Buds per spur

Source	df	F	P>F
TRT	15	89.64	<0.0001
Tree(TRT)	67	4.3	<0.0001
Section	2	410.29	<0.0001
TRT*Section	30	5.31	<0.0001

Flowers per bud

Source	df	F	P>F
TRT	15	30.84	<0.0001
Tree(TRT)	67	1.76	0.0028
Section	2	83.64	<0.0001
TRT*Section	30	3.78	<0.0001

Metamer lengths

Source	df	F	P>F
TRT	15	1.62	0.0775
Tree(TRT)	65	1.78	0.0028
Section	2	395.61	<0.0001
TRT*Section	30	0.73	0.8395

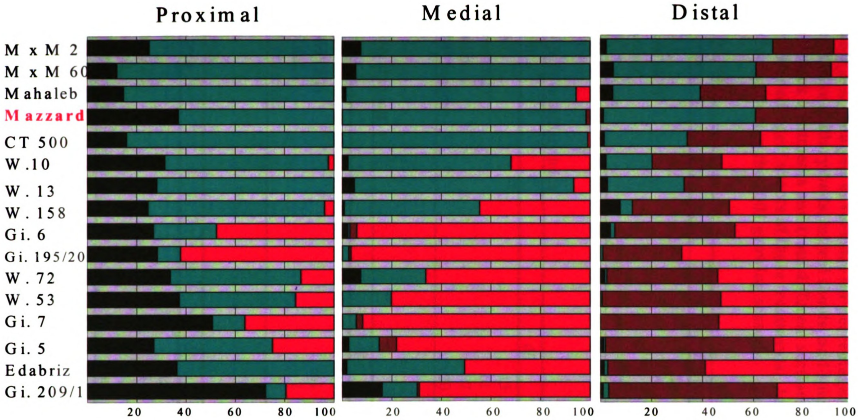

Figure 2-2: Percentage of nodes within each section (proximal, medial, distal) of a two-year-old 'Hedelfinger' sweet cherry shoot having no buds (black), only vegetative axillary buds (green), lateral shoots (brown), or spurs (red). Rootstocks are listed in order of decreasing vigor.

Table 2-7. Analysis of variance for percentages of blind nodes, single vegetative axillary buds, lateral shoots, and spurs from different rootstock treatments and from different sections within the two-year-old 'Hedelfinger' shoots of those treatments. Analyzed data were collected during the 2001 growing season.

Blind nodes

Source	df	F	P>F
TRT	15	4.58	<0.0001
Tree(TRT)	61	1.92	<0.0001
Section	2	199.86	<0.0001
TRT*Section	30	3.55	<0.0001

Vegetative axillary buds

Source	df	F	P>F
TRT	15	78.99	<0.0001
Tree(TRT)	61	1.97	<0.0001
Section	2	206.88	<0.0001
TRT*Section	30	7.85	<0.0001

Lateral shoots

Source	df	F	P>F
TRT	15	6.25	<0.0001
Tree(TRT)	61	1.68	<0.0001
Section	2	782.82	0.0017
TRT*Section	30	4.99	<0.0001

Spurs

Source	df	F	P>F
TRT	15	57.76	<0.0001
Tree(TRT)	61	2.23	<0.0001
Section	2	155.26	<0.0001
TRT*Section	30	10.82	<0.0001

Table 2-8. The effect of rootstock, listed in order of increasing vigor, on relative amounts of different node types (blind node, single vegetative axillary bud, lateral shoot, and spur) in the proximal section of the two-year-old shoots of 'Hedelfinger' sweet cherry. Shoots were analyzed as three sections (proximal, medial, and distal) of equal length. Counts were taken in the 2001 growing season, and are reported in each column as percentage of the total number of nodes (± standard errors).

	blind	vegetative	shoots	spurs	number of nodes
Gi. 209/1	72 ± 7.5	8 ± 3.2	0	20 ± 6.3	4 ± 0.3
Edabriz	36 ± 9.3	64 ± 9.3	0	0 ± 0.0	5 ± 0.3
W. 53	37 ± 7.9	47 ± 8.7	0	16 ± 7.1	4 ± 0.2
W. 72	34 ± 10.1	53 ± 10.4	0	13 ± 6.0	5 ± 0.3
Gi. 5	27 ± 5.8	48 ± 7.0	0	25 ± 7.3	4 ± 0.4
Gi. 7	51 ± 10.4	13 ± 7.8	0	36 ± 10.3	4 ± 0.4
Gi. 195/20	28 ± 6.4	9 ± 4.6	0	62 ± 5.3	5 ± 0.4
Gi. 6	27 ± 8.8	26 ± 9.0	0	48 ± 11.4	5 ± 0.4
W. 158	25 ± 7.6	72 ± 7.5	0	4 ± 3.8	4 ± 0.3
W. 10	31 ± 10.5	66 ± 10.1	0	2 ± 2.3	5 ± 0.5
W. 13	28 ± 7.8	72 ± 7.8	0	0 ± 0.0	5 ± 0.4
Mazzard	37 ± 8.2	63 ± 8.2	0	0 ± 0.0	5 ± 0.9
Mahaleb	15 ± 6.7	85 ± 6.7	0	0 ± 0.0	7 ± 0.8
MxM 2	25 ± 6.7	75 ± 6.7	0	0 ± 0.0	6 ± 1.7
MxM 60	12 ± 3.5	88 ± 3.5	0	0 ± 0.0	4 ± 0.9

Table 2-9. The effect of rootstock, listed in order of increasing vigor, on relative amounts of different node types (blind node, single vegetative axillary bud, lateral shoot, and spur) in the medial section of the two-year-old shoots of 'Hedelfinger' sweet cherry. Shoots were analyzed as three sections (proximal, medial, and distal) of equal length. Counts were taken in the 2001 growing season, and are reported in each column as percentage of the total number of nodes (± standard errors).

	blind	vegetative	branches	spurs	number of nodes
Gi. 209/1	16 ± 5.5	14 ± 4.5	1 ± 0.8	69 ± 8.8	6 ± 0.5
Edabriz	2 ± 1.8	47 ± 8.0	0 ± 0.0	51 ± 7.8	8 ± 0.5
W. 53	0 ± 0.0	20 ± 8.0	0 ± 0.0	80 ± 8.0	7 ± 0.5
W. 72	7 ± 7.4	26 ± 7.4	0 ± 0.0	66 ± 8.6	8 ± 0.5
Gi. 5	3 ± 1.9	12 ± 4.0	7 ± 3.6	78 ± 6.0	7 ± 0.8
Gi. 7	0 ± 0.0	6 ± 3.1	3 ± 1.7	92 ± 3.2	6 ± 0.5
Gi. 195/20	0 ± 0.0	3 ± 1.9	1 ± 0.8	97 ± 2.0	7 ± 0.5
Gi. 6	2 ± 1.5	1 ± 0.8	3 ± 2.1	94 ± 2.5	9 ± 0.6
W. 158	1 ± 0.8	55 ± 9.2	0 ± 0.0	45 ± 9.2	7 ± 0.6
W. 10	2 ± 1.6	66 ± 10.1	0 ± 0.0	32 ± 10.1	8 ± 0.8
W. 13	5 ± 2.6	89 ± 3.8	0 ± 0.0	7 ± 3.6	9 ± 0.7
Mazzard	0 ± 0.0	98 ± 1.6	2 ± 1.6	0 ± 0.0	9 ± 0.9
Mahaleb	1 ± 1.0	93 ± 3.9	0 ± 0.0	6 ± 3.4	11 ± 0.8
MxM 2	7 ± 4.3	93 ± 4.3	0 ± 0.0	0 ± 0.0	10 ± 1.2
MxM 60	5 ± 2.8	95 ± 2.8	0 ± 0.0	0 ± 0.0	6 ± 0.7

Table 2-10. The effect of rootstock, listed in order of increasing vigor, on relative amounts of different node types (blind node, single vegetative axillary bud, lateral shoot, and spur) in the distal section of the two-year-old shoots of 'Hedelfinger' sweet cherry. Shoots were analyzed as three sections (proximal, medial, and distal) of equal length. Counts were taken in the 2001 growing season, and are reported in each column as percentage of the total number of nodes (± standard errors).

	blind	vegetative	shoots	spurs	number of nodes
Gi. 209/1	1 ± 1.0	2 ± 1.5	66 ± 5.4	31 ± 5.0	7 ± 0.3
Edabriz	1 ± 0.9	2 ± 1.0	38 ± 5.2	59 ± 5.4	9 ± 0.5
W. 53	0 ± 0.0	1 ± 0.6	47 ± 5.0	53 ± 4.9	9 ± 0.5
W. 72	2 ± 1.7	1 ± 0.8	43 ± 5.5	54 ± 5.5	10 ± 0.7
Gi. 5	1 ± 1.2	1 ± 0.7	66 ± 4.8	32 ± 4.7	8 ± 0.9
Gi. 7	0 ± 0.0	0 ± 0.0	46 ± 9.0	54 ± 9.0	8 ± 0.7
Gi. 195/20	0 ± 0.0	1 ± 1.0	31 ± 5.9	68 ± 6.1	8 ± 0.5
Gi. 6	4 ± 2.6	2 ± 1.2	47 ± 8.5	47 ± 8.3	12 ± 1.5
W. 158	8 ± 5.2	5 ± 2.5	38 ± 5.9	50 ± 6.3	9 ± 0.5
W. 10	2 ± 1.4	18 ± 5.4	27 ± 4.5	52 ± 5.0	9 ± 0.7
W. 13	3 ± 1.5	30 ± 6.4	38 ± 4.0	29 ± 6.2	10 ± 0.5
Mazzard	1 ± 0.8	60 ± 4.6	36 ± 4.8	3 ± 1.2	11 ± 0.9
Mahaleb	5 ± 3.2	34 ± 6.0	26 ± 6.1	35 ± 3.9	14 ± 0.8
MxM 2	2 ± 1.2	65 ± 4.7	24 ± 5.3	8 ± 4.8	12 ± 1.7
MxM 60	5 ± 2.3	56 ± 3.7	30 ± 4.4	9 ± 4.0	7 ± 0.9

located in the medial section, with the exception of lateral shoots, which were still relatively few (Table 2-9). Blind nodes were also less common. On most of the rootstocks, shoots had < 7 % blind nodes; only Gi. 209/1 had a considerable amount, with 16 % of the total nodes being blind.

Trunk Cross-Sectional Area Increase and Spur Flowering Characteristics

Trunk cross sectional area increase (TCSAI) is an indicator of tree vigor or size. With the use of TCSAI, tree size at planting can be eliminated as a variable, and relative vigor in a single year can be quantified. The spur flowering characteristics assessed in this study (flower number per spur, and flower number per bud) were plotted against TCSAI during 1999 (the year that the shoots grew) and TCSAI during 2000 (the year that the flowers were initiated). The R^2 values for the relationship between floral bud number per spur and TCSAI were similar for 1999 and 2000. The regression equation describing floral bud number per spur to TCSAI was more linear in 2000. However, the R^2 values for the relationship between flower number per bud and TCSAI were higher for 2000 than 1999. Therefore, only the relationships for spur flowering characteristics versus 2000 TCSAI will be shown.

No strong relationship was found between TCSAI and flower number per bud or bud number per spur in the proximal section (Figures 2-3, 2-4). The floral bud number per spur in the medial section was related more to 2000 TCSAI (Figure 2-3) than to 1999 TCSAI (data not shown). Flower number per bud in the medial section also had a stronger relationship to 2000 TCSAI (Figure 2-4). Bud number per spur in the distal section showed the greatest relationship to TCSAI, with 2000 TCSAI having the highest R^2 (0.70) ($P<0.0001$) (Figure 2-3). This trend did not hold true for the flower number

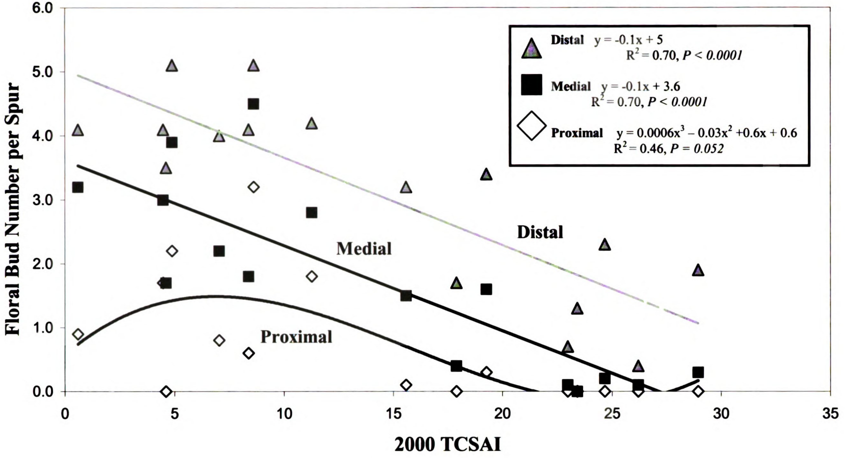

Figure 2-3: Floral bud number per spur in each section of two-year-old 'Hedelfinger' sweet cherry shoots versus 2000 TCSAI. Bud counts were made during spring 2001. Each point represents the average floral bud number per spur for the specified section of a single rootstock treatment.

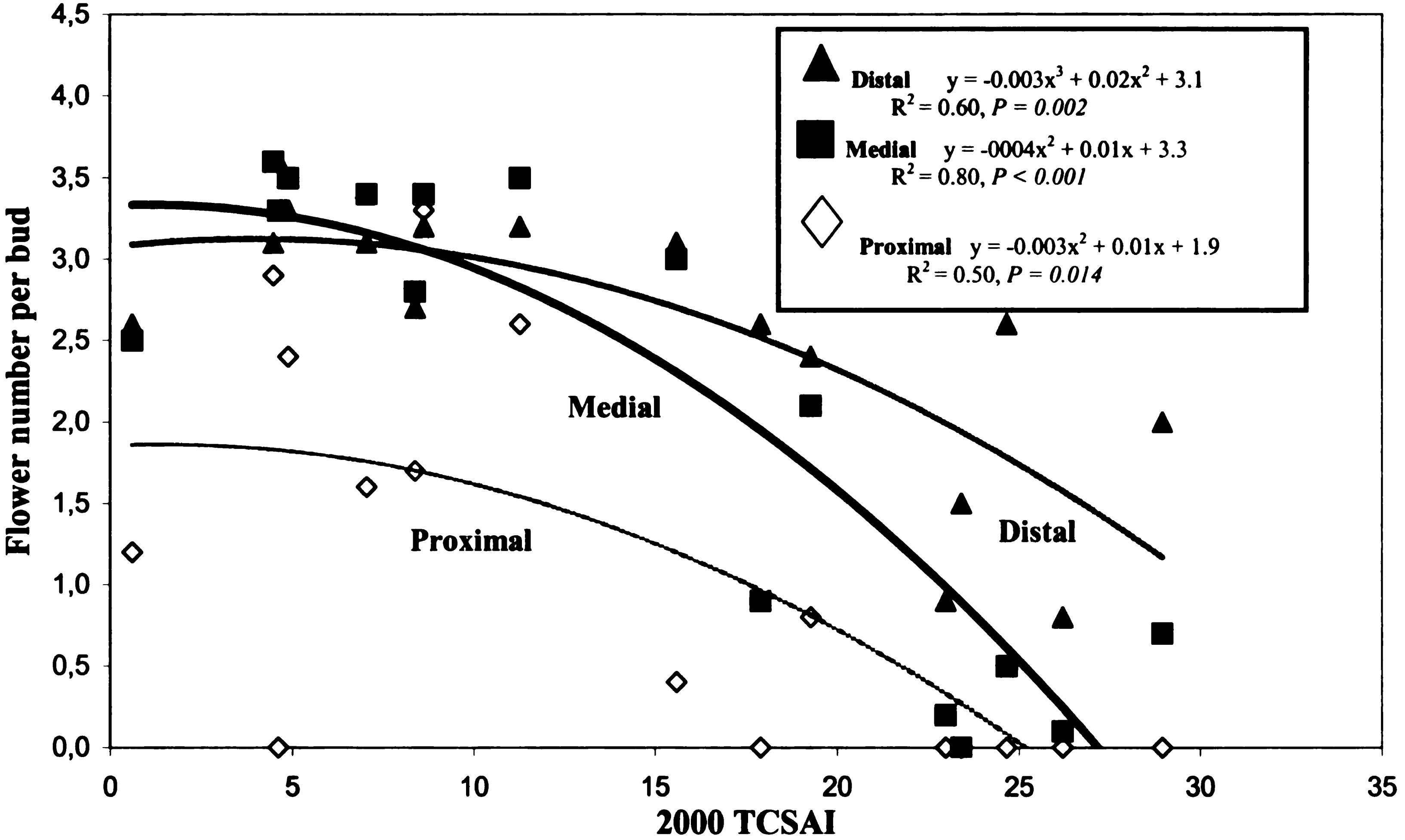

Figure 2-4: Flower number per bud of two-year-old 'Hedelfinger' sweet cherry shoots versus 2000 TCSAI. Flower counts were made during spring 2001. Each point represents the average flower number per bud for a single rootstock treatment.

per bud in the distal section, since 1999 TCSAI was related more to flower number per bud (data not shown) than was 2000 TCSAI (Figure 2-4).

