Modelling Biennial Bearing in Apple Trees

by

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DEDICATION

This is dedicated to the Lord Jesus Christ. He is

My Lord and my God (John 20:28)

Lord of all things (Acts 10:36)

The true God (1 John 5:20)

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ABSTRACT

Many commercially grown apple cultivars have a biennial cropping habit, producing many small fruit in one year and few or none in the following year. The production of fruits is known to inhibit flower initiation for the following year. This undesirable trait is frequently managed by removing (thinning) some flowers or young fruit in years of heavy flowering which improves the size of remaining fruits, but does not reliably improve flowering in the following year. The effect of thinning on flower initiation is not well understood. Two mathematical models are developed describing the relationship between flowering in one year and the next. The first models the effects of thinning on return bloom and attempts to define maximum repeatable flower number. The second models how proximity of growing points may impact biennial bearing and maximum annual flower number. This second model may be useful to advance research into biennial bearing in apple.

LIST OF ABBREVIATIONS AND SYMBOLS USED

APE   Analyses of Phylogenetics and Evolution package

CFI   Cumulative Floral Inhibition

FB   Flower Bud

GA   Gibberellic Acid

JA   Jasmonic Acid

KODA   9,10-Ketol-Octadecadienoic Acid

MaxAnn   Maximum Annual

SAM   Shoot Apical Meristem

ACKNOWLEDGEMENTS

Chapter 1: INTRODUCTION

This thesis is laid out in the following manner: a literature review which summarizes past work and current knowledge followed by two papers and a conclusion. The first paper (Chapter 5) has been published in the journal of HortScience 46(1):40-42 (Appendix B).

The yearly flowering of apple trees is a complex phenomenon known to be hormonally controlled. The developing fruits and buds which will produce next year's flowers are competing for resources on the tree. The process is difficult to understand and I offer two mathematical models to describe biennial bearing and maximum annual percent flowering.

Chapter 2: CARBON PARTITIONING

Annual vegetative growth in apple trees is a distribution pattern of carbon photosynthates partitioned among source and sink organs (Maggs 1963; Genard et al. 2008). Each partition of gained or expended carbon can be quantified as the dry matter mass of the seasonal growth of each tissue (Barlow 1969).

Many factors affect carbon partitioning in apple trees including cultivar and rootstock combination, reproductive growth and prolonged shading (Hansen 1967a; Heim et al. 1979; Corelli-Grappadelli et al. 1994; Genard et al. 2008). A heavy crop acts as a strong sink and limits the potential duration of vegetative growth, total leaf area and total carbon accumulation until the fruit are harvested when the main carbon sinks become carbon reserves, root growth and radial shoot growth (Barlow and Smith 1971; Giuliani et al. 1997).

2.1: CANOPY STRUCTURE AND ARCHITECTURE

The shoot architecture of an apple tree creates the canopy structure and source of energy from which fruits develop; the shoot system itself is largely determined by genotype and rootstock effects (Wareing 1970; Lauri et al. 2006). Physiology, morphology, horticultural practices and environmental influences all affect canopy structure (Willaume et al. 2004). A shoot can be defined as the woody tissue produced in the current season whereon leaves, fruits and buds will develop (Hansen 1967b; Sprugel et al. 1991).

Shoots can be classified as short spur shoots or elongated extension shoots based on total extension growth during a single season (Hansen 1967b). Many biennial cultivars such as Honeycrisp tend to have low-vigour, spur-type growth otherwise known as class III (Lespinasse 1980). The fruiting behaviour and pruning of this cultivar largely determines its branch architecture to be spur-type (Kikuchi and Shiozaki 2007).

Fig. 1. Photographs of short spur shoots and elongated extension shoots on Honeycrisp. Photographs taken in May 2011.

