AN ABSTRACT OF THE THESIS OF

Gordon Arthur Stevens, Jr. for the degree of Doctor of Philosophy

in Horticulture presented on February 24, 1983

Title: Hormonal Regulation of Fruit Set of Sweet Cherry (Prunus avium L.)

Abstract approved: _____
Dr. Melvin N. Westwood

Exogenous growth regulators and endogenous cytokinins produced by rootstocks were investigated with regard to their effects on fruit set of sweet cherry.

Exogenous applications of the cytokinin N_6 benzyladenine (N_6BA), the auxins 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and gibberellic acid (GA_3) increased fruit set with sweet cherry, even in the absence of normal embryo development. The phenoxy auxins, alone or combined with GA_3 were most effective. Naphthaleneacetic acid (NAA) reduced final fruit set. 2,4-D enhanced maturity of the fruit, while treatments containing GA_3 retarded maturity, induced fruit cracking and inhibited flowering and fruit set the following year. N_6BA appeared to counteract, in part, the inhibitory effects of GA_3 on flower initiation.

All treatments caused some leaf epinasty, with the GA_3 containing treatments most severe. GA_3 also enhanced shoot elongation of the current season's growth.

Two cytokinin-like substances believed to be zeatin and zeatin riboside were detected in the xylem sap of sweet cherry scion branches on trees grafted to different rootstocks. The greatest cytokinin activity occurred during bloom and decreased rapidly throughout the fruit development period. The higher yielding and fruit setting rootstocks produced higher levels of cytokinin in the xylem sap of scion branches at each date sampled.

The strongest correlations between the effects of rootstock on xylem sap cytokinin level and yield data were with fruit retention between first and final set and with yield efficiency. The predominant activity of the cytokinin translocated from the rootstocks appeared to be through prevention of senescence and abscission of fruit between early fruit set and harvest.

Hormonal Regulation of Fruit Set of Sweet Cherry (*Prunus avium* L.)

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed February 24, 1983

Commencement June 1983

APPROVED:

Professor of Horticulture in charge of major

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Date thesis is presented February 24, 1983

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DEDICATION

This dissertation is dedicated to my wife

Akiko Hayashi Stevens

A woman of grace, intelligence and caring, she has provided me with immeasurable support throughout this research program—financially, intellectually and, above all, emotionally. Words are so inadequate to express my appreciation to her.

ACKNOWLEDGMENTS

The author wishes to express his sincere appreciation to Dr. Melvin N. Westwood for guidance during this program. Appreciation is also extended to the Department of Horticulture for materials and space provided in the interests of this research. The author also wishes to express his appreciation to Dr. Rod V. Frakes and Dr. Kenneth Rowe for their assistance with statistical procedures, and Dr. Roy O. Morris and Dr. Joe B. Zaerr for their assistance, and access to laboratory facilities. Finally, the author acknowledges the support of Joy Stevens and Akiko Stevens.

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PREFACE

This dissertation is presented in two papers written in the format required by the Journal of the American Society for Horticultural Science.

Hormonal Regulation of Fruit Set of Sweet Cherry (*Prunus avium* L.)

INTRODUCTION

Fruit set and development on trees in orchard systems involve various genetic and physiological phenomena, and are controlled by a number of endogenous and external factors. Final fruit set, the number of fruit reaching harvestable maturity, is dependent upon the number of flower buds that develop per tree, the number of flowers fertilized, initial fruit drop, and pre-harvest fruit drop. Important factors affecting these processes are mineral nutrition, the rootstock-scion combination, pollination, certain environmental factors such as temperature and available moisture, and the balance of hormones in the tree. This hormone balance is of great importance in regulating fruit set and development, and is affected in turn by the other factors mentioned above.

Fruit set in *Prunus*, including sweet cherry, may be low due to environmental factors affecting pollination and fertilization. It has been found that poor fruit setting in 'Italian' prune is often attributed to cool weather during and following bloom which causes delayed pollen tube growth sufficiently long that ovule degeneration occurs and even in the event of fertilization fruit set is not promoted [181]. It is desirable to develop treatments effective in alleviating this reduction in fruit set.

Exogenous hormones and growth regulators have been shown to

increase fruit set in woody plants [43, 149, 192, 193], and in some cases parthenocarpy [39, 41, 43, 96, 146, 149]. The phenoxy auxins are particularly effective at promoting parthenocarpic fruit set in Prunus, alone or in combination with other hormones [41, 146]. Gibberellin (GA) has been shown to induce parthenocarpic set in Prunus [43] and this effect may be enhanced when phenoxy auxins are applied with GA [41, 146]. Although most studies involving exogenous gibberellins have been done using GA_3 [41, 42, 43], fruit of Prunus appear to contain a number of GA's including GA_3 and GA_{29} [85, 130, 150]. Exogenous cytokinins can be effective at promoting fruit set [192, 193] and parthenocarpy [39, 96]. The cytokinins most often used in exogenous growth regulator experiments are benzyladenine (BA), and 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA) [86, 190, 192, 193]. PBA is generally more active in higher plants than BA largely as a result of its greater solubility, penetration into plant tissues, and mobility within the plant [190, 193]. The principal naturally occurring auxin in higher plants is indoleacetic acid (IAA). Because this hormone is easily degraded by light and IAA oxidase, synthetic auxins have been developed which are more stable [121, 190]. Applications of auxins or cytokinins have been found to increase fruit growth [38, 40, 191, 193] and auxins often hasten maturity and stimulate color development. Exogenous gibberellins appear to inhibit flowering [35].

Rootstock affects scion physiology and development of Prunus

in many ways including vigor [198], leaf element content [30], fruit set [67], flowering and yield [197] and flower morphology [66].

Endogenous hormones are important regulators of most plant processes including fruit set and development [110, 130, 150, 178]. Cytokinins and cytokinin-like substances have been detected in fruit extracts [14, 50, 145, 178] and appear to play vital roles in fruit development [145].

Cytokinins have been shown to be produced in roots [60, 169], transported in the transpiration stream [125], and have been detected in the xylem sap of woody plants [114, 132, 170]. Cytokinin level in the xylem sap of scion branches appears to vary with rootstock [170] and this could have a significant effect on fruit set.

The research reported in this dissertation involves two aspects of the hormonal regulation of fruit set in sweet cherry.

The objectives of the exogenous growth regulator experiments were to determine the effects of various growth regulator treatments on fruit set, yield, fruit quality, return bloom and set, and vegetative growth of sweet cherry. The long-range objectives of these experiments were to provide information leading to the development of treatments that would enhance fruit set during years when fruit set is low, without causing adverse effects on fruit quality, return bloom and set or vegetative development.

The objectives of the xylem sap cytokinin experiments were to determine the effects of rootstock on endogenous cytokinin production, and the relationship between this phenomenon and the effects of rootstock on fruit setting. This would provide a means of determining, in part, the biochemical basis of rootstock effects on scion development. Studies of this nature are also valuable as a means of rootstock evaluation. Evaluating rootstocks exclusively by means of yield data is slow and laborious. Considerable time could be saved by use of rapid biochemical screening techniques.

REVIEW OF LITERATURE

Factors Affecting Fruit Set

Mineral Nutrition

Applications of magnesium and potassium to 'Elberta' peach trees [44] increased both the number of fruit harvested and the size of the fruit. No yield increases were obtained with the application of either element during the first five years after treatments were initiated, but thereafter the effects appeared to be accumulative. Kwong [97] found that nitrogen in the form of ammonium nitrate alone or combined with potassium sulfate significantly increased yields of 10-year-old 'Stanley' prunes. Increased potassium in the leaves did not affect yield unless leaf nitrogen was also increased. High rates of nitrogen with the addition of potassium sustained better yields under weather conditions unfavorable to fruit setting. Crotonylidene-di-urea fertilization [184] was found to increase the yield of 'Hakuto' white peach, however the increased yield was due to increased fruit size not set. Phosphorus applications of 300 to 600 g per tree to peach under orchard conditions [177] resulted in a positive fruit set and yield response, when compared to an untreated control. Prunes, when given a low regime of calcium (2 ppm) while under greenhouse sand culture [1] had most fruit drop after fruit set and the few that remained were smaller and misshapen.

In the same experiment, peach fruits were smaller, greener and firmer and had less soluble solids and red blush at 2 and 90 ppm calcium than at higher concentrations. Boron shortage or excess was found [89] to cause reduced pollen viability, fewer flower buds, reduced fruit set, and poor fruit quality.

Boron, when added to the pollen germination medium, almost doubled or more than doubled the germination percentage, while the length of the pollen tube was increased 2- to 10-fold depending upon the species [180]. The species studied were plum, peach, apricot, cherry, pear and apple. Manganese chelate when added to an agar medium increased pollen germination of 'Parson' prune with manganese concentrations less than 600 ppm [204]. Pollen tubes from control treatments burst at the apex, while tubes in the medium containing manganese did not burst. The treated pollen tubes were smooth, straight, hyaline, and longer than controls.

Sodium ferric ethylenediamine di-(O-hydroxyphenylacetate) (FeEDDHA) applied alone or in combination with disodium manganous ethylendiamine tetraacetate (MnEDTA) [155] significantly increased yields of iron-chlorotic 'Elberta' peach trees. The addition of the MnEDTA to the FeEDDHA had no significant effect on fruit size, but did increase the yield in Kg fruit per tree. These results indicate that increased yields were not due entirely to increased fruit size, but perhaps to final fruit set.

Mineral elements have been found to affect the uptake or metabolism of other elements [7, 45, 152, 153, 154, 155]. This

phenomenon is affected by rootstock [30]. The application of ammonium sulfate with FeEDDHA significantly depressed the uptake of manganese in 'Sungold' peach trees [153]. In a five-year study with 'Elberta' peach trees growing in a lakeland sand [45], potassium application to the soil increased the level of potassium in the foliage, and decreased the level of other cations. Magnesium application also increased magnesium levels in the leaves. Magnesium fertilization had little effect upon the availability of soil potassium, but potassium application had a greater effect on soil magnesium levels than did magnesium application. In an experiment with 'July Elberta' peach [154], FeEDDHA application depressed the concentration of manganese, zinc, phosphorus, potassium and nitrogen in the leaves, and reduced iron chlorosis. With 'Bing' cherry [7] results of sand culture and field experiments indicate that there is a one-sided effect of potassium on magnesium deficiency. Potassium application increased the severity of magnesium deficiency symptoms.

Levels of mineral elements vary in plant tissues, are higher in some tissues than others, and vary in quantity throughout the year. Boron content of the flower buds of sweet cherry 'Napoleon' [202] was found to reflect the amount in the soil. Total boron increased gradually as the bud enlarged and then rapidly as the flower reached full bloom. In many parts of the flower, particularly the sepals, anthers, styles and receptacles, the boron level was high. These blossom parts contained more boron during the bloom period than did the leaves and pedicels. The distribution

of mineral elements was found to vary widely in different tissues of 'Elberta' peach [46]. Levels of nitrogen, phosphorus, potassium and zinc were similar in both the fruit and foliage, while copper, iron, manganese, magnesium and calcium were much lower in the fruit than in the foliage. Concentrations of calcium and zinc in the twigs were similar to those in the foliage. Other elements were found to be lower in the twigs than in the foliage. The tissues with the lowest levels of all elements, except calcium, were the branches and trunk. With studies involving the seasonal fluctuations in a number of important nutrient elements in leaves and fruit of 'Elberta' peach [9], calcium and magnesium accumulated in the leaves throughout the cropping season, while nitrogen, phosphorus, and potassium declined. The fruit flesh showed a continual seasonal movement of elements into the tissue, and nitrogen, phosphorus, potassium and boron in the fruit followed closely the physiological changes in the developing fruit. Calcium and magnesium were found to decline in percentage in the fruit throughout the season.

The level of carbohydrates and certain mineral elements have been shown to vary between bearing and non-bearing fruit trees [53, 158]. With samples taken from bearing and non-bearing 'Sugar Prune' trees [53], the level of reducing substances was found to be greater on a dry weight basis in the bearing rather than the non-bearing trees after May 1. Before May 1, bearing trees showed a higher nitrogen content than the non-bearing, but after May 1,

the non-bearing trees were higher in total nitrogen. In this experiment starch was found to be higher in the non-bearing trees than in the bearing. In another experiment on the carbohydrate and mineral composition of bearing and defruited 'French' prune [158], the non-bearing shoots contained more starch and percent dry matter than the bearing ones during the period when fruits were growing rapidly and accumulating soluble solids. Analysis of the fruits showed that sorbitol was the predominant carbohydrate, followed by sucrose and reducing sugars during the ripening period.

Certain mineral elements are essential for the synthesis of plant hormones. In the synthesis of gibberellins, reactions from melvalonic acid to kaurene are catalyzed by enzymes which are dependent upon a divalent cation as well as ATP. In most cases Mg^{++} is the most effective cation, but sometimes Mn^{++} is most effective [121]. Manganese also appears to be an enzymatic cofactor in the synthesis of ethylene from 1-aminocyclopropane-1-carboxylic acid [95]. Nitrogen appears to be important in the synthesis of cytokinins [48, 169]. Reduced quantities of cytokinins were found in xylem sap and root extracts of sunflower when grown in a medium containing low levels of nitrogen [169]. Darrall and Wareing [48] found low levels of cytokinin-like compounds in leaves of Betula pendula Roth. receiving a low supply of nitrogen. Sycamore seedlings growing in a liquid culture containing deficient levels of phosphorus produced low levels of cytokinins in their xylem sap [56].

Some mineral elements appear to suppress the occurrence of growth inhibitors. Increased supply of potassium to water stressed wheat plants delayed the occurrence of abscisic acid (ABA) in the seed, and reduced its absolute value [69].

A relationship between mineral nutrition, stress and hormones is indicated by increased sensitivity of nitrogen deficient cotton plants to water stress [148]. This increased sensitivity had tow components; increased accumulation of ABA and increased stomatal sensitivity to ABA.

Rootstock

Rootstock has been shown [67] to affect fruit set in 'Italian' prune. Wangenheim prune seedlings, Brompton, Broad-leaved Mussel and Prune 72 clonal rootstocks had a strong positive effect on fruit set in 'Italian' prune, whereas Ackermann, Hunter IV, Marianna, Spilong Brocking and Damascena rootstocks had a negative effect. Tests with 'Italian,' 'Early Italian,' and 'Brooks' prunes [197] showed influences of rootstocks on a number of parameters including flowering and yield. In this study Myrobalan, Marianna, and several Prunus domestica L. roots resulted in heavier bloom, and bloom density than did peach root. The peach rootstock, however, resulted in greater yield efficiency than Myrobalan.