Flowers per Bud vs. Buds per Spur

Average flower number per bud was plotted against the floral bud number per spur, across all rootstocks. In all sections, the relationship between flower formation and bud formation was positive and strong (Figure 2-5). Rootstock affected both variables, with the strongest relationship ($R^2 = 0.93$) in the medial section ($P<0.0001$).

DISCUSSION

It is important to be able to predict the location and flowering characteristics of spurs within the canopy (Lespinasse and Lauri, 1996; Crabbe, 1984). A general conclusion of our study is that rootstock influenced both spur floral characteristics (bud number per spur and flower number per bud) and location of spurs along the shoot, results that also occurred in the 'Montmorency' trial (Chapter Three). This could be attributed, at least partially, to the rootstock effect on tree size (vigor); however, differences in TCSAI only accounted for ~ 2/3 to 3/4 of the variability in spur floral characteristics for 'Hedelfinger' sweet cherry.

In the Gisela / Giessen series, bud number per spur and the flower number per bud increased as tree size increased (Table 2-2), while those parameters generally decreased in other rootstocks as size increased. The importance of this finding is that when selecting rootstocks for a balanced vegetative to reproductive ratio, more must be considered than simply TCSAI or tree size, such as location of the spurs and quantity of flowers produced per spur.

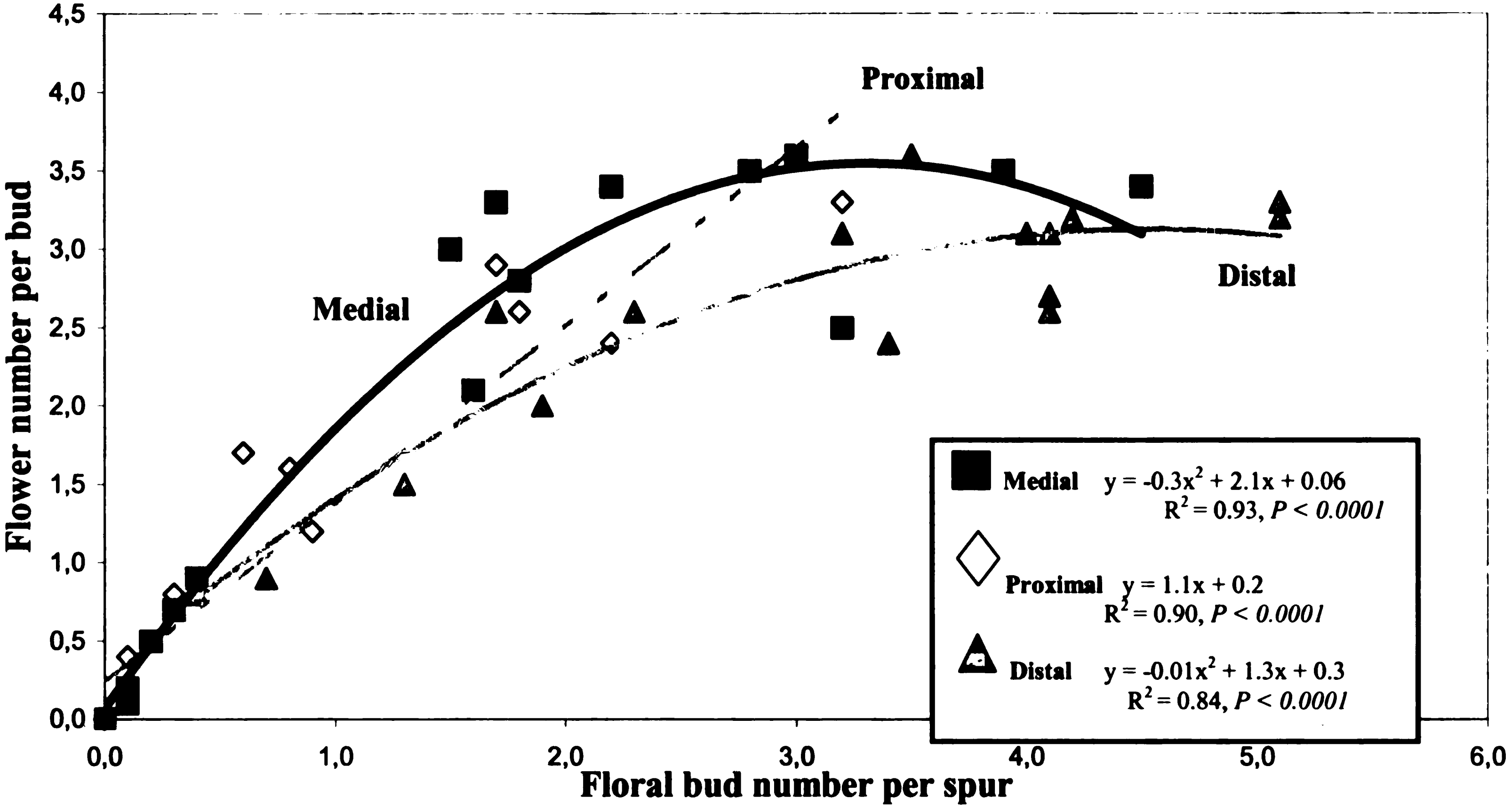

Figure 2-5: Flower number per bud of each shoot section plotted against its corresponding floral bud number per spur. Each point represents the average number of flowers per bud and floral buds per spur in the specified section of two-year-old 'Hedelfinger' sweet cherry shoots of a single rootstock treatment.

Another major effect of rootstock on spur flowering characteristics was the difference in numbers of flowers per bud and buds per spur between the proximal and distal sections of the two-year-old shoot. The two-year-old shoot had both the characteristic of being the first location of spur growth, as well as an indicator of where spurs will be produced in the future. Location within this two-year-old section affected both spur flowering characteristics. It seems reasonable that in larger trees with more dense canopies, the more proximal sections of the shoots would have fewer spurs because of within-canopy shading. However, in our trial, the trees were only in their fourth year, and therefore small enough to have very little within-canopy shading. Assuming there was not a strong effect of light, the increase in number of flowers per bud and buds per spur in the distal sections of the shoot relative to the proximal were probably due to internal or genetic factors. In the 'Montmorency' trial, the bud number per spur and flower number per bud were greatest in the proximal section, supporting the theory that the spur flowering characteristics were due to internal or genetic factors (Chapter Three).

Average metamer length did not predict metamer type in our study since, among rootstocks, there were differences in percentages of different metamers within each section (Figure 2-2), even though metamer length was the same among rootstocks. However, a limitation existed in the way that metamer length was assessed. The number of nodes was counted per section and the length of that section was taken. Metamer lengths were not equal throughout the shoot section (personal observation). In some cases, a very short metamer was adjacent to a long metamer. Because of this, a more accurate presentation of these lengths might be relative phyllotactic length. The length can only be relative since the actual number of phyllotactic units was not counted, and it must be assumed that nodes had the same orientation among rootstocks. An interesting observation would be to look at phyllotactic differences among rootstocks.

Even though average metamer length was the same between rootstocks, some general conclusions can be made about the location of different metamer types within the shoot. For trees on all rootstocks, there were no lateral shoots in the proximal sections. The medial sections also had a very small amount of lateral shoots. Almost all of the metamers in the proximal sections of trees smaller than those on W.158 were lateral shoots. Determining the location of different metamers within the shoot based on dividing the total section lengths into thirds may be a useful, if artificial, way to divide the shoot, and so could be useful in developing training sytems for different rootstocks. Earlier work in apple by Lespinasse and Lauri (1996) showed that neither distal nor proximal node number orientation predicted node type accurately.

Knowing the location of spurs and the spurs flowering characteristics could be helpful in managing the tree. The one variable not measured, which may be useful for future work, is the evolution of the branch, or how spurs within a branch age. We did not look at the way that the metamer types and quality change in these shoot sections over time, particularly as the tree canopy matures. This is also a major factor in tree management, since rootstock may also affect how the spurs age.

The effect of rootstock on spur flowering characteristics, independent of its effect on tree size, underscores the value of more accurate assessment of spur flowering characteristics on different rootstocks. Lespinasse and Lauri (1996) have noted that prediction of the distribution of specific metamers is one the three variables that must be understood before an accurate prediction and assessment of the tree's productive potential can be found. This is currently a critical step in the evaluation and adaptation of new precocious and/or vigor-controlling rootstocks for sweet cherry production.

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CHAPTER THREE

Rootstock Affects Floral Distribution and Patterning in 'Montmorency' Tart Cherry

Rootstock Affects Floral Distribution and Patterning in 'Montmorency' Tart Cherry.

ABSTRACT

The NC-140 regional tree fruit rootstock project established a trial of cherry rootstocks across North America in 1998 to test their performance with the tart cherry scion 'Montmorency'. One 'Montmorency' trial was established near Traverse City, MI. This plot was used to characterize rootstock influence on the development of flowers, buds, spurs, blind nodes, and lateral branches on two-year-old shoots. Both rootstock genotype and node location on the shoot influence bud number per spur and flower number per bud. Both floral bud number per spur and flower number per bud were influenced, in general, by rootstock. This effect was not completely due to the rootstock's effect on scion vigor. For the Gisela/Giessen and Weiroot series of rootstocks, the flower number per bud and bud number per spur increased with increasing vigor with the exception of Gi.6, whereas the other rootstock had decreasing flowers per bud and buds per spur with increasing vigor. Both variables had the lowest values in the distal areas of the shoot, which is opposite that in 'Hedelfinger' sweet cherry (Chapter Two). Rootstock genotype influenced location of lateral shoots, floral buds, and axillary vegetative buds within two-year-old shoots; however, the location of blind nodes was not affected by rootstock. Blind nodes occurred in all sections. These characteristics are useful for evaluating, and ultimately managing, production of `Montmorency' tart cherry on rootstocks that alter scion precocity and/or vigor.

INTRODUCTION

Cherry rootstocks have been selected for various reasons, including precocity, productivity, vigor control, disease tolerance, and adaptability to different soils. With some of these rootstocks, there is a potential problem of excessive cropping levels when grafted to very productive scion cultivars. Excessive cropping can result in poor fruit quality and stunted vegetative growth (Lang, 2000). In tart cherry, one of the potentially major problems associated with dwarfing rootstocks is the large proportion of flowers on the one-year-old laterals (Perry, 1996). On dwarfing rootstocks, there may be less growth and so less fruiting, since the fruiting potential is dictated by the annual vegetative growth that occurs. One approach to study this phenomenom, and potentially develop strategies to manage it, is to more precisely characterize how rootstock genotype influences tree architecture and flower placement on the scion; that is, how the development of different nodes can be used to assess how nodes are affected differentially within a shoot.

Pattern can be studied within a tree by dividing the tree either artificially, *e.g.*, empirically dividing a shoot into equal units of length, or naturally, *e.g.*, using biological growth parameters that are repeated throughout the tree or have an identical nature (Godin and Caraglio, 1998; White, 1984). Growth of angiosperm shoots can be described as a series of repeating units (metamers) that are formed sequentially by the apical meristem. In the most basic form, a metamer consists of a node with an associated leaf-like organ, a lateral meristem in the axil of the leaf, and the proximally located internode (White, 1984). It has been suggested that there is an ordered pattern of different metamers (Schultz and Haughn, 1991).

Distribution of different metamers within the plant plays a large part in the productive potential of tree fruit species (Lespinasse and Lauri, 1996). Metamer types in sweet cherry include blind nodes, lateral shoots, single vegetative buds, and spurs. Lespinasse and Lauri suggested that studying the patterns and distribution of different metamer types in fruit trees would result in several practical applications including the development of new tree growth habits through breeding, new training systems that reduce pruning and thinning, and a more rational approach to cultivation that could lead to an improvement in fruit quality characteristics. A better ability to predict flower location would also provide a more definitive base for the study of flower bud 'quality' (Crabbe, 1984).

Rootstocks are known to affect spur characteristics. Franken-Bembenek and Gruppe (1985) found that yield potential in sweet cherry was mainly dependent on rootstock. One of the main ways rootstock altered yield potential was by altering location of spur insertions. Schaumberg and Gruppe (1985) tested 'Hedelfinger' sweet cherry on some of the Giessen series rootstocks, and observed that the number of buds per spur was not altered, but that the number of flowers per bud was greatly affected by rootstock. The number of floral buds per spur depended greatly on the position of the spurs within the shoot. More distally located spurs produced a greater number of buds per spur. Other correlations have been made between location of the spur and the number of floral buds per spur in sweet cherry cultivars, indicating that position within the shoot affects spur flowering characteristics (Wustenberghs and Keulemans, 2000).

The limitations of the above trials were that they were done on a limited number of rootstocks, some rootstocks not in the trial may be more ideal for conditions in

Michigan, and most of the trials were not done with tart cherry. Location of different metamer types has not received a lot of attention in tart cherry trees, since they are harvested mechanically. With the recent U.S. introduction of a new tart cherry with fresh market potential, 'Balaton', it would be beneficial to understand how rootstocks affect tart cherry tree habit. A regional tree fruit rootstock (NC-140) trial has been established in the US to evaluate rootstocks for suitability in the US. Rootstock can significantly influence flower density, as well as vigor and yield efficiency. This is not enough information, however, to predict where the flowers will be borne within the canopy, which is important for predicting and managing the productive potential of the tree.

Spurs are first produced in the two-year-old shoot sections in tart cherry. This is because tart cherries initiate flowers in the summer before flowering occurs, after active growth has ceased (Diaz et al., 1981). The two-year old shoot section, then, is a good predictor of the distribution of different metamer types since it is the first area where spurs form.

Schaumberg and Gruppe (1985) reported that the effect of rootstock on the number of floral buds per spur was small on 'Hedelfinger' sweet cherry, but that there was a significant effect on the number of flowers per bud. They also noticed that the number of buds per spur was correlated strongly with the location of the spur within the branch. The number of buds per spur increased distally along two-, three-, and four- year old shoots.

Schaumberg and Gruppe's (1985) trials were in Germany, and so may not have accurately assessed rootstocks and scions examined in trials in the US. The objectives for this experiment were: (1) to assess the effect of rootstock and node location within two-year old wood on spur flowering characteristics (flower number per bud and floral bud number per spur) and (2) to assess the effect of rootstock on location of the different metamer types (blind nodes, single vegetative buds, lateral shoots, and spurs) in 'Montmorency' tart cherry.

MATERIALS AND METHODS

Plant Materials

As part of the NC-140 regional tree fruit rootstock project, a plot of 'Montmorency' tart cherry (*Prunus ceraus* L.) trees on 12 rootstocks was established in spring 1998 at Michigan State University's Northwest Horticultural Research Station (NWHRS) near Traverse City, Michigan. The trees were planted in an Emmet-Leelenau sandy loam in a randomized complete block design with 8 replications; however, a completely randomized design was used for this study since 12 rootstocks (see Table 3-1) and only 5 replications were used. The trees were trained to a modified central leader and drip irrigated. Fertilization and protective sprays were applied as by local standard recommendations.

Five trees per rootstock were selected in early spring 2001. Three shoots of comparable size and including second year wood were selected from each tree's central leader. Markings were made using paint so that the second-year section of each shoot was divided into three equal sections (proximal, medial, and distal) (Figure 3-1).

Table 3-1. Rootstocks, listed in order of increasing vigor, characterized in the 1998 NC-140 'Montmorency' tart cherry trial at the NWHRS near Traverse City, Michigan. Vigor values are based on 2001 TCSA. Genotypes are clonal selections unless otherwise noted.

Rootstock	Abbreviation	Origin	Genotype	Vigor (% Mahaleb)
Ahrensburg 209/1	Gi. 209/1	Giessen and Ahrensburg, Germany	*P. cerasus* 'Schattenmorelle' X *P. canescens*	22
Edabriz	Edabriz	France (INRA)	*P. cerasus*	26
Weiroot 53	W. 53	Weihenstephan, Germany	*P. cerasus*	40
Gisela 5	Gi. 5	Giessen, Germany	*P. cerasus* X *P. canescens*	41
Gisela 6	Gi. 6	Giessen, Germany	*P. cerasus X P. canescens*	54
Weiroot 158	W.158	Weihenstephan, Germany	*P. cerasus*	58
Weiroot 72	W. 72	Weihenstephan, Germany	*P. cerasus*	61
Gisela 7	Gi. 7	Giessen, Germany	*P. cerasus* X *P. canescens*	63
Giessen 195/20	Gi. 195/20	Giessen, Germany	*P. canescens* X *P. cerasus*	71
Weiroot 10	W. 10	Weihenstephan, Germany	*P. cerasus*	82
Weiroot 13	W. 13	Weihenstephan, Germany	*P. cerasus*	94
Mahaleb	Mahaleb	south and central Europe and Asia Minor	*P. mahaleb* seedling	100

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Data Collection

In 2001, data for each node along the two-year-old section of each tagged shoot were collected throughout the season. Each node was characterized according to its placement within the shoot section and its type. Node types used for this study were: (1) vegetative (axillary) bud; (2) spur; (3) lateral (axillary) shoot (or lateral); and (4) blind node.