The theory of plant modularity states that an apple tree canopy is a meta-population of individual branches (White 1979; Kikuchi and Shiozaki 2007). One important physiological aspect of modularity is that branches may be autonomous in some ways. Numerous ^{14}C studies confirm that branches are independent from one another in terms of demand and supply of photosynthates, water or nutrients (Hansen 1967a,b; 1969; Sprugel et al. 1991; Ben Mimoun et al. 1998). This supports the use of single branches as experimental units.

2.2: REPRODUCTIVE GROWTH

On older apple varieties such as Cortland and McIntosh flowering typically occurs in shoot apical meristems on spurs and elongated shoots (Wellensiek 1977; Buban and Faust 1982). On many modern cultivars such as Honeycrisp floral initiation also occurs on one year old lateral shoots (Tromp 2000). In Honeycrisp, most fruit buds form on one year spurs and are infrequent on elongated shoots (Nichols et al. in press). Fruiting spurs reach peak cropping efficiency and fruit quality at two years and then decline (Buban and Faust 1982; Lauri et al. 1997; Tromp 2000; Davis 2002). Their ability to bear marketable fruit increases with increased light exposure (Robinson et al. 1983; Rom and Ferree 1984).

Flower induction is the change of an apical meristem from being vegetative to reproductive; flower initiation is the time before the induced apical meristem shows a different morphology (Hanke et al. 2007). Temperate fruit trees complete an autonomous floral induction pathway dependent on unknown signals during the season

before anthesis (Abbott 1960; Wilkie et al. 2008). In contrast to the well-studied *Arabidopsis*, the flower induction pathways are not understood but may involve a combination of exogenous and endogenous stimuli (Tromp 2000). An old theory has been that a balance between available carbon and nitrogen determines whether vegetative growth or flower bud development prevails (Kobel 1954). Many experiments have been conducted to prove this, but there is no strong evidence for the theory (Tromp 2000). Other suggestions are that the accumulation of heat units or a change in temperature triggers floral induction (Tromp 1980; McArtney et al. 2001) but this has not been proven (Wilkie et al. 2008).

Before floral induction can occur, an apple tree bud must remain between successive leaf initiation stages for approximately seven days, have bracts and a minimum of sixteen nodes (Faust 1989). Defoliation experiments have shown that a shoot requires a critical number of leaves in order for flower induction to occur (Hennerty and Forshey 1971). Recently, Neilsen and Dennis (2000) reported that increased bourse shoot growth and leaf area contribute to floral initiation. This appears to be evidence for hormonal stimulation from the leaves but chemical analysis has not been able to support this theory (Wilkie et al. 2008).

Although flower initiation takes less than a week for any single bud, the whole tree takes about 100 days for all induction to occur (Tromp 2000). On spurs, flower bud induction occurs approximately 3 to 6 weeks after full bloom (Ryugo 1986). On

elongated shoots, it can begin anywhere from 6 weeks after bloom until the end of shoot growth (Luckwill 1970; Childers et al. 1995).

Flower bud differentiation is first apparent as a flattened tip at the end of the shoot. Transition from vegetative to flowering states is irreversible, in general; treatments imposed on the developing flower bud influence blossom quality in the next season but not blossom quantity (Tromp 2000).

Typically all flower parts except pollen sacs and ovules are completely formed by the end of the growing season and the onset of dormancy; fully developed flower buds have a rosette of 21 leaves and 5 flowers. In temperate climates, growth is stopped during the winter and resumes again during the cool spring months (Tromp 2000).

