DETERMINING THE GROWTH PATTERN AND CONTROLLING FRUITING OF YOUNG HIGHBUSH BLUEBERRIES

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ABSTRACT

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Highbush blueberries grown in Michigan often require seven to ten years from planting until peak fruit production. Preventing young plants from producing fruit for three years after planting increases vegetative growth and reduces this time interval. Manual removal of floral meristems is recommended, but labor intensive. Less costly methods to prevent fruiting are needed. Studies were conducted across several years to determine the efficacy of plant hormones in preventing fruiting and to determine the growth pattern of young highbush blueberry cultivars currently being planted. The majority of shoot growth occurred from May to July in the first and second growth flush. Floral buds were found on the first, second, and third growth flushes, with the majority on the second flush in 2012 and on the first flush in 2013. The pattern of floral bud distribution between growth flushes indicates a lengthy floral initiation time interval. Foliar gibberellins (GA) applications reduced the number of floral buds up to 49%, with the greatest reduction occurring when GA was applied multiple times from July to October. Auxin applications showed inconsistent results. Cytokinin (BA) applications in the spring reduced fruit set, but also reduced vegetative growth. Manual removal of floral structures resulted in more vegetative shoots per plant and canopy growth compared to non-treated plants.

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Background:

Blueberries are members of the family Ericaceae and are closely related to lingonberry (*Vaccinium vitis-idaea*), cranberry (*V. macrocarpon*), and sparkleberry (*V. arboreum*) (Song and Hancock, 2011). Blueberries are a diverse group of many species of economic importance, such as highbush (*V. corymbosum*), rabbiteye (*V. ashei*), and lowbush (*V. angustifolium*) blueberry (Scherm et al., 2001). Domestic highbush blueberries originated through the breeding program of Fredrick Coville at the start of the twentieth century (Moore, 1965). Significant crosses between blueberry species have also been made. Crosses between highbush and lowbush blueberry generated half-high blueberries (*V. corymbosum x V. angustifolium*), which can survive in colder winter environments than highbush blueberries (Albert et al., 2010). Crosses have also been made between the northern highbush blueberry and native southern blueberry species, including *V. darrowii*, to produce southern highbush cultivars, which have low chill requirements and are well adapted to conditions in the southern United States (Hancock, 2006; Ratnaparkhe, 2007).

The demand for blueberries has steadily increased, as American consumption went from 0.18 pounds per capita in 1980 to 0.95 pounds in 2009 (USDA, 2012). Due to increased demand, global production grew 30% from 2005 to 2010 (USDA, 2012). Blueberries have high concentrations of antioxidants and are widely perceived as providing health benefits (Giacalone et al., 2011; Yang et al., 2011). North America leads total production, but significant yields also come from South America, Europe, Australia, and Asia (Brazelton and Strik, 2007).

The majority of domestic highbush blueberry production comes from the Pacific Northwest, Michigan, and New Jersey (Finn et al., 2003; Strik and Yarborough, 2005). From 2010 to 2012 Michigan blueberry production averaged 89 million pounds, representing 35% of

United States production (USDA, 2012). However, 40% of Michigan's acreage contains older cultivars (USDA, 2012). With increasing market competition, growers may need to replant fields with modern cultivars, which can yield higher quality and quantities of fruit. However, young plants may take seven to ten years to reach maximum fruit production in Michigan's climate (Strik, 2007). If the establishment times were reduced, Michigan growers would be more likely to replace old cultivars with improved varieties and increase their advantage over competing regions. New management strategies are needed to decreases the time to full fruit production of young highbush to make replanting with new cultivars feasible.

Factors Affecting Growth:

Highbush blueberry plants in other regions, like the Pacific Northwest, often grow faster than those in Michigan. For example, three years after planting highbush blueberry plants of the same cultivar in Oregon were 80% taller, 104% wider, and had a 36% higher survival rate than plants grown in Michigan (Finn et al., 2003). Growth differences likely resulted from differing environmental factors. (Finn et al., 2003; Hancock et al., 1992; Moon et al., 1987). Michigan summer temperatures often exceed 30º C, while in Oregon temperatures rarely reach 30º C. The photosynthetic capabilities of highbush blueberries are inhibited and carbon assimilates were reduced 21 to 50% as temperatures increased from 20° C to 30º C (Hancock et al., 1992). During a study by Finn et al. (2003) winter temperatures in Oregon remained fairly steady at 0º C, while Michigan temperatures often fell under 0º C and to as low as -20º C. Winter injury can significantly limit blueberry establishment (Moore, 1994). In addition, the length of the growing season is different between these regions. Based on a growing degree model from March to October, Michigan receives about 5% fewer growing degree days than Oregon (NWS, 2013).

Finn et al. (2003) also observed bloom occurring three weeks earlier in Oregon than in Michigan. The combination of greater winter damage, decreased photosynthesis due to higher summer temperatures, and a shorter growing season may account for reduced plant growth in Michigan.

Blueberries have a shallow fibrous root system, which runs parallel to the surface and lacks root hairs (Gough, 1980). These traits reduce water absorption capabilities and make blueberries sensitive to drought (Gough, 1980). Irrigation with drip lines, micro sprinklers, and overhead irrigation are commonly used in blueberry production to promote growth (Bryla et al., 2011).

Soils may also influence the growth of highbush blueberries. Unlike many plants, blueberries grow best in highly acidic soils with a pH of 4.0 to 5.0 (Haynes and Swift, 1985). In addition to soil pH, soil nitrogen levels can alter plant growth. Blueberries can access both $NH_4^+$and NO_3^- from the soil and there is conflicting evidence on whether one form is preferred (Peterson et al., 1988; Rosen et al., 1990). Soil microorganisms can also alter blueberry growth. Plant parasitic nematodes are ubiquitous in nature and are a threat to agricultural crops (Lilley et al., 2012). In apples (*Malus domestica*), replant disease can commonly occur, resulting in stunted plant growth on a replanted site (Braun et al., 2010). Highbush blueberries planted on previously cultivated soils had reduced root systems and plant growth, compared to those on virgin soils (Blasing, 1988). Differences in growth were not attributed to soil nutrition, but may have resulted from plant pathogens (Blasing, 1988).

Highbush blueberries may produce fruit within a year of planting (Strik and Buller, 2005). However, plants that are prevented from fruiting often produce more vegetative growth. Highbush blueberry plants that had floral meristems removed during the first two years after planting had 44% greater root mass and 60% greater shoot mass (Strik and Buller, 2005).

Southern highbush cultivars also produced more vegetative mass when fruiting was prevented for the first two years after planting (Williamson and NeSmith, 2007).

Fruiting may reduce growth due to the allocation of photosynthates away from vegetative sinks. Although fruits can photosynthesize, they depend on the other sources for the majority of carbon assimilates (Birkhold et al., 1992). Young highbush blueberry plants allocate over half of total photosynthates towards flowers and fruiting (Pritts and Hancock, 1985). Vegetative growth, leaf area, and internal carbohydrate concentrations were reduced with increasing floral bud density in southern highbush blueberries (Maust et al., 1999). Similar trends probably occur in northern highbush blueberries.

Fruiting can also draw mineral nutrients away from vegetative growth. In *Banksia hookeriana*, 44% of total nitrogen and 65% of total phosphorus were within reproductive structures (Witkowski and Lamont, 1996). Similar competition probably exists in blueberries, as both vegetative and reproductive development depends on stored nitrogen reserves (Birkhold et al., 1992). In Michigan, highbush blueberries are often grown on sandy soils that are low in organic material and cation exchange capacity (Hanson and Retamales, 1992). These soils have increased leaching and a limited supply of available nutrients, which may increase competition between vegetative and reproductive development. Increasing fertilization applications may not help, due to the small root zone of young highbush blueberries and the possibility of killing roots with high salt contents from extra fertilizer applications (Bañados et al., 2012).

To prevent the competition between vegetative and reproductive growth for nutrients and photosynthates, the manual removal of floral buds is recommended (Pritts and Hancock, 1992). However, this process is highly labor intensive and costly (Strik and Buller, 2005). A more efficient method is needed to prevent early fruiting in highbush blueberries, which would reduce

the interval from planting to peak fruit production and allow growers the opportunity to plant modern cultivars.

Shoot growth cycle:

Shoots in highbush blueberries can originate from two types of buds. Most shoot come from axillary buds on the previous year's growth, but some may develop from adventitious or latent buds on the crown or roots of the plant (Bañados 2006; Gough et al., 1978). The shoot growth pattern of highbush blueberry is episodic and sympodial and is similar to the American elm (*Ulumus americana*) and lilac (*Syringa vulgaris*) (Gough et al., 1978). Each growth flush terminates with the abortion of the apical meristem (Gough et al., 1976). Two to five weeks after apical meristem abortion, a lateral meristem may commence shoot growth and form the next growth flush (Gough et al., 1978). This cycle may repeat itself, with the majority of shoot growth occurring in the late spring and early summer (Gough et al., 1976). The number of growth flushes is dependent on cultivar, environmental conditions, and plant health (Gough et al., 1976). Gough et al. (1976) found varieties that ripen fruit later in the season tended to have fewer growth flushes than early fruiting varieties.

Reproductive Development:

Reproductive development starts with floral induction, a process where signals to transition from a vegetative bud to a floral bud are produced and transported to the meristem (Samach and Smith, 2013). After the signal is perceived, floral initiation occurs, which is the transition from a vegetative meristem to an inflorescence meristem (Samach and Smith, 2013). Based on histological examinations Gough et al. (1978) found the first morphological evidence of floral initiation was the flattening of the meristem and formation of sepal primordial, which occurred six to ten weeks after full bloom during the fruit harvest period (Gough et al., 1978; Ye

et al., 2005). Floral primordia development continued and by October all flower parts formed (Gough et al., 1978). In March, meristematic division's resumed as floral buds concluded their development (Gough et al., 1978). Pollen grains formed in April and bloom occurred in May (Gough et al., 1978). After pollination, blueberry fruit development follows a double sigmoidal curve pattern; harvest of mature fruit occurs from July through September, depending on environmental conditions and cultivar (Finn and Luby, 1986).

Floral induction and initiation are complex physiological processes, controlled by many regulatory factors (Bowman et al., 2012). In *Arabidopsis*, floral induction is controlled in part by *FT* genes, which produce proteins in the leaves that are transported and accumulate in the meristem, where they interact with other factors, and lead to floral initiation (Taoka et al., 2011; Turck et al., 2008). Once floral initiation occurs, floral identity genes, such as *LFY*, *AP1*, and *CAL*, specify formation of floral organ formation (Piñeiro and Coupland, 1998).

In addition to genetic factors, other factors affect floral initiation. *Arabidopsis* mutants unable to metabolize starch had delayed floral initiation, suggesting floral initiation and starch metabolism may be related (Eimert et al., 1995). In avocado (*Persea americana*) the level of floral initiation was dependent on endogenous carbohydrate levels (Scholefield et al., 1985). Mineral nutrient levels also may alter floral initiation in plants. Excess nitrogen leads to increases in floral initiation per stem area in rabbiteye blueberries (Darnell et al., 1992). In tobacco (*Nicotiana tabacum*), floral initiation occurred once levels of nitrogen and carbohydrates increased in the apical meristem (Rideout et al., 1992). In sunflower (*Helianthus annuus*), nitrogen applications at the start of the floral initiation period increased the number of floral meristems (Steer and Hocking, 1983). In highbush blueberries, a drought stress during the floral initiation period resulted in fewer floral meristems (Mingeau et al., 2001).