Vegetative buds had two fates by the end of active growth that were easily distinguishable: 1) bud break occurred late in the 2001 season and a lateral shoot formed, or 2) the bud produced only leaves during the 2001 season, and so remained only a vegetative bud. Whether the bud remained vegetative or became a lateral shoot in 2001 was not assessed in this study. Assessment of the distribution of vegetative buds included all that were vegetative at the beginning of the season.

Three spur parameters were measured. Bud number in each spur was counted in April 2001 and the number of floral buds was determined by subtracting one (for the vegetative bud) from the total buds within the spur. Flowers within each spur were counted in spring 2001. Flower number per bud was estimated by dividing the flower number per spur by the floral bud number per spur.

The only parameters measured on nodes with lateral shoots were length of growth that occurred during 2000, and length of growth that occurred during 2001 (made in November 2001, after active growth had ceased and the leaves had dropped). Metamer

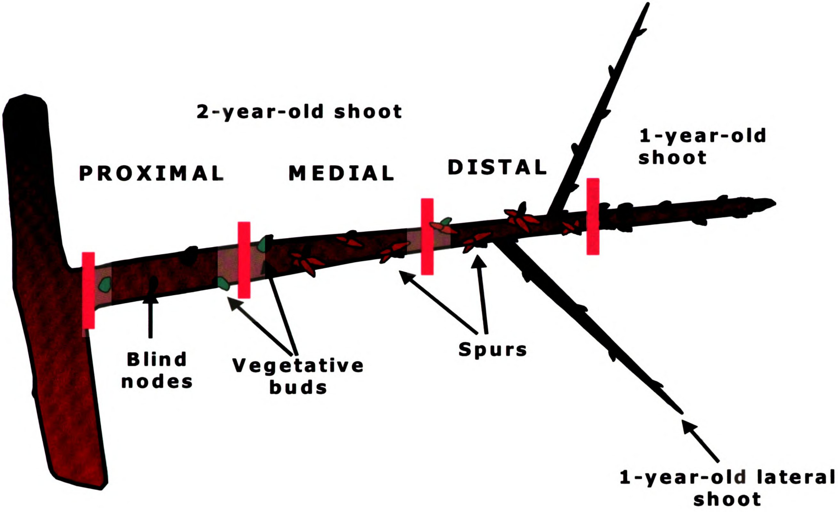

Figure 3-1: Illustration of a two-year-old (and associated one-year-old) cherry shoot. Red lines indicate the different sections by which the shoot was analyzed.

lengths were estimated for each section (proximal, medial, distal). Since shoots were split into three equal sections, the estimate was done using the following equation:

$$\text{Metamer length (section)} \equiv \frac{\text{total section length}}{\text{number of nodes within the section}}$$

Blind nodes were considered to be those that did not exhibit any active growth, whether vegetative or reproductive. Although there are different reasons for blind node development (Wustenberghs and Keulemans, 2001), these were not ascertained in this study. Most blind nodes of sweet cherry are due to the formation of solitary flower buds at the base of one-year-old shoots, so blind nodes in this study are most likely due to flowering during 2000 in the tagged shoots.

Trunk cross-sectional area (TCSA) is an indicator of tree size (Westwood and Roberts, 1970). Trunk cross-sectional area increase (TCSAI), then, indicates vigor of the scion. TCSAI measurements used in this study were taken as part of the NC-140 regional tree research project (Win and Jon, 2002).

Statistical Analysis

Statistical analyses were performed using the SAS proc glm program (SAS Institute, 1989). In all studies, pairwise comparisons and the analysis of variance were used in the comparisons among rootstocks. When comparing effects of rootstock on metamer location and flowering characteristics, all values were expressed as mean ± standard error. Statistical significance was calculated using Student's t-test. For the regression analyses, highest R^2 was used to determine the best fitting regression curve.

RESULTS

Floral Bud Number per Spur

In general, the more vigorous rootstocks had more floral buds per spur (Table 3-2). Spur formation occurred in all sections, except for the distal part of Gi.209/1 (which had no floral buds per spur) (Table 3-3, 3-4, 3-5). Spur location within the shoot section also correlated with floral bud number per spur. The highest number of floral buds per spur occurred in the medial section, and the lowest in the distal section. The floral bud number per spur increased with increasing tree size to a point and then decreased (Figure 3-2).

Flower Number per Bud

Rootstock affected the flower number per bud, and the more vigorous rootstocks generally had more flowers per bud (Tables 3-4). The fewest flowers per bud were in the distal sections (Table 3-5). In the smaller trees, there were generally more flowers per bud in the proximal section, while in the trees larger than those on Gi.7, the higher number of flowers per bud occurred in the medial section (Figure 3-3).

Metamer Lengths

Metamer lengths did not differ significantly among rootstocks, but did by location within the shoot (Table 3-6). The differences in metamer lengths were strongly related to location within the shoot. The main difference was observed between the proximal and distal sections, as well as between the medial and distal sections. Average metamer lengths were: 3.8 ± 0.09 for the proximal, 2.7 ± 0.04 for the medial, and 2.2 ± 0.04 for the distal sections. Metamer lengths in the proximal section ranged from 3.2 cm

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Table 3-2. The effect of rootstock, listed in order of increasing vigor, on the average number of flowers per bud and floral buds per spur (± standard error) on the entire two-year-old shoot section of 'Montmorency' tart cherry. Counts were taken in the 2001 growing season.

	Buds per spur	Flowers per bud
Edabriz	2.3 ± 0.4	2.1 ± 0.4
W. 53	3.5 ± 0.5	2.3 ± 0.3
Gi. 5	2.2 ± 0.4	1.7 ± 0.4
Gi. 6	3.0 ± 0.6	2.0 ± 0.4
W. 158	3.2 ± 0.4	2.6 ± 0.4
W, 72	3.7 ± 0.3	2.8 ± 0.3
Gi. 7	3.3 ± 0.4	2.7 ± 0.3
Gi. 195/20	3.9 ± 0.4	3.0 ± 0.3
W. 10	3.7 ± 0.4	2.5 ± 0.3
W. 13	3.2 ± 0.5	2.0 ± 0.3
Mahaleb	3.2 ± 0.3	2.7 ± 0.2

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Table 3-3. The effect of rootstock, listed in order of increasing vigor, on the average number of flowers per bud and floral buds per spur (± standard error) in the proximal part of two-year-old shoot section of 'Montmorency' tart cherry. Counts were taken in the 2001 growing season.

	Buds per spur	Flowers per bud
Gi. 209/1	2.4 ± 1.0	1.5 ± 0.5
Edabriz	2.3 ± 0.4	2.8 ± 0.5
W. 53	4.3 ± 0.5	3.0 ± 0.2
Gi. 5	3.2 ± 0.3	2.7 ± 0.5
Gi. 6	3.9 ± 0.3	3.0 ± 0.2
W. 158	3.5 ± 0.3	3.4 ± 0.3
W. 72	3.4 ± 0.4	3.4 ± 0.3
Gi. 7	3.8 ± 0.3	3.7 ± 0.4
Gi. 195/20	4.0 ± 0.1	3.4 ± 0.5
W. 10	3.6 ± 0.3	2.7 ± 0.2
W. 13	3.6 ± 0.4	2.3 ± 0.2
Mahaleb	2.6 ± 0.4	2.8 ± 0.1

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Table 3-4. The effect of rootstock, listed in order of increasing vigor, on the average number of flowers per bud and floral buds per spur (± standard error) in the medial part of two-year-old shoot section of 'Montmorency' tart cherry. Counts were taken in the 2001 growing season.

	Buds per spur	Flowers per bud
Gi. 209/1	1.0 ± 1.0	0.6 ± 0.6
Edabriz	3.9 ± 0.8	3.0 ± 0.6
W. 53	4.7 ± 0.7	2.9 ± 0.5
Gi. 5	2.7 ± 0.8	2.0 ± 0.6
Gi. 6	3.5 ± 1.4	2.0 ± 0.7
W. 158	4.7 ± 0.3	3.3 ± 0.2
W. 72	4.9 ± 0.2	3.3 ± 0.1
Gi. 7	4.0 ± 0.6	2.9 ± 0.5
Gi. 195/20	5.3 ± 0.4	3.6 ± 0.2
W. 10	4.9 ± 0.4	3.4 ± 0.3
W. 13	4.9 ± 0.2	2.8 ± 0.1
Mahaleb	4.0 ± 0.4	2.9 ± 0.2

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Table 3-5. The effect of rootstock, listed in order of increasing vigor, on the average number of flowers per bud and floral buds per spur (± standard error) in the distal part of two-year-old shoot section of 'Montmorency' tart cherry. Counts were taken in the 2001 growing season.

	Buds per spur	Flowers per bud
Gi. 209/1	0.0 ± 0.0	0.0 ± 0.0
Edabriz	0.8 ± 0.4	0.6 ± 0.3
W. 53	1.4 ± 0.6	0.8 ± 0.4
Gi. 5	0.6 ± 0.6	0.4 ± 0.6
Gi. 6	1.7 ± 1.0	1.1 ± 0.7
W. 158	1.7 ± 0.8	1.1 ± 0.6
W. 72	2.8 ± 0.8	1.7 ± 0.5
Gi. 7	1.9 ± 0.6	1.4 ± 0.4
Gi. 195/20	2.4 ± 0.7	1.9 ± 0.7
W. 10	2.6 ± 0.9	1.5 ± 0.6
W. 13	1.2 ± 0.8	0.7 ± 0.5
Mahaleb	3.1 ± 0.8	2.3 ± 0.7

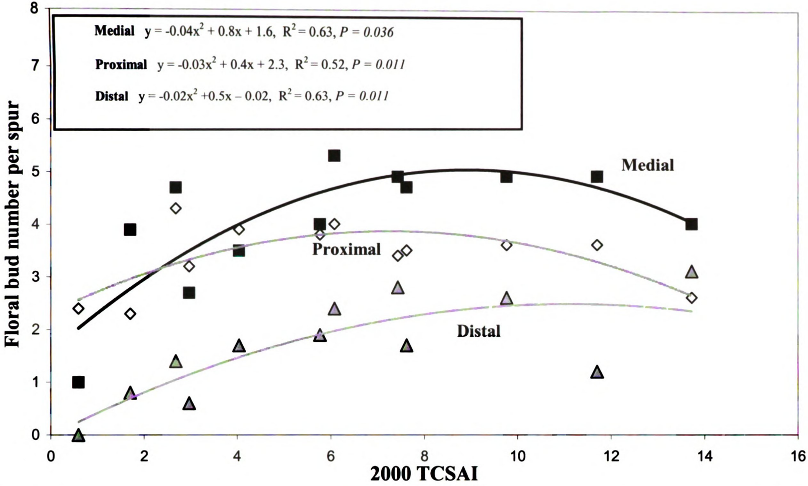

Figure 3-2: Floral bud number per spur in each section of two-year-old ‘Montmorency’ tart cherry shoots versus 2000 TCSAI. Bud counts were made during spring 2001. Each point represents the average floral bud number per spur for the specified section of a single rootstock treatment.

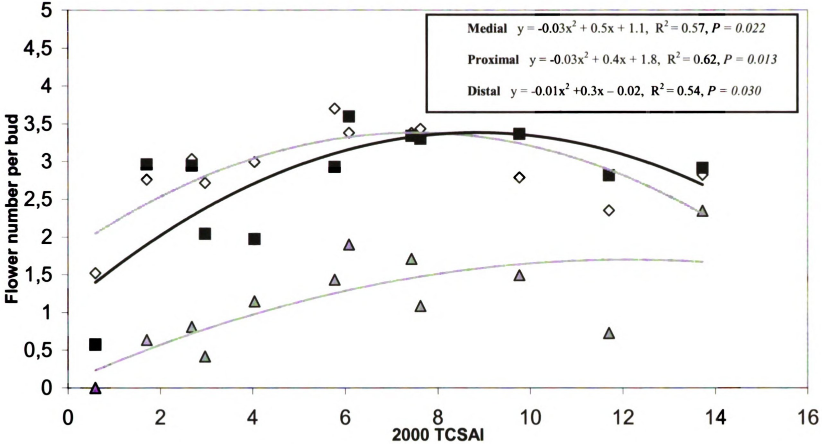

Figure 3-3: Flower number per bud in each section of two-year-old 'Montmorency' tart cherry shoots versus 2000 TCSAI. Flower counts were made during spring 2001. Each point represents the average flower number per bud for the specified section of a single rootstock treatment.

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Table 3-6: Analysis of variances for the number of buds per spur, flowers per bud and metamer lengths from different rootstock treatments and from different sections within the two-year-old shoots of those treatments. Analyzed data were collected during the 2001 growing season.

Buds per spur

Source	df	F	P>F
TRT	11	4.91	<0.0001
Tree(TRT)	52	1.38	0.0817
Section	2	48.35	<0.0001
TRT*Section	22	1.2	0.2636

Flowers per bud

Source	df	F	P>F
TRT	11	8.83	<0.0001
Tree(TRT)	52	2.52	<0.0001
Section	2	89.32	<0.0001
TRT*Section	22	1.33	0.1713

Metamer Length

Source	df	F	P>F
TRT	11	3.24	0.0003
Tree(TRT)	52	2.04	<0.0001
Section	2	328.45	<0.0001
TRT*Section	22	2.25	0.001

(Gi.209/1) to 4.3 cm (W.13), while the range was from 1.8 (Gi. 209/1) to 2.6 (W.13) in the distal section.

Distribution of Metamer Types

Distributions of four different metamer types (blind node, vegetative axillary bud, lateral shoot, spur) were analyzed as a percentage of the total number of metamers within each section (proximal, medial, and distal) (Figure 3-4). A significant effect of rootstock was observed in the relative amounts of vegetative (axillary) buds, blind nodes, and spurs (Table 3-7). However, rootstock genotype did not affect the percentage of lateral shoots. Location within the shoot affected distribution of metamer types. Very few vegetative (axillary) buds were present in any of the rootstock treatments or sections; in the medial and distal sections, vegetative buds never exceeded 5 % of the total metamers present (Tables 3-8, 3-9). Blind nodes were found in all sections. The greatest percent of nodes in the medial section were either lateral shoots or spurs, and in the distal section were lateral shoots and blind nodes (Figure 3-4). A relationship between vigor and distribution of specific metamer types was not apparent.

Trunk Cross-Sectional Area Increase and Spur Flowering Characteristics

Trunk cross sectional area increase (TCSAI) is an indicator of tree vigor or tree size. With the use TCSAI, tree size at planting can be eliminated as a variable, and relative vigor in a single year can be quantified. The spur flowering characteristics assessed in this study (floral bud number per spur, and flower number per bud) were plotted against TCSAI during 1999 (the year that the shoots grew) and TCSAI during 2000 (the year that the flowers were initiated). Only spur flowering characteristics plotted against 2000 TCSAI are presented. For both 1999 and 2000, there was no

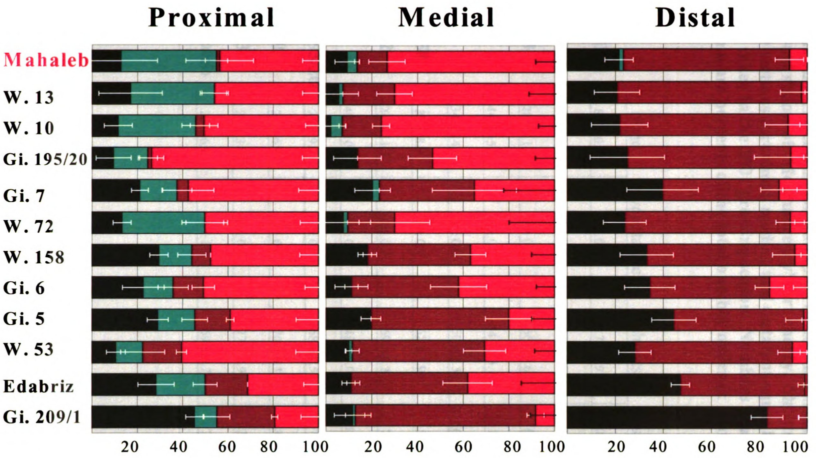

Figure 3-4: Percentage of nodes within each section (proximal, medial, distal) of two-year-old tart cherry shoot having: no buds (black), single vegetative axillary buds (green), lateral shoots (brown), or spurs (red).