Rootstocks have been shown [66] to influence flower morphology which appears to affect fruit set of 'Italian' prune. The

investigators found that the pistils and stamens of own-rooted trees were of equal length. Four rootstocks were found to cause a slight shortening of the pistils relative to the stamens. Fruit set was higher with trees having flowers with pistils shorter than or equal to the length of the stamens. Hand-pollination alleviated these differences.

Rootstocks have been found [30] to affect the leaf element content, and the efficiency of uptake of certain elements with 'Italian' prune. Trees on plum roots had higher levels of nitrogen, potassium, manganese and zinc in the leaves, and less boron and magnesium than trees on peach root. Plum clones, St. Julien A, Myrobalan 29-C and Myrobalan B were found to be more efficient in the uptake of calcium. There were negative correlations between certain elements with regard to uptake by different rootstocks. With most rootstocks positive correlations existed between nitrogen and calcium, nitrogen and magnesium, nitrogen and zinc, calcium and magnesium, calcium and boron and magnesium and boron.

When examined for phenolic composition [203], two ungrafted sweet cherry rootstocks, Mazzard (Prunus avium) and Prunus mahaleb differed in three phenolic groups; phenolic acids, coumarins, and flavonoids. Mazzard contained five acids; p-coumaric, o-coumaric, caffeic, p-coumarylquinic and chlorogenic, whereas Mahaleb contained mostly o-coumaric acid. Three flavenoids, dihydrowogonin, kaempherol and quercetin, were found in Mazzard,

while Mahaleb contained only kaempherol. Mahaleb contained considerable amounts of coumarin and herniarin, which were absent in Mazzard.

With tests conducted at several orchards comparing Mahaleb and Mazzard rootstocks for cherry [5], Mazzard proved to be the better of the two in general. Although Mahaleb often induced earlier and heavier bearing than Mazzard, Mazzard was usually more vigorous and survived better than Mahaleb. In another study [198] comparing Mahaleb, Mazzard and certain hybrid rootstocks for 'Montmorency' cherry, trees on F12/1 Mazzard roots were more vigorous and less productive than those on other stocks. With regard to yield efficiency P. mahaleb PI 163091 was superior, with most of the P. mahaleb introductions having higher yield efficiencies than the Mahaleb X Mazzard hybrids. Both Mahaleb and the hybrid stocks outyielded the Mazzard stocks. Stockton Morello has been reported to be a dwarfing rootstock for sweet cherry [196]. This stock has been shown to yield more efficiently during the first eight years than standard stocks [33].

Rootstock has been shown to influence the level of endogenous hormones in grafted systems. Skene and Antcliff [170] found considerable differences in the cytokinin content of xylem sap from grapevine rootstocks. The rootstocks with higher levels of cytokinin in the xylem sap were also higher yielding due to a greater number of berries per bunch. Grapevine rootstocks were also shown to differ regarding ABA level [160].

Pollination and Fertilization

Without pollination and fertilization, the Prunus fail to set fruit [183]. These fruit species produce many more flowers than will set fruit which grow to maturity. Kester and Griggs [91] noted that almond will set fruit from 25 to 40 percent of the initial number of flowers under favorable conditions, and that there is a significant positive correlation between the number of flowers cross-pollinated and the final fruit set. In another study by the same investigators [92], three periods of fruit drop were found with almond. The first drop took place during bloom or shortly thereafter and consisted largely of defective flowers. The second drop took place about a month after bloom and consisted of flowers with pea-sized ovaries which had not shed their floral tubes. Flowers that were not cross-pollinated, or those that were cross-pollinated but not fertilized, accounted for most of this drop. The third drop took place six to seven weeks after bloom and consisted of larger fruits that had shed their floral tubes. This was considered to be "June drop," the cause being one or more of a number of factors such as a shortage of hormones to oppose abscission.

In some cases, both cross-incompatibility and pollen tube growth appear to be regulated by hormones. It was found that the length, dry weight, rates of cell division, and average cell volume of embryos from the interspecific cross Phaseolus vulgaris

L. X Phaseolus acutifolius A. Gr. were considerably lower than those of the self-pollinated female parent during the first 22 days after pollination [131]. At this stage hybrid ovules showed signs of abortion. Ovules of selfed P. vulgaris contained appreciable quantities of cytokinin-like substances, while levels of cytokinin-like substances in the hybrid ovules were considerably lower. Pollen grains of lilly washed in distilled water produced shorter pollen tubes than unwashed pollen grains, and the addition of indoleacetic acid (IAA) and gibberellin (GA) increased pollen tube elongation of the washed pollen grains [159]. A mixture of both hormones increased pollen tube elongation more than did either hormone individually.

It has been found [58] that delayed development of the megagametophyte and irregularities in its development are not numerous enough to alone explain reduced fruit set in sweet cherry. It was found, however, that low temperatures inhibit the development of the megagametophyte. The primary cause of unsatisfactory fruit set was found to be due largely to the degeneration of egg cells increasing after anthesis. Within two days of anthesis [58], the proportion of functional embryo sacs was shown to rapidly decrease. In an experiment [58] in which flowers were pollinated at intervals following anthesis, pollination one day after anthesis was much more effective in accomplishing fertilization than was pollination two days after anthesis.

Tukey [183] found that the fruit of cherry and peach develop

in three clearly marked stages: Stage I, rapid increase of the pericarp from about the time of fertilization to mid-season; Stage II, retarded increase during mid-season; and Stage III, second rapid increase to fruit maturity. Destruction of the embryo early in Stage II resulted in a termination of fruit development, shrivelling and abscission. Wounding of the fleshy and stony percarp, without injuring the seed, did not alter the growth rate of the fruit. These results indicate that abortion of the embryo is a factor which affects pericarp development.

There appears to be a relationship between endogenous cytokinin and embryo development [131, 145], and embryo abortion may be due, at least in part, to sub-optimal levels of growth promoting hormones.

Ethylene at low concentrations (.01 to 10 ppm) stimulated pollen germination and pollen tube growth, but high concentrations (100 to 1000 ppm) were found to inhibit both processes [25]. Auxin appears to be involved in the synthesis of ethylene and may, in fact, induce ethylene production or evolution in plant tissues [121]. It is possible that endogenous ethylene produced through the action of auxin stimulates pollen tube growth or pollen germination.

Certain mineral elements such as boron and manganese [180, 204] have also been found to increase pollen germination when added to the germinating medium.

Hormones appear to play essential roles in fertilization and

embryo development [103, 115, 126, 145, 159, 173]. Powell and Pratt [145] detected cytokinin activity in the endosperm and embryo of developing fruits of peach, and suggested that cytokinins are necessary for normal development of the ovule. Smith and van Staden found a close relationship between endogenous cytokinins of the endosperm of Zea mays kernels and embryo development [173]. Removal of the endosperm resulted in fewer embryos developing, and a decrease in the growth rate of the embryos which appeared to be dependent upon the endosperm for their endogenous cytokinins. This view was supported by the fact that applied zeatin or a cytokinin glucoside could partly substitute for the endosperm. Growth of Phaseolus coccineus embryos at the heart-shaped and middle cotyledonary stages was greatly promoted by both zeatin and zeatin riboside [12]. Gibberellin A_1 was identified in suspensors of heart-shaped embryos of Phaseolus coccineus [3].

Auxins are also involved in pollination and fertilization, and auxins are present in large quantities in the pollen of many species [103]. Muir [126] found a sudden increase in auxin in the ovary of tobacco flowers within two days of pollination, while unpollinated ovaries remained low in auxin. He concluded that auxin in the ovary was produced as a result of the pollen stimulus. Lund [115] also using tobacco showed that 20 hours after pollination, auxin synthesis occurred primarily in the tip of the style, and gradually moved downward until the pollen reached the ovary,

after which the ovary base was the site of most auxin synthesis.

The Environment

Environmental factors such as temperature and available moisture affect fruit set and development in a number of ways. Correlations between the average flower bud drop of a number of peach varieties, and fall and winter temperatures have been found to be significant [23, 194]. Results of experiments with 14 varieties of peach [23] indicate that above-average temperatures in late September and early October may cause the breakdown which results in bud drop. Late October and November temperatures were not found to be as important. December and January temperatures were found to be just as important in relation to bud drop as those in early fall. In December, the minimum temperatures were more highly correlated with bud drop than were the maximums, while the reverse was true in January. In another experiment [194], subjecting buds to higher maximum temperatures, higher mean temperatures, and higher humidity failed to induce bud drop. However, raising minimum temperatures of 'Redglobe' peach buds to 7.2°C during the winter increased bud mortality, indicating that high minimum temperatures during December and January are a principal cause of flower bud drop in peach. Dormancy is brought about in at least some species by high ABA and low GA levels, and the emergence from dormancy by high GA and low ABA levels [121]. GA has been shown to substitute for the chilling

requirements for flowering of carrot [99]. Bud drop following warm winters may be due partly to inadequate chilling and high levels of ABA relative to GA.

The effects of temperature during flowering and the early stages of fruit development are also important [174, 181]. It has been found that the cause of poor fruit setting in 'Italian' prune in some years [181] is attributed to its genetically determined sensitivity to cool weather following bloom. The cool temperature was shown to delay pollen tube growth, fertilization, and early embryo and endosperm development, sufficiently long that the ovule begins to degenerate. This degeneration of the ovule begins in the nucellus at the chalazal end, such that even in the event of fertilization, fruit set is not promoted. Optimum temperatures for pollen tube growth in some almond cultivars was found to vary somewhat depending upon genotype [174].

In studying the effect of water regime on the morphological differentiation of the flower buds of apricot [2], sixteen irrigations at 450, 500 or 600 m^3/ha were applied between May and October. Flower bud differentiation was delayed by the heaviest treatment, but the density of flower buds and flowers, fruit set, and fruit yield per tree were highest with this treatment. Prolonged periods of dry-soil conditions during July, August, and September [21, 22] affected 'Royal' apricot trees by limiting the number of flower buds differentiated, delaying the time of differentiation of many of the flower buds, and by slowing the rate of

development of buds which were differentiated at about the normal time. These results were found after both mild and cooler winter seasons.

Hormones, and specifically the balance of promoters and inhibitors, are important biochemical determinants of plant responses to environmental and stress factors. Cytokinins have been shown to enhance flower development [127, 145] and inflorescence growth [127, 128]. Cytokinin synthesis appears to be reduced under stress. Root exudate obtained from water stressed sunflower plants [84] contained significantly less cytokinin activity than exudate of control plants. A root temperature of 20°C resulted in greater cytokinin production with grape than did 30°C [171]. Growth inhibitors such as ABA and ethylene appear to increase under unfavorable environmental conditions. Red pine and paper birch seedlings exposed to sulfur dioxide exhibited an increased production of ethylene [93]. ABA was shown to increase with water stress in rice seedlings [73]. ABA was shown to increase in Euphorbia lathyrus L. in response to either waterlogging or deficit, and endogenous ethylene increased in response to waterlogging [165].

With cotton, water deficit and fruit load were investigated in relation to endogenous ABA level in the fruit [68]. Both ABA concentration and the rate of fruit abscission increased with water deficit, and decreased with relief of stress by irrigation. Water deficit appeared to have a greater effect than crop load on ABA

content of the fruit.

It appears that environmental factors affect endogenous hormone levels, and the hormone balance, and that this affects a number of plant processes including fruit set.

Cultural Practices

Pruning date appears to affect the flowering density, fruit set, fruit size and yield of peaches [47]. A significant reduction in the number of blooms occurred from May and July pruning as a result of excessive vegetative growth, when trees were pruned at this time in Georgia [47]. Winter pruning resulted in increased cold injury to blossoms which resulted in a reduction in fruit set. A high fruit set was obtained from some summer and early fall pruning treatments. In another experiment dealing with the interaction between pruning severity, soil nitrogen application, and irrigation on peach fruiting in North Carolina [8], light pruning, moderate to high nitrogen levels, and irrigation resulted in a greater number of fruit developing per tree, and in larger fruit, than did more severe pruning, low nitrogen levels and no irrigation.

The application of (2-chlorethyl) phosphonic acid (ethephon) to sweet cherry, plum, and peach [55] at rates of 250 and 500 ppm in September or October delayed bloom and thereby reduced spring freeze injury. These beneficial effects were offset, however, by such deleterious effects as bud abscission or failure to open, reduced fruit set, and gummosis.

Wound healing following pruning was shown to be enhanced by applications of IAA, indolebutyric acid (IBA), benzyladenine (BA) and GA [13].

Because pruning is a dwarfing process that reduces the potential bearing surface, it is logical to assume that hormones such as cytokinins that are produced in roots [60, 169] would be increased relative to the bearing surface of the tree. This would provide a greater quantity of cytokinin for each developing fruit.

Hormones and Growth Regulators

Exogenous hormone applications may enhance either natural or parthenocarpic set or fruit retention. Aqueous solutions of GA, GA + 2,4-dichlorophenoxyacetyl methionine (2,4-DM) and GA + IBA were applied as sprays to 'Bing' cherry in an attempt to promote parthenocarpy [149]. Pollination was prevented either by emasculation or by enclosing the blossoms in muslin bags. Only treatments involving the combination of GA + 2,4-DM were effective in promoting parthenocarpic set and development to maturity. The ultimate growth curve and size and shape of the parthenocarpic fruit were similar to those of pollinated fruits. All treatments containing GA alone or in combination with 2,4-DM caused inhibition of both flower and vegetative bud development on current seasons growth. In another experiment by Crane et al. [43], using 'J. H. Hale' peach, aqueous sprays of GA from 250 to 1000 ppm applied to emasculated blossoms promoted parthenocarpic fruit set.

The sets were significantly greater than that resulting from pollination. Although the parthenocarpic fruits were smaller in diameter than pollinated ones, they were longer, contained longer endocarps, and matured a week or more earlier. In further studies on growth regulator induced parthenocarpy in 'Bing' cherry, Crane and Hicks [41] found that GA in combination with a number of auxins, naphthaleneacetic acetic acid (NAA), 2,4-DM, 4-chlorophenoxyacetic acid (PCPA) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were effective in promoting parthenocarpic set. All treatments, however, stimulated the spurs to form long shoots that were devoid of flower buds the following year. Primer and Crane [146] promoted parthenocarpic fruit sets of 5 to 15 percent in three varieties of apricots by spraying weekly with 2,4,5-T at concentrations of 7.5 to 50 ppm. Parthenocarpic fruits were only 40 percent as large as fertilized fruits.