Hormones:

Plant hormones are organic molecules that regulate activities in plants (Nemhauser et al., 2006). Gibberellins (GA) are the largest class of plant hormones and include numerous forms that differ in their tetracyclic dierpene structure (Iqbal et al., 2011). Floral initiation is promoted by GA in some species, and repressed in others (Sharp et al., 2010). In many woody plants, decreases in GA levels are required prior to floral initiation occurring (Sharp et al., 2010). In biennial producing apple plants, seeds within developing fruit may inhibit floral initiation by producing and secreting GA into the shoot (Dennis and Neilsen, 1999).

Application of GA can affect the number of flower buds formed. In *Rhododendron* the application of GA prevented floral initiation, while inhibitors of GA synthesis increased the process (Sharp et al., 2010). GA applications reduced floral initiation in apples, sweet cherries (*Prunus avium*), mangos, (*Mangifera indica*) and apricots (*Prunus armeniaca* cv. Royal) (Bradley and Crane, 1960; Luckwill, 1970; Turnbull et al., 1996; Lenahan et al., 2006). The response of highbush blueberries to GA have varied from reduction in reducing the number of flower buds 0 to 95% relative to non-treated plants (Retamales et al., 2000; Black and Ehlenfeldt, 2007). The application of paclobutrazol, which blocks GA production, increased the number of floral meristems in highbush and lowbush blueberries (Lewis and Hu, 1993; Ehlenfeldt, 1998).

Several factors could explain the inconsistent results of experiments of highbush blueberries to GA applications. In sour cherry leaves, GA was absorbed for only the first three hours after application. The absorption rates was dependent on temperature, with greater absorption at 25°C than at 15°C or 35°C (Knoche et al., 1992). Different temperatures during the application of GA could change the absorption rates and thereby alter effectiveness.

Timing of GA applications may also alter plant response. GA applications did not prevent floral development in *Citrus sinensis* after sepal primordia had formed (Lord and Eckard, 1987) or in *Fuchsia hybrids* after floral primordial were initiated (Sachs et al., 1967). The period of floral initiation in highbush blueberries is unclear, but is thought to occur approximately six to nine weeks after full bloom (Gough et al., 1978). Mainland and Eck (1969) applied GA to highbush blueberries during full bloom and reduced the number of floral buds, while Black and Ehlenfeldt (2007) found the greatest reduction in induction when GA was applied in September. Retamales et al. (2000) also found reductions in the number of floral buds when applying GA 18 weeks after full bloom. An ideal application timing of GA for highbush blueberries is not known.

The variable response of highbush blueberries to GA may also be a result of the environment in which plants were grown, as greenhouse grown plants often show greater response than field grown plants. Mainland and Eck (1969) found GA applications significantly reduced the number of floral buds in greenhouse treated plants, but not in field grown plants. Retamales et al. (2000) also found significant reductions in the number of flower buds in nursery plants, but not in field grown plants. Mesquite plants (*Prosopis juliflora*), grown in greenhouses had less cuticle development compared to those in the field, which likely resulted from changes in ultra violet irradiation, soil moisture, humidity, or wind (Hull, 1958). Similar trends in leaf cuticle development could exist in blueberries. This could alter GA absorption rates and account for variable responses in previous studies.

Plant responses are also affected by the type of GA applied. There are over one hundred GA forms found in plants, but only a portion are biologically active (MacMillian, 2002). GA with hydroxylation of the C_3 position and double bonds at the $C_{(1,2)}$ or $C_{(2,3)}$ were most

effective in preventing floral initiation in cherries (Oliveira and Browning 1993). In apples, GA_4 had no effect or increased floral initiation, while GA_3 or $GA_{(4+7)}$ prevented the process depending on concentration and application timing (Li et al., 1995; Looney et al., 1985; Meador and Taylor 1987; Tromp 1982). In *Clerodendrum thomsoniae*, GA_3 prevented floral initiation whereas GA_7 had the opposite effect (Koranski et al., 1979). In highbush blueberries, GA_3 and $GA_{(4+7)}$ were both effective in preventing floral initiation (Retamales et al., 2000; Black and Ehlenfeldt, 2007).

Auxins are another class of plant hormones that can influence floral initiation (Muday and DeLong 2001). In pineapple (*Ananas comosus*) 0.001% auxin, applied prior to floral initiation increased the number of floral meristems, whereas 0.1% prevented floral development (Clark and Kearns, 1942). In Cocklebur (*Xanthium*) low auxin concentrations (50 $mg \cdot L^{-1}$) had no effect on floral initiation, but high concentrations (500 $mg \cdot L^{-1}$) prevented the process (Bonner and Thurlow, 1949). In *Chenopodium rubrum* auxin applied prior to inductive photoperiods prevented floral initiation, while applications after inductive photoperiods increased initiation (Seidlova and Khatoon 1976). In pecans (*Carya illnoinensis*) auxin applications prevented pistillate flower formation and an auxin transport inhibitor increased floral initiation (Wood, 2011).

Plant hormones may also co-regulate floral initiation. The application of GA in apple and bayberry (*Morella pensylvanica*) prevented floral initiation and increased the synthesis and transport of auxin (Callejas and Bangerth, 1997; Li et al., 2003). Developing highbush blueberry fruit treated with GA showedz a tenfold increase in endogenous auxin (Mainland and Eck 1971).

Floral initiation may require an optimal level of endogenous auxin, which can be altered by GA applications (Li et al., 2003). Auxins may also regulate the production of GA. In peas (*Pisum sativum* L.) application of an auxin increased the expression of a key enzyme in GA biosynthesis (Ngo et al., 2002).

Photoperiod:

Photoperiod, the length of the light period, is an important signal for plants (Wilkie et al., 2008). Plants are classified based on their response to photoperiod as either short day, long day, or day neutral (Wilkie et al., 2008). Flowering of short day plants is promoted under long dark periods, while short dark periods promote flowering in long day plants; flowering in day neutral plants is not affected by photoperiod (Mockler et al., 1999).

Blueberries are considered short day plants (Darnell, 1991). Lowbush blueberries placed under 16 h photoperiods produced no floral meristems, while those in 14 h or less photoperiods did (Hall et al., 1961). In northern highbush blueberries, floral buds formed in 8 h photoperiods, but not in 16 h photoperiods (Bañados and Strik, 2006). Southern highbush blueberries produced floral meristems in 8 h photoperiods but not in 16 h or 8 h photoperiods with a night interruption (Spann et al., 2003). In rabbiteye blueberries the effects of photoperiod on floral initiation was cultivar specific and quantitative, not qualitative, showing that flower buds could form under non-inductive photoperiods (Darnell, 1991). These studies demonstrate that in very short or long photoperiods formation of floral buds is strongly regulated. However, day lengths under field conditions in Michigan (latitude 43º N) do not reach these levels, but range from 15 to 13 h in July and August when floral initiation is thought to occur. Short day photoperiods (< 11 hr) do not occur in Michigan until October. Effects of photoperiod on highbush blueberry development appear variable, with floral buds forming even under long day conditions potentially as a result

of other pathways co-regulating the process. Pescie et al. (2011) found that southern highbush blueberries growing in Argentina initiate floral buds in both long (15 h) and short (8 h) photoperiods.

Temperature:

Temperature can also alter floral development. Some plants, such as winter wheat, flower in response to extended periods of cold temperatures, a phenomenon known as vernalization (Simpson and Dean, 2002). Plants may respond differently to lowered ambient temperatures during floral initiation periods. Grape (*Vitis vinifera*) plants grown at 30º C had increased numbers of floral primordia, while those at 20º C produced none (Buttrose, 1969). Both ambient temperature and photoperiod influenced floral initiation in short-day strawberries (*Fragaria ananassa*) (Ito and Saito, 1962). Strawberry plants held at 9º C initiated floral meristems, while plants at 30º C did not regardless of photoperiod (Ito and Saito, 1962). Plants grown between 17º C and 24º C initiated floral meristems at photoperiods of 4 to 12 h, but did not at photoperiods of 16 h or more (Ito and Saito, 1962).

Likewise, both photoperiod and temperature may regulate floral initiation in blueberries. Lowbush blueberries in 11 h photoperiods initiated more floral meristems at 21º C, than at 10º C (Hall and Ludwig 1961). Southern highbush blueberries in 8 h photoperiods initiated flower buds at 21º C, but produced 50% fewer flower buds at 28º C (Spann et al., 2004). Variable weather patterns could alter the timing of floral initiation from year to year. In Michigan's climate summer temperatures often reach 30° C and could suppress or shift the timing of floral bud initiation.

Fruit Thinning:

Another strategy to prevent fruit production on young plants is to remove or thin flowers and fruit. Plants may naturally abort flowers or fruits, depending on the species (Bangerth 2000). A lack of carbohydrates may lead to fruit thinning, as apple trees thinned their crop load as sugar levels fell below a threshold level (Beruter and Droz, 1991). However, other studies found no relationship between carbohydrate reserves and thinning in apples (Abruzzese et al., 1995). Another possibility is that changes in hormone concentrations result in thinning (Wertheim 2000). Auxin translocated from the leaf may prevent the activation of the abscission zone (Bangerth 2000; Schroder et al., 2013). If auxin is not translocated, ethylene will activate the abscission zone and lead to fruit or flowering abscission (Bangerth 2000). In a similar theory, termed the auxin-auxin balance, auxin transported from specific regions of the leaf regulates thinning (Schroder et al., 2013).

Artificial thinning is used in many crops to balance vegetative and reproductive growth. In apple, thinning improves the size and quality of the remaining fruit, prevents alternate bearing, and reduces harvest costs (Ebert and Bangerth, 1982; Schroder et al., 2013). Chemical thinning can be accomplished by applying agents that damage floral structures or alter hormone levels (Wertheim 2000; Williams 1994). Endothall, carbaryl, and hydrogen cyanamide reduced fruit numbers in apples (Greene 2002; Williams et al., 1995; Fallahi et al., 1992). Hydrogen cyanamide, cytokinin, and carbaryl reduced fruit set in rabbiteye blueberries (Williamson and NeSmith, 2007; Cartagena et al., 1994), while in highbush blueberries ammonium thiosulfate, BA, and N-(2-chloro-4-pyridyl)-N-phenyl urea reduced fruit set, but also inhibited vegetative growth (Koron and Stopar, 2004). Several factors can affect the efficacy of thinning agents, including application coverage, temperature, humidity, concentration, and plant developmental stage.

Finding more efficient methods to prevent early fruiting and increase plant growth is important for Michigan's blueberry industry. Early fruit production can result in the transmission of pollen borne viruses, reduced plant size and vigor due to competition for photosynthates and nutrients, and an increased time period until peak fruit production. Inhibiting the transition from a vegetative to floral meristem or removing flowers or fruit may be viable options to increase plant growth of young highbush blueberries.

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CHAPTER 2: APPLICATION OF GA AND AUXIN IN YOUNG HIGHBUSH BLUEBERRY TO PREVENT FLORAL INITIATION

Abstract:

Preventing young blueberry (*Vaccinium corymbosum* L.) plants from fruiting can increase their vegetative growth. Gibberellins (GAs) and auxins have prevented floral induction in other species. The purpose of this work was to determine the inhibitory effects on floral initiation with GAs or auxins in new plantings of highbush blueberries. Foliar sprays were applied in several experiments with different application intervals and concentrations; efficacy was determined by counting the number of floral meristems formed. Two GA forms (GA_3, GA_{4+7}) and concentrations (200, 400 $mg \cdot L^{-1}$) were equally effective in preventing floral induction. GA applications in July and August were more inhibitive than those in September and October. Four or eight sprays applied at 1 to 2 week intervals was effective to inhibit initiation by as much as 49% compared to non-treated controls. Auxin treatments did not consistently reduce floral initiation.