Table 3-7. The effect of rootstock, listed in order of increasing vigor, on relative amounts of different node types (blind node, single vegetative axillary bud, lateral shoot, and spur) in the distal section of the two-year-old shoots of 'Montmorency' tart cherry. Shoots were analyzed as three sections (proximal, medial, and distal) of equal length. Counts were taken in the 2001 growing season. Numbers reported in each column are percents of the total number nodes (± standard errors).

	blind	vegetative	shoots	spurs	number of nodes
Gi. 209/1	83 ± 6.0	0 ± 0.0	17 ± 5.9	0 ± 0.0	6 ± 0.7
Edabriz	47 ± 9.5	0 ± 0.0	52 ± 8.8	1 ± 1.0	6 ± 0.4
W. 53	28 ± 11.7	0 ± 0.0	66 ± 9.6	6 ± 3.1	8 ± 0.5
Gi. 5	44 ± 15.5	0 ± 0.0	54 ± 15.1	1 ± 1.5	6 ± 0.7
Gi. 6	34 ± 14.9	0 ± 0.0	50 ± 7.5	16 ± 10.2	7 ± 0.4
W. 158	33 ± 8.9	0 ± 0.0	62 ± 5.9	5 ± 5.2	7 ± 0.4
W. 72	24 ± 11.1	0 ± 0.0	69 ± 9.2	7 ± 2.6	7 ± 0.3
Gi. 7	40 ± 10.5	0 ± 0.0	48 ± 6.0	12 ± 5.8	6 ± 0.7
Gi. 195/20	25 ± 9.2	0 ± 0.0	68 ± 7.5	7 ± 2.6	7 ± 0.3
W. 10	22 ± 6.8	0 ± 0.0	70 ± 6.1	8 ± 3.0	8 ± 0.2
W. 13	21 ± 3.9	0 ± 0.0	77 ± 2.9	2 ± 1.4	7 ± 0.7
Mahaleb	22 ± 6.6	2 ± 1.8	69 ± 3.7	7 ± 3.4	8 ± 1.0

Table 3-8. Analysis of variance for percentages of blind nodes from different rootstock treatments and from different sections within the two-year-old 'Montmorency' shoots of those treatments. Analyzed data were collected during the 2001 growing season.

Blind nodes

Source	df	F	P>F
TRT	11	2.53	0.0134
Tree(TRT)	47	2.36	0.0002
Section	2	32.47	<0.0001
TRT*Section	22	1.46	0.1096

Vegetative axillary buds

Source	df	F	P>F
TRT	11	3.31	0.002
Tree(TRT)	47	1.18	0.2474
Section	2	126.87	<0.0001
TRT*Section	22	2.74	0.0004

Lateral shoots

Source	df	F	P>F
TRT	11	1.42	0.1968
Tree(TRT)	47	1.06	0.3972
Section	2	102.58	<0.0001
TRT*Section	22	4.4	<0.0001

Spurs

Source	df	F	P>F
TRT	11	3.25	0.0023
Tree(TRT)	47	2.05	0.0017
Section	2	178.35	<0.0001
TRT*Section	22	3.17	<0.0001

Table 3-9. The effect of rootstock, listed in order of increasing vigor, on relative amounts of different node types (blind node, single vegetative axillary bud, lateral shoot, and spur) in the medial section of the two-year-old shoots of 'Montmorency' tart cherry. Shoots were analyzed as three sections (proximal, medial, and distal) of equal length. Counts were taken in the 2001 growing season. Numbers reported in each column are percents of the total number nodes (± standard errors).

	blind	vegetative	shoots	spurs	number of nodes
Gi. 209/1	12 ± 5.4	1 ± 1.1	79 ± 8.0	8 ± 8.3	3 ± 0.4
Edabriz	11 ± 8.6	0 ± 0.0	51 ± 7.8	38 ± 11.1	5 ± 0.6
W. 53	10 ± 6.7	1 ± 1.4	58 ± 3.8	31 ± 7.0	7 ± 0.5
Gi. 5	20 ± 10.5	0 ± 0.0	60 ± 10.6	20 ± 8.4	5 ± 0.3
Gi. 6	11 ± 7.8	0 ± 0.0	47 ± 18.4	42 ± 22.3	5 ± 0.4
W. 158	18 ± 11.9	0 ± 0.0	45 ± 15.5	37 ± 19.9	6 ± 0.6
W. 72	8 ± 4.1	2 ± 1.9	21 ± 6.7	70 ± 10.1	6 ± 0.5
Gi. 7	20 ± 7.2	3 ± 3.0	42 ± 12.3	35 ± 8.0	6 ± 0.3
Gi. 195/20	14 ± 4.3	0 ± 0.0	33 ± 10.2	53 ± 10.0	6 ± 0.2
W. 10	2 ± 1.3	5 ± 3.1	17 ± 9.2	76 ± 8.7	6 ± 0.4
W. 13	6 ± 3.9	2 ± 1.8	23 ± 10.8	70 ± 14.5	6 ± 0.5
Mahaleb	9 ± 8.0	4 ± 4.2	13 ± 3.7	73 ± 10.5	7 ± 0.7

difference in the relationship between TCSAI and floral bud number per spur. The R^2 value for the relationship of flower number per bud to TCSAI, however, was higher for 2000 than 1999.

The proximal section showed the strongest relationship, perhaps due to the greater concentration of spurs in this area (Table 3-10). There is a general trend in which both floral bud number per spur and flower number per bud increased with vigor to a point and then decreased, resulting in a curvilinear relationship between spur characteristics and vigor. This point for both flowers per bud and buds per spur occurred at TCSAI values of ~8 cm^2 (1999) and 6 to 8 cm^2 (2000).

Flowers per Bud vs. Buds per Spur

Average flower number per bud was plotted against floral bud number per spur for the proximal, medial, and distal sections, across all rootstocks. Rootstock affected both of these variables positively (Figure 3-5). The weakest relationship occurred within the proximal section ($R^2 = 0.32$, $p=0.054$); the R^2 values for the other sections were higher than 0.89. As the bud number per spur increased, the number of flowers per bud also increased.

DISCUSSION

It is important to be able to predict the location and flowering characteristics of spurs within the canopy (Lespinasse and Lauri, 1996; Crabbe, 1984). Although tart cherries flower extensively on axillary buds of one-year-old lateral shoots (Thompson, 1996), only flowering on spurs is sustainable. Flowering on the one-year-old lateral

Table 3-10. The effect of rootstock, listed in order of increasing vigor, on relative amounts of different node types (blind node, single vegetative axillary bud, lateral shoot, and spur) in the proximal section of the two-year-old shoots of 'Montmorency' tart cherry. Shoots were analyzed as three sections (proximal, medial, and distal) of equal length. Counts were taken in the 2001 growing season. Numbers reported in each column are percents of the total number nodes (± standard errors).

	blind	vegetative	shoots	spurs	number of nodes
Gi. 209/1	45 ± 16.2	10 ± 3.2	26 ± 15.0	19 ± 7.0	3 ± 0.3
Edabriz	28 ± 14.0	22 ± 9.3	19 ± 6.2	31 ± 7.1	4 ± 0.2
W. 53	11 ± 6.2	12 ± 8.7	17 ± 6.1	61 ± 5.7	4 ± 0.4
Gi. 5	29 ± 7.7	16 ± 10.4	16 ± 5.3	39 ± 7.1	4 ± 0.4
Gi. 6	23 ± 3.7	13 ± 7.0	13 ± 11.4	51 ± 8.7	4 ± 0.4
W. 158	30 ± 3.9	14 ± 7.8	8 ± 8.3	48 ± 8.0	4 ± 0.6
W. 72	13 ± 4.0	36 ± 4.6	0 ± 0.0	50 ± 8.2	4 ± 0.2
Gi. 7	21 ± 9.2	16 ± 9.0	5 ± 5.0	58 ± 5.9	4 ± 0.3
Gi. 195/20	9 ± 4.6	15 ± 7.5	2 ± 1.7	74 ± 9.9	4 ± 0.3
W. 10	12 ± 4.1	34 ± 10.1	4 ± 2.3	51 ± 10.1	5 ± 0.2
W. 13	17 ± 8.0	37 ± 7.8	0 ± 0.0	46 ± 6.5	5 ± 0.4
Mahaleb	13 ± 3.8	42 ± 7.5	1 ± 1.4	44 ± 7.7	5 ± 0.7

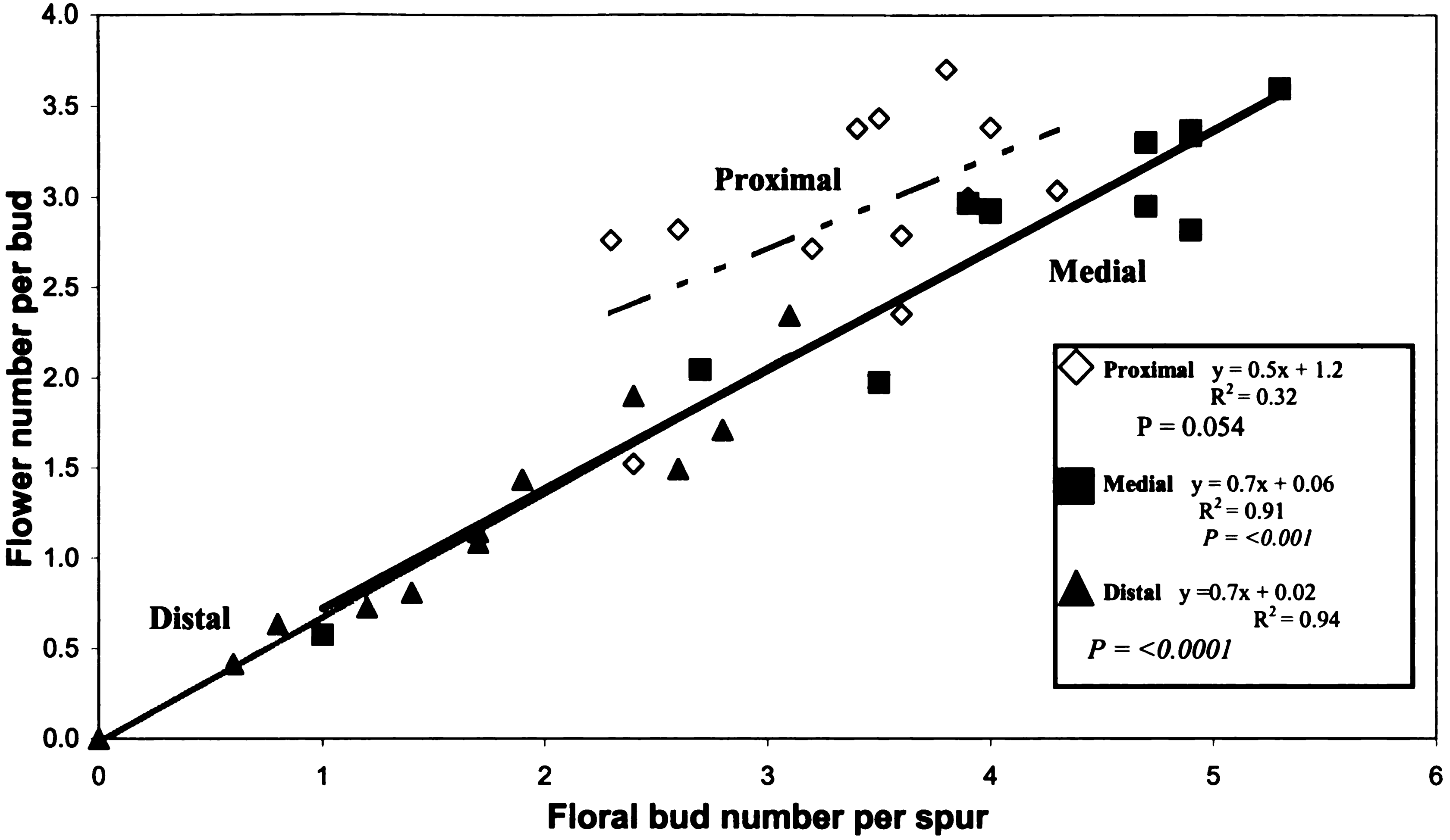

Figure 3-5: Flower number per bud of a shoot section plotted against its corresponding floral bud number per spur. Each point represents the average number of flowers per bud and buds per spur in the specified section of the two-year-old 'Montmorency' tart cherry shoots of a single rootstock treatment.

shoots does account for more precocious flowering, since the earliest flowering of spurs is on two-year-old shoots (Thompson, 1996). However, dwarfing rootstocks have already been shown to reduce the time to flowering in 'Montmorency' (Perry, 1996). Further acceleration in time to flowering may not be beneficial since tart cherry trees currently are harvested mechanically, and trees must reach a certain size before they are large enough to withstand the mechanical harvesting equipment (Nugent, 2001).

A general conclusion of our study is that rootstock influenced both spur floral characteristics (floral bud number per spur and flower number per bud), as well as the location of spurs and lateral shoots within the two-year-old `Montmorency' tart cherry shoot. The same conclusion was also drawn from the 'Hedelfinger' sweet cherry study (Chapter Two). This could be attributed, at least partially, to the rootstock effect on tree size (TCSAI); however, TCSAI accounted for only 1/2 to 2/3 of the variability in spur floral characteristics for 'Montmorency' tart cherry.

In the Gisela/Giessen series, floral bud number per spur and flower number per bud increased as tree size increased (Table 3-2). This trend also occurred in the Weiroot series, although to a lesser extent compared to the Gisela series.

The balance between vegetative and reproductive growth is important for reasons other than fruit size and yield. Overcropping is thought to be one of the contributing factors to the soft tart cherry problem in Michigan (Nugent, 2001). Vigorous Weiroot and Gisela/Giessen rootstocks alleviate some of this problem, since with an increase in reproductive growth, there is also an increase in vegetative growth.

Another major effect of rootstock on spur flowering characteristics was the effect of location on both numbers of flowers per bud and buds per spur. The two-year-old shoot had both the characteristic of being the first location of spur growth, as well as an indicator of where spurs will be produced in the future. In tart cherries, it is important to identify spur location as well as how many buds and flowers occur on that spur. Location within the two-year-old shoot affected both spur flowering characteristics. A lower floral bud number per spur and flower number per bud were found in the distal sections of all rootstock treatments (Table 3-5), which is probably due to the very low quantity of spurs in this area (Figure 3-4).

Metamer length did not predict metamer type in our study. Metamer length differed not only between treatments, but also between sections and was different according to the section x treatment interaction. Metamer length did decrease distally along the shoot in 'Montmorency', as well as in 'Hedelfinger', but there does not appear to be a relationship between this change in metamer length and location or quantity of any of the metamer types assessed (Figure 3-4; Chapter Two).

General conclusions can be made about where certain metamers are located, or where certain metamer types are not found. For example, vegetative axillary buds are not found in the medial or distal sections. Very few, if any, lateral shoots are found in the proximal section, except on the less vigorous rootstocks, which could be due to the shorter length of the two-year-old shoot. It is also interesting to note that blind nodes are found in all sections, but mainly in the distal section, which was where the fewest spurs formed. So, even if the distal section of the shoot appears more productive, this is not the case, since the more sustainable fruiting occurs in the medial and proximal sections.

Knowing the location of spurs and the spurs flowering characteristics could be helpful in managing the tree. The one variable not measured that may be useful for future work is the evolution of the branch, or how spurs within a branch age. Branch evolution in this trial may be implied since tree habit tends to repeat itself, such that one-year-old shoots may repeat pattern of the shoots in this trial (Tomlinson, 1983). Spur evolution, however, is something that we cannot predict from this trial. Whether the spurs will continue to produce the same number of buds and flowers, and at what point these spurs will change the number of buds and/or flowers they produce, is not yet known. A second question arises as to whether the same amount of flowering will occur in the one-year-old shoots, causing the same relative amount of blind node production in the succeeding years. We did not look at the way that the metamer types and quality change in these shoot sections over time. This is a major factor in tree management, though, since rootstock may also affect how well the spurs age.

This effect of rootstock on location of different metamer types, independent of the rootstock effect on tree size, implies a need to more accurately assess tree growth habit in tart cherry. Lespinasse and Lauri (1996) have noted that prediction of the distribution of specific metamers is one the three variables that must be understood before an accurate prediction and assessment of the tree's productive potential can be found. This is currently a critical step in the evaluation and adaptation of new precocious and/or size-controlling rootstocks for tart cherry production.