Flower buds emerge at bud break as a fine silver fuzz of leaf tissue at the tip of the swollen bud followed by the emergence of leaves (Barlow 1969). The leaf rosette opens to reveal a small green cluster of five flowers which swell up, expose their pink petals and extend away from the leaves. The center (king) blossom opens first followed by the other flowers (Davis 2002). Any period of two or three warm days and nights can greatly speed up flowering stages (Fig. 2; Lakso et al. 2001).
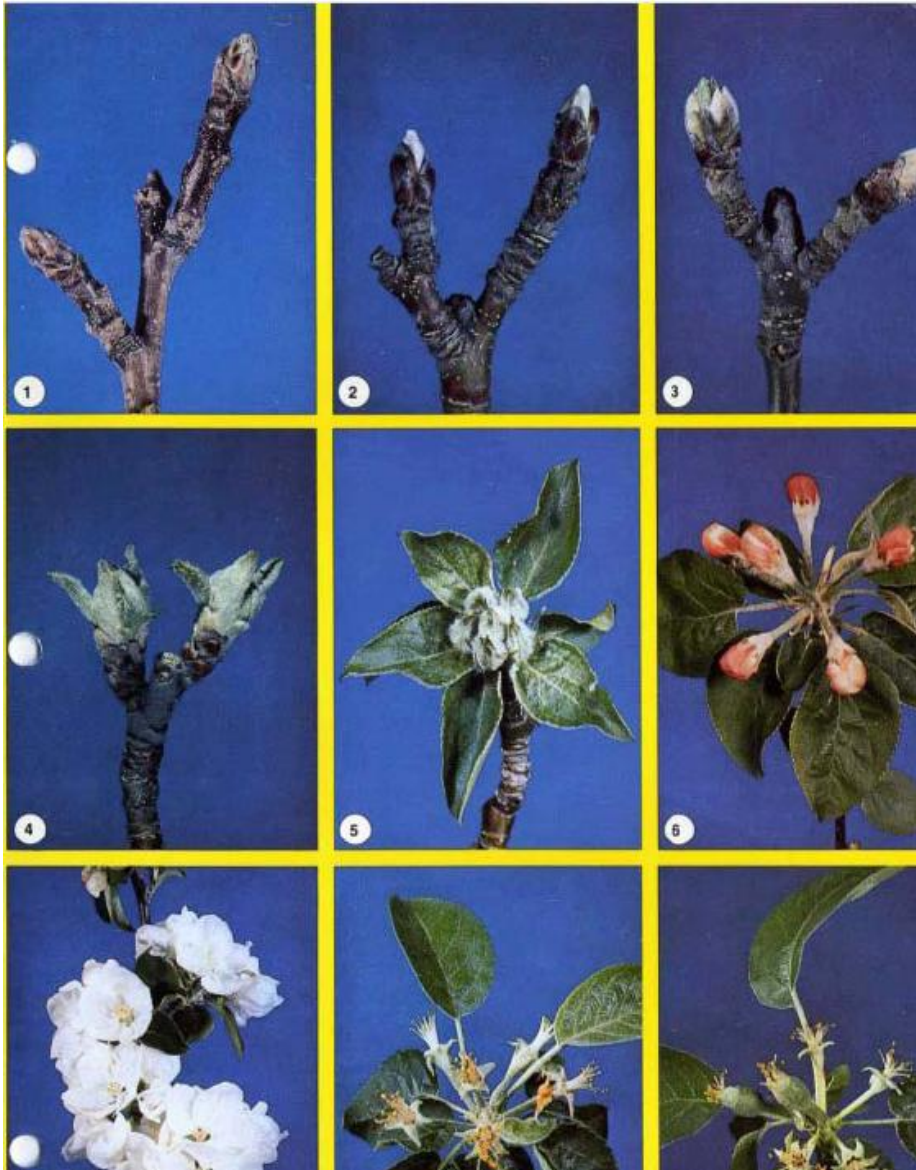
Fig. 2. An illustration of the stages of an apple tree flower cluster. Figure taken from Chapman and Catlin (1976).

The king blossom is commonly the first to be pollinated and fertilized; flowers that are pollinated and fertilized are said to set fruit and are then called fruitlets. Some of these fruitlets naturally abscise from the tree at a specific time, known as June drop in temperate climates (Meland and Gjerde 1993).

The ability of a flower bud to produce a mature fruit depends on light exposure and age of the spur wood; increased light exposure can improve its ability to bear marketable fruit (Rom and Ferree 1984). A spur is at its peak cropping efficiency in its second year and productivity declines after five years (Tromp 2000; Davis 2002).