Introduction:

The global production of highbush blueberries (*Vaccinium corymbosum*) has increased 400% from 1975 to 2004 (Brazelton and Strik 2007). In short season growing regions like Michigan, blueberry plants may not reach full fruit production for 7 to 10 years after planting (Finn et al.,2003; Strik, 2007). Slow establishment rates deter growers from removing older plants, while newer cultivars may produce higher yields and superior fruit (Hancock et al., 2008).

Growth of new plants can be accelerated by preventing fruit production, as young highbush blueberries allocate over 50% of photosynthates to fruiting (Pritts and Hancock, 1985). Manually removing blueberry floral buds for two years after planting increased shoot length and mass (Strik and Buller, 2005) and reduces the exposure of plants to pollen borne diseases

(Bristow and Martin, 1998). Manual removal of flower buds is recommended (Pritts and Hancock, 1992), but is labor intensive.

Gibberellin (GA) applications reduced floral induction in apples, sweet cherries, and mangos (Lenahan et al., 2006; Lobos and Yuri, 2006; Luckwill, 1970; Turnbull et al., 1996), but the response of blueberries has been variable and cultivar dependent, ranging from 0-95% floral inhibition (Black and Ehlenfeldt, 2007**;** Retamales et al., 2000). Previous studies in blueberries indicated that floral induction was inhibited by GA_3 and GA_{4+7} at concentrations of 150 to 400 $mg \cdot L^{-1}$ (Black and Ehlenfeldt, 2007; Retamales et al., 2000). Single applications inhibited floral induction (Retamales et al., 2000), but multiple sprays applied over several weeks were most inhibitive (Black and Ehlenfeldt, 2007).

GAs are expected to be most inhibitory when applied prior to floral initiation. Applications did not prevent floral development in *Citrus sinensis* after sepal primordia had formed (Lord and Eckard, 1987). Blueberry floral initiation is thought to occur from July to September (Bañados and Strik, 2006**;** Gough et al., 1978), but the timing may be influenced by shoot growth. Blueberries produce episodic and sympodial vegetative flushes. Tamada (1997) concluded from anatomical changes that floral induction on primary shoots of 'Jersey' bushes in Japan (latitude 35.6 N) progressed for two to six weeks after apical abortion, which corresponded to mid-July to the end of August (day length of 14.1 to 12.8 h). If this model is true for all shoots, floral induction could occur from July to October on multiple growth flushes.

However, floral initiation is also influenced by environmental factors. In growth chamber studies, floral induction was strongly suppressed by 14 to 16 h photoperiods **(**Hall et al., 1963; Bañados and Strik, 2006). The amount of floral initiation was proportional to the duration of

exposure to short days (Darnell, 1991) and was suppressed by high temperatures (Spann et al., 2004).

Auxin may also affect floral initiation alone or in combination with GAs. In pineapple (*Ananas comosus*) low concentrations of auxin (10 $mg \cdot L^{-1}$) increased floral initiation, whereas high concentrations (1,000 $mg \cdot L^{-1}$) prevented it (Clark and Kearns, 1942). High concentrations of auxin also prevented floral development in cocklebur (*Xanthium*) (Bonner and Thurlow, 1949). In pecans (*Carya illnoinensis*) auxin applications prevented normal floral initiation by disrupting carpel formation (Wood, 2011). In apple and bayberry, GAs prevented floral initiation and increased the synthesis and transport of auxin (Callejas and Bangerth, 1997; Li et al., 2003). Developing highbush blueberry fruit treated with GAs contained ten times more endogenous auxin than non-treated fruit **(**Mainland and Eck, 1971). Auxins may also regulate the production of GA in some species (Ngo et al., 2002). In summary, both GA and auxin have shown to inhibit floral initiation in some species, while the results regarding young highbush cultivars currently being planted remain absent. The purpose of this work was to determine the inhibitory properties of GAs and auxins in preventing floral initiation in young highbush blueberries currently being widely planted and to determine an ideal application interval and concentration.

Materials and Methods:

Study 1

Commercial blocks of *Vaccinium corymbosum* cultivars 'Elliott', 'Liberty', and 'Aurora' plants established in 2009 in Gobles, MI (42 º22' N, 85 º54' W) were used. Plots containing five plants of similar size were assigned one of seven treatments, with six replicates in a randomized complete block design. Treatments consisted of a water-sprayed control and GA_{4+7} (ProVide,

Valent BioSciences Corp. Libertyville, IL) or GA_3 (ProGibb, Valent BioSciences Corp. Libertyville, IL) applied early (10 and 17 Aug. 2009), mid-season (26 Aug. and 3 Sept.), or late (13 and 21 Sept.). Sprays were applied at 400 $mg \cdot L^{-1}$ active ingredient (a.i.) with a handheld sprayer to the point of runoff. The number of floral buds per plant was recorded once they began to swell in April 2010.

Study 2

This experiment was conducted in commercial fields near Lacota, Michigan (42° 24' N, 86° 07' W) that were planted in 2008 ('Elliott') or 2009 ('Draper', 'Aurora', 'Liberty'). Plants with similar size and vigor were assigned one of six treatments, in a randomized complete block design with eight single-plant replicates. Treatments were a water-sprayed control and 400 $mg \cdot L^{-1}$ a.i. GA_{4+7} applied at two week intervals either early (21 July. to 1 Sept, 2010), late (8 Sept. to 20 Oct., 2010), or early and late (21 July to 20 Oct. 2010). The sixth treatment consisted of sprays on the early and late dates at 200 $mg \cdot L^{-1}$ a.i. All treatments were applied to the foliage until the point of runoff.

Flower bud number per plant was recorded in April, 2011. On 13 May 2011, the length of dead branches was recorded as a measure of winter cold injury and the number of flowers per bud was determined on five randomly selected buds per plant. On 7 July 2011, berry number per bush was recorded and bush height and width in the least and greatest dimensions were measured. Canopy volume was estimated as the product of these dimensions.

Study 3

A three-year-old planting at Michigan State University Horticultural Teaching and Research Center in Holt, Michigan (42º 67' N, 84º 48' W) was used. Three treatments were replicated on eight single-bush plots: 1) non-treated control; 2) GA_{4+7} at 400 $mg \cdot L^{-1}$ applied

weekly from 25 July to 30 Aug. 2011 (early); 3) GA_{4+7} at the same rate applied weekly from 2 Sept. to 9 Oct. 2011 (late). Treatments were repeated in separate sections of 'Liberty' and 'Draper'. Flower buds per plant were counted in Nov. 2011 after leaves abscised. Average shoot length was also recorded by randomly selecting one main branch per plant and measuring the length of each shoot.

Study 4

Commercial fields of 'Liberty' and 'Chandler' planted in 2011 and 2012 near Coopersville, MI (43º 06' N, 85º 93' W) were used. Plants of similar size and vigor were assigned one of nine treatments with six single-plant replicates in a randomized complete block design. Treatments were a nontreated control and 1-naphthalenacetic acid (Fruitone, AMVAC, Los Angeles, CA) applied at biweekly intervals early (14 July to 11 Aug. 2012) or late (25 Aug. to 22 Sept. 2012) at 0.2, 2.0, 20, or 200 $mg \cdot L^{-1}$ a.i. All treatments were applied with a hand held sprayer to the foliage until the point of runoff. The number of floral buds per plant was recorded as previously described. Plants with obvious deer browsing injury were excluded during data collection.

Data from all studies was analyzed with SAS 9.3 (SAS Institute, Cary, NC) as randomized complete block designs with cultivar as the main factor and form and/or timing as sub-plot factors. PROC Glimmix was used to determine statistical significance for floral meristems and flowers per meristem, while PROC Mixed was used to determine significance for shoot length and canopy volume. When significant interactions ($P<0.05$) were found, means separation was performed with PDIFF in LSMEANS statement.

Results:

Study 1

A study was performed to determine the efficacy of different GA forms and applications intervals on the inhibition of floral initiation in several highbush blueberry cultivars. Flower bud numbers were affected by GA application time, (Table 2.1) but not by GA form (GA_3 or GA_{4+7}) or the interactions between GA time, form, or cultivar (Table 2.2). All GA application times reduced flower bud number relative to the control, but the middle timing (26 Aug., 3 Sep.) provided a greater inhibition (43% reduction) than the early timing (22%) or late timing (21%).

Study 2

From the results of Study 1 a subsequent study was performed to determine if longer GA application intervals would lead to greater reductions in floral initiation in recently planted highbush blueberry cultivars. In addition, possible differences in response between GA concentrations was also tested. The time of GA application affected flower bud and berry numbers, while GA rate affected the number of flowers per bud (Table 2.3). The effects of concentration and all interactions between cultivar, time, and concentration were not significant (Table 2.4). The greatest reductions in flower bud and berry numbers resulted from earlier applications (49% reduction) and early plus late timings (42%). The 400 $mg \cdot L^{-1}$ rate reduced the number of flowers per bud relative to the control. Treatments did not affect the length of dead branches per bush (overall mean 32 cm) or canopy volume (39 m^3).

Study 3

Based on the results from study 2, a subsequent study was performed to determine if greater frequency of GA applications would affect the suppression of floral initiation in highbush blueberry cultivars. The analysis of variance indicated that flower bud numbers were significantly reduced by weekly applications of GA_{4+7} in July and August (121 per plant)

compared to numbers on non-treated plants (218) and those treated in September and October (194). The interaction of GA application time and cultivar was not significant, indicating that 'Liberty' and 'Draper' responded similarly. Average shoot length (8.4 cm) was not affected by the treatments.

Study 4

Flower bud numbers were significantly affected by the interaction of cultivar, application timing, and concentration (Table 2.5). Auxin applications had inconsistent effects on flower bud numbers. The highest rate of auxin applied from August to September decreased the number of flower buds in 'Liberty', (10 per plant) but not in 'Chandler' (19) relative to the controls. Other timings and concentrations did not reduce floral induction.

Discussion:

These results are similar to those reported by Black and Ehlenfeldt, 2007 and Retamales et al. 2004 in that GAs inhibit floral initiation in highbush blueberries. Although two sprays at two week intervals reduced flower bud numbers (Table 2.1), maximum inhibition (49%) was achieved from eight applications applied from late July through October (Table 2.3). Weekly sprays (Study 3) did not reduce flower buds more than bi-weekly applications over a similar time frame. Black and Ehlenfeldt (2007) also found multiple applications from July through September most effective, but were able to achieve as high as 95% inhibition. Cultivars responded similarly to GA, even though they ranged in harvest times from relatively early ('Draper') to very late ('Aurora', 'Elliott').