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APPENDIX A

Table A1. Comparison of buds per spur values in the proximal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	1.000	<.0001	0.005	<.0001	<.0001	<.0001	1.000	1.000	1.000	1.000	0.320	1.000	0.765	0.016	0.114
Edabriz		<.0001	0.005	<.0001	<.0001	<.0001	1.000	1.000	1.000	1.000	0.320	1.000	0.765	0.016	0.114
Gi.195/20			<.0001	<.0001	<.0001	0.002	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.209/1				0.010	0.006	0.000	0.005	0.004	0.009	0.005	0.075	0.004	0.013	0.779	0.314
Gi.5					0.877	0.136	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.006	0.001
Gi.6						0.179	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.004	0.001
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Mahaleb								1.000	1.000	1.000	0.320	1.000	0.765	0.016	0.114
Mazzard									1.000	1.000	0.299	1.000	0.755	0.012	0.101
MxM2										1.000	0.348	1.000	0.778	0.023	0.134
MxM60											0.320	1.000	0.765	0.016	0.114
W.10												0.299	0.486	0.150	0.518
W.13													0.755	0.012	0.101
W.158														0.034	0.193
W.53															0.474

Table A2. Comparison of buds per spur values in the medial section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.826	0.593	0.523	0.632	0.000	0.497	0.000	<.0001	<.0001
Edabriz		<.0001	<.0001	<.0001	0.000	<.0001	<.0001	<.0001	<.0001	<.0001	0.688	0.000	0.609	0.148	0.709
Gi.195/20			<.0001	<.0001	<.0001	0.106	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.209/1				0.518	0.255	0.021	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.002	<.0001
Gi.5					0.621	0.004	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.011	0.001
Gi.6						0.001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.038	0.003
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Mahaleb								0.445	0.398	0.486	0.000	0.652	0.000	<.0001	<.0001
Mazzard									0.871	0.972	<.0001	0.204	<.0001	<.0001	<.0001
MxM2										0.851	<.0001	0.194	<.0001	<.0001	<.0001
MxM60											<.0001	0.239	<.0001	<.0001	<.0001
W.10												0.001	0.912	0.066	0.452
W.13													0.001	<.0001	<.0001
W.158														0.051	0.392
W.53															0.321

Table A3. Comparison of buds per spur values in the distal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.001	<.0001	<.0001	<.0001	<.0001	<.0001	0.255	<.0001	0.003	<.0001	0.001	0.073	0.011	<.0001	<.0001
Edabriz		<.0001	0.056	0.045	0.018	<.0001	<.0001	<.0001	<.0001	<.0001	0.926	<.0001	0.414	0.081	0.070
Gi.195/20			0.001	0.002	0.005	0.963	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.001	0.004
Gi.209/1				0.917	0.622	0.001	<.0001	<.0001	<.0001	<.0001	0.045	<.0001	0.006	0.928	0.930
Gi.5					0.697	0.002	<.0001	<.0001	<.0001	<.0001	0.036	<.0001	0.005	0.850	0.996
Gi.6						0.006	<.0001	<.0001	<.0001	<.0001	0.014	<.0001	0.001	0.575	0.724
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.002	0.005
Mahaleb								<.0001	0.055	0.001	<.0001	0.541	0.000	<.0001	<.0001
Mazzard									0.016	0.300	<.0001	<.0001	<.0001	<.0001	<.0001
MxM2										0.159	<.0001	0.153	<.0001	<.0001	<.0001
MxM60											<.0001	0.002	<.0001	<.0001	<.0001
W.10												<.0001	0.468	0.066	0.058
W.13													<.0001	<.0001	<.0001
W.158														0.011	0.010
W.53															0.868

Table A4. Comparison of marginal buds per spur values of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.595	0.000	0.037	0.003	<.0001	0.517	0.000	<.0001	<.0001
Edabriz		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.772	<.0001	0.552	0.001	0.031
Gi.195/20			<.0001	<.0001	<.0001	0.006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.209/1				0.233	0.222	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.042	0.005
Gi.5					0.977	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.002	0.000
Gi.6						<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.002	0.000
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Mahaleb								0.002	0.111	0.015	<.0001	0.926	<.0001	<.0001	<.0001
Mazzard									0.190	0.536	<.0001	0.002	<.0001	<.0001	<.0001
MxM2										0.480	<.0001	0.116	<.0001	<.0001	<.0001
MxM60											<.0001	0.015	<.0001	<.0001	<.0001
W.10												<.0001	0.376	0.004	0.058
W.13													<.0001	<.0001	<.0001
W.158														0.000	0.007
W.53															0.373

Table A5. Comparison of flowers per bud values in the proximal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	1.000	<.0001	0.012	<.0001	<.0001	<.0001	1.000	1.000	1.000	1.000	0.095	1.000	0.402	0.001	0.001
Edabriz		<.0001	0.012	<.0001	<.0001	<.0001	1.000	1.000	1.000	1.000	0.095	1.000	0.402	0.001	0.001
Gi.195/20			<.0001	0.422	0.146	0.072	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.000	0.002
Gi.209/1				<.0001	0.001	0.006	0.012	0.008	0.018	0.012	0.427	0.008	0.096	0.377	0.282
Gi.5					0.491	0.277	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.003	0.012
Gi.6						0.666	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.021	0.055
Gi.7							<.0001	<.0001	<.0001	<.0001	0.001	<.0001	<.0001	0.071	0.142
Mahaleb								1.000	1.000	1.000	0.095	1.000	0.402	0.001	0.001
Mazzard									1.000	1.000	0.081	1.000	0.381	0.001	0.001
MxM2										1.000	0.115	1.000	0.429	0.002	0.002
MxM60											0.095	1.000	0.402	0.001	0.001
W.10												0.081	0.402	0.109	0.081
W.13													0.381	0.001	0.001
W.158														0.016	0.012
W.53															0.811

Table A6. Comparison of flowers per bud values in the medial section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.622	0.403	0.330	0.539	0.001	0.339	<.0001	<.0001	<.0001
Edabriz		0.953	0.066	0.599	0.734	0.750	<.0001	<.0001	<.0001	<.0001	0.010	<.0001	0.498	0.942	0.296
Gi.195/20			0.058	0.642	0.780	0.794	<.0001	<.0001	<.0001	<.0001	0.009	<.0001	0.462	0.989	0.271
Gi.209/1				0.014	0.023	0.030	0.000	<.0001	<.0001	<.0001	0.383	0.001	0.254	0.056	0.518
Gi.5					0.845	0.847	<.0001	<.0001	<.0001	<.0001	0.001	<.0001	0.218	0.652	0.115
Gi.6						0.994	<.0001	<.0001	<.0001	<.0001	0.003	<.0001	0.295	0.791	0.160
Gi.7							<.0001	<.0001	<.0001	<.0001	0.004	<.0001	0.319	0.805	0.179
Mahaleb								0.177	0.151	0.269	0.005	0.658	<.0001	<.0001	<.0001
Mazzard									0.819	0.846	<.0001	0.061	<.0001	<.0001	<.0001
MxM2										0.692	<.0001	0.057	<.0001	<.0001	<.0001
MxM60											0.000	0.111	<.0001	<.0001	<.0001
W.10												0.012	0.055	0.008	0.160
W.13													<.0001	<.0001	0.000
W.158														0.453	0.683
W.53															0.265

Table A7. Comparison of flowers per bud values in the distal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.041	0.199	0.954	0.269	0.157	0.142	0.227	0.000	0.042	0.001	0.720	0.962	0.244	0.307	0.854
Edabriz		0.444	0.029	0.300	0.467	0.561	0.001	<.0001	0.000	<.0001	0.017	0.037	0.375	0.303	0.081
Gi.195/20			0.162	0.813	0.942	0.853	0.013	<.0001	0.001	<.0001	0.101	0.195	0.904	0.790	0.303
Gi.209/1				0.222	0.123	0.112	0.228	<.0001	0.039	0.001	0.751	0.912	0.203	0.261	0.806
Gi.5					0.745	0.667	0.019	<.0001	0.002	<.0001	0.139	0.267	0.912	0.967	0.396
Gi.6						0.904	0.008	<.0001	0.001	<.0001	0.075	0.152	0.842	0.726	0.255
Gi.7							0.008	<.0001	0.001	<.0001	0.069	0.137	0.759	0.652	0.229
Mahaleb								0.009	0.363	0.026	0.394	0.191	0.019	0.027	0.186
Mazzard									0.125	0.763	0.001	<.0001	<.0001	<.0001	0.000
MxM2										0.227	0.088	0.031	0.002	0.003	0.035
MxM60											0.002	0.000	<.0001	<.0001	0.001
W.10												0.673	0.128	0.169	0.602
W.13													0.242	0.308	0.884
W.158														0.885	0.360
W.53															0.436

Table A8. Comparison of marginal flowers per bud values of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	<.0001	<.0001	0.000	<.0001	<.0001	<.0001	0.678	0.007	0.082	0.020	0.008	0.562	<.0001	<.0001	<.0001
Edabriz		0.001	0.385	0.001	0.002	0.006	<.0001	<.0001	<.0001	<.0001	0.055	<.0001	0.675	0.181	0.757
Gi.195/20			<.0001	0.740	0.523	0.431	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.026	0.003
Gi.209/1				<.0001	<.0001	0.000	<.0001	<.0001	<.0001	<.0001	0.253	0.001	0.665	0.024	0.256
Gi.5					0.747	0.623	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.000	0.046	0.005
Gi.6						0.854	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.001	0.091	0.011
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.002	0.148	0.021
Mahaleb								0.022	0.176	0.054	0.002	0.312	<.0001	<.0001	<.0001
Mazzard									0.449	0.774	<.0001	0.001	<.0001	<.0001	<.0001
MxM2										0.638	<.0001	0.019	<.0001	<.0001	<.0001
MxM60											<.0001	0.003	<.0001	<.0001	<.0001
W.10												0.027	0.132	0.001	0.035
W.13													0.000	<.0001	<.0001
W.158														0.080	0.481
W.53															0.339

Table A9. Comparison of internode length values in the proximal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.185	0.270	0.039	0.934	0.191	0.711	0.119	0.202	0.866	0.315	0.831	0.446	0.406	0.500	0.934
Edabriz		0.821	0.492	0.159	0.937	0.091	0.004	0.008	0.278	0.021	0.299	0.033	0.032	0.512	0.182
Gi.195/20			0.356	0.236	0.875	0.142	0.008	0.016	0.383	0.036	0.408	0.057	0.055	0.668	0.262
Gi.209/1				0.032	0.422	0.015	0.000	0.001	0.078	0.002	0.086	0.003	0.004	0.171	0.043
Gi.5					0.164	0.774	0.140	0.234	0.805	0.356	0.771	0.499	0.454	0.449	0.996
Gi.6						0.091	0.004	0.007	0.293	0.019	0.314	0.031	0.031	0.545	0.189
Gi.7							0.233	0.372	0.605	0.525	0.574	0.707	0.645	0.297	0.791
Mahaleb								0.723	0.102	0.577	0.093	0.384	0.464	0.027	0.165
Mazzard									0.170	0.819	0.156	0.587	0.680	0.049	0.266
MxM2										0.265	0.966	0.374	0.342	0.640	0.811
MxM60											0.246	0.773	0.861	0.094	0.387
W.10												0.349	0.319	0.673	0.778
W.13													0.916	0.144	0.530
W.158														0.134	0.484
W.53															0.472

Table A10. Comparison of internode length values in the medial section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.511	0.753	0.624	0.185	0.755	0.115	0.457	0.712	0.696	0.857	0.988	0.530	0.226	0.484	0.195
Edabriz		0.731	0.240	0.502	0.708	0.356	0.931	0.751	0.818	0.633	0.546	0.953	0.578	0.966	0.497
Gi.195/20			0.413	0.311	0.987	0.206	0.668	0.968	0.925	0.893	0.779	0.765	0.369	0.700	0.317
Gi.209/1				0.062	0.400	0.034	0.206	0.368	0.387	0.498	0.634	0.242	0.080	0.223	0.072
Gi.5					0.283	0.801	0.559	0.309	0.389	0.252	0.217	0.448	0.909	0.530	0.963
Gi.6						0.182	0.642	0.953	0.910	0.901	0.782	0.741	0.340	0.676	0.291
Gi.7							0.403	0.201	0.272	0.163	0.142	0.307	0.714	0.379	0.848
Mahaleb								0.683	0.756	0.573	0.493	0.881	0.638	0.965	0.550
Mazzard									0.953	0.856	0.741	0.786	0.369	0.717	0.316
MxM2										0.825	0.722	0.855	0.451	0.788	0.389
MxM60											0.877	0.660	0.302	0.603	0.260
W.10												0.567	0.260	0.519	0.225
W.13													0.522	0.918	0.447
W.158														0.607	0.878
W.53															0.523

Table A11. Comparison of internode length values in the distal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	edabriz	gi19520	gi2091	gi5	gi6	gi7	mahaleb	mazzard	mxm2	mxm60	w10	w13	w158	w53	w72
ct500	0.9008	0.9663	0.6068	0.9686	0.8303	0.5466	0.9297	0.7399	0.6536	0.9511	0.8807	0.7065	0.9297	0.7184	0.9854
edabriz		0.9343	0.5191	0.932	0.7304	0.6324	0.9709	0.644	0.5713	0.8524	0.974	0.8054	0.8314	0.6276	0.8919
gi19520			0.5764	0.9977	0.7961	0.5749	0.9633	0.7069	0.6252	0.9175	0.9122	0.7395	0.8962	0.6871	0.9537
gi2091				0.5784	0.7526	0.2534	0.5441	0.8479	0.9869	0.6522	0.5231	0.3505	0.6726	0.89	0.6428
gi5					0.7984	0.573	0.961	0.7091	0.6271	0.9198	0.91	0.7372	0.8985	0.6893	0.9558
gi6						0.399	0.7592	0.9017	0.7907	0.8806	0.7211	0.5356	0.9027	0.8711	0.8555
gi7							0.6067	0.3368	0.3097	0.5066	0.6755	0.7999	0.4896	0.3359	0.5574
mahaleb								0.6715	0.5948	0.8811	0.9466	0.776	0.8599	0.6536	0.9191
mazzard									0.8768	0.7887	0.6402	0.4577	0.8104	0.9644	0.7699
mxm2										0.6958	0.57	0.4128	0.7146	0.9132	0.6829
mxm60											0.8352	0.6595	0.9786	0.7648	0.9684
w10												0.8436	0.8155	0.6242	0.8731
w13													0.6393	0.4517	0.7097
w158														0.7853	0.9483
w53															0.7477

Table A12. Comparison of marginal internode length values of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.752	0.666	0.077	0.403	0.484	0.142	0.168	0.447	0.896	0.516	0.978	0.308	0.259	0.846	0.431
Edabriz		0.908	0.149	0.250	0.712	0.075	0.091	0.276	0.867	0.335	0.787	0.178	0.149	0.902	0.278
Gi.195/20			0.185	0.206	0.803	0.058	0.071	0.227	0.782	0.280	0.705	0.143	0.120	0.812	0.233
Gi.209/1				0.009	0.259	0.001	0.002	0.009	0.127	0.015	0.103	0.004	0.004	0.116	0.014
Gi.5					0.117	0.524	0.585	0.909	0.358	0.851	0.414	0.884	0.770	0.303	0.998
Gi.6						0.026	0.033	0.127	0.603	0.169	0.530	0.073	0.062	0.619	0.142
Gi.7							0.927	0.436	0.130	0.410	0.158	0.603	0.730	0.097	0.547
Mahaleb								0.494	0.153	0.463	0.184	0.671	0.800	0.116	0.605
Mazzard									0.396	0.935	0.458	0.785	0.675	0.336	0.917
MxM2										0.457	0.922	0.275	0.233	0.959	0.384
MxM60											0.522	0.733	0.631	0.399	0.861
W.10												0.325	0.275	0.877	0.440
W.13													0.873	0.223	0.890
W.158														0.187	0.781
W.53															0.333

Table A13. Comparison of percentage of blind node values in the proximal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.001	0.043	<.0001	0.081	0.052	<.0001	0.825	0.001	0.195	0.522	0.030	0.061	0.156	0.001	0.007
Edabriz		0.215	<.0001	0.146	0.329	0.024	0.001	0.821	0.121	0.000	0.402	0.185	0.088	0.868	0.701
Gi.195/20			<.0001	0.809	0.890	0.001	0.031	0.141	0.637	0.008	0.757	0.913	0.599	0.151	0.422
Gi.209/1				<.0001	<.0001	0.001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5					0.724	0.000	0.058	0.093	0.800	0.018	0.598	0.896	0.776	0.099	0.309
Gi.6						0.003	0.037	0.237	0.578	0.012	0.879	0.815	0.544	0.253	0.553
Gi.7							<.0001	0.042	0.000	<.0001	0.003	0.000	0.000	0.033	0.011
Mahaleb								0.000	0.147	0.694	0.022	0.043	0.114	0.000	0.005
Mazzard									0.080	<.0001	0.294	0.121	0.054	0.950	0.547
MxM2										0.064	0.474	0.712	0.995	0.085	0.246
MxM60											0.006	0.012	0.042	<.0001	0.001
W.10												0.686	0.433	0.314	0.654
W.13													0.681	0.130	0.373
W.158														0.057	0.204
W.53															0.581