Crane [38] found that applications of the tri-ethyl amine salt of 2,4,5-T at concentrations of 25 and 50 ppm to 'Tilton' apricot at the initiation of pit hardening, reduced preharvest fruit drop to 6.6 and 6.4 percent of the total crop, respectively, as compared to 52.9 percent drop from unsprayed trees. In addition, the spray treatments increased the rate of fruit growth and size. The 'Stewart' apricot, characterized by fruit drop resulted from seed abortion, responded to applications of 2,4,5-T at 25, 50 and 100 ppm at the initiation of pit hardening which resulted in reduced fruit drop. Post-bloom application of gibberellic acid

(GA_3) to open pollinated peach trees prevented seed development in some fruit, and also resulted in the persistence and maturation of both seeded and seedless fruit on the same trees [176]. NAA and naphthaleneacetamide (NAAM) were shown [76] to be somewhat effective in controlling fruit drop of a number of stone fruits.

A reduction in fruit set and final yield may occur if frost injury is severe. An increase in resistance of 'Royal' apricot fruit to low temperature injury was obtained [37] by a spray application of 2,4,5-T about 15 hours before a frost that occurred approximately 40 days after full bloom. In addition, the sprayed trees dropped 83.9 percent less fruit that were injured by frost than did the unsprayed trees. It was also noted during the same experiment that spraying 2,4,5-T to severely frosted 'Tilton' apricot fruit, in which the ovules were killed, induced the fruit to grow to normal size when sprayed two days after the frost occurred.

Spray applications of 2,4,5-T at concentrations ranging from 25 to 1000 ppm markedly increased the growth rate of apricots, which, as a consequence, matured a maximum of 18 days earlier and were a maximum of 10.5 percent larger in diameter than unsprayed fruits [40]. The chemicals, to be effective, were applied at the beginning of pit hardening. It was also shown later [36] that spray applications of the trialkylamine and ammonium salts of 2,4,5-T, to 'Stewart,' 'Derby' and 'Royal' cultivars of apricot resulted in increased fruit size, hastened maturity, and stimulated red color

development. GA when applied to peach trees in August [35] at 200 ppm resulted in flower thinning, bloom delay, and increased hardiness near bloom. A morphological study of buds after treatment indicated the GA delayed flower initiation, delayed development after initiation, resulted in smaller buds, and delayed microsporogenesis. GA at 500 ppm applied twice, once at full bloom and again eight days later, brought about parthenocarpic sets in almond, apricot, and peach, but failed to induce parthenocarpy in cherry or plum [42]. The GA treatment also caused an inhibition of flower bud differentiation and retarded vegetative bud development in the leaf axils of current seasons shoots. GA application resulted in the complete abscence of flower buds on spurs of the almond, apricot, cherry and plum. Buds of peach, however, appeared unaffected.

The promotion of vegetative growth of 'Redhaven' and 'Golden Jubilee' peach trees with potassium gibberellate (KGA) [59] was associated with sharply reduced numbers of flower buds, delayed differentiation and smaller buds during the dormant season. Cold hardiness of the buds was increased. Shoot growth which continued through August at the higher concentrations of KGA was less hardy in fall and during the dormant season than untreated shoots or those receiving the lower concentrations.

Flowers on KGA-treated trees appeared normal the following spring, and a higher percentage set fruit than on unsprayed control limbs. Because of the reduced number of flower buds resulting from

the treatment the previous summer, no additional thinning was needed and fruits were larger at harvest than with hand-thinned trees.

Applications of N-dimethylaminosuccinamic acid (Alar) at 2000 ppm in July resulted in a slight increase in flower bud formation [59].

Williams and Flook [201] found that a hormone mixture containing GA, 1,3-diphenylurea and NAA when applied between full bloom and petal fall to apple increased final fruit set and the number of pollen tubes penetrating to the ovary. Parthenocarpy was not involved as the fruits were seeded.

Benzyladenine (BA) was found to increase fruit set in muskmelon [86]. In this experiment BA was compared with GA and NAAM. The BA treated group as a whole had 37.5 percent fruit set as compared to less than one percent in the other groups, and fruit was set with BA even with fruit already set on plants growing under stress conditions. It was mentioned that BA treatment appeared to prevent senescence and abscission zone formation. Applications of the synthetic cytokinin 6-(benylamino)-9-(2-tetrahydropyranyl)-9H purine (PBA) to poorly setting varieties of grape significantly increased berry set over control clusters [192]. There was also a greater number of seedless berries on treated clusters. In the same experiment, emasculating flowers at bloom reduced fruit set dramatically in untreated control clusters, but had little effect on clusters dipped in the cytokinin at 500 ppm. It was suggested

that cytokinins play a significant role in fruit set and development. In another experiment with grape [193], PBA and BA both greatly increased fruit set and in some instances fruit growth. PBA also resulted in the set of berries on emasculated flowers. PBA was more effective than BA because of its greater solubility. Parthenocarpic fruit set was induced in apple with a mixture of PBA, GA, and NAA when applied at two weeks after petal fall, to flowers that had stamens, styles, petals and the tips of sepals removed by decapitation at "pink bud" stage to prevent pollination [96]. The cytokinin alone did promote parthenocarpic set but the highest set was obtained with the hormone mixture. Cytokinins also appear to affect fruit growth [39, 191]. Solutions of PBA when applied with a wetting agent to grape clusters at four days after bloom resulted in a significant increase in berry size [191]. Crane [39] produced parthenocarpic fruit set in fig by application of cytokinins, auxins or gibberellins. It was mentioned that these hormones appear to be required for fruit growth, and can be supplied by a source other than the seed.

Extensive work has been done to isolate growth substances from fruits to determine the possible effects these substances may have on fruit set and development. Levels of gibberellin (GA)-like substances were determined in sour cherry seed in relation to fruit development [130]. The presence of two GA-like substances coincided with development of the nucellus, endosperm, and embryo, and the early development of the pericarp, but not

with subsequent development of the pericarp. One of the GA-like components resembled GA_3. Reed and Martin [150] established the presence of GA_{29} in the extracts of pericarp of immature fruits of French prune by combined gas chromatography-mass spectrometry. Jackson and Coombe [85] found with apricot fruit sampled at intervals after anthesis that endogenous concentrations of GA-like substances in the seed, endocarp and mesocarp correlates well with growth rates in these tissues between anthesis and maturity, with the greatest GA activity in all parts of the fruit at 20 days after anthesis. The seed had the greatest GA activity and the endocarp the least. Activity in the endocarp declined to a low level concurrently with cessation of growth and sclerification of the tissue, 30 to 45 days after anthesis. Activity in the seed remained high and did not decline until the interval 35 to 60 days after anthesis, during which seed growth rate declined and stopped. The mesocarp, the only tissue which grew after 60 days, contained the highest level of GA at that time. The gibberellins in the apricot seed and pericarp were more polar than GA_3. It was found that with 'Palora' peach [116] abscising fruits at June drop had low levels of a growth promoter, and high levels of an inhibitor, thought to be ABA. ABA was isolated from sour cherry fruit and identified to determine the changes in the level of the inhibitor during fruit development [50]. Inhibitor levels in the seed paralleled those in the pericarp and were, in general, directly related to the growth rate of the fruit. Higher levels

were found during the initial rapid growth (Stage I) than during the retarded growth phase (Stage II), increased in State III, and then decreased in the final stages of maturity.

Cytokinins have been extracted from various fruit tissues from a number of plant species [16, 105, 110, 145, 178, 205]. In an experiment with peach [145] to determine cytokinin activity in the embryo and endosperm of developing fruits, appreciable activity was measured by growth of callus on tobacco pith explants. There was an indication that the endosperm was a richer source of cytokinin than the embryo. The data showed that cytokinins exist in peach seeds for at least three months after fertilization. It was suggested that cytokinins are necessary for normal development of the ovule, and thus play a vital role in the middle as well the early stages of peach fruit development, since the development of the pericarp is dependent on the ovule well into the middle period of fruit development. Letham [105] purified factors inducing cell division in plum fruitlets. Two cell division promoting substances were recovered from the extracts. One of the factors, present only at very low levels, appeared to be zeatin. The second factor was identified as myo-inositol which usually promoted cell division only in the presence of the first factor. Three cytokinins were detected in the extracts of apple fruit bud organs [110]. The activity of fruitlet extracts was slightly greater than the activity of pedicel extracts, and considerably greater then the activities of extracts of other organs. One cytokinin was

identified as either zeatin or a closely related compound. Two other cytokinins exhibited chromatographic behavior of zeatin riboside and zeatin ribotide. There are other reports of cytokinin-like substances present in the extracts of apple fruitlets [16, 205]. The unidentified cytokinins were found to have properties very similar to zeatin [205]. Taylor et al. [178] found endogenous cytokinin-like activity in extracts of developing fruit of the orchid Epidendrum ibaguense. Cytokinins declined during the first 30 days after pollination and then began to accumulate until very high levels were reached in the mature fruit and seed. The increase in cytokinin activity coincided with the onset of embryo development. GA levels declined in the fruit throughout development, although high activity was observed in the mature seed.

It is valuable to ascertain whether the predominant source of cytokinins in fruits comes from the fruits themselves or from other sources such as the roots. Indirect evidence suggests that seeds are capable of producing cytokinins, but that these cytokinins are not the same as those that form the majority of the cytokinin activity of many fruits. Blumenfeld and Gazit [14] found that avocado cotyledon callus tissue was capable of synthesizing cytokinin. When extracts were chromatographed from the fifth generation of callus grown on a kinetin-free medium, two regions of cytokinin activity were found, but compared to cytokinin from fruits and seeds, which had three zones of cytokinin activity, the first zone

of activity which contains the nucleotides was not present.

It is also possible that cytokinins present in fruits and seeds come from outside sources. Hoad et al. demonstrated [80] that removal of fruit from potted grape cuttings increased the concentration of a cytokinin glucoside in the leaf tissue, and decreased the level of extractable GA-like substances. The cytokinin detected was the glucoside of zeatin riboside. Because zeatin riboside and not the glucoside is present in grape xylem sap, the authors concluded that this cytokinin was synthesized in the leaves.

Many plant tissues appear to form glucosides of either endogenous or exogenously applied cytokinins [54, 77, 81, 113, 138, 139, 186, 187]. These cytokinin glucosides may represent storage forms of the hormone. Zeatin and zeatin riboside occur in leaves [186, 187], particularly under conditions of active leaf growth and development. In mature and aging leaves, the levels of these cytokinins tend to fall, and that of a cytokinin glucoside increases [77, 113, 186, 187]. Van Staden [187] showed that during the time course of the growing season, Ginkgo biloba leaves undergo both quantitative and qualitative changes in their cytokinin content. In young expanding leaves the major cytokinins co-chromatographed with zeatin and zeatin riboside. In mature and aging leaves, most of the cytokinin activity was due to slow-moving compounds that could be hydrolyzed by B-glucosidase. After hydrolysis the active compounds co-chromatographed with zeatin and zeatin

riboside. It was suggested that the cytokinin glucosides were formed when the xylem transported cytokinins were metabolized (inactivated) in the leaves. A number of plant tissues have demonstrated the ability to convert N_6 benzyladenine (N_6BA) into a stable, persistent derivative. This metabolite was identified as 6-benzylamino-7-glucofuranosyl purine on the basis of UV and mass spectra and associated data [54]. When [3H]-zeatin was supplied to the roots of Zea mays seedlings [139], the principal metabolite formed was 9-glucosylzeatin. This glucoside together with 7-glucosylzeatin accounted for about 90 percent of the radioactivity in the form of extractable metabolites. Other metabolites were produced including zeatin riboside. When zeatin was supplied to radish seedlings, with roots excised, the principal metabolite formed was a glucose derivative of zeatin. This metabolite was termed raphanatin, which was active as a cytokinin, and occurred primarily in the cotyledons of the seedlings [138]. Cytokinins occur in the phloem sap primarily as glucosides [121].

It has been postulated that at least one major mode of cytokinin action in many cytokinin mediated plant responses, including fruit set, is through the induction of cell division [104, 109]. Cytokinin-like substances were detected in the extracts of fruitlets of a number of species, particularly apple, peach and plum [104]. The cytokinins acted synergistically with auxin to promote cell division. Cytokinin activity of apple fruitlet extracts was highest during the period of intense cell division, and declined

just before the cessation of the cell division period. With plum fruitlets, the highest activity occurred at the onset of active cell division. Extracts of apple fruitlets showed evidence of a cytokinin-like substance present at the time of cessation of cell division, and again 24 days later [116]. The authors concluded that the presence of stimulants of cell division after the cessation of intense cell division suggests that these stimulants may have roles in fruit other than cell division.

Cytokinins also appear to inhibit senescence and abscission, and direct source to sink relationships [31, 102, 124, 133]. Application of BA [102] to leaves of red kidney bean (Phaseolus vulgaris) seedlings with roots removed, stimulated growth and inhibited senescence of treated leaves, and retarded growth and induced the senescence of untreated leaves on the same plant. It was suggested that the effect of the cytokinin in this experiment may be due to mobilization of metabolites from untreated portions of the plant to the treated portion. Mothes and Englebrecht [124] applied non-labeled kinetin, and labeled amino acids to isolated leaves of Nicotinia rustica. The radioactive amino acids migrated to the kinetin-treated parts of the leaves. Leaves kept in the dark for long periods, or starved, or the white parts of variegated leaves often did not show this effect. It was presumed, therefore, that ATP was necessary for the process. Similar labeling experiments indicate that cytokinin activity is restricted to the locale to which it is applied [64], and cytokinins appear to be relatively

immobile in tissues [121].

The action of cytokinins to retard senescence may not be related entirely to effects on mobilization, but rather to a direct effect by maintaining active tissues. Applications of kinetin to detached leaves of Xanthium pennsylvanicum Wall. [133] temporarily arrested senescent changes, and maintained a relatively high ratio of RNA (or protein) to DNA. The kinetin appeared to operate directly and was not dependent upon accumulation of metabolites from untreated tissues. Kinetin induced the incorporation of [14C]-leucine into protein and [14C]-orotic acid into RNA, indicating a stimulation of both RNA and protein synthesis. Kinetin has been shown to inhibit leaf abscission of bean explants when applied at 10^{-3} M, while GA promoted abscission [31].