Timing of GA sprays affected floral initiation in each study. Highbush blueberries are short day plants; floral induction was inhibited by 14-16 h photoperiods and promoted in 10 h

photoperiods (Hall et al., 1963; Bañados and Strik, 2006). Black and Ehlenfeldt (2007) suggested that optimum timing for GA may be during inductive photoperiods. However, in southern highbush blueberries, floral initiation occurred in both long and short day photoperiods (Pescie, 2011). In current studies, photoperiods were longer than 14 hr until 7 Aug. and 1 Aug. (Black and Ehlenfeldt; 2007). Although treatments included multiple sprays over different time periods, the most effective was mid-August to early September (Tables 2.1, 2.3), consistent with previous reports (Black and Ehlenfeldt, 2007; Retamales et al., 2000). Interestingly, GA applied prior to inductive day lengths also reduced flower bud numbers (Black and Ehlenfeldt, 2007; Retamales et al., 2000). In addition, greenhouse grown blueberries treated with GA during bloom (to induce parthenocarpy) produced fewer flower buds (Mainland and Eck, 1969). GA sprays could result in elevated tissue levels and activity for several weeks after application (Samach and Smith, 2013). The impact of photoperiods ranging from 14 to 12 h on highbush blueberries floral initiation is somewhat unclear, but may partially control floral initiation in field conditions.

The fact that multiple GA sprays over several weeks provided the greatest reduction in flower bud numbers suggests that induction occurs over a lengthy time period. Blueberries can produce multiple shoot flushes, each terminating in apical abortion. In Michigan, most primary flushes terminate growth in June, while later flushes may grow into September, depending on the cultivar, environment, and plant health (Gough et al., 1978). Tamada (1997) suggested that blueberries initiate flower buds only for two to six weeks after apical abortion. If this is true, plants may initiate flower buds from July to October, depending on the timing of growth flushes. It is unclear how photoperiod affects this timing, since many primary shoots terminate growth in June, several weeks before photoperiods become inductive.

GA_3 and GA_{4+7} were equally effective in preventing floral initiation (Table 2.2), which is consistent with earlier blueberry studies (Retamales et al., 2000; Black and Ehlenfeldt 2007). Over 100 forms of GAs occur naturally, but only a few are biologically active (MacMillan, 2002). GA structures with a hydroxylation of the C_3 position and double bonds of $C_{(1,2)}$ or $C_{(2,3)}$ were most effective at preventing floral induction in cherry (Oliveira and Browning 1993). GA_3 and GA_7 have a C_3 hydroxylation and $C_{-1,2}$ double bond, while GA_4 has C_3 hydroxylation (Oliveira and Browning 1993).

Concentrations of 200 or 400 $mg \cdot L^{-1}$ GA were effective in preventing floral initiation (Table 2.4). This is supported by Retamales et al. (2000) who found rates of 150 or 300 $mg \cdot L^{-1}$ equally effective. Black and Ehlenfeldt (2007) found rates of 400 $mg \cdot L^{-1}$ more effective than 200 $mg \cdot L^{-1}$, but no different than 600 $mg \cdot L^{-1}$. In apples, inhibition of floral initiation was dependent on GA concentrations in a linear manner (Greene, 1993).

In our preliminary study, auxin applications did not consistently reduce floral initiation (Table 2.5). In 'Liberty' the highest rate applied from August to September reduced floral initiation compared to the non-treated control, but other rates and timings had no effect, and 'Chandler' showed no floral inhibition to any auxin treatment. It is unclear why auxin had minimal effect on induction in this study, but substantial effects were observed in other species (Clark and Kearns, 1942; Bonner and Thurlow, 1949). Salisbury (1955) suggested that auxin needs to be applied prior to inductive photoperiods to prevent floral initiation. In the current study, photoperiods were longer than 14 h during the early treatment, but shorter than 14 h during the late treatment period.

In summary, these studies indicate that GA partly inhibits floral initiation in young highbush blueberries currently being widely planted. The effect is consistent across several cultivars, but multiple sprays over several weeks are needed for maximum effect. Four to eight applications may be impractical since GA products are relatively expensive. The growth benefits from partially inhibiting fruiting needs to be determined. This preliminary study indicated that auxin did not inhibit flowering, but further studies are needed to determine its commercial potential.

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CHAPTER 3: IMPACT OF THINNING ON FRUIT SET AND VEGETATIVE GROWTH IN YOUNG HIGHBUSH BLUEBERRIES

Abstract:

Highbush blueberries often require seven to ten years to reach full fruit production in Michigan's climate. Thinning fruit has been used in other plants to balance reproductive and vegetative growth. The purpose of this study was to determine the efficacy of removing flowers and fruit to promote vegetative growth in highbush blueberries. Experiments were performed in 2012 and 2013 and the effect on fruiting and vegetative growth was measured. In 2012, foliar applications of 6-benzyladenine (BA) reduced fruit set, but also decreased the number of vegetative shoots relative to the control, while applications of carbaryl had no affect; manually thinning reduced fruit set and doubled the number of shoots and canopy growth. In 2013, manually thinning treatments of 0, 50, or 100% of flower buds did not affect shoot growth. Flower thinning can be used to enhance vegetative growth in young highbush blueberries, but the impact is also dependent on how heavily plants are fruiting. Further studies are needed to determine whether other agents can reduce fruit set without adversely affecting vegetative growth.

Introduction:

Highbush blueberries can produce fruit within a year of planting, but often do not reach maximum fruit production for an additional six to nine years (Strik, 2007; Finn et al., 2003; Black and Ehlenfeldt, 2007). Preventing fruiting on young plants can increase growth, decrease the exposure of plants to pollen borne diseases, and reduce the interval from planting to peak fruit production (Strik and Buller, 2005; Pritts and Hancock, 1992). Highbush blueberry plants that had their floral meristems manually removed for the first two seasons after planting had 44% greater root mass and 60% greater shoot mass (Strik and Buller, 2005). Added growth may

result from a reduction in competition for carbon assimilates, as fruiting accounts for half of available photosynthates in young highbush blueberries (Pritts and Hancock, 1985). The removal of floral meristems on young plants is recommended (Pritts and Hancock, 1992), but highly labor intensive (Strik and Buller, 2005). More efficient strategies are desired to prevent fruiting in young highbush blueberries.

Fruit thinning is a complex process involving both environmental and internal factors (Dennis 2000; Schroder et al., 2013). Plants may abort a portion of their flowers depending on environmental conditions (Bangerth 2000). The mechanisms controlling thinning are unclear, but may involve assimilate supply, sink activity, seed number, ethylene production, and polar auxin transport (Schroder et al., 2013). Artificial thinning, by either chemical or mechanical means, has been used to balance vegetative and reproductive growth in other plants (Schroder et al., 2013). In apples, thinning improves the size and quality of the remaining fruit and prevents alternate bearing cycles (Ebert and Bangerth, 1982). Chemical treatments of hydrogen cyanamide, cytokinin, and carbaryl successfully reduced fruit set in rabbiteye blueberries (Williamson and NeSmith, 2007, Cartagena et al., 1994), while in highbush blueberries ammonium thiosulfate, BA, and N-(2-chloro-4-pyridyl)-N-phenyl urea reduced fruit set, but also inhibited vegetative growth (Koron and Stopar, 2006). The purpose of this study was to determine the efficacy of different agents for removing flowers and fruit and promoting vegetative growth on highbush blueberries.

Materials and Methods:

All studies were conducted at Michigan State University's Horticultural Teaching and Research Center in Holt, Michigan (42º 67' N, 84º 48' W).

Study 1

Study one was conducted in 2012 using four-year-old 'Bluecrop' plants of similar size and vigor. A completely randomized block design was used, which included four treatments with eight single bush replicates. Treatments included foliar sprays of BA (MaxCell, Valent Bioscience Corp., Libertyville, IL) at 400 $mg \cdot L^{-1}$ or carbaryl (Sevin SL, Bayer Environmental Sciences, Research Triangle Park, NC) at 800 $mg \cdot L^{-1}$. Sprays were applied with handheld sprayer till the point of runoff. Other treatments included a non-treated control and a manual removal (hand thinning) of all fruiting structures. Plants were treated on 24, May, about two weeks after petal fall.

To measure fruit set, flowers were counted on four randomly selected shoots per plant on 2, April and viable berries were counted on the same branches on 18, June, just before maturity. Fruit set was calculated by dividing berry number by flower number. The number of vegetative shoots per plant was counted on 13, June and 21, Aug. Canopy volume growth was estimated by recording the height and width of the plants in the greatest and smallest dimensions to the nearest inch on 22, May and 21, Aug.

Study 2

Study 2 was conducted in 2012 using mature plants from the cv. 'Jersey'. Experimental units consisted of 10 to 30 cm long branches, which had flower buds. Seven uniform branches were randomly selected on each of ten bushes, and seven treatments were assigned to one branch on each bush. Treatments included carbaryl, BA, and a non-treated control; all applications were applied as previously described on 17, 24, 30 May, with petal fall on 14, May. Fruit set was determined as previously described on 18, June.

Study 3

Study 3 was performed in 2013 with one-year-old ‘Duke’ plants of similar size and vigor. Plants were placed into a randomized complete block design, consisting of three treatments and eight single plant replicates. Treatments were a non-treated control, a 50% hand thinning of all floral structures, or a 100% hand thinning of floral structures applied on 14, May once floral buds had opened. Netting was placed over all bushes to prevent bird damage. Berries were hand harvested, counted, and weighed on 8, 14, and 22, of July. The number and length of vegetative shoots per plant were recorded on 26, Aug.

Data from all studies were analyzed with SAS 9.3 (SAS Institute, Cary, NC), as a randomized complete block design. For study 1 and 3, treatment was the main factor and for study 2 treatment was the main factor and timing was a sub-plot factor. PROC Glimmix was used to determine statistical significance for fruit set, number of vegetative shoots, and berry number, while PROC Mixed was used to determine statistical significance for canopy volume, fruit weight, and shoot length. When treatment effects were significant based on ANOVA table ($P<0.05$), differences between treatment means were analyzed by LSMEANS test.

Results:

Experiments were performed to determine the efficacy of thinning treatments to reduce fruit set in highbush blueberries. Hand thinning and BA treatments significantly reduced fruit set compared to the control, while carbaryl did not (Table 3.1). Canopy growth under hand thinning treatment was significantly greater compared to all other treatments, an approximate 150% increase compared to the control. Treatments also significantly affected the number of vegetative shoots per plant (Table 3.2). BA treatment resulted in a reduction in the number of vegetative shoots per plant compared to all other treatments on 13, June. Hand thinning resulted

in an increase in the number of vegetative shoots per plant immediately after treatment and a near doubling at the end of the experiment compared to control.

Study 2 was performed to determine the efficacy of thinning treatments applied to individual shoots at different time intervals. Treatment timing, form, and the interaction of timing and form did not significantly affect fruit set for study 2 (Table 3.3).

Study 3 was performed to determine the effect of different thinning rates on the vegetative growth of young highbush blueberries. For study 3 treatments affected the number of berries per plant, but not the number of shoots, shoot length, or average berry weight (Table 3.4). Plants receiving the 100% thinning treatment had significantly fewer berries per plant than those under the 50% thinning or non-treated control.

Discussion:

In 2012, BA significantly reduced fruit set when applied to the entire canopy and decreased the number of shoots per plant (Tables 3.1 and 3.2). These results are similar to those of Koron and Stopar (2006), who found that two forms of cytokinins, BA and CPPU, reduced fruit set and stunted shoot growth in highbush blueberry. Koron and Stopar (2006) also used similar timings and concentrations of BA and found that concentrations of 200 $mg \cdot L^{-1}$ BA effectively reduced fruit set, but had negative impacts on growth. At concentrations of 20 $mg \cdot L^{-1}$ BA had no effect on either fruit set or shoot growth. It appears that the act of stressing the plant to induce thinning, also negatively impacts shoot growth and thereby negates possible benefits for increased shoot growth.