Table A14. Comparison of percentage of blind node values in the medial section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.793	1.000	0.008	0.697	0.488	0.950	0.847	0.906	0.295	0.379	0.845	0.485	0.860	1.000	0.258
Edabriz		0.793	0.018	0.900	0.655	0.852	0.950	0.888	0.422	0.548	0.961	0.668	0.937	0.793	0.388
Gi.195/20			0.008	0.697	0.488	0.950	0.847	0.906	0.295	0.379	0.845	0.485	0.860	1.000	0.258
Gi.209/1				0.025	0.086	0.013	0.017	0.012	0.188	0.069	0.023	0.052	0.016	0.008	0.155
Gi.5					0.739	0.759	0.853	0.790	0.489	0.636	0.868	0.762	0.840	0.697	0.457
Gi.6						0.544	0.619	0.564	0.734	0.927	0.639	0.955	0.608	0.488	0.718
Gi.7							0.902	0.960	0.343	0.442	0.898	0.548	0.915	0.950	0.309
Mahaleb								0.939	0.397	0.515	0.992	0.629	0.987	0.847	0.364
Mazzard									0.353	0.457	0.934	0.569	0.953	0.906	0.317
MxM2										0.779	0.420	0.675	0.389	0.295	0.999
MxM60											0.541	0.870	0.504	0.379	0.762
W.10												0.652	0.980	0.845	0.389
W.13													0.618	0.485	0.652
W.158														0.860	0.355
W.53															0.258

Table A15. Comparison of percentage of blind node values in the distal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.968	0.810	0.953	0.947	0.481	0.870	0.627	0.949	0.919	0.567	0.961	0.902	0.306	0.810	0.977
Edabriz		0.845	0.923	0.980	0.465	0.902	0.606	0.918	0.892	0.547	0.991	0.872	0.297	0.845	0.947
Gi.195/20			0.769	0.866	0.358	0.950	0.474	0.764	0.756	0.416	0.864	0.720	0.210	1.000	0.799
Gi.209/1				0.902	0.521	0.828	0.674	0.995	0.961	0.615	0.918	0.949	0.343	0.769	0.978
Gi.5					0.451	0.922	0.589	0.898	0.874	0.530	0.990	0.852	0.286	0.866	0.928
Gi.6						0.411	0.806	0.524	0.590	0.846	0.482	0.560	0.812	0.358	0.517
Gi.7							0.536	0.824	0.809	0.480	0.917	0.781	0.259	0.950	0.855
Mahaleb								0.678	0.742	0.948	0.620	0.721	0.605	0.474	0.665
Mazzard									0.965	0.619	0.914	0.954	0.346	0.764	0.974
MxM2										0.690	0.890	0.993	0.422	0.756	0.943
MxM60											0.565	0.661	0.637	0.416	0.609
W.10												0.871	0.321	0.864	0.942
W.13													0.376	0.720	0.929
W.158														0.210	0.347
W.53															0.799

Table A16. Comparison of percentage of vegetative axillary bud values in the proximal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.007	<.0001	<.0001	<.0001	<.0001	<.0001	0.736	0.002	0.273	0.580	0.021	0.127	0.044	<.0001	<.0001
Edabriz		<.0001	<.0001	0.019	<.0001	<.0001	0.004	0.727	0.200	0.001	0.845	0.253	0.543	0.017	0.132
Gi.195/20			0.903	<.0001	0.017	0.699	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.209/1				<.0001	0.014	0.621	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5					0.022	<.0001	<.0001	0.046	0.001	<.0001	0.017	0.001	0.004	0.997	0.453
Gi.6						0.054	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.020	0.004
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Mahaleb								0.001	0.175	0.845	0.011	0.073	0.024	<.0001	<.0001
Mazzard									0.112	0.000	0.601	0.136	0.342	0.042	0.241
MxM2										0.115	0.299	0.796	0.475	0.001	0.010
MxM60											0.005	0.039	0.011	<.0001	<.0001
W.10												0.381	0.704	0.015	0.108
W.13													0.609	0.000	0.009
W.158														0.003	0.039
W.53															0.443

Table A17. Comparison of percentage of vegetative axillary bud values in the medial section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.503	0.793	0.436	0.520	<.0001	0.196	<.0001	<.0001	<.0001
Edabriz		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.029	<.0001	0.600	<.0001	0.004
Gi.195/20			0.108	0.229	0.903	0.777	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.017	0.002
Gi.209/1				0.692	0.187	0.214	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.459	0.122
Gi.5					0.338	0.389	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.253	0.054
Gi.6						0.890	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.044	0.007
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.048	0.007
Mahaleb								0.686	0.869	0.959	0.000	0.557	<.0001	<.0001	<.0001
Mazzard									0.592	0.713	<.0001	0.311	<.0001	<.0001	<.0001
MxM2										0.828	0.002	0.712	<.0001	<.0001	<.0001
MxM60											0.000	0.507	<.0001	<.0001	<.0001
W.10												0.002	0.099	<.0001	<.0001
W.13													<.0001	<.0001	<.0001
W.158														<.0001	0.001
W.53															0.388

Table A18. Comparison of percentage of vegetative axillary bud values in the distal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	<.0001	<.0001	<.0001	<.0001	0.001	<.0001	0.681	0.000	<.0001	0.001	0.062	0.864	<.0001	<.0001	<.0001
Edabriz		0.846	0.992	0.725	0.786	0.677	<.0001	<.0001	<.0001	<.0001	0.050	0.000	0.992	0.809	0.842
Gi.195/20			0.838	0.870	0.651	0.813	<.0001	<.0001	<.0001	<.0001	0.030	<.0001	0.841	0.961	0.988
Gi.209/1				0.717	0.793	0.670	<.0001	<.0001	<.0001	<.0001	0.051	0.000	1.000	0.801	0.834
Gi.5					0.553	0.939	<.0001	<.0001	<.0001	<.0001	0.022	<.0001	0.723	0.907	0.889
Gi.6						0.518	0.000	<.0001	<.0001	<.0001	0.121	0.001	0.797	0.620	0.656
Gi.7							<.0001	<.0001	<.0001	<.0001	0.022	<.0001	0.676	0.849	0.835
Mahaleb								0.003	0.000	0.006	0.029	0.570	<.0001	<.0001	<.0001
Mazzard									0.387	0.732	<.0001	0.000	<.0001	<.0001	<.0001
MxM2										0.237	<.0001	<.0001	<.0001	<.0001	<.0001
MxM60											<.0001	0.001	<.0001	<.0001	<.0001
W.10												0.093	0.056	0.027	0.038
W.13													0.000	<.0001	<.0001
W.158														0.805	0.837
W.53															0.976

Table A19. Comparison of percentage of lateral shoot values in the proximal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.974	1.000	0.985	0.913	0.506	0.878	0.967	0.925	0.955	1.000	0.952	0.938	0.949	1.000	1.000
Edabriz		0.974	0.960	0.940	0.495	0.856	0.943	0.951	0.933	0.974	0.976	0.914	0.925	0.974	0.976
Gi.195/20			0.985	0.913	0.506	0.878	0.967	0.925	0.955	1.000	0.952	0.938	0.949	1.000	1.000
Gi.209/1				0.900	0.524	0.895	0.982	0.911	0.969	0.985	0.938	0.954	0.965	0.985	0.985
Gi.5					0.452	0.799	0.884	0.989	0.879	0.913	0.968	0.854	0.867	0.913	0.918
Gi.6						0.619	0.545	0.460	0.587	0.506	0.500	0.559	0.558	0.506	0.526
Gi.7							0.914	0.810	0.935	0.878	0.842	0.939	0.930	0.878	0.884
Mahaleb								0.895	0.986	0.967	0.923	0.973	0.983	0.967	0.969
Mazzard									0.890	0.925	0.978	0.865	0.878	0.925	0.929
MxM2										0.955	0.915	0.990	0.999	0.955	0.957
MxM60											0.952	0.938	0.949	1.000	1.000
W.10												0.895	0.906	0.952	0.954
W.13													0.990	0.938	0.941
W.158														0.949	0.952
W.53															1.000

Table A20. Comparison of percentage of lateral shoot values in the medial section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.974	0.845	0.870	0.095	0.934	0.678	0.967	0.646	0.955	1.000	0.952	0.938	0.949	1.000	1.000
Edabriz		0.874	0.898	0.108	0.912	0.706	0.943	0.675	0.933	0.974	0.976	0.914	0.925	0.974	0.976
Gi.195/20			0.977	0.139	0.797	0.818	0.819	0.789	0.820	0.845	0.906	0.787	0.802	0.845	0.854
Gi.209/1				0.139	0.820	0.800	0.843	0.771	0.842	0.870	0.928	0.813	0.826	0.870	0.878
Gi.5					0.114	0.239	0.099	0.234	0.128	0.095	0.140	0.086	0.095	0.095	0.114
Gi.6						0.650	0.966	0.621	0.982	0.934	0.894	0.991	0.982	0.934	0.937
Gi.7							0.660	0.978	0.674	0.678	0.745	0.630	0.645	0.678	0.694
Mahaleb								0.629	0.986	0.967	0.923	0.973	0.983	0.967	0.969
Mazzard									0.646	0.646	0.717	0.598	0.614	0.646	0.664
MxM2										0.955	0.915	0.990	0.999	0.955	0.957
MxM60											0.952	0.938	0.949	1.000	1.000
W.10												0.895	0.906	0.952	0.954
W.13													0.990	0.938	0.941
W.158														0.949	0.952
W.53															1.000

Table A21. Comparison of percentage of lateral shoot values in the distal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.030	0.650	<.0001	<.0001	0.002	0.000	0.480	0.067	0.303	0.846	0.798	0.041	0.047	<.0001	0.001
Edabriz		0.084	<.0001	<.0001	0.243	0.108	0.006	0.736	0.004	0.047	0.025	0.896	0.891	0.057	0.265
Gi.195/20			<.0001	<.0001	0.006	0.001	0.253	0.166	0.154	0.794	0.501	0.111	0.120	0.000	0.005
Gi.209/1				0.900	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5					<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.6						0.731	0.000	0.140	0.000	0.003	0.002	0.198	0.203	0.583	0.914
Gi.7							<.0001	0.053	<.0001	0.000	0.000	0.083	0.087	0.840	0.635
Mahaleb								0.014	0.707	0.372	0.686	0.008	0.010	<.0001	0.000
Mazzard									0.009	0.101	0.053	0.837	0.846	0.025	0.150
MxM2										0.231	0.462	0.005	0.006	<.0001	0.000
MxM60											0.664	0.064	0.071	<.0001	0.002
W.10												0.033	0.037	<.0001	0.001
W.13													0.994	0.042	0.215
W.158														0.045	0.221
W.53															0.484

Table A22. Comparison of percentage of spur values in the proximal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.903	<.0001	0.009	0.000	<.0001	<.0001	0.901	0.999	0.993	1.000	0.674	0.958	0.394	0.024	0.067
Edabriz		<.0001	0.007	0.000	<.0001	<.0001	0.997	0.904	0.908	0.903	0.598	0.945	0.340	0.019	0.055
Gi.195/20			<.0001	<.0001	0.030	0.001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.209/1				0.330	0.001	0.012	0.008	0.010	0.022	0.009	0.045	0.009	0.091	0.683	0.497
Gi.5					0.012	0.116	0.000	0.000	0.002	0.000	0.004	0.000	0.008	0.162	0.106
Gi.6						0.313	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.000	<.0001
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.003	0.002
Mahaleb								0.903	0.907	0.901	0.602	0.943	0.347	0.022	0.059
Mazzard									0.994	0.999	0.679	0.958	0.403	0.027	0.072
MxM2										0.993	0.714	0.957	0.457	0.049	0.106
MxM60											0.674	0.958	0.394	0.024	0.067
W.10												0.644	0.704	0.098	0.194
W.13													0.375	0.023	0.064
W.158														0.187	0.336
W.53															0.765

Table A23. Comparison of percentage of spur values in the medial section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.586	0.907	0.923	0.904	<.0001	0.447	<.0001	<.0001	<.0001
Edabriz		<.0001	0.012	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.026	<.0001	0.679	<.0001	0.028
Gi.195/20			<.0001	0.013	0.558	0.624	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.019	<.0001
Gi.209/1				0.134	0.003	0.001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.004	0.084	0.815
Gi.5					0.096	0.059	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.843	0.093
Gi.6						0.907	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.131	0.002
Gi.7							<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.084	0.001
Mahaleb								0.517	0.566	0.509	0.000	0.844	<.0001	<.0001	<.0001
Mazzard									0.994	0.999	<.0001	0.389	<.0001	<.0001	<.0001
MxM2										0.993	0.000	0.446	<.0001	<.0001	<.0001
MxM60											<.0001	0.379	<.0001	<.0001	<.0001
W.10												0.001	0.072	<.0001	<.0001
W.13													<.0001	<.0001	<.0001
W.158														<.0001	0.011
W.53															0.058

Table A24. Comparison of percentage of spur values in the distal section of 'Hedelfinger' sweet cherry two-year old shoots from 16 different rootstocks. P-values show significance of each interaction.

	Edabriz	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	Mazzard	MxM2	MxM60	W.10	W.13	W.158	W.53	W.72
CT 500	0.003	<.0001	0.283	0.509	0.311	0.017	0.673	<.0001	0.000	<.0001	0.033	0.233	0.043	0.025	0.022
Edabriz		0.150	<.0001	0.000	0.099	0.650	0.001	<.0001	<.0001	<.0001	0.533	<.0001	0.392	0.452	0.589
Gi.195/20			<.0001	<.0001	0.003	0.064	<.0001	<.0001	<.0001	<.0001	0.048	<.0001	0.023	0.026	0.053
Gi.209/1				0.685	0.050	0.001	0.536	0.000	0.008	0.004	0.002	0.907	0.003	0.001	0.001
Gi.5					0.112	0.003	0.825	<.0001	0.003	0.001	0.007	0.601	0.009	0.004	0.004
Gi.6						0.235	0.174	<.0001	<.0001	<.0001	0.322	0.039	0.401	0.320	0.270
Gi.7							0.007	<.0001	<.0001	<.0001	0.859	0.001	0.701	0.791	0.932
Mahaleb								<.0001	0.002	0.001	0.015	0.463	0.019	0.010	0.009
Mazzard									0.493	0.356	<.0001	0.000	<.0001	<.0001	<.0001
MxM2										0.900	<.0001	0.011	<.0001	<.0001	<.0001
MxM60											<.0001	0.006	<.0001	<.0001	<.0001
W.10												0.002	0.847	0.944	0.926
W.13													0.002	0.001	0.001
W.158														0.892	0.767
W.53															0.862

Table A25. Comparison of buds per spur values in the proximal section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.049	0.972	0.288	0.061	0.084	0.743	0.130	0.135	0.165	0.020	0.182
Gi.195/20		0.064	0.379	0.853	0.817	0.097	0.657	0.645	0.572	0.722	0.481
Gi.209/1			0.326	0.080	0.104	0.782	0.157	0.162	0.195	0.028	0.216
Gi.5				0.463	0.516	0.452	0.663	0.675	0.752	0.218	0.831
Gi.6					0.955	0.121	0.781	0.767	0.686	0.578	0.585
Gi.7						0.154	0.831	0.818	0.739	0.558	0.642
Mahaleb							0.228	0.235	0.280	0.043	0.312
W.10								0.986	0.904	0.425	0.808
W.13									0.917	0.415	0.822
W.158										0.358	0.908
W.53											0.283

Table A26. Comparison of buds per spur values in the medial section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.098	0.001	0.148	0.636	0.870	0.894	0.241	0.260	0.510	0.349	0.244
Gi.195/20		<.0001	0.004	0.036	0.153	0.127	0.641	0.610	0.337	0.488	0.583
Gi.209/1			0.065	0.004	0.001	0.001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5				0.317	0.124	0.116	0.013	0.015	0.045	0.024	0.011
Gi.6					0.539	0.544	0.106	0.116	0.269	0.166	0.103
Gi.7						0.971	0.333	0.355	0.636	0.458	0.343
Mahaleb							0.296	0.317	0.595	0.418	0.302
W.10								0.965	0.621	0.821	0.951
W.13									0.652	0.855	0.987
W.158										0.788	0.649
W.53											0.861