As fruitlet development proceeds, hormones are produced. The most well-known hormones involved in apple flowering and fruiting are auxins, gibberellins and cytokinins. Auxins and gibberellins are produced after fertilization by developing seeds and promote fruit growth but inhibit flower bud initiation (Chan and Cain 1967; Callejas and Bangerth 1997; Zhao et al. 2009). Jasmonic acid and 9,10-ketol-octadecadienoic acid are produced in apple trees and are known to be associated with flower development in other plants (Kong et al. 2005; Yokoyama et al. 2005; Kittikorn et al. 2010, 2011). It may be that the ratio of inhibitor to promoter hormones determines flowering (Luckwill 1970; Kittikorn et al. 2010).

There also appear to be inhibitor hormones produced during anthesis (Bobb and Blake 1938). Singh (1948) removed 86% of the flower buds at bud break and at pink bloom from trees in their on year, 35 (bud break) and 15 days (pink bloom) before full bloom, respectively. Removing flower buds at bud break resulted in 45% of the buds in the following year producing flowers while removing flower buds at pink bloom resulted in only 25.7% of buds producing flowers on Miller's Seedling apple.

Chapter 3: BIENNIAL BEARING

Adequate regular yields of good quality fruits are essential for modern fruit growers; however, many fruit tree species suffer from biennial or alternate bearing (Davis 2002). This is a self-recurring oscillation in yearly fruit number between yields of numerous small, poor quality fruits and, in alternate years, a few large fruits or no fruits at all. The problem can be viewed as a failure to produce enough flowers every year causing the total marketable yield over the lifetime of a biennial tree to be less than that of a regular bearing apple tree (Kraybill et al. 1925; McCormick 1933; Bobb and Blake 1938; Hoffman 1947; Dennis 2003; Pavičić et al. 2004).The years of heavy flowering and cropping are called 'on' years and the years of light flowering and cropping are called 'off' years (Davis 2002). Fig. 3 shows the difference between on and off years of Honeycrisp at king bloom stage.

Fig. 3. An illustration of on and off years of Honeycrisp apple trees. Images taken in May 2011 during king bloom stage in the Annapolis valley, Nova Scotia.

The biennial bearing pattern reduces the overall yield of many apple cultivars not only because the 'off' year crops are negligible but also because in 'on' years many fruits are not marketable (Davies 1950). Small fruits (<115g) and diseased fruits are called culls and represent a significant loss for the grower (Dennis 2003).

Environmental factors such as spring frost and diseases as well as endogenous factors such as cultivar, rootstock and hormones associated with anthesis and seed development can all induce biennial bearing (Singh 1948, Chan and Cain 1967; Jonkers 1979; Monselise and Goldschmidt 1982; Dennis and Neilsen 1999). Many management techniques including fertilizing, pruning, defoliating and thinning have been tried to ensure regular yields of marketable apples (Singh 1948; Davies 1950; Williams and Edgerton 1974; Davis 2002). Response to chemical thinners is extremely variable. Endogenous factors such as carbohydrate demands, leaf area and percent bloom as well as exogenous factors such as temperature, humidity and sunlight all influence the response to chemical thinners (Lakso et al. 2008).

Early studies demonstrated that the proximity of neighbouring flower clusters directly inhibits return bloom (Bobb and Blake 1938) and the number of leaves on nearby shoots promotes it (Haller and Magness 1926, 1933). Other local influences on fruiting include the growth of terminal, lateral and bourse shoots (Abbott 1960; Hansen 1967a; Kikuchi and Shiozaki 2007). Honeycrisp is a biennial cultivar which tends to produce so much fruit that it inhibits shoot growth. However, new extension shoots can be encouraged to grow if fruit buds or whole spurs are removed (Nichols et al. in press). Pruning spur wood encourages new growth which bears fruit after two or three years. This suggests there may be confounding effects from year to year of treatments in previous years.