Applications of cytokinins to rabbiteye blueberries in similar concentrations as the current study reduced fruit set, without inhibiting vegetative growth (Cartagena et al., 1994).

Differences in response may have resulted from species specific reactions or bud break patterns. Vegetative buds in highbush blueberries begin growing before floral buds open (Bell, 1950), whereas rabbiteye blueberries flowering and fruit set occur prior to vegetative growth in (Maust et al., 1999). This difference in timing may have exposed highbush blueberry vegetative shoots to higher concentrations of cytokinins resulting in injury.

Foliar treatments to individual shoots did not affect fruit set (Table 3.3). Similar results were found in apples, as BA applied to leaves and canopy was more effective at thinning than those applied to fruit alone (Greene et al., 1992; Schroder et al., 2013). This may be a result of insufficient coverage or absorption. Thinning may be regulated by polar auxin movement from the apical region of the leaf (Schroder et al., 2013). If auxin is expressed in high enough concentrations, activation of the abscission zone ensues leading to abscission (Schroder et al., 2013). In roots, cytokinins regulates polar auxin transport by altering expression of auxin transport components (Ruzicka et al., 2009). Similar processes could regulate auxin transport in leaves. BA applied to individual shoots may not increase auxin transport to sufficient levels compared to when BA was applied to the entire canopy. This could explain inconsistent thinning effects of canopy applied versus shoot applied BA.

In 2012, hand thinning significantly increased the number of vegetative shoots per plant and canopy volume growth compared to control plants (Tables 3.1 and 3.2). These results are in agreement with Strik and Buller (2005), who found removing floral meristems for two consecutive years promoted shoot growth. Maust et al. (1999) also found an inverse relationship between floral meristem density and canopy establishment in southern highbush blueberries. However, in 2013 hand thinning of 0, 50, or 100% of floral structures did not affect shoot number or length per plant (Table 3.4). This may be due to a small number of floral meristems

(12) or berries (10) per plant. Reduced fruiting stress, would result in a reduced response to manual thinning. In addition, plants in current study appeared to exhibit nitrogen deficiency, which would hinder growth and obscure differences between treatments.

Preventing fruiting on young highbush blueberries can result in greater plant growth. This study demonstrated that thinning flowers can be an effective way to increase the number of vegetative shoots and canopy growth. Thinning should be done prior to or soon after pollination, as fruit pericarp tissue begins rapid growth and competes with vegetative growth for resources (Finn and Luby, 1986). The potential for increased vegetative growth is also dependent upon the number of floral buds set and cultural factors. From the current study, plants that averaged ten flower buds did not respond to thinning treatment. Growers may use this information and incorporate into management model, in which plants that average ten or fewer flower buds would not require thinning treatment. Chemical thinning could be a labor saving alternative to manually thinning, but further studies are needed to determine if other agents, concentrations, or timings may reduce fruit set without negatively affecting vegetative growth.

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CHAPTER 4: THE SHOOT GROWTH PATTERN AND DISTRIBUTION OF FLOWER BUDS IN YOUNG HIGHBUSH BLUEBERRIES GROWN IN MICHIGAN

Abstract:

The patterns of shoot growth and floral initiation in young highbush blueberries grown in Michigan are not fully understood. Highbush blueberries exhibit episodic and sympodial growth, which commences in the spring and ceases with the abortion of the apical meristem. Two to six weeks later a distal, lateral meristem may commence growth, forming the next growth flush. This process may be repeated throughout the season. The purpose of this experiment was to describe the chronology of shoot growth flushes over the growing season and to determine the distribution of flower buds between growth flushes. In 2012 and 2013, the time of shoot growth initiation and termination, final shoot length, and flower bud numbers were recorded on eight plants of recently planted 'Duke', 'Draper', and 'Liberty'. The majority of shoot growth occurred from May to July in the first and second flushes. In 2012, the majority of flower buds developed on secondary shoots, whereas in 2013 the majority of flower buds were located on primary shoots. Primary shoots that initiated later growth flushes produced fewer flower buds than those that did not. This pattern of shoot growth creates a lengthy floral initiation time period, based on the when shoot growth ceases and whether subsequent growth flushes occur.

Introduction:

Highbush blueberries in short growing regions may need seven to ten years to reach maximum fruit production (Strik, 2007) whereas bushes in longer growing seasons often exhibit faster establishment (Finn et al., 2003). Preventing young blueberry plants from fruiting can increase their vegetative growth (Strik and Buller, 2005; Williamson and NeSmith, 2007). Flowers and flower buds are often removed manually from young plants, but this is time

consuming (Strik and Buller, 2005). Inhibiting flower bud formation could be a low-cost alternative to manual removal. Gibberellin (GA) applications have prevented flower bud formation in other species (Lenahan et al., 2006; Turnbull et al., 1996; Bradley and Crane, 1960), but results have been inconsistent in highbush blueberries (Black and Ehlenfeldt, 2007; Retamales et al., 2000). GA effectiveness appears to be dependent on application timing, as GA was ineffective once the transition from a vegetative to a reproductive meristem occurred (Lord and Eckard, 1987; Sachs et al., 1967). However, this timing in highbush blueberry is not well understood.

Reproductive development begins with floral induction, as signals are transported from the leaves to the vegetative meristem (Samach and Smith, 2013). When these signals are perceived, floral initiation, the transition from a vegetative meristem producing leaves and shoots to a reproductive meristem, producing sepals, petal, stamens, and carpels, occurs and can be observed as the formation of floral primordia (Bowman et al., 2012; Samach and Smith, 2013). Based on histological examination, Gough et al. (1978) found that floral initiation in highbush blueberries occurred six to nine weeks after full bloom. Tamada (1997) also found floral initiation occurring about nine weeks after full bloom and linked the timing of initiation to shoot growth patterns. Floral initiation began two weeks after the current shoot ceased growth and was completed after one month (Tamada, 1997).

Temperature and photoperiod also influence floral development in blueberries. Southern highbush blueberries (*Vaccinium corymbosum* interspecific hybrid) formed flower buds at 21°C, but numbers were greatly reduced at 28°C (Spann et al., 2004). Photoperiod also influences floral development in blueberries. Lowbush blueberries (*Vaccinium angustifolium*) in 16 h photoperiods produced no floral meristems, while those in 12, 10, or 8 h photoperiods did (Hall

et al., 1961). Likewise, northern highbush blueberries produced floral buds at photoperiods of 8 h, but not 16 h (Bañados and Strik, 2006). Southern highbush blueberries in 8 h photoperiods produced floral meristems, while those in 16 h or 8 h photoperiods plus a night interruption did not (Spann et al., 2003). These studies indicate that floral bud induction is strongly regulated by photoperiod. However, natural photoperiods in Michigan (latitude 43° N) during July and August, when floral initiation is thought to occur, range from 15 to 13 h. Other studies have shown more variable results of floral initiation in response to photoperiod. Pescie et al. (2011) found southern highbush blueberries growing in Argentina exhibiting two distinct floral initiation periods, with flower buds developing under both long day (16 h) and short day photoperiods (8 h). This study was performed in Buenos Aires (34°S, 59°W), which may have different environmental conditions than plants grown in Michigan. These studies indicate that floral development in field grown blueberries likely occurs even under long day conditions.

The shoot growth pattern of highbush blueberries is episodic and sympodial (Gough et al., 1978). Most shoots originate from axillary buds on the previous seasons growth, but some shoots develop from adventitious buds near the crown or roots (Bañados 2006). Growth flushes terminate in the abortion of the apical meristem (Gough et al., 1978). This stage is referred to as black tip because the blackened shoot apex and subtending leaf and stem tissue are easily visible (Gough et al., 1978). After two to five weeks, a distal meristem may begin growing and form a second shoot or flush (Gough et al., 1978). The number of growth flushes is dependent upon plant health, environmental conditions, and cultivar, with later fruiting varieties producing fewer growth flushes than early and mid-season varieties (Gough et al, 1976). Growth flushes may develop flower buds at different times, with later flushes producing fewer flower buds (Bañados, 2006). Gough et al. (1976) observed highbush blueberries in Rhode Island to have flower buds

interspersed on all flushes and higher numbers on thicker diameter shoots and observed up to five growth flushes on a given plant. The purpose of this experiment was to describe the chronology of shoot growth flushes over the growing season and to determine the distribution of flower buds between growth flushes.

Materials and Methods:

Study 1

Study was conducted in 2012 and 2013 using one-year-old 'Liberty' plants at commercial planting near Coopersville, MI (43º 06' N, 85º 93' W) and three-year-old 'Draper' and 'Duke' plants at a planting at Michigan State University Horticultural Teaching and Research Center (HTRC) in Holt, MI (42º 67' N, 84º 48' W). Eight plants of similar size from each cultivar were randomly selected in the spring prior to bud break. Plants at HTRC were managed under organic practices, with manual removal of weeds, and solid set irrigation, while plants in Coopersville were managed under conventional practices and irrigated with drip lines. Each new shoot was tagged as it began growing, and shoot length was measured at approximately two week intervals thereafter. Flush number was also determined for each axillary meristem by observing apical meristem abortion evidence of a blackened shoot apex and subtending leaf and stem tissue and subsequent shoot growth. The first flush of shoots originated from axillary buds on the previous year's shoots. Shoots in the second flush originated from axillary buds on the current-season first shoots. The third flush of shoots originated from axillary buds on the current-season second flush of shoots. Petal fall was recorded in the spring after full bloom, once half the flowers abscised. The number of floral meristems on each shoot was recorded in late fall, once the

enlarged, rounded floral meristems could be distinguished from the smaller, pointed vegetative buds.

Study 2

Fifteen three-year-old plants of 'Bluecrop' and 'Elliott' were randomly selected at the HTRC. One major branch was randomly selected from each plant, brought indoors and separated into individual growth flushes based on visual evidence of apical meristem abortion and subsequent shoot growth. Evidence of apical meristem abortion consisted of circular rings on the shoot, which was the abscission layer from the black tip event. The total shoot length and number of floral meristems from each flush was recorded as previously described.

Growing degree days and temperature data for 2012 and 2013 was obtained from automated weather stations (http://www.agweather.geo.msu.edu/mawn/) located nearest to study locations at West Olive, MI (42º 97' N, 86º 07' W) or at Holt, MI (42º 67' N, 84º 48' W). The Baskerville-Emin growing degree model with a base temperature of 50° F was used to calculate the number of growing degree days for each month from January to October. For 2012 and 2013, the average maximum air temperate was determined from May to October, by using the mean function feature.

Data from each study was statistically analyzed with SAS 9.3 (SAS Institute, Cary, NC) with flush number as the main factor. PROC Glimmix was used to determine statistical significance for the number of floral meristems, while PROC Mixed was used to determine statistical significance for shoot length. When significant interactions ($P<0.05$) were found, means separation was performed with PDIFF in LSMEANS statement.

Results:

The growth pattern observed was episodic and sympodial (Figure 4.1). In 2012, the first growth flush started in April and concluded by June (Figure 4.2). The second growth flush occurred from May to July, while the third flush grew from July to September (Figure 4.2). About 80% of the primary shoots in 'Draper' and Liberty' exhibited black tip formation by 22, May. Primary shoots of Duke reached 80% dieback by 4, June. Black tip formation was observed on 80% of all secondary shoots in 'Draper' and 'Duke' by 9, July and in 'Liberty' by 24, July. A third growth flush was first observed on 25, July.