Protein synthesis appears necessary for the synthesis of cytokinins. Reduced quantities of cytokinins were found in xylem sap and root extracts of sunflower when grown in a media containing low levels of nitrogen [169]. It was suggested that cytokinin synthesis in the root tip might depend upon protein synthesis, rather than cell division. Colchicine inhibited mitosis yet had little effect on cytokinin content, whereas chloramphenicol and cycloheximide reduced root growth and cytokinin production.

Cytokinins also appear to affect flowering and flower development. It has been shown [127] that flowers failed to develop and atrophy soon after bud burst on woody grape vine cuttings in the absence of roots. Inflorescences were retained on pre-rooted cuttings and on un-rooted cuttings treated with 6-benzylamino purine

or PBA. The applied cytokinins also stimulated inflorescense growth [127, 128]. Tendrils produced from shoot tips of grape grown *in vitro* developed into inflorescenses when BA or PBA was applied directly to the tendril tips [32]. A cytokinin requirement was found [143] for pistil development of heraphroditic flowers of 'Concord' grape in the absence of roots when cultured *in vitro*. It has been suggested that cytokinins are necessary for normal ovule development in peach [145]. Several synthetic cytokinins were found to promote the development of female organs on flowers from staminate grape vines [83]. Clusters from "male" vines treated with GA or IAA showed no change in sex expression.

Hormone Studies with Xylem Sap

Hormones in Xylem Sap

Xylem sap has been extracted from many woody and herbaceous plants for the determination of plant hormones.

A number of studies have been done involving the presence of inhibitors including ABA in the xylem map of woody plants [17, 49, 51, 52, 100]. Davidson and Young [51] identified ABA by gas chromatography in the xylem sap of sugar maple, Chinese gooseberry, apple, peach, willow and *Tecomaria capensis* during the winter. The quantity of ABA varied considerably between species. The same authors studied the seasonal flux of ABA in the xylem sap of peach [52], and found a marked increase in levels occurring in

autumn during leaf fall, and high levels in winter. The level decreased at bud swell and remained low throughout the summer. A possible relationship with dormancy was suggested. Experiments with willow [17] showed that there is one inhibitor present in the phloem sap which increases as the plants enter dormancy, and two inhibitors, one of which is ABA, and another unidentified, in the xylem sap. It was suggested that increasing levels of ABA in both xylem and phloem sap was directly related to the rate of synthesis within the leaves. Browning [24] studied the changes in GA levels of the xylem sap and flower buds of coffee in relation to the release of this tropical species from dormancy. GA levels increased rapidly in the buds, but remained unchanged in the xylem sap. It was mentioned that resumption of active growth leading to flowering was probably regulated by release of free GA from bound forms in the buds. GA-like activity was detected in the xylem sap of sycamore-maple and birch in spring immediately prior to bud burst [151].

Luckwill and Whyte [114] found xylem sap extracted from mature apple trees to contain two major hormones. One was a cytokinin, active in delaying senescence of isolated oat leaf segments and in stimulating cell division in soybean callus. The greatest cytokinin activity (200-300 µg/l kinetin equivalents), occurred at or slightly preceding full bloom and decreased to very low levels in summer and ceased in fall. No activity was detected during winter. The other substance was a highly polar compound that was not

identified. GA was also sometimes detected in trace amounts (0.0001 ppm) from samples taken in early spring.

Kende [90] found two cytokinin-like substances in the root exudate of sunflower plants. One of the substances was very active at promoting cell division in the soybean callus bioassay (Factor II), while the other was only slightly promotive (Factor I). After hydrolysis, Factor I was converted to a substance with activity similar to Factor II. Cytokinin-like activity was found in the xylem sap of sycamore and birch in the spring [151]. For this experiment 45 ml of sap was collected from each species. The cytokinins had the properties of zeatin, its nucleoside and nucleotide.

Alvim et al. [4] detected cytokinin and ABA in the xylem sap of willow. Growth in the spring was preceded by decreasing levels of ABA, and an increase in cytokinin activity. The onset of dormancy was associated with increased ABA content of the sap, and decreased cytokinin activity. Cytokinin activity increased abruptly at floral bud burst, with a second peak at leaf bud burst, and thereafter fell to low levels during the summer. Partition chromatography of the sap indicated that most of the cytokinin activity was due to a zeatin riboside-like compound. Hewett and Wareing [78] detected cytokinins in the xylem sap and vegetative buds of Populus X robusta. From non-detectable levels in winter, parallel increases in cytokinin levels in both the sap and buds occurred in February and March. The maximum activity in the sap

preceded the maximum attained in the buds by three weeks. Partition chromatography indicated that at least five cytokinins were present in the buds, two of which were probably zeatin and zeatin riboside. The main activity in the sap was due to zeatin riboside. The same authors [77] found cytokinins in the xylem sap and leaves of Populus X robusta in mid-summer. Two cytokinin fractions were detected: fraction Z in the xylem sap and leaves in mid-summer; fraction N found in older and senescent leaves. As leaves aged, their cytokinin levels decreased, with yellow senescent leaves having only one detectable cytokinin (Fraction N) thought to be a glucoside. Removal of the shoot apex resulted in increased cytokinin (Fraction N) levels in the leaves when compared to intact plants. It was suggested that once the apical sink activity has ceased, cytokinins in the xylem sap are diverted to the leaves and converted to cytokinin glucoside, possibly a storage form of the hormone.

Niimi and Torikata [132] detected cytokinins in the xylem sap of grape vines from April to July. Purse et al. [147] isolated cytokinins from the xylem sap of sycamore-maple sampled in spring. The cytokinins, trans-zeatin riboside, trans-zeatin, and dihydrozeatin were positively identified by combined gas chromatography-mass spectrometry.

Rootstocks also vary with regard to cytokinin production and levels in their xylem sap, which might affect fruit set and development with grafted systems. Considerable differences were noted

between three grape vine rootstocks regarding cytokinin levels in the xylem sap of seedlings and scion branches of grafted systems [170]. No differences existed between these rootstocks with regard to their effect on vegetative growth of the scion. One rootstock, however, significantly depressed yield due primarily to fewer berries per bunch and per vine. This rootstock also resulted in much lower levels of cytokinin in the xylem sap of scion branches, both on a concentration basis and in terms of the total amount passing to the shoot each day. Two peaks of cytokinin activity were observed, one at zeatin and another at its nucleoside. The authors mentioned that their results support the hypothesis that hormones of root origin are implicated in effects of rootstocks on scion development.

Environmental and stress factors also appear to affect cytokinin levels in xylem sap [56, 84, 166, 167, 171]. Root exudate obtained from water-stressed sunflower plants [84] contained significantly less cytokinin activity than exudate of control plants. A root temperature of 20°C resulted in greater cytokinin production than did 30°C with grape grown in well-aerated liquid culture [171]. Phosphorus supply to sycamore seedlings grown in aerated liquid nutrient solutions had an affect on vegetative growth and xylem sap cytokinin level [56]. It was found that the growth retardant (2-chloroethyl) trimethylammonium chloride (CCC) induced swollen root tips on seedlings of grape as well as on plants grown from cuttings, when applied to the growth medium [166].

Both the concentration and absolute amounts of cytokinin activity in the xylem sap were increased by CCC. It was postulated that the swelling of the root tips was a response to increased cytokinin synthesis in the roots induced by the CCC treatment.

Research has been done to elucidate whether the presence of cytokinins in xylem sap is due exclusively to cytokinin production by the roots or due simply to recirculation of cytokinins produced by other plant tissues [26, 27, 60, 125, 167, 169, 195]. Recirculation of cytokinins is not supported by the work of Skene [167] who failed to detect a rise in the level of extractable cytokinins in grape vine shoots distal to a bark girdle. Kende's [90] observation that the quantity of cytokinins in the xylem sap of decapitated sunflower plants did not decrease over a four-day period is in agreement with the concept that cytokinins are synthesized in the roots, and the presence of cytokinins in the xylem sap is due to their synthesis by the roots. Further evidence in support of the concept that cytokinins are produced by roots comes from a study with excised grape shoots [144], in which sustained shoot development was observed only when cytokinins were present in the medium. If roots developed, the requirement for an exogenous source of cytokinin was nullified. More direct evidence that cytokinins are produced in roots comes from time course studies on the rooting of leaf cuttings of Phaseolus vulgaris [60]. Initially, no cytokinin could be detected. Activity first appeared in the combined extracts of roots and petioles shortly after the formation

of roots and did not appear in the leaf blades until several days later. Burrows and Carr [27] noticed a strong correlation between metabolic activity of sunflower root apices and the total cytokinin content of the xylem sap. Flooding the root systems reduced root activity which reduced the levels of detectable cytokinins in the xylem sap. Experiments using labeled amino acids with rooted leaf cuttings, show an accumulation of the label in roots and along leaf veins, implying a synthesis of cytokinins in the roots and its translocation upward in the vascular system [64]. Weiss and Vaadia [195] found high levels of two cytokinin-like fractions in the extracts of root apices (0-1 mm from the tip) of sunflower. Physiologically older root tissue (1-3 mm from the tip) yielded very little cytokinin activity. Extracts from roots of chicory (Cichorium intybus L.) [26] produced crystalline trans-zeatin riboside, as indicated by precise comparison of the isolated substance with pure zeatin riboside standard. The authors suggested that based upon their results, it was unlikely that the isolated cytokinin came from the degradation of T-RNA, but rather that it probably represented a separate pool of cytokinin.

The root to shoot transport of cytokinins had been studied with intact seedlings of Citrus aurantium L. with root tips replaced with an uptake solution containing [14C]-N_6 benzyladenine [125]. The transport of the labeled cytokinin was predominantly in the transpiration stream and into the leaves.

Xylem Sap Collection and Assay for Hormones

Xylem sap has been removed from various species of woody plants in the interest of studying the contents of the sap for either growth substances or nutrients. A variety of methods has been experimented with and employed to extract the xylem sap.

A simple means of obtaining xylem sap involves decapitation of the stem or trunk and collecting the exuding sap [70, 87, 88, 90]. Jones, when studying the effects of rootstock [87] and interstock [88] on the mineral element composition of apple xylem sap, obtained sap samples by decapitating trees in spring. About 2 cm of bark was removed from immediately below the cut surface, and a vertical sleeve of polyethylene tubing was attached round the circumference of the stump with adhesive tape. A watertight seal was obtained by placing a silicone rubber preparation between the wood and polyethylene. Sap was then removed from the polyethylene tubes by pipette. Very similar techniques were used to obtain xylem sap from apple for studies on sorbitol content [70] and sunflower for studies on kinetin-like substances in the root exudate [90].

Centrifugation has also been used to extract xylem sap from stems [65, 157]. Bennett, Anderssen and Milad [11] developed two methods of xylem sap extraction, one based on displacement of water and the other by displacement by gas. With the displacement by water technique, the procedure consisted of forcing water

at one-half to one atmosphere pressure into one end of a cut branch and catching the liquid expelled at the opposite end. Variations of this method have been developed [164].

The gas displacement technique involves placing one end of the branch in a suction chamber and utilizing atmospheric pressure to force out the sap. The suction chamber consists of a heavy glass T into which the branch is inserted at one open end, and a tube leading to an exhaust pump is inserted into the other open end. The sap is collected in the collecting vessel between the open ends. As the displaced sap reaches an end wall, displacement stops. Sections of stem must, therefore, be cut at regular intervals to keep the sap moving through the branch. The observed advantage of the gas displacement method over the water displacement method was the absence of possible mixing of sap with a displacing fluid.

Although liquid displacement techniques have been used recently [24], gas displacement techniques have been used and developed more extensively. Bollard [15] developed a method of extracting xylem sap for apple tree nutrition studies, which is a simplification of the gas displacement method of Bennett [11]. For this technique, shoots were stripped of bark for 5 cm from the base and set in a rubber bung in a 500 ml filtering flask. A test-tube (150 x 15 mm), supported on cotton-wool inside the flask, was set under the proximal end of the shoot. Sap was extracted by reducing pressure inside the flask to a vacuum of 24 inches of

mercury and then cutting successive sections off the distal end of the shoot. Each shoot yielded an average of 3.5 ml of sap. Samples were immediately filtered through paper, as samples went brown unless wood fragments were removed, and those kept for later determinations were frozen. This method and modifications of it have been used extensively where xylem sap extraction has been necessary for the study of xylem sap for mineral elements, sugars, growth substances or other chemical constituents [4, 10, 18, 34, 78, 119, 120, 156, 161, 162, 168, 182, 200].

A number of chemical and biological tests have been used to identify gibberellins or gibberellin-like activity in xylem sap and other plant extracts [24, 29, 71, 141, 162]. It has been shown [29] that 17 gibberellins can be separated and identified by gas-liquid chromatography of the methyl esters and trimethyl silyl ethers of the methyl esters. Gibberellins A_1, A_3 and A_9 have been isolated [71] using data of physical analysis (mass spectrometry, fluorescence, melting point) of these substances, together with the results of various bioassays.

A number of biological assays exist for GA determination. One of the main plant responses to gibberellins is shoot elongation, and two bioassays, the dwarf corn test [190], and the Pisum sativum assay [19], are based upon this response. The lettuce hypocotyl test [63], although not highly sensitive, is a rapid and easily performed test compared to some other tests. Other GA bioassays include the cucumber hypocotyl test [20], the dwarf pea

bioassay [94, 112], the barley endosperm test [135], the rice seedling test [129], and the Rumex leaf senescence test [199].

A number of methods of determining the presence of auxins, and separating them have been developed [79, 190]. Chemical methods of separating and obtaining auxins from plant extracts are paper chromatography (both ascending and descending chromatography, thin layer chromatography, column chromatography, spectrophotofluorometry and gas chromatography. Each method has its advantages and specific uses. The biological determination of auxins involves a number of bioassays. Some of the more important tests, as reviewed by Weaver [190], are the Avena curvature test, the Avena straight growth test, the Avena first internode test, the split-pea test, pea-stem section test, the root-formation test, the Ageratum petiole curvature test, the root-inhibition test, and the tomato ovary test.

Ice cold perchloric acid has frequently been used to extract nucleotides, and cytokinin metabolites [98], but some loss of zeatin occurs with this procedure [79]. Ion exchange methods are often employed in extracting cytokinins, particularly when using plant tissues. The recovery of small quantities of cytokinin bases and nucleosides from strong cation exchange resins are not quantitative [188], and when used in the H^+ form cause some cytokinin breakdown [57]. Vreman and Corse [188] have advocated use of the weak cation exchange resin Duolite CS-101 with cytokinin samples applied in aqueous solutions at pH 10. Hillman [79]

suggests the use of cellulose phosphate as a cation exchange material for cytokinin applications.