Chapter 4: MATHEMATICAL MODELING

Mathematical models are helpful tools to simplify complex biological systems and provide meaningful results for scientists, and fruit growers (Marcelis et al. 1998). Models take on numerous forms and are broadly classified according to the mathematics used (linear algebra, differential equations, etc.), whether time is accounted for (static or dynamic) and the result (deterministic or probabilistic). The model assumptions are then critiqued to best fit real-world results and refine predictions (Mesterton-Gibbons 2007).

Models describe either underlying mechanisms (mechanistic models) or real-world, evidence (empirical models) to some degree. No model is completely mechanistic or empirical. A truly mechanistic model would completely describe the reason for everything and a truly empirical model would be correct for no reason at all (Nestorov et al. 1999).

A useful model captures the essence of a system and responds to hypothetical situations. Developing a mathematical model sifts away details until only essential aspects remain. Once developed, it is essential but often difficult to understand what the model actually represents (Meerschaert 2007).

4.1: CONCEPTUAL FRAMEWORK OF FIRST MODEL

Field work on Honeycrisp undertaken in summer 2009 under the supervision of Dr. Deborah Buszard (Dalhousie), Charles Embree (AAFC, Kentville) and Mr. Doug Nichols

(NSFGA) in the Annapolis valley and observation of data from the ten-year NC-140 'Royal Gala' rootstock trial in Geneva, NY (Barritt et al. 1997) and the six-year fertigation trial in Summerland, BC (Neilsen et al. 2009) were the starting point for thinking about better understanding apple tree biennial bearing and the effect of thinning using mathematical modeling. This builds on the undergraduate honors project (Pellerin 2009).

Chapter 5: A THEORY OF BLOSSOM THINNING TO CONSIDER MAXIMUM ANNUAL FLOWER BUD NUMBERS ON BIENNIAL APPLE TREES

Brian P. Pellerin, Deborah Buszard, David Iron, Charles G. Embree, Richard P. Marini, Douglas S. Nichols, Gerald H. Neilsen and Denise Neilsen

5.1: ABSTRACT

Tree fruit growers use chemical and mechanical thinning techniques in an attempt to maintain regular annual flower production and maximum repeatable yields of varieties susceptible to biennial bearing. If the percentage of floral buds an apple tree could produce without causing yield depression in subsequent years was known, it would be possible to better manage crop thinning regimes. This study proposes that thinning is a partial transfer of potential flower buds from one year to the next year and estimates the maximum repeatable sequence of flower buds without biennial bearing. The conceptual framework is tested on a 50 year simulation with 0 to 100% transfer of thinned flower buds. Results indicate that the maximum repeatable sequence of flower buds rises sharply when the final years of the orchard approach and declines when the percent transfer of thinned buds is near 0%.

5.2: INTRODUCTION

Regular yields of good quality fruits are essential for modern apple growers; however many cultivars such as Honeycrisp suffer alternate bearing and more precisely, biennial

bearing (Davis 2002). This recurring oscillation in fruit number between numerous small, poor quality fruits in one year and, in alternate years, a few large fruits or no fruits at all causes the total marketable yield over the lifetime of a biennial tree to be less than that of a regular bearing apple tree (Dennis 2003; Pavičić et al. 2004). The problem can be viewed as a failure to produce sufficient flower buds annually.

While cultivar is the main factor in biennial bearing, other endogenous factors such as rootstock and hormones associated with anthesis and seed development and environmental factors such as drought, spring frost and diseases can all induce biennial bearing in apple trees (Singh 1948; Landsberg and Thorpe 1975; Wilkie et al. 2008). In years of heavy bloom, fruit growers may remove excess flowers and fruitlets chemically or by hand to increase fruit size of the current crop and encourage return bloom (Davies 1950; Wertheim 2000). To increase flower bud initiation, timing of flower removal is critical: it is most effective to remove whole flower buds, including leaves, during winter dormancy (termed bud or spur extinction by Lauri et al. (1995)) and least effective to remove only the flowers or fruitlets during or after flowering because anthesis and seed development both significantly impact return bloom (Singh 1948; Dennis 2000; Nichols et al. in press).