In 2013, the first flush of growth began in May and continued until July (Figure 4.3). The second flush grew from July through August, while the third flush grew from August to September (Figure 4.3). 80% of primary shoots in 'Duke' exhibited black tip on 19 June and in 'Liberty' by 6 July and in 'Draper' on 5 June. 80% black tip formation of secondary shoots varied considerably by cultivar, and was observed on 4 July (Draper), 21 July (Duke) and 11 August (Liberty).

In 2012, there was significantly more shoot length in the first and second flushes than in the third flush (Table 4.1). In 2013, the shoot length in the first flush was greatest, accounting for 71 to 90% of all shoot growth (Table 4.2). For 'Draper' and 'Duke' in 2012, there were significantly more floral buds in the second flush, than in the first or third flush, accounting for 61 to 72% of all floral buds (Table 4.1). In 2012, average shoot length per growth flush was not affected in 'Draper' and 'Liberty', but the third flush was significantly shorter than the first or second flush in 'Duke' (Table 4.1). Similar trends were found in 2013 as 'Liberty' and 'Duke' had significantly shorter average shoot lengths in the third growth flush than other growth flushes (Table 4.2).

A study was conducted to determine if the number and length of shoots and flushes and position of flower buds could be determined more easily by examining dormant branches collected after the growing season. Similar trends in shoot growth were observed by examining dormant branches of 'Bluecrop' and 'Elliott' (Table 4.3). The first growth flush accounted for a significantly greater amount of shoot length (57 to 80% of total growth) than later flushes (Table 4.3). The third flush resulted in minimal amounts of shoot growth, representing only 5 and 1% of total growth in 'Bluecrop' and 'Elliott', respectively (Table 4.3). In 'Bluecrop' the greatest number of floral meristems was in the second flush, while in 'Elliott' the greatest number of floral meristems was in the first growth flush.

The 2012 growing season was warmer and started earlier than in 2013. In March of 2012, 400 growing degree days accumulated, compared to March of 2013 when less than 5 growing degree days accumulated. (Table 4.4). Average maximum air temperature was higher in 2012 compared to 2013. The greatest monthly difference occurred during July, with 2012 averaging about 6° C higher temperatures than 2013 (Table 4.5). In 2012, petal fall was observed on 12 May, whereas in 2013 it was observed on 5 June.

The event of multiple growth flushes affected the distribution of floral buds on primary shoots. Primary shoots that underwent multiple flushes had significantly fewer flower buds those that did not have subsequent growth flushes during 2012 (Table 4.6) and 2013 (Table 4.7).

Discussion:

Growth patterns were episodic and sympodial, as described by Gough et al., (1978). In 2012, 49, 41, and 10% of shoot growth was associated with the first, second, and third flushes, respectively (Table 4.1) In 2013, the first, second and third flushes accounted for 79, 19 and 2% of shoot growth (Table 4.2). These results are in agreement with Bañados (2006) who found

80% of shoot biomass within the primary flush, and about 96% of all shoots were primary or secondary flushes. This is also in agreement with Gough et al. (1978) who found the greatest amount of growth occurring from late spring to early summer and Mingeau et al. (2001) who found the greatest shoot elongation rates occurred between early June and the middle of July. Mingeau et al. (2001) was likely viewing the first and second growth flush during that time period. A reduced amount of shoot growth occurring later in the growing season may be a result of decreasing photoperiods. Photoperiods were over 14 h in July and declined to 12 h in September. Highbush blueberries in continuous 8 h photoperiods produced fewer growth flushes and 80% less shoot biomass than plants in 16 h photoperiods (Bañados and Strik; 2004). Whether plants in current studies exhibited similar reductions in growth as a result of decreasing photoperiods is not known. Another possible explanation for a reduction in late season growth may be decreased available photosynthates. Highbush blueberries are fairly heat sensitive, and increasing temperatures from 20°C to 30°C reduced carbon assimilation 22 to 51% (Hancock et al., 1992). In both years, average maximum temperatures were highest in July, with recordings above or near 30°C (Table 4.5). In 2012, there were 16 days with daily maximum temperatures at or above 30°C, whereas in 2013 there were 7 days of similar conditions (http://www.agweather.geo.msu.edu/mawn/). This could lead to decreases in available photosynthates, resulting in reductions of later growth flushes. A final possibility could be the effect of fruiting stress on shoot growth. However, 'Liberty' plants had all flowers removed in the spring, while 'Duke' and 'Draper' plants had partial fruit removal, thereby reducing the potential impact on shoot growth.

The weather conditions differed in 2012 compared to 2013. The most striking difference was the much warmer weather in March of 2012 compared to 2013 (Table 4.4), which resulted in

early plant development in 2012. Petal fall occurred on 12 May in 2012 and on 5 June in 2013. Shoot growth also started earlier in 2012 compared to 2013. In addition, average maximum temperatures appeared higher in 2012 compared to 2013 (Table 4.5). These factors could have resulted in differences in the shoot growth pattern between 2012 and 2013. High temperatures in 2012 could have caused plant stress and led to faster apical abortion of primary shoots. Evidence of this can be seen when 80% black tip formation of primary shoots occurred. In 2012, 80% of primary shoots underwent apical abortion by 22 May, about two weeks after petal fall. However, in 2013 80% of primary shoots underwent apical abortion by 4 July, about a month after petal fall. Faster abortion of primary shoots would have resulted in a decrease in the percentage of shoot growth in the first flush during 2012 compared to 2013, which was also observed (Tables 4.1 and 4.2). Another possibility is that by starting shoot growth earlier in 2012, there was a greater amount of time for multiple growth flushes to occur. This seems unlikely as Bañados (2006) observed mature plants in Oregon, which have a long growing season, to have only 5% of shoots within the third flush. However, Bañados (2006) did not describe the timing of shoot growth, so the time interval of growth flushes is not known.

The distribution of floral buds within growth flushes differed between 2012 and 2013. In 2012, the second flush had significantly more flower buds (51 to 72% of all flower buds) than the first or the third. However, in 2013 there was significantly more flower buds in the first and second flushes of ‘Duke’, ‘Draper’, and ‘Liberty’ than in the third. The third growth flush generally had the fewest flower buds, with 9 to 43% in 2012, and 1 to 12% in 2013. These results are supported by Bañados (2006), who hypothesized that later growth flushes would produce fewer flower buds. Cultivars exhibit similar trends in floral bud distribution, even though varieties included early (Duke), midseason (Draper) and late (Liberty) ripening types.

The number of flower buds on primary shoots was affected by subsequent growth flushes. Primary shoots that did not undergo additional growth flushes had greater numbers of floral buds than primary shoots that did undergo subsequent growth flushes (Tables 4.6 and 4.7). This is in agreement with Bañados (2006), who found flower buds located on the distal portions of the last growth flush. However, Gough et al. (1976) observed flower buds interspersed on all flushes and not only on the distal portion of shoots. The reason for this discrepancy is not clear, but in the current studies flower buds on the second and subsequent third growth flush were observed. Subsequent growth flushes appear to inhibit floral initiation on primary shoots, thereby ensuring that flowers and fruit will be near the apex of the branch.

Floral initiation in highbush blueberries appears to occur over an extended time period, based on the timing and number of growth flushes. Tamada (1997) observed flower bud initiation on primary shoots, which did not undergo subsequent flushes, starting two weeks after shoot growth ceased. Based on this model, plants in the current study would begin initiating flower buds on primary shoots during June (2012) or July (2013). It is not known if later growth flushes exhibit a similar floral initiation timeline. However, even if initiation began immediately after a shoot ceased growth, flower bud initiation would not occur until the end of July on second growth flushes and in August on the third growth flush. In addition, subsequent growth flushes appear to inhibit flower bud formation on primary shoots, which also indicates that highbush blueberries initiate flower buds at separate intervals. Southern highbush blueberries undergoing floral initiation months apart, depending on whether flower buds were formed on the first or second growth flush (Pescie et al., 2011). This may partially explain results of Retamales et al. (2000) who found variable inhibition of floral initiation with GA application. Due to long floral

initiation interval, GA would likely have to be repeatedly applied from May through October to observe more complete inhibition of floral initiation.

In summary, highbush blueberries shoot growth occurs in flushes from April to September, with the majority of growth occurring early in the growing season. The timing of growth flushes varied between seasons and was associated with growing degree accumulation in the spring and maximum ambient temperature in the summer. Flower buds were found on the first, second, and third flushes, however the number of flower buds on primary shoots was inhibited when subsequent growth flushes occurred. Due to this growth pattern, floral initiation in highbush blueberries appears to occur from June to September. From current studies, it appears that shoots did not cease growth after a set shoot length or node number. Further studies could be needed to determine if bio-regulators could delay the process of apical abortion and thereby increase shoot growth and plant establishment.

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APPENDIX

Introduction:

Highbush blueberry plants in Michigan have a lengthy plant establishment time from planting prior to reaching peak fruit production (Strik, 2007). Preventing fruiting on young plants reduces this interval and increases vegetative growth (Strik and Buller, 2005). Manual removal of flower buds is recommended, but labor intensive (Pritts and Hancock, 1992). More efficient ways to prevent fruit production on young plants are needed.

Floral initiation, which is the transition from a vegetative meristem to an inflorescence meristem, is a complex process (Samach and Smith, 2013). Some plants use photoperiod to regulate the timing of floral initiation (Wilkie et al., 2008). Plants may be classified based on their flowering response as either long day, short day, or day neutral. Under long nights, short day plants flowering is promoted, while with long day plants flowering is promoted in short dark periods; day neutral plants flower development is not affected by photoperiod (Mockler et al., 1999).

Blueberries are thought to be a short day plant, with floral induction occurring under long dark periods (Darnell, 1991). Lowbush blueberries placed under 16 h photoperiods produced no floral meristems, while those in 14 h or less photoperiods did (Hall et al., 1961). In northern highbush blueberries, floral buds formed in 8 h photoperiods, but not in 16 h (Bañados and Strik, 2006). Southern highbush blueberries produced floral meristems in 8 h photoperiods but not in 16 h or 8 h photoperiods with a night interruption (Spann et al., 2003). In rabbiteye blueberries the effects of photoperiod on floral initiation was cultivar specific and quantitative, not qualitative, showing that flower buds could form under non-inductive photoperiods (Darnell, 1991). Day lengths under field conditions in Michigan (latitude 43º N) range from 15 to 13 h in July and August when floral initiation is thought to occur. Altering the photoperiodic conditions

of highbush blueberries could be a labor saving alternative to reduce fruiting in young highbush blueberries. The purpose of this experiment was to determine if supplemental lighting could inhibit floral initiation in field grown highbush blueberries by interrupting plants photoperiod response.

Material and Methods:

A study was performed at Michigan State HTRC in Holt MI. Sixteen four-year-old highbush blueberry 'Bluecrop' plants were randomly selected and assigned into one of two treatments, either a non-treated control or a light interruption treatment. Light interruption was accomplished by using two 200W halogen lights per plant, with lighting directed to the plant canopy. Light intensities at canopy level were 2 $uMol \cdot m^{-2} \cdot sec^{-1}$. Each plant was separated by three foot by three foot black plastic squares, which prevented light pollution between plants. Lights were turned on between midnight and 4 am daily from July to October. The number of flower buds was recorded per plant in the fall after leaf senesce. Data was analyzed with SAS 9.3.