Solvent partitioning is usually a necessary step in the isolation of cytokinins, and a number of different organic solvents are useful [79, 108].

Chromatography has also been used in extracting cytokinins. The LH-20 Sephadex column has been the most widely used recently, and when using 35 percent ethanol as the eluant, has been extremely useful for purifying cytokinin samples [6, 82, 189]. Thin layer chromatography (TLC) has been extensively used for cytokinins, and has been shown to possess high resolving power for cytokinins of very similar structure [111, 136, 137, 142]. Gas chromatography has been used successfully as a sensitive means of identifying cytokinins [123]. The trimethylsilyl derivatives of isopentenyladenine, dihydrozeatin, zeatin, isopentenyladenosine, and zeatin riboside were separated by this gas chromatographic method. A large number of purine cytokinins which have been reported to be naturally occuring were separated by gas-liquid chromatography [185]. High-pressure liquid chromatography (HPLC) is a relatively new tool for cytokinin analysis [28, 122, 179] but will undoubtedly become a major one in the future. Carnes et al. [28] showed that HPLC with a Bondapak C_{18}/Porsil B column achieved the same resolution of cytokinins as Sephadex LH-20, but in 1/30 of the separation time. Thomas [179] used HPLC to separate and isolate cytokinins from cabbage tissue. Zeatin,

N^6-(Δ^2-isopentenyl) adenine, their related ribonucleotides, and kinetin were separated using a simple isocratic elution with 0.025 M borate buffer at pH 6.8 and 4 percent (v/v) 1-butanol. Morris et al. [122] achieved quantitative recovery of cytokinins at the nanomolar level using a trace-enrichment protocol involving adsorption onto octadecyl-silica columns followed by elution with ethanol. Where further fractionation was desired, the eluate was evaporated and partitioned against ethylacetate: t-butanol: Tris-Cl (0.01 M, Ph 8.0), 4:2:2 (v/v) and the organic phase retained and chromatographed using HPLC. The HPLC fractions were tested for activity using the cucumber cotyledon bioassay [62].

Cytokinins have been positively identified by UV spectroscopy [101], and mass spectroscopy [72, 163], but the small quantities of cytokinins obtainable from most plant sources limit the use and efficacy of these techniques.

The soybean callus bioassay has been used very extensively as a method of determining cytokinins in xylem sap or other plant extracts [4, 74, 75, 140, 168, 170]. The methods most often used for performing this bioassay are those of Miller [117, 118], or a modification of those methods [74]. Another bioassay [90] using the capacity of kinetin to delay chlorophyl degration has also been used.

A relatively new cytokinin bioassay that is quite sensitive, and rapid, is based upon the induction of chlorophyl formation in cucumber cotyledons [61, 62]. Other growth substances and sucrose

do not appear to cause the increase in chlorophyl levels making this bioassay highly selective for cytokinins.

Other cytokinin bioassays include the radish or Xanthium test [134], tobacco tissue culture assay [172], the radish cotyledon expansion test [107], and the carrot phloem bioassay [106].

SUGGESTIONS FOR FUTURE RESEARCH

After reviewing the literature, it is apparent that many questions regarding the hormonal regulation of fruit set and development of sweet cherry are yet to be answered.

It is known that hormones such as the cytokinins are produced in roots, but the effects of these hormones on fruit setting in sweet cherry remains untested. The physiological or biochemical basis of rootstock effects on yield differences with sweet cherry also remains to be elucidated. The effects of hormones produced or stored in leaves and buds on fruit setting needs investigation. The exact modes and mechanisms of action of hormones in regulating fruit set, and the interaction of these hormones on a molecular basis are not fully understood, and remain to be elucidated. For example, little is known about the molecular basis of the activity of free cytokinins. The metabolism of plant hormones in the fruit also needs investigating. Because flowering affects fruit setting indirectly, the complex hormonal regulation of flowering including the interaction between hormones as they affect flowering needs to be determined. In the long term, a thorough understanding of the sequence of hormonal activity and interaction between hormones in relation to fruiting from flowering to final set needs to be achieved.

There is also a need to develop exogenous hormone or growth regulator treatments that would enhance fruit set in years when

fruit set potential is low, without causing adverse effects to fruit quality or vegetative development.

The following research will attempt to add to our knowledge of the hormonal regulation of fruit set of sweet cherry.

CHAPTER I

Effects of Exogenously Applied Growth Regulators on Fruit Set and Sustained Fruit Development of Sweet Cherry (*Prunus avium* L.)[1]

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Additional index words. Hormone, crop density, yield

Abstract

Growth regulators were tested individually and in various combinations for effectiveness in promoting fruit set in sweet cherry. N_6 Benzyladenine (N_6BA), 2,4,-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and gibberellic acid (GA_3) increased fruit set and the retention of fruit even in the absence of normal embryo development, with the phenoxy auxins alone or in combination with GA_3 being the most effective. Naphthaleneacetic acid (NAA) reduced final fruit set. 2,4-D enhanced maturity of the fruit, while treatments containing GA_3 retarded maturity, induced fruit cracking and inhibited flowering and fruit set the following year. All treatments caused some leaf epinasty, with the GA_3 containing treatments most severe.

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[1]Received for publication _____. Oregon Agricultural Experiment Station Technical Paper No. _____. From the Ph.D. dissertation of the senior author.

Introduction

Fruit set in Prunus, including sweet cherry, may be low due to environmental factors affecting pollination and fertilization. It has been found that poor fruit setting in 'Italian' prune is often attributed to cool weather during and after bloom which causes delayed pollen tube growth sufficiently long that ovule degeneration occurs and even in the event of fertilization, fruit set is not promoted [181]. It is desirable to develop treatments effective in alleviating this reduction in fruit set.

Various growth regulators have been shown to increase fruit set in woody plants [43, 149, 192, 193], and in some cases parthenocarpy [39, 41, 43, 96, 146, 149]. The phenoxy auxins are particularly effective at promoting parthenocarpic fruit set in Prunus, alone or in combination with other hormones [41, 146]. Gibberellin (GA) has been shown to induce parthenocarpic set in Prunus [43], and this effect may be enhanced when phenoxy auxins are applied with GA [41, 149]. Synthetic cytokinins can be effective at promoting fruit set [192, 193], parthenocarpy [39, 96], and fruit growth [191, 193].

Fruits set parthenocarpically by hormone treatment usually develop normally but are often smaller than fertilized ones [43, 146]. It was noted, however, that 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) induced fruits of apricot in which the ovules were killed by frost injury to grow to normal size [37]. Growth regulators have also been shown to reduce mid-season fruit drop [38,

76]. Applications of synthetic auxins or cytokinins have been found to increase fruit growth [38, 40, 191, 193], and auxins often hasten maturity and stimulate color development [36, 40, 43]. Exogenous gibberellins inhibit flowering by inhibiting flower initiation, and delay flower bud differentiation, but appear to increase hardiness near bloom [35].

The present paper deals with screening growth regulators, applied alone or in various combinations for effectiveness at promoting fruit set and yield, without causing adverse effects on fruit quality, return bloom and set or vegetative development.

Materials and Methods

Initial Screening Experiments

The initial experiments conducted in 1977 and 1978 involved testing auxins, GA_3 and cytokinins individually at different concentrations and in various combinations for effectiveness at promoting fruit set and their effects on fruit quality.

Growth regulators tested in the first experiment (1977) were GA_3, NAA, 2,4-D, 2,4,5-T, and N_6BA. The growth regulators were applied as aqueous sprays with the surfactant B 1956 (77 percent modified phthalic glycerol alkyd resin in ethylene dichloride) to the point of run-off to individual limb units of mature trees of sweet cherry (Prunus avium L.) cv. Napoleon grafted to clonal F12/1 Mazzard rootstock. The treatments were applied 18 days after full bloom during the shuck split stage when the fruit were approximately 3 to 8 mm in diameter, hereafter referred to as the shuck split stage. Flowers on one-half of each branch were depetalled prior to open bloom to prevent pollination by bees, and one-half of the test plot received 18.8 g per tree soil applied boron the previous year. This gave two boron and two petal treatments within each growth regulator treatment. Two observations were made for each treatment combination. Data were analyzed as a completely randomized design with factorial arrangement of treatments.

Parameters investigated were percent first fruit set at 30

days after full bloom, and final set at 85 days after full bloom. The weight of 20 mature oven-dried kernels was determined for each treatment as an indication of extended development of fruits with aborted embryos. To more closely approximate the normal distribution, percentage data were transformed using the arcsin of the square root of the percentage for analysis of variance. Data are presented as true percentages.

The second screening experiment (1978) tested 6-furfurylamino purine (kinetin), 6-(benzylamino)-9-(2-tetrahydropyranyl)-9H-purine (PBA), 2,4-D, N_6BA and GA_3 alone and in pairs. Growth regulators were applied as aqueous sprays without surfactant 16 days after full bloom (shuck split stage) to individual limb units of mature trees of sweet cherry cvs. 'Bada' grafted to clonal F12/1 Mazzard, and 'Napoleon' grafted to the Mazzard X Prunus mahaleb L. hybrids MxM 44 and MxM 79, and clonal F12/1 Mazzard rootstocks. One limb unit on each of four trees, one from each genetic combination, was used for each treatment. Percent final fruit set at 73 days after full bloom was subjected to analysis of variance as a randomized block design with different rootstock-scion combinations as blocks. Fifty fruit collected at random with equal numbers from each genetic combination were used for fruit weight, fruit removal force and soluble solids comparisons. Percentage data were transformed using the arcsin of the square root of the percentage for analysis of variance. Data are presented as true percentages.

Whole Tree Experiments

Two treatments that showed promise during the initial experiments, and a sequential treatment approximating the sequence of activity of endogenous hormones in the fruit, were tested using whole mature trees of sweet cherry cvs. 'Napoleon' and 'Bada' on F12/1 Mazzard rootstock. Treatments tested were 25 ppm 2,4-D applied at the shuck split stage (16 days after full bloom), 25 ppm 2,4-D with 200 ppm GA_3 applied at the shuck split stage, a sequential treatment of 200 ppm GA_3 at full bloom, 200 ppm N_6BA at the shuck split stage and 25 ppm 2,4-D 50 days after full bloom, and a water control applied at the shuck split stage. Twelve trees were used with each cultivar, with three trees per treatment per cultivar. Data were analyzed as a randomized block design with cultivars as blocks.

Parameters investigated were first and final fruit set calculated as crop density (fruit/cm^2 limb cross-sectional area), two limbs per tree, yield efficiency (g/cm^2 trunk cross-sectional area), fruit quality determinations and vegetative growth responses. First set was taken 57 days after full bloom, and final set was taken 84 days after full bloom. Fruit color, leaf chlorosis and leaf epinasty were rated on a scale of 0-5. With fruit color "0" indicated no red color, and "5" indicated full red blush color. A rating of "0" represented no epinasty or chlorosis and "5" indicated severe epinasty or chlorosis. A

sample from each treatment was brined, using a commercial SO_2 brine (1 percent SO_2, and 0.3 percent Ca^{++} by weight, pH 2.5), for six months to determine effects of treatments on brined quality.

Carry-over Effect Studies

The year following treatment (1980), the trees treated in 1979 were used to determine carry-over effects of the growth regulators. Parameters investigated were percent final fruit set at 81 days after full bloom, yield efficiency, crop density, fruit quality, return bloom as indicated by bloom density (flowers/cm^2), and vegetative growth responses. Yield efficiency measurements were made with whole trees. Percent final fruit set, bloom and crop density were based upon limb unit counts, two limbs per tree. Percentage data were transformed using the arcsin of the square root of the percentage for analysis of variance. Data are presented as true percentages.

Results and Discussion

Initial Screening Experiments

The screening experiments of 1977 and 1978 showed 2,4-D and 2,4,5-T, alone or combined with GA_3 to be most effective at promoting fruit set (Tables I.1 and I.2).

In the first screening experiment (1977) all growth regulators and combinations of growth regulators promoted fruit set above that of the control except treatments containing NAA which significantly reduced final set. The only treatments with significantly greater fruit set than the control were 25 ppm 2,4-D and 200 ppm GA_3 with 25 ppm 2,4-D at final set (Table I.1). N_6BA alone or combined with GA_3 promoted initial set much more strongly than final set. This may be due to rapid utilization of this cytokinin by the fruit, and a continuous supply or multiple applications may be required for sustained fruit setting responses to N_6BA. In 1978 N_6BA and PBA were more effective at enhancing fruit set than kinetin (Table I.2).

In 1977, all treatments except NAA at 50 ppm caused a reduction in average kernel weight compared to the control. Visual inspection showed many kernels to be small and shrunken, and in some cases only a small papery-like structure was present. The correlation between percent final fruit set and kernel weight was negative ($r = -0.66$, Table I.1), and highly significant, indicating that some of the increase in fruit set caused by the hormones was

TABLE I.1. Effects of growth regulators on set and sustained development of sweet cherry fruit

Treatment	% First[z] fruit set	% Final[y] fruit set	Mg/20 oven dried kernels
2,4-D, 25ppm	74	64a[x]	452
GA_3, 200 ppm + 2,4-D, 25 ppm	71	63a	529
2,4,5-T, 25 ppm	70	59ab	378
GA_3, 100 ppm	64	57ab	562
GA_3, 100 ppm + 2,4-D, 25 ppm	67	57ab	421
GA_3, 200 ppm + 2,4,5-T, 25 ppm	64	57ab	456
GA_3, 200 ppm + N_6BA, 100 ppm	67	56abc	460
N_6BA, 200 ppm	68	55abc	542
GA, 100 ppm + 2,4,5-T, 25 ppm	63	54abc	382
N_6BA, 100 ppm	67	54abc	513
GA_3, 100 ppm + N_6BA, 100 ppm	71	54abc	594
GA_3, 200 ppm	65	53abc	467
GA_3, 200 ppm + N_6BA, 200 ppm	63	49abcd	484
GA_3, 100 ppm + N_6BA, 200 ppm	59	47abcd	622
CONTROL (H_2O + surfactant)	56	43bcd	682
NAA, 25 ppm	63	43bcd	510
GA_3, 100 ppm + NAA, 25 ppm	58	37cde	548
NAA, 50 ppm	65	31de	692
GA_3, 200 ppm + NAA, 25 ppm	63	22e	527
GA_3, 200 ppm + NAA, 50 ppm	56	11f	665
GA_3, 100 ppm + NAA, 50 ppm	75	10f	676
Correlation between % final set and kernel weight = -0.66**			

**Significant at the 1% level.

[z]First set taken 30 days after full bloom.