Table A27. Comparison of buds per spur values in the distal section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.066	0.360	0.834	0.278	0.175	0.005	0.031	0.632	0.296	0.500	0.013
Gi.195/20		0.009	0.050	0.415	0.638	0.384	0.755	0.189	0.441	0.260	0.587
Gi.209/1			0.498	0.053	0.031	<.0001	0.004	0.183	0.062	0.129	0.001
Gi.5				0.214	0.134	0.004	0.024	0.510	0.230	0.398	0.010
Gi.6					0.745	0.079	0.254	0.576	0.991	0.717	0.155
Gi.7						0.175	0.434	0.398	0.763	0.510	0.302
Mahaleb							0.586	0.026	0.096	0.042	0.731
W.10								0.105	0.280	0.151	0.828
W.13									0.585	0.851	0.057
W.158										0.721	0.179
W.53											0.087

Table A28. Comparison of marginal buds per spur values of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.002	0.015	0.729	0.151	0.061	0.057	0.006	0.075	0.076	0.024	0.004
Gi.195/20		<.0001	0.001	0.074	0.219	0.179	0.730	0.188	0.186	0.398	0.681
Gi.209/1			0.044	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5				0.087	0.034	0.032	0.003	0.042	0.043	0.013	0.002
Gi.6					0.610	0.637	0.152	0.675	0.679	0.361	0.147
Gi.7						0.952	0.375	0.930	0.926	0.699	0.380
Mahaleb							0.324	0.975	0.970	0.643	0.326
W.10								0.330	0.327	0.616	0.960
W.13									0.996	0.635	0.333
W.158										0.631	0.330
W.53											0.635

Table A29. Comparison of flowers per bud values in the proximal section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.216	0.014	0.928	0.625	0.061	0.899	0.958	0.409	0.179	0.583	0.197
Gi.195/20		<.0001	0.204	0.438	0.535	0.264	0.256	0.050	0.918	0.507	0.995
Gi.209/1			0.023	0.004	<.0001	0.010	0.016	0.114	<.0001	0.004	<.0001
Gi.5				0.578	0.060	0.832	0.890	0.481	0.170	0.540	0.186
Gi.6					0.156	0.718	0.680	0.198	0.378	0.934	0.420
Gi.7						0.079	0.081	0.010	0.605	0.201	0.513
Mahaleb							0.946	0.344	0.221	0.669	0.244
W.10								0.400	0.216	0.635	0.238
W.13									0.039	0.189	0.041
W.158										0.444	0.909
W.53											0.493

Table A30. Comparison of flowers per bud values in the medial section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.206	<.0001	0.066	0.039	0.944	0.915	0.423	0.764	0.501	0.972	0.432
Gi.195/20		<.0001	0.003	0.002	0.202	0.173	0.656	0.135	0.569	0.214	0.604
Gi.209/1			0.006	0.006	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5				0.887	0.090	0.083	0.012	0.140	0.017	0.084	0.011
Gi.6					0.057	0.049	0.006	0.093	0.009	0.052	0.005
Gi.7						0.975	0.404	0.825	0.478	0.974	0.413
Mahaleb							0.367	0.842	0.439	0.947	0.373
W.10								0.292	0.901	0.423	0.958
W.13									0.352	0.799	0.295
W.158										0.498	0.939
W.53											0.433

Table A31. Comparison of flowers per bud values in the distal section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.012	0.204	0.660	0.282	0.110	<.0001	0.087	0.853	0.364	0.726	0.025
Gi.195/20		<.0001	0.005	0.132	0.372	0.372	0.436	0.026	0.120	0.038	0.703
Gi.209/1			0.425	0.023	0.007	<.0001	0.005	0.164	0.039	0.122	<.0001
Gi.5				0.144	0.052	<.0001	0.040	0.550	0.198	0.450	0.011
Gi.6					0.563	0.013	0.486	0.400	0.905	0.498	0.237
Gi.7						0.070	0.909	0.176	0.505	0.231	0.581
Mahaleb							0.089	0.002	0.013	0.003	0.183
W.10								0.142	0.435	0.190	0.664
W.13									0.489	0.875	0.050
W.158										0.593	0.213
W.53											0.073

Table A32. Comparison of marginal flowers per bud values of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.004	<.0001	0.171	0.763	0.050	0.038	0.138	0.587	0.093	0.618	0.013
Gi.195/20		<.0001	<.0001	0.002	0.370	0.358	0.174	0.001	0.243	0.022	0.601
Gi.209/1			<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5				0.278	0.002	0.001	0.007	0.427	0.004	0.075	<.0001
Gi.6					0.025	0.018	0.077	0.798	0.050	0.432	0.006
Gi.7						0.987	0.640	0.017	0.784	0.159	0.679
Mahaleb							0.614	0.012	0.762	0.137	0.676
W.10								0.054	0.847	0.343	0.368
W.13									0.034	0.319	0.004
W.158										0.255	0.484
W.53											0.060

Table A33. Comparison of internode length values in the proximal section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.114	0.267	0.496	0.005	0.003	0.150	0.006	<.0001	0.017	<.0001	0.002
Gi.195/20		0.010	0.031	0.263	0.175	0.836	0.267	0.034	0.439	0.044	0.170
Gi.209/1			0.681	<.0001	<.0001	0.013	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5				<.0001	<.0001	0.040	0.001	<.0001	0.003	<.0001	<.0001
Gi.6					0.766	0.164	0.968	0.274	0.755	0.323	0.790
Gi.7						0.105	0.806	0.445	0.559	0.508	0.965
Mahaleb							0.172	0.016	0.310	0.021	0.098
W.10								0.313	0.736	0.364	0.831
W.13									0.178	0.920	0.400
W.158										0.213	0.571
W.53											0.462
W.72											

Table A34. Comparison of internode length values in the medial section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.185	0.012	0.086	0.283	0.265	0.278	0.203	0.095	0.600	0.412	0.938
Gi.195/20		0.277	0.732	0.746	0.821	0.753	0.939	0.766	0.435	0.616	0.162
Gi.209/1			0.447	0.136	0.181	0.139	0.235	0.420	0.057	0.105	0.010
Gi.5				0.487	0.562	0.493	0.669	0.964	0.253	0.390	0.073
Gi.6					0.928	0.991	0.803	0.517	0.617	0.839	0.250
Gi.7						0.936	0.879	0.594	0.572	0.778	0.235
Mahaleb							0.811	0.524	0.610	0.831	0.245
W.10								0.703	0.473	0.665	0.178
W.13									0.272	0.415	0.081
W.158										0.777	0.550
W.53											0.371

Table A35. Comparison of internode length values in the distal section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.431	0.094	0.745	0.727	0.384	0.809	0.905	0.034	0.586	0.800	0.437
Gi.195/20		0.019	0.287	0.649	0.936	0.577	0.532	0.199	0.816	0.610	0.964
Gi.209/1			0.195	0.045	0.015	0.057	0.091	<.0001	0.034	0.065	0.016
Gi.5				0.511	0.252	0.578	0.674	0.019	0.405	0.579	0.286
Gi.6					0.590	0.915	0.837	0.073	0.832	0.937	0.668
Gi.7						0.522	0.482	0.229	0.754	0.554	0.897
Mahaleb							0.915	0.058	0.753	0.981	0.592
W.10								0.060	0.691	0.901	0.544
W.13									0.130	0.073	0.166
W.158										0.781	0.844
W.53											0.626

Table A36. Comparison of marginal internode length values of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.033	0.874	0.680	0.014	0.004	0.111	0.018	<.0001	0.046	0.006	0.029
Gi.195/20		0.028	0.099	0.848	0.489	0.534	0.822	0.034	0.884	0.567	0.954
Gi.209/1			0.585	0.013	0.004	0.093	0.016	<.0001	0.039	0.006	0.025
Gi.5				0.054	0.019	0.267	0.061	<.0001	0.130	0.025	0.093
Gi.6					0.593	0.390	0.964	0.041	0.729	0.683	0.792
Gi.7						0.176	0.641	0.149	0.399	0.904	0.432
Mahaleb							0.391	0.004	0.636	0.220	0.551
W.10								0.058	0.709	0.730	0.768
W.13									0.023	0.118	0.022
W.158										0.470	0.924
W.53											0.510

Table A37. Comparison of percentage of blind node values in the proximal section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.065	0.096	0.943	0.576	0.479	0.126	0.100	0.292	0.906	0.081	0.123
Gi.195/20		0.001	0.056	0.195	0.251	0.748	0.839	0.485	0.064	0.917	0.695
Gi.209/1			0.111	0.027	0.019	0.002	0.001	0.010	0.146	0.001	0.001
Gi.5				0.528	0.436	0.109	0.086	0.263	0.960	0.070	0.106
Gi.6					0.882	0.328	0.274	0.598	0.519	0.233	0.335
Gi.7						0.406	0.343	0.698	0.433	0.296	0.417
Mahaleb							0.906	0.692	0.119	0.829	0.955
W.10								0.612	0.095	0.921	0.857
W.13									0.267	0.549	0.720
W.158										0.078	0.117
W.53											0.777

Table A38. Comparison of percentage of blind node values in the medial section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.779	0.939	0.387	0.983	0.350	0.869	0.384	0.619	0.505	0.930	0.734
Gi.195/20		0.838	0.558	0.796	0.512	0.656	0.251	0.447	0.687	0.713	0.527
Gi.209/1			0.430	0.956	0.391	0.809	0.344	0.569	0.552	0.869	0.674
Gi.5				0.399	0.945	0.303	0.084	0.191	0.881	0.341	0.215
Gi.6					0.361	0.852	0.372	0.605	0.518	0.913	0.717
Gi.7						0.272	0.073	0.170	0.829	0.307	0.189
Mahaleb							0.480	0.733	0.411	0.939	0.866
W.10								0.745	0.139	0.434	0.569
W.13									0.271	0.679	0.844
W.158										0.454	0.312
W.53											0.804

Table A39. Comparison of percentage of blind node values in the distal section of 'Montmorency' tart cherry two-year old shoots from 11 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.031	0.001	0.787	0.208	0.462	0.013	0.014	0.015	0.193	0.063	0.018
Gi.195/20		<.0001	0.058	0.357	0.149	0.731	0.750	0.686	0.451	0.755	0.911
Gi.209/1			0.000	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Gi.5				0.321	0.641	0.026	0.027	0.029	0.294	0.111	0.037
Gi.6					0.597	0.207	0.216	0.205	0.908	0.542	0.283
Gi.7						0.075	0.079	0.079	0.540	0.256	0.106
Mahaleb							0.980	0.936	0.282	0.512	0.805
W.10								0.918	0.293	0.529	0.826
W.13									0.273	0.486	0.753
W.158										0.645	0.375
W.53											0.661

Table A40. Comparison of percentage of axillary vegetative bud values in the proximal section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.232	0.033	0.331	0.121	0.331	0.000	0.022	0.009	0.215	0.073	0.005
Gi.195/20		0.336	0.822	0.720	0.822	<.0001	0.001	0.000	0.910	0.545	<.0001
Gi.209/1			0.236	0.545	0.236	<.0001	<.0001	<.0001	0.427	0.720	<.0001
Gi.5				0.559	1.000	<.0001	0.001	0.001	0.745	0.407	0.000
Gi.6					0.559	<.0001	0.000	<.0001	0.821	0.806	<.0001
Gi.7						<.0001	0.001	0.001	0.745	0.407	0.000
Mahaleb							0.146	0.369	<.0001	<.0001	0.276
W.10								0.631	0.001	<.0001	0.663
W.13									0.000	<.0001	0.928
W.158										0.647	0.000
W.53											<.0001

Table A41. Comparison of percentage of axillary vegetative bud values in the medial section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	1.000	0.838	1.000	1.000	0.591	0.436	0.365	0.756	1.000	0.792	0.721
Gi.195/20		0.838	1.000	1.000	0.591	0.436	0.365	0.756	1.000	0.792	0.721
Gi.209/1			0.838	0.838	0.739	0.566	0.482	0.907	0.847	0.953	0.886
Gi.5				1.000	0.591	0.436	0.365	0.756	1.000	0.792	0.721
Gi.6					0.591	0.436	0.365	0.756	1.000	0.792	0.721
Gi.7						0.810	0.711	0.844	0.612	0.784	0.837
Mahaleb							0.897	0.672	0.463	0.606	0.648
W.10								0.586	0.393	0.520	0.554
W.13									0.768	0.950	0.991
W.158										0.804	0.738
W.53											0.935

Table A42. Comparison of percentage of axillary vegetative bud values in the distal section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	1.000	1.000	1.000	1.000	1.000	0.737	1.000	1.000	1.000	1.000	1.000
Gi.195/20		1.000	1.000	1.000	1.000	0.737	1.000	1.000	1.000	1.000	1.000
Gi.209/1			1.000	1.000	1.000	0.737	1.000	1.000	1.000	1.000	1.000
Gi.5				1.000	1.000	0.737	1.000	1.000	1.000	1.000	1.000
Gi.6					1.000	0.737	1.000	1.000	1.000	1.000	1.000
Gi.7						0.737	1.000	1.000	1.000	1.000	1.000
Mahaleb							0.737	0.752	0.752	0.737	0.726
W.10								1.000	1.000	1.000	1.000
W.13									1.000	1.000	1.000
W.158										1.000	1.000
W.53											1.000

Table A43. Comparison of percentage of lateral shoot values in the proximal section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.154	0.570	0.794	0.648	0.250	0.148	0.207	0.140	0.410	0.893	0.103
Gi.195/20		0.048	0.243	0.330	0.780	0.984	0.867	0.895	0.599	0.196	0.884
Gi.209/1			0.408	0.307	0.088	0.046	0.069	0.046	0.176	0.483	0.027
Gi.5				0.845	0.373	0.235	0.316	0.218	0.563	0.900	0.173
Gi.6					0.486	0.320	0.419	0.294	0.693	0.748	0.245
Gi.7						0.765	0.911	0.693	0.792	0.309	0.662
Mahaleb							0.851	0.910	0.586	0.189	0.901
W.10								0.772	0.713	0.260	0.748
W.13									0.533	0.177	1.000
W.158										0.486	0.494
W.53											0.135

Table A44. Comparison of percentage of lateral shoot values in the medial section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.129	0.022	0.438	0.727	0.430	0.002	0.005	0.027	0.640	0.564	0.009
Gi.195/20		0.000	0.023	0.240	0.462	0.103	0.191	0.421	0.331	0.037	0.284
Gi.209/1			0.122	0.009	0.002	<.0001	<.0001	<.0001	0.009	0.082	<.0001
Gi.5				0.262	0.119	0.000	0.001	0.004	0.232	0.843	0.001
Gi.6					0.659	0.006	0.014	0.057	0.890	0.355	0.023
Gi.7						0.019	0.043	0.135	0.781	0.173	0.067
Mahaleb							0.741	0.458	0.013	0.000	0.521
W.10								0.667	0.029	0.001	0.767
W.13									0.094	0.006	0.865
W.158										0.313	0.046
W.53											0.001

Table A45. Comparison of percentage of lateral shoot values in the distal section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.179	0.004	0.854	0.869	0.773	0.150	0.128	0.051	0.441	0.251	0.139
Gi.195/20		<.0001	0.245	0.132	0.104	0.923	0.857	0.484	0.616	0.842	0.938
Gi.209/1			0.002	0.007	0.009	<.0001	<.0001	<.0001	0.001	<.0001	<.0001
Gi.5				0.727	0.637	0.208	0.180	0.074	0.550	0.335	0.197
Gi.6					0.902	0.109	0.092	0.035	0.355	0.190	0.099
Gi.7						0.085	0.071	0.027	0.298	0.152	0.076
Mahaleb							0.933	0.543	0.554	0.767	0.981
W.10								0.596	0.502	0.704	0.912
W.13									0.256	0.375	0.513
W.158										0.754	0.553
W.53											0.775

Table A46. Comparison of percentage of spur values in the proximal section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.000	0.254	0.465	0.064	0.014	0.239	0.068	0.187	0.144	0.006	0.061
Gi.195/20		<.0001	0.001	0.031	0.124	0.005	0.029	0.014	0.021	0.207	0.021
Gi.209/1			0.063	0.003	0.000	0.022	0.004	0.018	0.012	0.000	0.003
Gi.5				0.258	0.079	0.652	0.269	0.525	0.437	0.042	0.263
Gi.6					0.525	0.495	0.979	0.665	0.771	0.360	0.951
Gi.7						0.189	0.508	0.303	0.374	0.779	0.469
Mahaleb							0.512	0.833	0.724	0.112	0.515
W.10								0.683	0.791	0.347	0.974
W.13									0.892	0.196	0.694
W.158										0.250	0.807
W.53											0.310

Table A47. Comparison of percentage of spur values in the medial section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.144	0.006	0.088	0.707	0.778	0.001	0.001	0.005	0.911	0.478	0.002
Gi.195/20		<.0001	0.002	0.276	0.082	0.060	0.035	0.138	0.136	0.031	0.101
Gi.209/1			0.267	0.002	0.012	<.0001	<.0001	<.0001	0.012	0.036	<.0001
Gi.5				0.038	0.152	<.0001	<.0001	<.0001	0.133	0.313	<.0001
Gi.6					0.511	0.003	0.002	0.013	0.640	0.279	0.006
Gi.7						0.000	0.000	0.002	0.878	0.669	0.001
Mahaleb							0.811	0.766	0.001	<.0001	0.740
W.10								0.601	0.001	<.0001	0.561
W.13									0.005	0.001	0.998
W.158										0.578	0.002
W.53											0.000

Table A48. Comparison of percentage of spur values in the distal section of 'Montmorency' tart cherry two-year old shoots from 12 different rootstocks. P-values show significance of each interaction.