Results and Discussion:

A study was performed to determine if floral initiation could be inhibited in field grown highbush blueberries by altering the photoperiod response using night interruption lighting. Based on ANOVA table, no significant differences in the number of flower buds per plant was found between the non-treated control (135) or light interrupted plants (109). The use of light interruption would have simulated 16 h photoperiods by disrupting the long dark period, but floral initiation still occurred. These results contradict previous work of Spann et al. (2004)

who found that floral initiation in southern highbush blueberries was inhibited with night interruption lighting.

There are several possible reasons to explain the discrepancy between studies. One possibility is that the light intensity of 2 $uMol \cdot m^{-2} \cdot sec^{-1}$ was not intense enough to activate the phytochromes in highbush blueberries. Spann et al. (2003) who was able to inhibit floral initiation with night interruption lighting had greater light intensities, of 75 $uMol \cdot m^{-2} \cdot sec^{-1}$. Another potential issue is whether enough of the plant canopy was receiving night interruption lighting in current study. In summary, the use of night interruption lighting was not able to inhibit floral initiation in field grown highbush blueberries in Michigan. Further studies could be performed to investigate if higher light intensities could inhibit floral initiation in field grown highbush blueberries.

Table 2.1 Effect of GA applied at 400 $mg \cdot L^{-1}$ during 2009 on floral meristem numbers in 2010. Data are means of three cultivars (Aurora, Elliott, Liberty) and two GA forms (GA_3, GA_{4+7})

Application dates	Flower buds/plant	
Control	69	a[z]
10, 17 Aug.	54	b
26 Aug., 3 Sep.	39	c
13, 21 Sep.	54	b

[z]Means followed by the same letter are not significantly different based on LS Means test at $P \leq 0.05$.

Table 2.2 ANOVA results for 2009 study based on differences in the number of flower buds per plant in response to different effects

Effect	*P* value
Form	0.27
Variety x form	0.47
Timing	0.001
Variety x timing	0.104
Form x timing	0.45
Variety x form x timing	0.19

Table 2.3 Effect of GA_{4+7} application time and concentration ($mg \cdot L^{-1}$ a.i. as ProVide) during 2010 on plant development in 2011[z]. Data are means of four cultivars (Elliott, Draper, Aurora, Liberty)

GA application	Flower buds/ plant		Flowers/ bud		Berries/ Plant	
Time						
None	92	a[z]	4.8		182	a
Early (21July, 4, 18 Aug., 1Sep.)	57	b	4.2		132	bc
Late (8, 22 Sep., 6, 20 Oct.)	76	a	4.5		162	ab
Early and late (all dates)	47	b	4.2		105	c
Concentration[y]						
0	92		4.8	a	182	
200	54		4.7	ab	133	
400	58		4.1	b	124	

[z]Means within a column group not followed by the same letter are significantly different at $P \leq 0.05$ based on LS Means test

Table 2.4 ANOVA results for 2010 study based on differences in the number of flower buds per plant in response to different effects

Effect	*P* value
Concentration	0.30
Variety x concentration	0.36
Timing	0.01
Variety x timing	0.53
Concentration x timing	0.57

Table 2.5: Effect of auxin applications on floral induction in 'Liberty' and 'Chandler' highbush blueberries grown near Coopersville, MI

Cultivar	Application time[z]	Rate[y]	Flower buds/plant
Chandler	-	0	21 de[x]
Chandler	early	0.2	24 bcde
Chandler	early	2	40 abc
Chandler	early	20	24 bcde
Chandler	early	200	33 abcd
Chandler	late	0.2	27 abcde
Chandler	late	2	20 de
Chandler	late	20	27 abcde
Chandler	late	200	19 de
Liberty	-	0	40 abc
Liberty	early	0.2	40 ab
Liberty	early	2	28 abcde
Liberty	early	20	44 a
Liberty	early	200	30 abcde
Liberty	late	0.2	34 abcd
Liberty	late	2.0	41 ab
Liberty	late	20	24 cde
Liberty	late	200	10 e

[z] Application made biweekly from 14 July to 11 Aug. (early) or biweekly from 25 Aug. to 22 Sept. (late).

[y] Rate is $mg \cdot L^{-1}$ of a.i. of Fruitone, NAA

[x] Means within a column not followed by the same letter are significantly different at $P \leq 0.05$ based on LS Means test

Table 3.1: Effect of thinning treatments on 'Bluecrop' highbush blueberry fruit set and canopy growth in Holt, MI during 2012

Treatment	Fruit set (%)		Canopy growth (cm^3)	
Control	55	a[z]	559	b
carbaryl[x]	63	a	1341	b
BA[y]	5	b	485	b
Hand thinning	0	b	4051	a

[z]Means with a column followed by different letters are significantly different at $P \leq 0.05$ based on LS Means test.

[y] BA (Maxcell) applied at 400 $mg \cdot L^{-1}$ a.i.

[x]Carbaryl (Sevin SL) applied at 600 $mg \cdot L^{-1}$ a.i.

Table 3.2: Effect of thinning treatments on number of vegetative shoots in 'Bluecrop' highbush blueberry grown in Holt, MI during 2012

Treatment	Shoots / plant 13 June		Shoots / plant 21 Aug.	
Control	95	b[z]	135	b
carbaryl[x]	94	b	136	b
BA[y]	59	c	134	b
hand thinning	163	a	260	a

[z]Means within a column followed by the different letters are significantly different at $P \leq 0.05$ based on LS Means test.

[y]BA (Maxcell) applied at 400 $mg \cdot L^{-1}$ a.i.,

[x]Carbaryl (Sevin SL) applied at 600 $mg \cdot L^{-1}$ a.i.

Table 3.3: Effect of BA and carbaryl foliar sprays to individual shoots in May to June on fruit set in 'Jersey' highbush blueberries grown in Holt, MI during 2012

Treatment[z]	Application date[y]	Fruit Set (%)
Control	.	63[x]
BA	17 May	45
Carbaryl	17 May	56
BA	24 May	58
Carbaryl	24 May	54
BA	30 May	58
Carbaryl	30 May	56

[z] BA (Maxcell) applied at 400 mg·L^{-1} a.i., carbaryl (Sevin SL) applied at 600 mg·L^{-1} a.i.

[y] Anthesis occurred on 14, May

[x] No significant differences between treatments or application dates, based on ANOVA at $P \leq 0.05$

Table 3.4: Effect of manual removal of flowers at different rates on 1 year old 'Duke' highbush blueberries grown in Holt, MI in 2013

Treatment[y]	Berries/ plant		Berry weight (g)	Vegetative shoots/plant	Shoot length (cm/plant)
Control	10	a[z]	1.6	41	216
50%	6	a	1.5	45	211
100%	0	b	-	46	213

[z] Means within a column followed by different letters are significantly different at $P \leq 0.05$ based on LS Means test.

[y] Treatments are amounts of floral buds manually removed

Figure 4.1: Diagram of the shoot growth cycle of highbush blueberries exhibiting (a) first growth flush (dashed line) during June, (b) second growth flush (dotted line) during July, and (c) third growth flush (dash dot line) during August. Solid line is previous year's growth. Circles are approximate position and numbers of flower buds on the first, second, and third growth flush

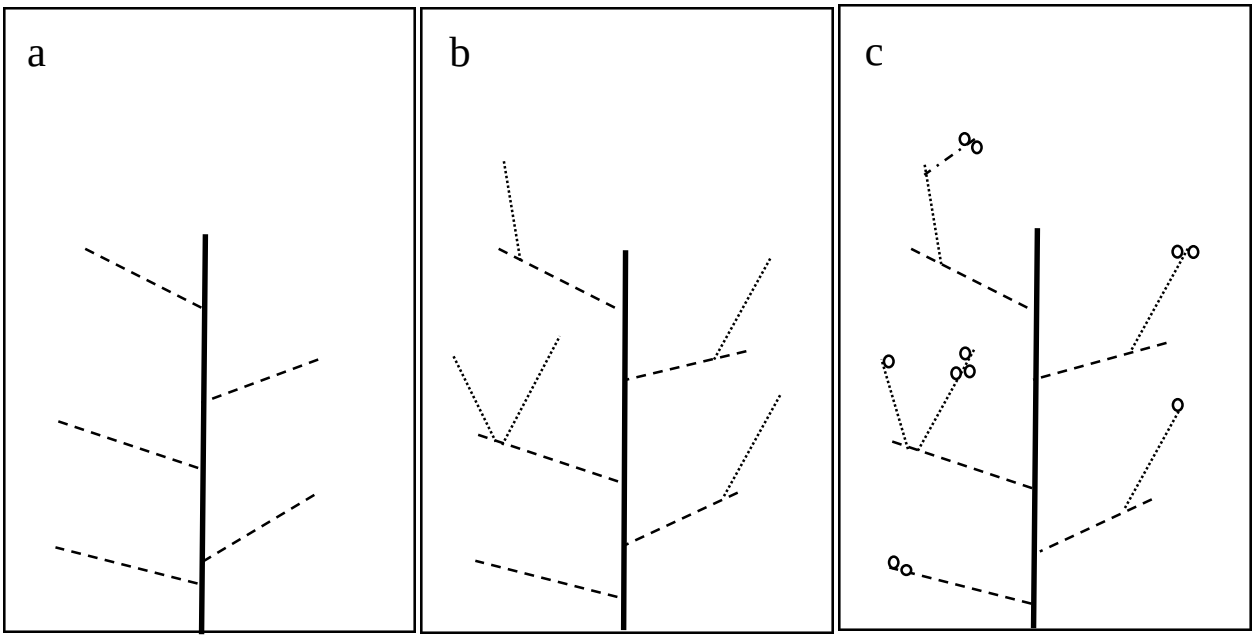

Figure 4.2: Timing of 2012 shoot growth within the first, second, or third flush in three young highbush blueberry cultivars grown in Holt, (Duke, Draper) or Coopersville, MI (Liberty)