[y]Final set taken 85 days after full bloom.

[x]Mean separation by Duncan's multiple range test, 5% level.

TABLE I.2. Effects of growth regulators on fruit set, size and maturity with sweet cherry[y]

Treatment	% Final[z] fruit set	Fruit removal force (g)	% Soluble solids	Fruit weight g/50 fruit
GA_3, 200 ppm + 2,4-D, 35 ppm	79a	1054abc	14.3	354
GA_3, 200 ppm + 2,4-D, 25 ppm	68ab	1025abcd	14.2	383
2,4-D, 35 ppm	64abc	985bcde	11.3	292
GA_3, 200 ppm + 2,4-D, 15 ppm	55bcd	1006abcd	13.5	312
2,4-D, 15 ppm	53bcd	959cdef	12.3	327
N_6BA, 400 ppm	50cd	864fgh	13.0	306
2,4-D, 25 ppm	49cd	1052abc	14.1	322
N_6BA, 200 ppm + GA_3, 200 ppm + 2,4-D, 25 ppm	48cd	837gh	15.8	370
N_6BA, 100 ppm	46cd	936def	13.2	320
PBA, 200 ppm + GA_3, 200 ppm + 2,4-D, 25 ppm	45d	1008abcd	12.4	292
Kinetin, 200 ppm	44d	941def	15.1	341
CONTROL (H_2O)	44d	890efg	15.4	353
N_6BA, 200 ppm	44d	859fgh	15.1	364
Kinetin, 400 ppm	44d	1094a	11.7	318
N_6BA, 300 ppm	43d	983cde	14.3	337
Kinetin, 300 ppm	40d	784h	14.9	357
Kinetin, 100 ppm	39d	1087ab	14.3	360

[z]Final set taken 73 days after full bloom.

[y]Mean separation, within columns, by Duncan's multiple range test, 5% level.

due to sustained development of fruits with aborted embryos.

Removal of petals prior to open bloom significantly reduced both initial and final set, probably by limiting insect pollination (Table I.3). Soil boron application, the year prior to treatment, had no effect on fruit set (Table I.3). No portion of the experimental plot, however, was deficient in boron at the time of the experiment.

In 1978, most treatments caused a reduction in soluble solids and fruit weight, and an increase in the fruit removal force. N_6BA applied alone was the only substance that appeared to reduce the fruit removal force consistently (Table I.2).

Whole Tree Experiments

With whole tree experiments (1979) 2,4-D applied alone at 25 ppm resulted in the highest first and final fruit set measured as crop density, and the highest yield efficiency (Table I.4). GA_3 with 2,4-D increased fruit set and yield efficiency but not significantly compared to the control. The sequential treatment which was made in an attempt to augment the natural flux of endogenous hormones in the fruit, reduced fruit set and yield. This treatment also caused many small aborted fruits to persist on the tree up to harvest. The sequential treatment and 2,4-D alone significantly reduced fruit weight. The 2,4-D treatment may have caused a reduction in fruit weight due to retention of fruit with aborted embryos or through a reduction on overall fruit size as a

TABLE I.3. Effects of soil boron and blossom depetalling on fruit set of sweet cherry

Treatment[z]	% First fruit set	% Final fruit set
Soil boron at 18.8 g per tree	65 ns	47 ns
No boron	66	46
Petals present	68*	50**
Petals removed at pre-bloom	62	44

*Significant at the 5% level.

**Significant at the 1% level.

[z]Soil boron and petals determinations were averaged over all hormone treatments.

TABLE I.4. Effects of growth regulators on fruit set, yield, fruit quality, and vegetative responses of sweet cherry[S]

Treatment	First[y,w] fruit set	Final[x,w] fruit set	Yield[v] efficiency	Fruit weight g/100 fruit	Fruit removal force (g)	% soluble solids	Fruit[u] color scale 0-5	Cracked fruit/ 100 fruit	Brine test (% fruit with mottling)	Leaf[t] epinasty scale 0-5	Leaf[t] chlorosis scale 0-5
2,4-D, 25 ppm	73a	71a	298a	648b	563b	15.2	4.1	4	30	1.0	1.1
GA_3, 200 ppm + 2,4-D, 25 ppm	54ab	48b	237b	712a	678a	15.4	1.7	18	15	3.4	2.2
Sequential treatment[z]	25c	23c	119c	640b	585b	16.2	3.7	10	0	3.7	3.0
CONTROL (H_2O)	45bc	44bc	228b	704a	659a	15.9	4.1	8	20	0.1	1.1

[z]Sequential treatment consisted of GA_3, 200 ppm at full bloom, N_6BA, 200 ppm 16 days after full bloom (shuck split stage), and 2,4-D, 25 ppm 50 days after full bloom.

[y]First fruit set taken 57 days after full bloom.

[x]Final fruit set taken 84 days after full bloom.

[w]First and final set are presented as crop density (fruit/cm^2 limb cross-sectional area).

[v]Yield efficiency was computed as g/cm^2 trunk cross-sectional area.

[u]Fruit color ratings were subjective with "0" indicating no red color and "5" indicating full red blush color.

[t]Leaf epinasty and chlorosis were subjective with "0" indicating no epinasty or chlorosis and "5" indicating severe epinasty or chlorosis.

[S]Mean separation, within columns, by Duncan's multiple range test, 5% level.

result of heavier crop density. GA_3 retarded fruit maturity as indicated by a lack of fruit color development, reduced soluble solids and a high fruit removal force. The application of 2,4-D alone slightly enhanced maturity as indicated by fruit color and a low fruit removal force. Soluble solids were low with 2,4-D alone during the whole tree and screening experiments, and it appears that 2,4-D may have a direct effect on reducing soluble solids, separate from effects on fruit maturity. The sequential treatment reduced the fruit removal force, and resulted in good color development even though it contained GA_3. It is likely that 2,4-D being applied at 50 days after full bloom enhanced fruit maturity with the sequential treatment, perhaps by stimulating endogenous ethylene production. The treatments containing GA_3 induced fruit cracking and caused moderate to severe epinasty and chlorosis. 2,4-D alone depressed cracking and caused only slight leaf epinasty and no chlorosis, but increased brown mottling of brined fruit while GA_3 containing treatments decreased mottling. Mottling was slight when present. GA_3 also enhanced shoot elongation of the current season's growth.

Carry-over Effect Studies

2,4-D alone caused increases in percent final fruit set, bloom density, and crop density the year after treatment, but these increases were not significant compared to the control (Table I.5). The treatments containing GA_3 showed a strong

TABLE I.5. Carry-over effects of growth regulators on sweet cherry fruit set, yield, return bloom and fruit quality[t]

Treatment[z]	% Final[x] fruit set	Crop[w] density	Bloom[v] density	Yield[u] .efficiency	Fruit weight g/50 fruit	Fruit removal force (g)	% Soluble solids	Cracked fruit/50 fruit
2,4-D, 25 ppm	52	34a	64a	132ab	362	539b	16.0	2
GA_3, 200 ppm + 2,4-D, 25 ppm	37	6b	14c	52c	418	568ab	17.2	6
Sequential treatment[y]	49	22a	44b	111b	395	594a	16.3	6
CONTROL (H_2O)	47	26a	54ab	159a	374	562ab	15.2	3

[z]Treatments were made the previous year (1979).

[y]Sequential treatment consisted of GA_3, 200 ppm at full bloom, N_6BA, 200 ppm 16 days after full bloom (shuck split stage), and 2,4-D, 25 ppm 50 days after full bloom.

[x]Final fruit set was taken 81 days after full bloom.

[w]Crop density was computed as fruit/cm^2 limb cross-sectional area.

[v]Bloom density was computed as flowers/cm^2 limb cross-sectional area.

[u]Yield efficiency was computed as g/cm^2 trunk cross-sectional area.

[t]Mean separation, within columns, by Duncan's multiple range test, 5% level.

negative carry-over effect regarding return bloom and set, and some trees were almost devoid of flowers. Despite the presence of GA_3, the sequential treatment showed no significant differences from the control regarding return bloom, fruit set and yield. An important difference between this treatment, and GA_3 with 2,4-D, is the presence of N_6BA. Gibberellins, when applied exogenously, have been shown to inhibit flower initiation and to delay differentiation [35, 42, 59]. Conversely, cytokinins appear to be necessary for normal flower development [143], and stimulate inflorescence growth [127, 128]. It is logical to postulate that N_6BA counteracted, at least in part, the effect of GA_3 by promoting normal flower initiation.

Differences in fruit weight were not significant and were inversely related to crop density. The slight differences observed were probably due only to the competitive effects of crop load. No chlorosis or epinasty was noted during the year following treatment.

These experiments indicate that synthetic cytokinins, GA_3, 2,4-D, and combinations of these substances will increase fruit set of sweet cherry. Only treatments containing 2,4-D increased fruit set significantly compared to controls. All of these growth regulators were capable of promoting fruit set to maturity in the absence of normal seed development, with the 2,4-D containing treatments being most effective. It appears, therefore, that the primary action of these substances is through fruit retention, and that the growth regulators are capable of maintaining fruit

growth and preventing senescence and abscission by augmenting endogenous hormones produced by the seeds and fruit tissues. 2,4-D also appears to promote flowering with sweet cherry, as indicated by increased bloom density the year following treatment. GA_3 inhibited flowering, and this effect appears to be counteracted in part by cytokinin. The sequential treatment failed to promote normal fruit set, and caused aborted fruits to remain on the tree up to harvest. Gibberellins have been shown to prevent normal seed development [176], and it is likely that GA_3 had this effect with the sequential treatment. The aborted fruits would persist on the tree due to the prevention of abscission as a result of the growth regulator sprays.

Certain endogenous hormones such as the cytokinins probably have several functions regarding fruit development such as promoting cell division in the early stages and promoting strong sink activity later. These hormones would, therefore, be needed throughout the course of fruit development and would have no single period of activity.

2,4-D caused no adverse carry-over effects, and only minimal adverse effects on fruit quality and vegetative growth. 2,4-D or substances similar to 2,4-D in activity appear promising for use in the future as growth regulator sprays to enhance fruit set during low set years.

CHAPTER II

Fruit Set and Cytokinin Activity in the Xylem Sap of Sweet Cherry (*Prunus avium* L.) as Affected by Rootstock[1]

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Additional index words. Hormone, growth substance, flowering, yield

Abstract

Using a trace-enrichment procedure involving solvent partitioning and high pressure liquid chromatography (HPLC), followed by bioassay; two cytokinin-like substances believed to be zeatin and zeatin riboside were detected in the xylem sap of sweet cherry. The levels of cytokinin varied between rootstocks with the higher yielding and fruit setting rootstocks producing the highest cytokinin levels. Most of the cytokinin activity occurred in the spring during bloom and early fruit set. A relationship between cytokinin level in the xylem sap and fruit set is proposed.

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[1]Received for publication _____. Oregon Agricultural Experiment Station Technical Paper No. _____. From the Ph.D. dissertation of the senior author.

Introduction

Rootstock affects scion physiology and development of Prunus in many ways including vigor [198], leaf element content [30], fruit set [67], yield and flowering [197] and flower morphology [66].

Endogenous hormones are important regulators of most plant processes including fruit set and development [110, 130, 150, 178]. Cytokinins and cytokinin-like substances have been detected in fruit extracts from a number of species [14, 110, 145, 178], and appear to play vital roles in fruit development [145]. Exogenously applied synthetic cytokinins have been shown to promote fruit set [86, 193, 201] and in some instances fruit growth [191, 193]. Parthenocarpic fruit set has been achieved with cytokinin application [96]. Cytokinins have been shown to be produced in roots [60, 169], transported in the transpiration stream [125], and have been detected in the xylem sap of woody plants [114, 132, 170]. Cytokinin level in the xylem sap also appears to vary with rootstock [170].

In the current study, five rootstocks for sweet cherry were compared for cytokinin levels in the xylem sap of scion branches during flowering and fruit development. These data were correlated with flowering, fruit set, and yield data for each rootstock to determine if a relationship exists between cytokinin production by the rootstocks and these parameters.

Materials and Methods

Xylem Sap Collection

Flower and fruit-bearing scion branches were collected in the early morning at full bloom, mid-season (47 days after full bloom), and immeidately prior to final set (67 days after full bloom), from mature trees of 'Napoleon' sweet cherry grafted to five different rootstocks. The rootstocks studied were the fully vigorous clonal F12/1 Mazzard (Prunus avium), the Mazzard X P. mahaleb hybrid clones MxM 14 and MxM 18 both intermediate in vigor, the vigorous MxM 88, and the semi-dwarfing rootstock Stockton Morello (Prunus cerasus). The branches were taken directly to the laboratory where the xylem sap was collected by means of the gas displacement method of Bollard [16]. The sap was immediately filtered to remove insoluble debris on Whatman 1 filter paper and frozen at -20°C. A total of 50 ml of sap was collected for each sample from 15 scion branches, three on each of five trees. Each branch yielded approximately 3.5 ml of sap.

Cytokinin Trace-enrichment and HPLC

All glassware that came in contact with the xylem sap was silanized with 5 percent (v/v) dichlorodimethyl silane in toluene prior to use.

The cytokinins were concentrated and extraneous substances eliminated by use of a trace-enrichment protocol involving solvent

partitioning and HPLC modified as follows from Morris et al. [122]. The xylem sap was thawed and partitioned three times (v/v) with distilled hexane at both pH 8.5 and 3.0. The partitioned sap (aqueous phase) was then roto-evaporated for 10 minutes to remove remaining hexane and loaded onto Sep-Pak C_{18} cartridges (Waters Assoc.). The cartridges were eluted with 4 ml reagent methanol, and the eluate then evaporated to dryness under reduced pressure. Ethylacetate, t-butanol and 0.1 M tris (2-amino-2-hydroxymethyl-1,3-propanediol) pH adjusted to 8.0 with HCL, 4:2:1 (v/v), were shaken and equilibrated. Samples were dissolved in 2 ml of the equilibrated organic phase and partitioned three times against 0.8 ml of the aqueous phase with the resulting organic phase retained and dried under a stream of nitrogen. The remaining solid was dissolved in 55 µl of reagent methanol and applied to an Altex Ultrasphere ODS (C_{18}) column and eluted with a linear gradient of reagent methanol (15-100 percent) and 0.2 M acetic acid adjusted with triethyl amine to pH 3.3, in the following elution profile: 15 percent methanol for one minute, 15 to 80 percent methanol in 30 minutes, and 80 to 100 percent methanol in three minutes for a total program of 34 minutes at a flow of 2 ml per minute. Cytokinin fractions were collected based upon known standards of t-zeatin, t-zeatin riboside, c-zeatin, c-zeatin riboside, N^6-(Δ^2-Isopentenyl) adenosine, and N^6-(Δ^2-Isopentenyl) adenine, and pooled for bioassay.