	Gi.195/20	Gi.209/1	Gi.5	Gi.6	Gi.7	Mahaleb	W.10	W.13	W.158	W.53	W.72
Edabriz	0.567	0.928	0.960	0.161	0.300	0.535	0.500	0.894	0.702	0.617	0.536
Gi.195/20		0.507	0.601	0.405	0.642	0.962	0.919	0.684	0.875	0.941	0.984
Gi.209/1			0.888	0.136	0.260	0.477	0.444	0.827	0.640	0.555	0.476
Gi.5				0.177	0.324	0.568	0.533	0.932	0.738	0.653	0.572
Gi.6					0.712	0.432	0.464	0.234	0.346	0.365	0.395
Gi.7						0.677	0.716	0.398	0.551	0.590	0.641
Mahaleb							0.957	0.651	0.839	0.903	0.976
W.10								0.615	0.800	0.861	0.931
W.13									0.813	0.735	0.658
W.158										0.930	0.855
W.53											0.923

APPENDIX B

LEAFY project:

1. **Rationale**

1.1 History

The idea behind this project was to find a gene that could be involved in the early stages of flower induction in sweet cherry.

The LEAFY (LFY) gene of *Arabidopsis thaliana* is a well-conserved gene in many plant species, including gymnosperms and angiosperms, and appears to be involved in the transition to flowering (see section 1.2)

1.2 LEAFY gene

The LFY gene and its activity has been one of the most promising genes for increasing precocity as well as flower density in trees (Egea-Cortines and Weiss, 2001). LFY is well-conserved, and its homologues have been found in many diverse species. The location of LFY was found to be on chromosome 5 of Arabidopsis (Schulz and Haughn, 1991). Mellerowicz et al. (1998) showed that, although LFY homologues are found in pre-angiosperm species, some differences exist in their sequences and, subsequently, proteins. The proline-rich and acidic domains in LFY and FLORICAULA (FLO) [the snapdragon (*Antirrhinum* spp.) homologue of LFY], whose presence indicates that they are transcription factors, are not evident in conifers. However, the C-terminal part of the protein, whose function is not yet known, is highly conserved between conifers and angiosperms (Mellerowicz et al., 1998). Blazquez et al. (1997) demonstrate that LFY is both necessary and sufficient for the initiation of individual flowers. LFY expression and flower formation are not strictly coupled, because LFY expression in the wild-type Arabidopsis precedes flower formation (Blazquez et al., 1997). However, upregulation of the LFY promoter appears to be an indicator of subsequent flowering, because failure to flower in short days in the GA-deficient ga1-3 mutant goes hand in hand with the elimination of LFY upregulation (Blazquez et al., 1998). LFY also appears to suppress leaf development (Weigel et al., 1992; Weigel and Meyerowitz, 1993). LFY is of primary importance in the transition from the vegetative to reproductive phase of meristems: *lfy* mutants of Arabidopsis have leaves in the locations where the first flowers form and later-arising flowers are replaced by structures with partial shoot characteristics (Blazquez et al., 1997). In *lfy* mutants, normal flowers are never produced. Instead of flowers, *lfy* mutant shoots resemble lateral shoots in that they produce an indeterminate number of metamers with elongated internodes, bract-like organs, tertiary lateral shoots, and spiral phyllotaxy (Schultz and Haughn, 1991). Blazquez et al. (1997) also observed that LFY is expressed in lateral primordia continuously from the vegetative to reproductive phase, changing only in intensity. Quantitative increases in LFY expression to a threshold level are a major factor in the transition to flowering (Blazquez et al., 1998). Blazquez et al. (1997) suggest that LFY is expressed in emerging leaf primordia because this is where floral induction is effective, as these primordia have the ability to make the transition to become floral once LFY activity reaches a critical level.

When transformed into other species, LFY still specifies a floral fate. Weigel and Nilsson (1995) have demonstrated that in aspen, a perennial tree, LFY is sufficient to determine floral fate in lateral meristems and increase precocity. When transformed into aspen, LFY was sufficient to trigger flower initiation. Aspen normally flowers after 8 years, but transgenic LFY aspen flowered within just a few months of germination (Coupland, 1995). Constitutive expression of LFY in aspen results in solitary flowers being produced in the axils of normal leaves, as well as the number of vegetative leaves being limited, the shoot apical meristem being turned prematurely into a terminal flower, and precocious flower development (Weigel and Nilsson, 1995). In citrus, the juvenile phase normally lasts from 6 to 20 years, but in both LFY and AP1 transgenic citrus trees flowers were initiated in the first year (Pena et al, 2001). In both cases, trees flowered in consecutive years, as well as under the control of environmental signals, and LFY and AP1 expression were found in the leaves. The LFY transgenic lines, compared to the controls, had a weeping growth habit and thin stems, reduced leaf size and a curling of the leaves. Citrus flower buds normally give rise to a range of inflorescence types, including solitary flowers to mixed flowers to leaf racemes, and all of these inflorescence types were found in the transgenic LFY citrus lines. Transgenic AP1 trees on the other hand had a more normal growth habit than the transgenic LFY lines (Pena et al., 2001).

LFY homologues and LFY-like genes have been identified and cloned in a diverse range of plant species (Weigel and Nilsson, 1995). Although LFY is well-conserved, its role in transition to flowering is not very conserved. Overexpression of PTLF, the homologue of LFY in *Populus trichocarpa* (poplar), resulted in no early flowering phenotype or other differences as compared to the control trees, even though when PTLF was transformed into Arabidopsis, it caused flowering to occur 5 days earlier than the control (Rottmann et al., 2000). Even without the early-flowering phenotype of PTLF in poplar, the strongest PTLF expression was in the lateral floral meristems. FLORICAULA (FLO), the LFY homologue in Antirrhinum majus, shares 70% amino acid identity. Both LFY and FLO are expressed in the floral meristem prior to initiation of floral organ primordial, while expression at later stages of floral development is less conserved (Coen et al., 1990; Weigel et al., 1992). The LFY homologue cloned in *Pinus radiata*, PRFLL, shares 53% similarity with LFY (Mellerowicz, 1998). Expression of PRFLL was found in vegetative buds of juvenile, adolescent, and mature trees, but not in vascular cambium, roots or secondary needles. PRFLL mRNA was detected in buds [in which cone and shoot primordia will develop] and in developing male cones, but not in developing female cones. Expression was particularly high in buds of the axillary meristems prior to their differentiation as male cones, which is consistent with PRFLL being involved in determination of male cone primordia. (Mellerowicz et al., 1998). Southerton et al. (1998) have isolated a LFY homologue, ELF, in Eucalyptus (*Eucalyptus L'Her.* spp.) whose sequence and expression pattern in floral primordia is very similar to LFY and FLO. A LFY homologue, LtLFY, has also been found in the grass species *Lolium tementulum* (Gocal et al., 2001). LtLFY has only 56% amino acid identity with LFY. Like the other LFY homologues, the C-terminal region of LtLFY is more highly conserved than the amino-terminal region. In *Lolium tementulum*, AP1 homologue expression precedes LtLFY expression, implying that the regulatory pathway of floral initiation may not be well-conserved in monocots (Gocal et al., 2001).

1.3 Brief overview

The idea behind this project was to isolate a gene in sweet cherry that had homology to the Arabidopsis LFY gene, and to see if it was upregulated during the flower initiation period in floral buds. As an addition to this experiment, we also looked at the same sweet cherry scion cultivar ('Hedelfinger') on two different rootstocks: a vigorous rootstock with little to no flowering ('Mazzard'), and a nonvigorous rootstock that has already begun to flower ('Gisela 6') to see if upregulation of LFY occurred in both of these rootstock/scion combinations similarly or differently.

2. Materials and Methods

2.1Plant materials

Sweet cherry (*Prunus avium* L.) 'Hedelfinger' trees grafted on Gisela 6 (Gi.6) and Mazzard rootstocks were used in this study. Trees were four years old and located at the Michigan State University's Northwest Michigan Horticulture Research Station near Traverse City, Michigan.

2.2 *Plant tissue collection*

Buds were collected from one-year-old shoots of 'Hedelfinger' trees on Gi.6 and Mazzard. Seven buds from each of eight trees on Gi.6 and eight buds from each of seven trees on Mazzard were collected on the following dates and stored at –80° C until RNA was extracted:

17 May 2001
5 June 2001
22 June 2001
4 July 2001
17 July 2001
14 August 2001

'Hedelfinger' buds were collected in mid-March 2002 (these were used to prime for the conserved regions of LFY)

Young leaves, sepals, petals, and floral buds were collected in spring 2002 for use as controls.

2.3 *RNA extraction*

Total RNA was isolated from 'Hedelfinger' buds and leaves using a method based on hot borate and proteinase K adapted from Hunter and Reid (2001). Approximately 1 gram of frozen tissue was ground into a fine powder. Crushed tissue was added to hot borate buffer (0.2 M sodium borate decahydrate, 30 mM EGTA, 1% (w/v) SDS, 1% (w/v) sodium deoxycholate, at 80° C) and vortexed for 30 s in a 14 mL Falcon 2059 tube. Proteinase K (37.5 µL of 0.75mg per 5 mL) was added to the tube and incubated horizontally on a shaking air incubator for 1.5 h at 42° C. 0.08 volumes of 2 M KCl was added and tubes were incubated horizontally on ice with shaking for 30 min. Tubes were centrifuged at 12,000 rpm for 20 min at 4° C, and supernatant was decanted into new sterile 2059 Falcon tubes. One volume of 4 M LiCl was added and tubes were incubated overnight at 4° C. RNA pellet was precipitated by centrifuging at 12,000 rpm for 30 min. at 4° C. Pellet was resuspended in 630µL H_2O/70 µL of 3M sodium acetate, precipitated with 1 volume of isopropanol, and pelleted by centrifugation at 16,000 x g for 30 min at 4°C. RNA pellet was washed with 70% ethanol, resuspended in 100 µL double-distilled RNase-free H_2O, and stored at -20°C. One gram of frozen tissue produced 25.4 µg of RNA at 25.4 µg/µL.

2.4 *RT-PCR*

First strand cDNA synthesis of total RNA from 1 gram of bud samples (*2.2*) was performed using SUPERSCRIPT™ Reverse Transcriptase according to protocol (). RNase was added to resultant cDNA mix to a final concentration of 10 ng/ µL, and solution was incubated at 37° C for 10 min. cDNA was purified using QIAquick® PCR Purification Kit (Qiagen, 2001), and eluted in 40 µL buffer EB(10mM Tris-Cl, pH 8.5). This was used as a template to amplify the conserved 763 bp fragment of Arabidpsis thaliana LFY homologue cDNA. Primer sequences were: 5'- ATG AAR

GAY GAD GAR MTY GAN GA -3' and 5'- BCA RAG CTG RCG NAR YTT NGT KGG MAC RTA CCA AAT -3'.

PCR protocol: 0.1 µL cDNA, 0.5 dNTPs, 0.5 µL pol, 2.5 µL buffer, and H_2O to 25µL was gently mixed and PCR was done as follows: 2 min at 94° C, 25 x (30 sec at 94° C; then 2 min 30 sec at 68° C), 2 min 30 sec at 68° C. These were electophoresed through 2% agarose.

2.5 Southern analysis

A southern analysis was performed according to a protocol based on Church and Gilbert (1984). The gel (see 2.4) was blotted onto a nylon membrane. Figure A-1 shows, with an arrow, the 923 bp fragment. Arabidopsis LFY probe was denatured, labeled with radioactive phosphorous, and hybridized to blots overnight (> 8h) at 55°C in hybridization buffer (1mM EDTA pH 8.0, 250 mM $Na_2HPO_4(7H_2O)$, 7%SDS, adjusted to pH 7.4 with H_3PO_4). Blots were rinsed twice in the wash solution (1mM EDTA pH 8.0, 40 mM $NaHPO_4$, 1% SDS) for 30 min at 55°C.

2.6 Ligations

The 923 bp band was extracted from the 2% agarose gel using QIAquick® Gel Extraction Kit (Qiagen, 2001), and eluted in 50 µL Buffer EB. Ligations were done using the pGEM-T and easy vectors and the 2X Rapid Ligation Buffer (in each tube, mix 5 µL 2X Rapid Ligation Buffer, 1 µL pGEMT Easy Vector, 1 µL T4 DNA Ligase, deionized water to a final volume of 10 µL, and either 3µL of the 923 bp band, or the positive control, or the negative control. Tubes were incubated overnight at 4° C). 3 µL of ligations were mixed with 1 mL LB and competent cells, and then they were zapped with voltage. Cells were grown for 1 hour at 37°C on a shaker. These were then centrifuged in 1 mL tubes a few times. Supernatant was taken off, and ~ 100 µL of solution plus cells was left. The cells were resuspended with a pipette, and spread on a LB/carbenicillan/X-Gal plates. Plates were stored at 37°C upside down.

Two white patches were collected from the plate. These 2 single well-isolated colonies were collected into tubes with 3 mL LB culture medium and 3µL carbenicillin 100. These tubes were incubated overnight at 37°C in a shaking incubator. A cleared lysate was produced from these colonies, and DNA was isolated and purified according to Promega.

DNA was digested with EcoRI enzyme (200 ng DNA, 2 µL EcoRI 10X buffer, 0.25 µL EcoRI, 1 µL BSA, H_2O to 20µL at 37°C for 1 hour). These were electrophoresced through 2% agarose.

Sequencing was done at the Genomics Technology Support Facility (Michigan State University; http://www.genomics.msu.edu).

2.7 Northern Analysis

A northern analysis was performed according to a protocol based on Church and Gilbert (1984). The gel was blotted onto a nylon membrane. The sequenced 923 bp region was used as a probe, and was denatured, labeled with radioactive phosphorous, and hybridized to blots overnight (> 8h) at 55°C in hybridization buffer (1mM EDTA pH 8.0, 250 mM $Na_2HPO_4(7H_2O)$, 7%SDS, adjusted to pH 7.4 with H_3PO_4). Blots were rinsed twice in the wash solution (1mM EDTA pH 8.0, 40 mM $NaHPO_4$, 1% SDS) for 30 min at 55°C.

3.1 Results

3.1 Identification of a LFY homolog

Primers for LFY were made in areas that were well-conserved in many species and used to prime sweet cherry cDNA. Table B-1 shows sequence obtained that has 86% identity with *Malus x domestica* AFL2, the apple LFY homolog.

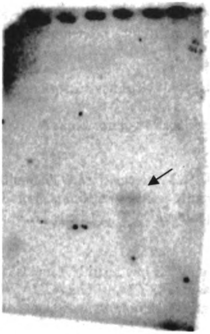
Table B-1: Sequence of 923 bp region (see section 2-6 and 2-7) used as a probe for the northern analysis. Sequence shows 86% identity with *Malus x domestica* AFL2 mRNA.

Sequence:

gacatgatgagtagcctctctcagatattcaggtgggatttgcttgtgggtgagaggtacggtatcaaagcggccgtcagagcag
agcgtcgccgcctcgatgaccaggactcgaggcgccgcccc...gtctccggcgacaccaccaccaatgccctagatgctctc
tcccaagaagggttgtcagaggagccggtgcaacaagagaaggagatggtggggaccggcggaggggccgcgtgggaag
tggtggcgtctgcaggggagaagcggaagaagcagcgaaggacgaaaaatgggcaatataggaattttaatggcatcggaag
ggggcataataataatgatcataatgagggtgtggacgacgaggacgacaacgacatggacgatatgaatgggcacgggaac
ggtggaggagggggggttgccgagcgagagagtgagggagcacccgttcattgtgactgatcctgaggaggtggcacgtggc
aaaaagaacggcctagattacctcttccatctctacgagcagtgccgtgatttcttgatccaggtccaaaacattgcagaggagcg
cggtgaaaaatgtccaaccgaggtaacaaaccaagtgtgtatgtttgccaaaaaggcanggggcagctacatcaacaagccaa
aaatgcgacacta

Figure B-1: Southern blot (see section *2-5*). Arrow shows the 763 bp fragment.

Figure B-2: Northern Analysis (see section *2-7*) of bud samples collected during 2002 (see section *2-2*). Arrow shows the Gisela 6, August 14th 923 bp band, which was the only one observed in the northern analysis.



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