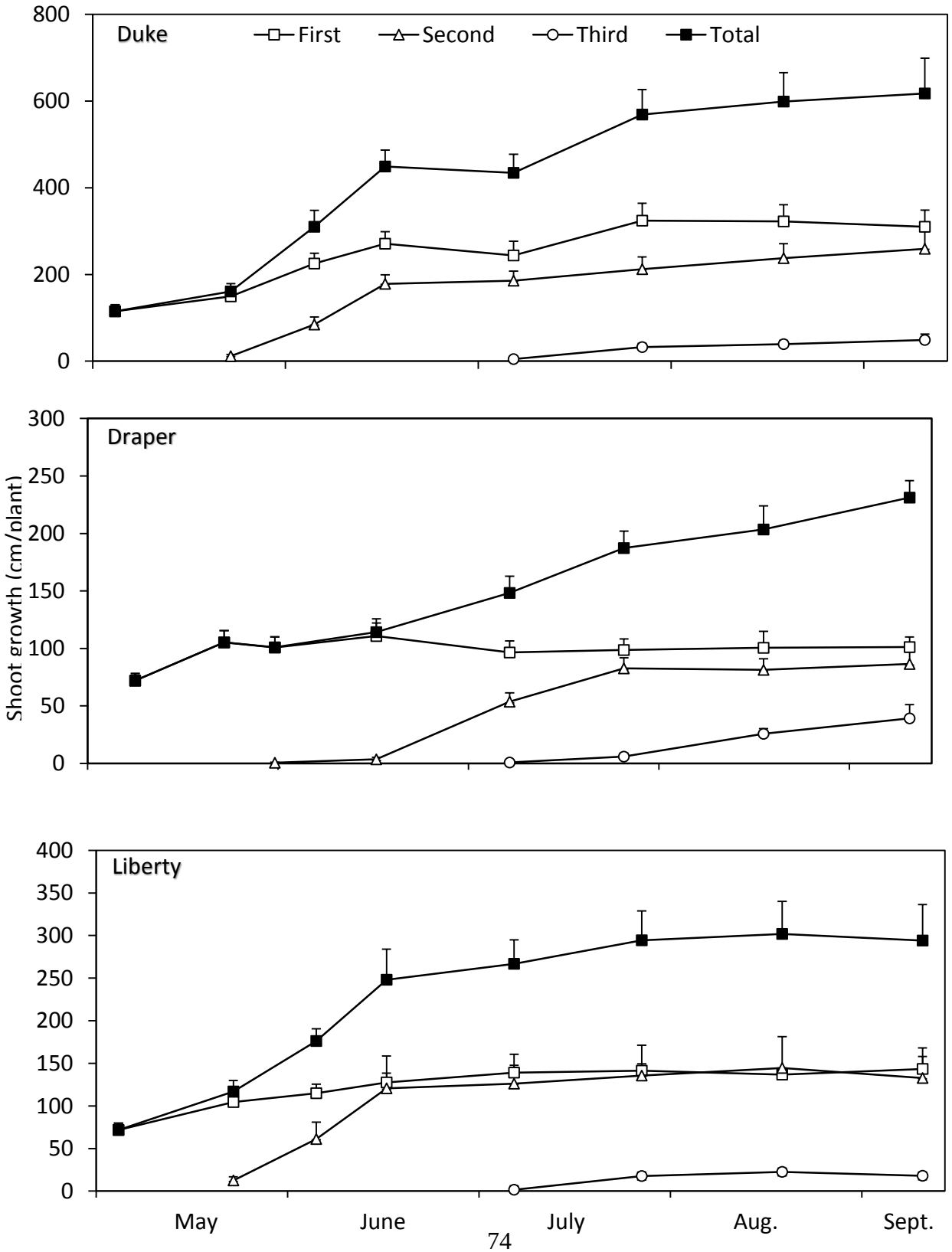

Figure 4.3: Timing of 2013 shoot growth within the first, second, or third flush in three young highbush blueberry cultivars grown in Holt (Duke, Draper) or Coopersville, MI (Liberty)

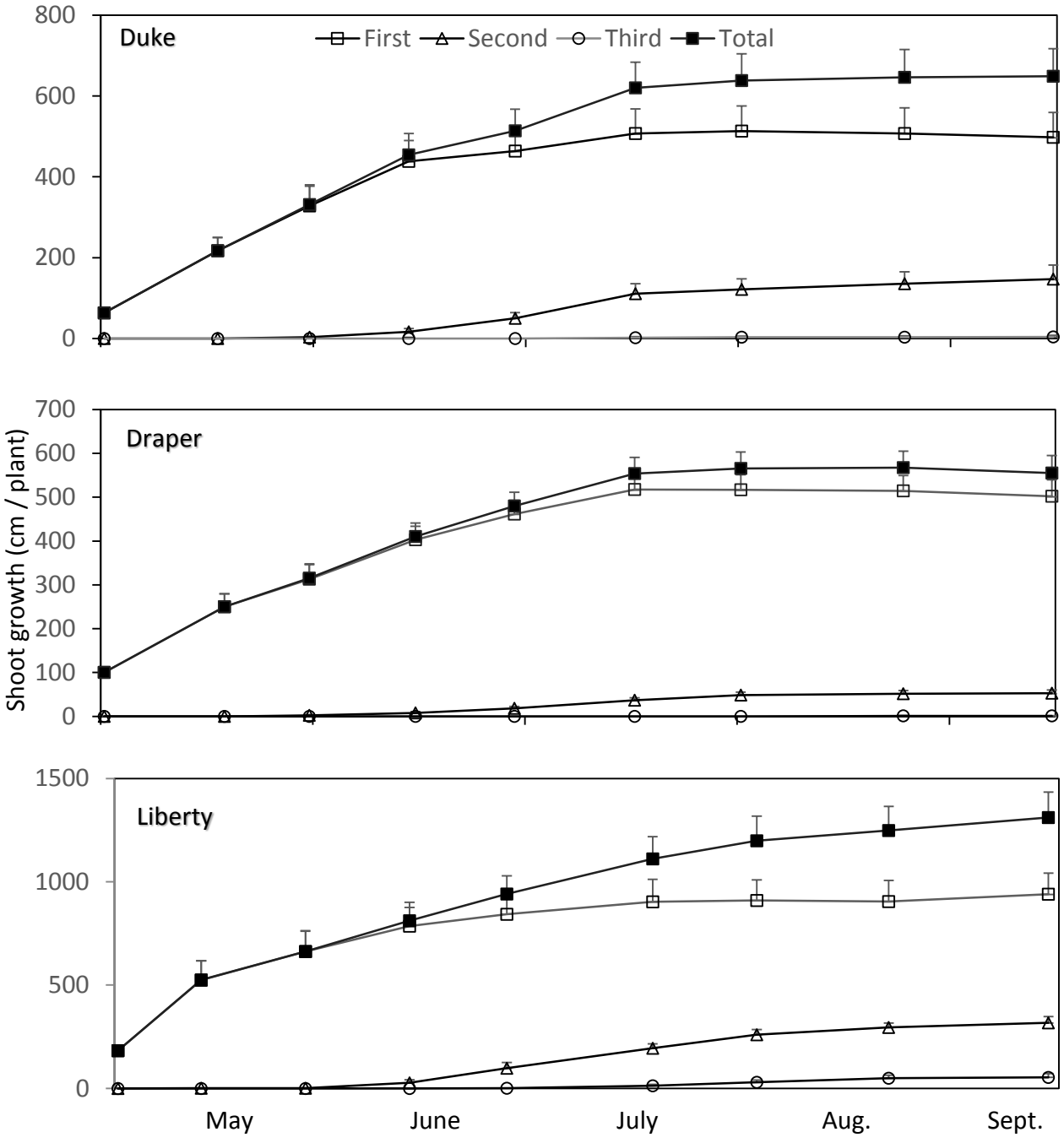

Table 4.1. Shoot growth and floral bud numbers associated with the first, second and third growth flushes of highbush blueberry cultivars grown in Coopersville, MI. ('Liberty') or Holt, MI. ('Duke', 'Draper') in 2012

Cultivar	Vegetative flush[z]	Shoot length per plant (cm)	Average shoot length (cm)	Floral buds per plant
Draper	first	144 a[y]	5.5	20 b
	second	125 a	5.1	78 a
	third	17 b	5.5	10 b
Duke	first	332 a	8.9 a	39 b
	second	254 a	8.2 a	105 a
	third	51 b	5.7 b	28 b
Liberty	first	94 a	1.9	2 b
	second	82 a	2.3	19 a
	third	30 b	2.2	16 a

[z]First flush shoots originated from axillary buds on previous year's shoots. Second flush shoots originated from axillary buds on current-season first shoots. Third flush shoots originated from axillary buds on current-season second shoots.

[y]Means within the same column and cultivar followed different letters are significantly different at P <0.05 based on LS Means test.

Table 4.2. Shoot growth and floral bud numbers associated with the first, second and third growth flushes of highbush blueberry cultivars grown in Coopersville, MI. ('Liberty') or Holt, MI. ('Duke', 'Draper') in 2013

Cultivar	Vegetative flush[z]	Shoot length per plant (cm)	Average shoot length (cm)	Floral buds per plant
Draper	first	502 a[y]	7.1	92 a
	second	53 b	9.1	22 b
	third	1 b	2.5	2 c
Duke	first	485 a	12.6 a	55 a
	second	147 b	9.2 a	63 a
	third	4 c	3.3 b	3 b
Liberty	first	1074 a	14.9 a	82 a
	second	362 b	5.4 b	109 a
	third	62 c	4.9 b	26 b

[z]First flush shoots originated from axillary buds on previous year's shoots. Second flush shoots originated from axillary buds on current-season first shoots. Third flush shoots originated from axillary buds on current-season second shoots.

[y]Means within the same column and cultivar followed different letters are significantly different at P<0.05 based on LS Means test.

Table 4.3. Shoot growth and floral bud numbers associated with the primary, secondary and tertiary growth flushes during 2012 of 'Bluecrop and Elliott' highbush blueberry cultivars grown in Holt, MI. by inspecting dormant branches

Cultivar	Vegetative flush[z]	Shoot length per plant (cm)		Average shoot length (cm)	Floral buds per plant	
Bluecrop	first	125	a[y]	4.6	19	b
	second	82	b	4.5	33	a
	third	13	c	4.2	8	c
Elliott	first	216	a	4.0	79	a
	second	53	b	4.6	30	b
	third	1	b	3.2	0.3	b

[z]first flush shoots originated from axillary buds on previous year's shoots. Second flush shoots originated from axillary buds on current-season first shoots. Third flush shoots originated from axillary buds on current-season second shoots.

[y]Means within the same column and cultivar followed different letters are significantly different at P <0.05 based on LS Means test.

Table 4.4 Growing degree days[z] for 2012 and 2013 at weather stations in West Olive and Holt, MI

Month	West Olive		Holt	
	2012	2013	2012	2013
January	2	3	1	0
February	0	0	0	0
March	202	1	193	4
April	91	66	88	74
May	385	383	386	389
June	562	518	569	507
July	832	635	782	633
August	589	566	580	542
September	370	403	360	358
October	132	167	154	162

[z]Basekerville-Emin growing degree model with base temperature of 50 °F, data from Enviro-weather, (http://www.agweather.geo.msu.edu/mawn/)

Table 4.5 Average maximum air temperatures from May to October in 2012 and 2013 at West Olive, MI and Holt, MI[z]

Month	West Olive		Holt	
	2012	2013	2012	2013
May	23.3	22.3	23.4	22.8
June	27.8	25.2	27.2	24.8
July	32	26.8	31.2	26.8
August	27.5	26.2	27.3	25.8
September	23.2	23.1	22.9	22.4
October	16	17.1	16.9	17.4

[z]Temperature measured in °C, data from the mean function at Enviro-weather (http://www.agweather.geo.msu.edu/mawn/)

Table 4.6: Number of flower buds on primary shoots with or without subsequent growth flushes in highbush blueberries grown at Coopersville, MI (Liberty) or Holt, MI (Duke, Draper) during 2012

Cultivar	Primary shoot without subsequent growth flushes	Primary shoot with subsequent growth flush
Duke	2.1 a[z]	0.1 b
Draper	1.3 a	0.3 a
Liberty	2.4 a	0.8 b

[z]Means within the same row followed different letters are significantly different at $P < 0.05$ based on LS Means test.

Table 4.7: Number of flower buds on primary shoots with or without subsequent growth flushes in highbush blueberries grown at Coopersville, MI (Liberty) or HTRC (Duke, Draper) during 2013

Cultivar	Primary shoot without subsequent growth flushes	Primary shoot with subsequent growth flush
Duke	2.4 a[z]	0.8 b
Draper	1.3 a	0.3 b
Liberty	2.1 a	0.06 b

[z]Means within the same row followed different letters are significantly different at $P < 0.05$ based on LS Means test.

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