The remaining portion of the HPLC program was also pooled for bioassay. Individual fractions were collected independently from an additional sample from F12/1 Mazzard rootstock at mid-season (May 26) to determine the relative contribution of each cytokinin to total activity. The samples were evaporated to dryness under reduced pressure and frozen at -20°C.

The extraction procedure, prior to chromatography, was tested for cytokinin recovery by adding approximately 30,000 cpm of [14C]-kinetin to a test sample at the beginning of solvent partitioning. Additional test samples were run to determine the importance of HPLC as a cleanup procedure.

Bioassay

Relative cytokinin activity was measured with the cucumber cotyledon bioassay adapted as follows from Fletcher and McCullagh [62]. Cucumber seeds cv. 'Straight Eight' were planted in vermiculite saturated with a rearing solution of 0.1 mM FeEDTA, 1.2 mM Ca^{++} and 0.4 mM Mg^{++}, and incubated in the dark for four days at 29°C. Dry HPLC samples were taken up in 10 µl dimethyl sulfoxide (DMSO) in 2 ml of bioassay greening buffer containing 10 mM PO_4^{---} and 3.0 mM Ca^{++}, pH 5.0. The sample solution was divided into five aliquots of 0.1 ml each in cavities of plexiglass imbibition trays. While working under a green safe light, cotyledons were removed from seedlings and placed in cavities, proximal end down, one cotyledon pair per cavity for a 16-hour dark imbibition period

at 29°C. After imbibition, the trays were placed under fluorescent lamps (6X [15 watt F15T8/CW cool white Westinghouse]) at a distance of 15 cm for two hours. Immediately after light treatment, cotyledons were placed in test tubes (125 x 15 mm) racked in dry ice. Frozen cotyledons were ground in 4.0 ml of tetrahydrofuran/2-ethyxyethanol (1:1 v/v) with a Brinkman Instruments polytron, centrifuged for five minutes and the relative chlorophyll content of the supernatant determined by measuring the difference in absorbance readings at 665 and 698 nm on a spectrophotometer.

All samples were tested simultaneously during four independent bioassay determinations. Concentration curves based upon known standards of zeatin at 0-100 µm were developed for each determination, and the activity of the samples was measured against the standard curves. The average of the four determinations for each sample was converted to µg/l zeatin equivalents and used in the factorial analysis of variance with the rootstock x dates interaction as the error term. Dilutions were not made, as preliminary tests indicated that activities of the concentrated samples were in the lower range of detection by the cucumber assay.

Effects of Rootstock on Fruit Set and Yield

Trees used in this study were mature trees with the same rootstocks compared for xylem sap cytokinin level: Stockton Morello, MxM 14, MxM 18 and MxM 88, grafted to 'Bada,' 'Lambert,'

'Napoleon,' 'Marx' and 'Corum' scion cvs. in approximately equal numbers. F12/1 Mazzard was not included in this study because of differences in pruning and culture that would complicate comparisons with the other rootstocks. Parameters investigated were percent first and final fruit set taken approximately 30 and 60 days after full bloom, respectively, percent first set remaining to final set $\left(\frac{\text{no. fruit at final set}}{\text{no. fruit at first set}} \times 100\right)$, percent bloom $\left(\frac{\text{flower buds}}{\text{flower + leaf buds}} \times 100\right)$, bloom and crop density, yield efficiency, and bloom ratings based on three- and four-year averages with a rating scale of 0-10, each point approximating 10 percent bloom. Fruit set, bloom and crop density were examined using individual limb units, one limb per tree. Yield efficiency and bloom ratings were done on whole trees.

To more closely approximate the normal distribution, percentage data were transformed using the arcsin of the square root of the percentage prior to analysis of variance. Data are presented as true percentages. Due to an unequal number of observations per rootstock, individual LSD tests were performed to compare rootstock means where significant differences were obtained with the analysis of variance.

Results

Preliminary Tests

The solvent extraction protocol appears satisfactory based upon the recovery of labeled cytokinin (Table II.1).

The use of HPLC is valuable both as a means of cytokinin fractionation and as a cleanup procedure. Test samples bioassayed following solvent extraction without HPLC showed greater activity in the bioassay when diluted v/v than when undiluted. Chromatographed samples with fractions collected at the retention times of known standards and pooled for bioassay showed greater activity when undiluted and only half that activity when diluted v/v. Unchromatographed samples were also more yellow in color. It is likely that inhibitors not removed from samples during solvent extraction were eliminated during chromatography when fractions were collected. This is possible when inhibitors present in the xylem sap samples have different concentration response curves than the cytokinins.

Xylem Sap Studies

Both date and rootstock significantly affected the cytokinin level in the xylem sap (Table II.2). The greatest cytokinin activity occurred during bloom and decreased rapidly throughout the fruit development period. The higher yielding and fruit setting rootstocks produced higher levels of cytokinin in the

TABLE II.1. Recovery of [14C]-kinetin from solvent extracted 50 ml sample of sweet cherry xylem sap

Recovery (cpm)		% Recovery
Initial count	28,932	----
Final count (sample)	21,436	74.1
Final count (waste solvent)	6,475	22.4
Final count (sample + solvent)	27,911	96.5

TABLE II.2. Effect of rootstock and date on cytokinin level in the xylem sap of sweet cherry[x]

Rootstock	µg/l zeatin equivalents at date			
	Full bloom (4-12)	Mid-[z] season (5-26)	Before[y] final set (6-15)	Rootstock $\bar{X}$
Stockton Morello	232	87	22	114a
M x M 18	189	61	14	88ab
M x M 14	184	56	14	85ab
F12/12 Mazzard	168	52	13	78bc
M x M 88	116	21	4	47c
Dates $\bar{X}$	178f	55g	13h	

[z]Sampled at 47 days after full bloom.

[y]Sampled at 67 days after full bloom.

[x]Mean separation, within the same source of variation, by Duncan's multiple range test, 5% level.

xylem sap of scion branches at each date sampled. There was no rootstock X date interaction, as declines in activity throughout the season were nearly parallel (Figure II.1). Fractionation of a sample of F12/1 Mazzard at mid-season (May 26) showed that approximately 72 percent of the cytokinin activity co-chromatographed with t-zeatin riboside, and 28 percent co-chromatographed with t-zeatin. Other fractions and the pooled remaining portion of the HPLC program yielded less than one percent of the total activity.

Effects of Rootstock on Fruit Set and Yield

Significant differences between rootstocks were found with percent final set, and yield efficiency (g fruit/cm^2 trunk cross-sectional area) (Table II.3). Stockton Morello had the highest yield efficiency and final fruit set. MxM 14 and MxM 18 were intermediate and very similar, and MxM 88 had low percent final set and very low yield efficiency. Other parameters where differences were noted, but these differences were not significant are percent first fruit set, percent final/first set, and crop density. Less differences were noted at first set, and MxM 88, the rootstock with the lowest final set and yield, had slightly higher first set than MxM 14. Although bloom density followed a similar pattern as final set with the MxM hybrid rootstocks, Stockton Morello, the rootstock with the highest fruit set and yield, had low bloom density. A greater number of flowers of Stockton Morello

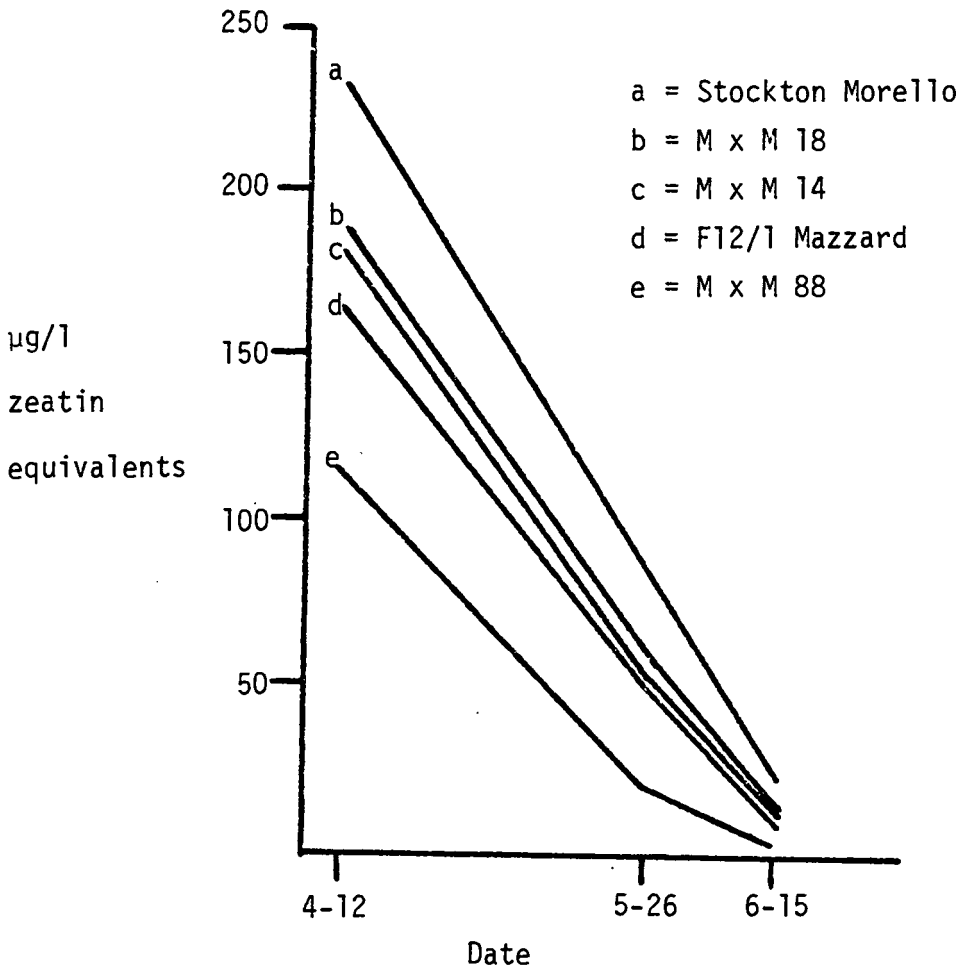

Figure II.1. Relationship between rootstock, date and cytokinin level in the xylem sap of sweet cherry

TABLE II.3. Effects of rootstock on flowering, fruit set and yield of sweet cherry[t]

Rootstock	% First[z] fruit set	% Final[y] fruit set	% Final/ first fruit set	% bloom	Crop[x] density	Bloom[w] density	Yield[v] efficiency	3-yr[u] bloom rating	4-yr[u] bloom rating
Stockton Morello	80	72a	90	60	40	55	106a	6.4	5.7
M x M 18	67	57ab	85	60	44	79	71b	6.7	6.0
M x M 14	62	52b	83	61	35	66	72b	6.8	6.7
M x M 88	64	50b	75	64	25	55	30c	6.9	6.2

[z]First fruit set was taken approximately 30 days after full bloom.

[y]Final fruit set was taken approximately 60 days after full bloom.

[x]Crop density was computed as fruit/cm^2 limb cross-sectional area.

[w]Bloom density was computed as flowers/cm^2 limb cross-sectional area.

[v]Yield efficiency was computed as g fruit/cm^2 trunk cross-sectional area.

[u]Bloom ratings were subjective with "0" indicating 0% bloom, and "10" indicating 100% bloom.

[t]Mean separation, within columns, by individual LDS tests, 5% level [175].

developed into harvestable fruit, however, and crop density was high with this rootstock. The percent of first set developing to final set (percent final/first set) almost paralleled yield density, and appears to have the greatest effect on yielding differences between these rootstocks. Little if any differences occurred between rootstocks regarding flowering parameters such as percent bloom and bloom ratings.

Correlations between Yield Parameters and Xylem Sap Cytokinin Level

The strongest correlations between the effects of rootstock on xylem sap cytokinin level and yield data were with percent final/first set and yield efficiency (Table II.4). Strong, positive but not significant correlations were found with percent first and final set and crop density. No correlation was found between cytokinin level and bloom density, and percent bloom showed a negative correlation with cytokinin level.

TABLE II.4. Correlations between xylem sap cytokinin level and flowering, fruit set and yield

Parameter	Correlation coefficient (r)
% First fruit set	.75
% Final fruit set	.87
% Final/first fruit set	.98*
Bloom density	.15
Crop density	.82
Yield efficiency	.99**
% Bloom	-.89

*Significant at the 5% level.

**Significant at the 1% level.

Discussion

Fruit retention appeared to have the greatest impact on yield differences between these rootstocks. Fruit set and specifically the retention of fruits set initially (percent final/first set) and yield efficiency were also most closely related to differences in cytokinin production by the rootstocks. Stockton Morello had the highest fruit set and yield, and highest cytokinin levels in the xylem sap. MxM 14 and MxM 18 had similar, relatively high fruit set and yield and cytokinin levels. MxM 88 had low fruit set and yield and low levels of cytokinin. These results support the known effects of cytokinins. It is well known that cytokinins inhibit senescence and abscission, and promote RNA and protein synthesis. A possible means by which cytokinins inhibit senescence may be directly related to the maintenance of active tissues. Cytokinins also induce cell division and are probably essential regulators of fruit development during the cell division period. Normal development of floral organs, including ovules, is also dependent upon cytokinin. Cytokinins also direct source to sink relationships by making tissues strong sinks for mineral elements and other metabolites including amino acids.

Considerable fruit is shed by stone fruit species between pollination and harvest. Many of these are unfertilized flowers or fruits with aborted embryos, but some are weaker fruits that

gradually senesce and abscise. It is likely that cytokinins are important in all phases of fruit development from flowering to final set. The following sequence is proposed: At first cytokinins are needed to promote flower and ovule development before and after fertilization. Cytokinins are then necessary during the cell division period of fruit growth. Later, cytokinins are necessary to maintain active tissues by enhancing protein synthesis and preventing senescence and abscission, together with promoting strong sink activity in the fruit allowing the fruit to draw metabolites from other parts of the plant. In this process cytokinins, acting with other growth promoters, oppose the effects of ethylene and abscisic acid on senescence and abscission. The end-product of these processes is the setting of a greater number of fruits from fertilized flowers, and the prevention of abscission of these fruits prior to harvest.

Previous work indicates that roots are a major source of cytokinins in roots and shoots [27, 60, 144, 169], and that the predominant cytokinin produced by the roots and translocated in the xylem sap is zeatin riboside [26, 132, 147]. Fruits also appear to be good sinks for cytokinins produced in roots. Removal of fruit was found to increase the amount of cytokinin in the leaves of grape [80]. This is probably due to the loss of the fruit as a major competitive sink.

Because the rootstock effect on xylem sap cytokinin level was most closely correlated with the percent of first set

developing to final set and yield efficiency, the predominant activity of the cytokinins from the xylem sap was probably through maintaining active tissues and strong source to sink activity of the fruit. This would prevent senescence and abscission of the fruit between early set and harvest. The higher fruit setting and yielding rootstocks had higher levels of cytokinin in their sap at all stages of fruit development.

Cytokinins from the roots probably do not act alone. Endogenous promoters such as auxins or gibberellins produced by seeds and fruit tissues must also have important effects on fruit set. The greater supply of cytokinins from certain rootstocks would give fruit on these grafted systems an additional source of a major growth promoter and a better probability of retention.

Studies of this nature are valuable both as a means of determining the biochemical basis of rootstock effects on scion development, and as a potential means of screening rootstocks. Evaluating rootstocks exclusively by means of yield data is slow and laborious. Considerable time could be saved by use of rapid biochemical screening techniques.

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APPENDIX

TABLE A.1. Analysis of variance table for statistical analysis of effects of growth regulators, boron, and depetalling (1977) on percent first fruit set of sweet cherry[z]

Source of variation	SS	df	MS	F
Treatments	6,571.22	83	79.17	0.82 ns
Growth regulators (G)	1,611.04	20	80.55	0.83 ns
Boron (B)	17.90	1	17.90	0.18 ns
Petals (P)	620.39	1	620.39	6.39*
G X B	1,397.18	20	69.86	0.79 ns
G X P	1,322.93	20	66.15	0.68 ns
B X P	3.62	1	3.62	0.04 ns
G X B X P	1,598.16	20	79.91	0.82 ns
Error	8,154.83	84	97.08	
Total	14,726.05	167		
n		168		

*Significant at the 5% level.

[z]Data transformed using the arcsin of the square root of the percentage.

TABLE A.2. Analysis of variance table for statistical analysis of effects growth regulators, boron, and depetalling (1977) on percent final fruit set of sweet cherry[z]

Source of variation	SS	df	MS	F
Treatments	22,195.69	83	267.42	2.98**
Growth regulators (G)	18,456.17	20	922.81	10.28**
Boron (B)	51.58	1	51.58	0.57 ns
Petals (P)	648.10	1	648.10	7.22**
G X B	1,277.09	20	63.85	0.71 ns
G X P	1,079.15	20	53.96	0.60 ns
B X P	82.89	1	82.89	0.92 ns
G X B X P	600.71	20	30.04	0.33 ns
Error	7,537.05	84	89.73	
Total	29,732.74	167		
n		168		

**Significant at the 1% level.

[z]Data transformed using the arcsin of the square root of the percentage.

TABLE A.3. Analysis of variance table for statistical analysis of effects of growth regulators (1978) on percent final fruit set of sweet cherry[z]

Source of variation	SS	df	MS	F
Blocks (B)	76.10	3	25.37	0.65 ns
Treatments (T)	2,712.72	16	169.54	4.35**
B X T (error)	1,868.86	48	38.93	
Total	4,657.68	67		
n		68		

**Significant at the 1% level.

[z]Data transformed using the arcsin of the square root of the percentage.

TABLE A.4. Analysis of variance table for statistical analysis of effects of growth regulators (1978) on the fruit removal force (g) of sweet cherry

Source of variation	SS	df	MS	F
Between treatments	6,640,916.50	16	415,057.28	8.33**
Within treatments	41,522,014.00	833	49,846.36	
Total	48,162,930.00	849		

**Significant at the 1% level.

TABLE A.5. Analysis of variance table for statistical analysis of effects of growth regulators (1979) on first fruit set (fruit/cm^2 limb cross-sectional area) of sweet cherry

Source of variation	SS	df	MS	F
Blocks (B)	16.90	1	16.90	0.05 ns
Treatments (T)	7,046.53	3	2,348.84	6.45**
B X T	650.41	3	216.80	
Within treatments	5,827.80	16	364.24	
Total	13,541.64	23		

**Significant at the 1% level.

TABLE A.6. Analysis of variance table for statistical analysis of effects of growth regulators (1979) on final fruit set (fruit/cm^2 limb cross-sectional area) of sweet cherry

Source of variation	SS	df	MS	F
Blocks (B)	4.52	1	4.52	0.02 ns
Treatments (T)	7,059.47	3	2,353.16	7.84**
B X T	611.05	3	203.68	
Within treatments	4,803.97	16	300.25	
Total	12,479.01	23		

**Significant at the 1% level.

TABLE A.7. Analysis of variance table for statistical analysis of effects of growth regulators (1979) on yield efficiency (g/cm^2 trunk cross-sectional area) of sweet cherry

Source of variation	SS	df	MS	F
Blocks (B)	10,751.40	1	10,751.40	6.79*
Treatments (T)	100,068.13	3	33,356.04	21.07**
B X T	20,522.77	3	6,840.92	
Within treatments	23,327.79	16	1,582.99	
Total	156,670.09	23		

*Significant at the 5% level.

**Significant at the 1% level.

TABLE A.8. Analysis of variance table for statistical analysis of effects of growth regulators (1979) on fruit weight (stem-off g/100 fruit) of sweet cherry

Source of variation	SS	DF	MS	F
Between treatments	8,192.50	3	2,730.83	7.79*
Within treatments	1,402.63	4	350.66	
Total	9,595.13	7		

*Significant at the 5% level.

TABLE A.9. Analysis of variance table for statistical analysis of effects of growth regulators (1979) on the fruit removal force (g) of sweet cherry

Source of variation	SS	df	MC	F
Blocks (B)	243,049.00	1	243,049.00	11.64**
Treatments (T)	933,450.00	3	311,150.00	14.90**
B X T	42,794.70	3	14,264.90	
Within treatments	8,185,796.00	392	20,882.13	
Total	9,405,089.70	399		

**Significant at the 1% level.

TABLE A.10. Analysis of variance table for statistical analysis of effects of growth regulators (1979) on soluble solids of sweet cherry fruit

Source of variation	SS	df	MS	F
Between treatments	2.77	3	0.92	0.54 ns
Within treatments	20.45	12	1.70	
Total	23.22	15		

TABLE A.11. Analysis of variance table for statistical analysis of effects of growth regulators (1979) on fruit cracking (cracked fruit/100 fruit) of sweet cherry

Source of variation	SS	df	MS	F
Between treatments	234.00	3	78.00	0.50 ns
Within treatments	630.00	4	157.50	
Total	864.00	7		

TABLE A.12. Analysis of variance table for statistical analysis of carry-over effects of growth regulators (1980) on percent final fruit set of sweet cherry

Source of variation	SS	df	MS	F
Blocks (B)	302.82	1	302.82	7.00**
Treatments (T)	275.98	3	91.98	2.13 ns
B X T	58.43	3	19.48	
Within treatments	692.43	16	43.28	
Total	1,329.66	23		

[z]Data transformed using the arcsin of the square root of the percentage.

**Significant at the 1% level.

TABLE A.13. Analysis of variance table for statistical analysis of carry-over effects of growth regulators (1980) on crop density (fruit/cm^2 limb cross-sectional area) of sweet cherry

Source of variation	SS	df	MS	F
Blocks (B)	219.80	1	219.80	2.33
Treatments (T)	2,475.19	3	825.06	8.74**
B X T	151.25	3	50.42	
Within treatments	1,510.00	16	94.38	
Total	4,356.24	23		

**Significant at the 1% level.

TABLE A.14. Analysis of variance table for statistical analysis of carry-over effects of growth regulators (1980) on bloom density (flowers/cm^2 limb cross-sectional area) of sweet cherry

Source of variation	SS	df	MS	F
Blocks (B)	1.35	1	1.35	0.01 ns
Treatments (T)	8,531.06	3	2,843.69	12.06**
B X T	116.94	3	38.98	
Within treatments	3,774.23	16	235.89	
Total	12,423.58	23		

**Significant at the 1% level.

TABLE A.15. Analysis of variance table for statistical analysis of carry-over effects of growth regulators (1980) on yield efficiency (g/cm^2 trunk cross-sectional area) of sweet cherry

Source of variation	SS	df	MS	F
Blocks (B)	107.70	1	107.70	0.14 ns
Treatments (T)	37,673.28	3	12,557.76	16.64**
B X T	5,953.31	3	1,984.44	
Within treatments	12,077.21	16	754.83	
Total	55,811.50	23		

**Significant at the 1% level.

TABLE A.16. Analysis of variance table for statistical analysis of carry-over effects of growth regulators (1980) on fruit weight (g/50 fruit) of sweet cherry

Source of variation	SS	df	MS	F
Between treatments	3,635.59	3	1,211.86	0.67 ns
Within treatments	7,233.13	4	1,808.28	
Total	10,868.72	7		

TABLE A.17. Analysis of variance table for statistical analysis of carry-over effects of growth regulators (1980) on fruit removal force (g) of sweet cherry

Source of variation	SS	df	MS	f
Blocks (B)	983,072.20	1	983,072.20	55.73**
Treatments (T)	152,478.70	3	50,826.23	2.88*
B X T	30,962.80	3	10,320.93	
Within treatments	6,914,930.00	392	17,640.13	
Total	8,081,443.70	399		

*Significant at the 5% level.

**Significant at the 1% level.

TABLE A.18. Analysis of variance table for statistical analysis of carry-over effects of growth regulators (1980) on soluble solids of sweet cherry fruit

Source of variation	SS	df	MS	F
Between treatments	8.15	3	2.72	3.09 ns
Within treatments	10.57	12	0.88	
Total	18.72	15		

TABLE A.19. Analysis of variance table for statistical analysis of carry-over effects of growth regulators (1980) on fruit cracking (cracked fruit/50 fruit) of sweet cherry

Source of variation	SS	df	MS	F
Between treatments	34.50	3	11.50	5.11 ns
Within treatments	9.00	4	2.25	
Total	43.50	7		

TABLE A.20. Analysis of variance table for statistical analysis of effects of rootstock and date on xylem sap cytokinin levels of sweet cherry scion branches

Source of variation	SS	df	MS	F
Rootstock (R)	6,927.27	4	1,731.82	5.51*
Date (D)	73,118.55	2	36,594.28	116.46**
R X D (error)	2,513.66	8	314.21	
Total	82,559.48	14		

*Significant at the 5% level.

**Significant at the 1% level.

TABLE A.21. Analysis of variance table for statistical analysis of effects of rootstock on percent first fruit set of sweet cherry[z]

Source of variation	SS	df	MS	F
Between rootstocks	520.77	3	173.59	2.12 ns
Within rootstocks	2,615.17	32	81.72	
Total	3,135.94	35		

[z]Data transformed using the arcsin of the square root of the percentage.

TABLE A.22. Analysis of variance table for statistical analysis of effects of rootstock on percent final fruit set of sweet cherry

Rootstock	Stockton Morello	MxM 18	MxM 14	MxM 88
Mean[z]	58	49	46	45
df	4	11	9	8
n	5	12	10	9

Source of variation	SS	df	MS	F
Between rootstocks	631.22	3	210.41	3.10*
Within rootstocks	2,171.71	32	67.87	
Total	2,802.93	35		

[z]Means transformed using the arcsin of the square root of the percentage.

*Significant at the 5% level.

TABLE A.23. Analysis of variance table for statistical analysis of effects of rootstock on percent first fruit set developing to final set (% final/first set) of sweet cherry[z]

Source of variation	SS	df	MS	F
Between rootstocks	599.19	3	199.73	1.78 ns
Within rootstocks	3,593.36	32	112.29	
Total	4,192.55	35		

[z]Data transformed using the arcsin of the square root of the percentage.

TABLE A.24. Analysis of variance table for statistical analysis of effects of rootstock on percentage bloom of sweet cherry

Source of variation	SS	df	MS	F
Between rootstocks	37.51	3	12.50	0.26 ns
Within rootstocks	1,538.34	32	48.07	
Total	1,575.85	35		

TABLE A.25. Analysis of variance table for statistical analysis of effects of rootstock on crop density (fruit/cm^2 limb cross-sectional area) of sweet cherry

Source of variation	SS	df	MS	F
Between rootstocks	1,797.58	3	599.19	1.45 ns
Within rootstocks	13,265.08	32	414.53	
Total	15,062.66	35		

TABLE A.26. Analysis of variance table for statistical analysis of effects of rootstock on bloom density (flowers/cm^2 limb cross-sectional area) of sweet cherry

Source of variation	WW	df	MS	F
Between rootstocks	3,586.19	3	1,195.40	0.85 ns
Within rootstocks	44,763.31	32	1,398.85	
Total	48,349.50	35		

TABLE A.27. Analysis of variance table for statistical analysis of effects of rootstock on yield efficiency (g/cm^2 trunk cross-sectional area) of sweet cherry

Source of variation	SS	df	MS	F
Between rootstocks	23,393.69	3	7,797.90	7.17**
Within rootstocks	30,435.97	28	1,087.00	
Total	53,829.66	31		

**Significant at the 1% level.

TABLE A.28. Analysis of variance table for statistical analysis of effects of rootstock on bloom (rating scale of 0-10) of sweet cherry (3-year average)

Source of variation	SS	df	MS	F
Between rootstocks	1.56	3	0.52	0.63 ns
Within rootstocks	29.61	36	0.82	
Total	31.17	39		

TABLE A.29. Analysis of variance table for statistical analysis of effects of rootstock on bloom (rating scale of 0-10) of sweet cherry (4-year average)

Source of variation	SS	df	MS	F
Between rootstocks	5.14	3	1.71	1.17 ns
Within rootstocks	52.79	36	1.47	
Total	57.93	39		