Removing flowers in one year can be viewed as partially transferring potential flowers and fruit to the subsequent year; this has been demonstrated in numerous studies on thinning biennially bearing apple cultivars described in the review by Dennis (2000). Hypothetically there must be an optimal thinning regime which would provide good

fruit size and consistent return bloom and yield every year, leading to predictable annual harvest and maintenance of healthy trees.

Observation of data from the ten-year NC-140 'Royal Gala' rootstock trial in Geneva, NY (Barritt et al. 1997) and the six-year fertigation trial in Summerland, BC (Neilsen et al. 2009) provided the conceptual framework behind this study. The data show distinct year to year fluctuations in yield. Potentially higher yields in one year might be considered to be "transferred" to a year of subsequently lower yield by thinning. The purpose of this paper is to model the effects of thinning on yearly flower bud number and determine the maximum annual number of flower buds on an apple tree for the life of the orchard. The maximum annual number of flower buds is defined as the largest number of flower buds an apple tree could produce in any year and maintain or increase numbers in every year thereafter.

5.3: THEORY AND MODEL

Biennial apple trees produce alternate numbers of few or many flower buds each year. To regulate the number of flower buds, fruit growers remove (thin) some when they are too numerous. For this model the percentage of floral buds is assumed to be a random value between 0 and 50% and in alternate years between 50 and 100%. The percentage of floral buds is defined as the number of floral buds relative to the total number of buds on a tree (Lombard and Richardson 1982). This assumes that the biennial tendency of a mature apple tree is constant.

Suppose the number of flower buds (FB) is recorded for three consecutive years as FB_1, FB_2, FB_3.

Thinning done in any year i removes α_i flower buds from FB_i leaving $\overline{FB_i} = FB_i - \alpha_i$. Suppose for α_i flower buds removed, $\alpha_i b$ are added to the next year where b is the percentage of floral buds removed in one year and transferred to the next as a result of thinning. The parameter b is a measure of thinning effectiveness. The value $b=1.0$ represents 100% transfer of floral buds removed in one year to the next as a result of thinning before anthesis. The value $b=0.0$ represents 0% transfer of floral buds removed in one year to the next as a result of thinning after fruit set, which is ineffective at improving return bloom (Singh 1948; Dennis and Neilsen 1999). Thinning in the first year increases the number of flower buds in the next year which would decrease the number of flower buds in the year after and affect every upcoming year in the following way: $FB_1 - \alpha_1, FB_2 + \alpha_1 b, FB_3 - \alpha_1 b$. This model fixes the value of b to be the same for all years in order to show how different thinning regimes influence the maximum annual flower bud number.

To prevent biennial bearing, there should be no decrease in flower bud numbers from year to year. This restricts the maximum annual percentage of flower buds for the first year to be $\overline{FB_1} = MIN\{FB_1, (FB_2 + FB_1 * b)/(1+b), (FB_3 + FB_2 * b)/(1+b)\}$ and the general equation to be

$$\overline{FB_i} = MIN\{FB_i + \alpha_{i-1} * b, (FB_{i+1} + FB_i * b)/(1+b), \ldots (FB_n + FB_{n-1} * b)/(1+b)\}$$ [1]

ensuring that $\overline{FB_1} \le \overline{FB_2} \le . .\le \overline{FB_n}$ and maximizing the values beginning from

$\overline{FB_1}$ to $\overline{FB_n}$. A simulation of 50 seasons was run as a macro in Excel 2007 with parameter

b run from 0 to 1 at intervals of 0.01 and replicated 10,000 times with half the seasons

beginning with a random value between 0 and 0.5 and half beginning with a random

value between 0.5 and 1.

5.4: RESULTS

Values for maximum annual percent of flower buds from years 2 to 50 were plotted

using SigmaPlot 11.0 (Fig. 2). The first value is unique to the number of years and

considered an artefact of the model; therefore, this value is ignored in the results.
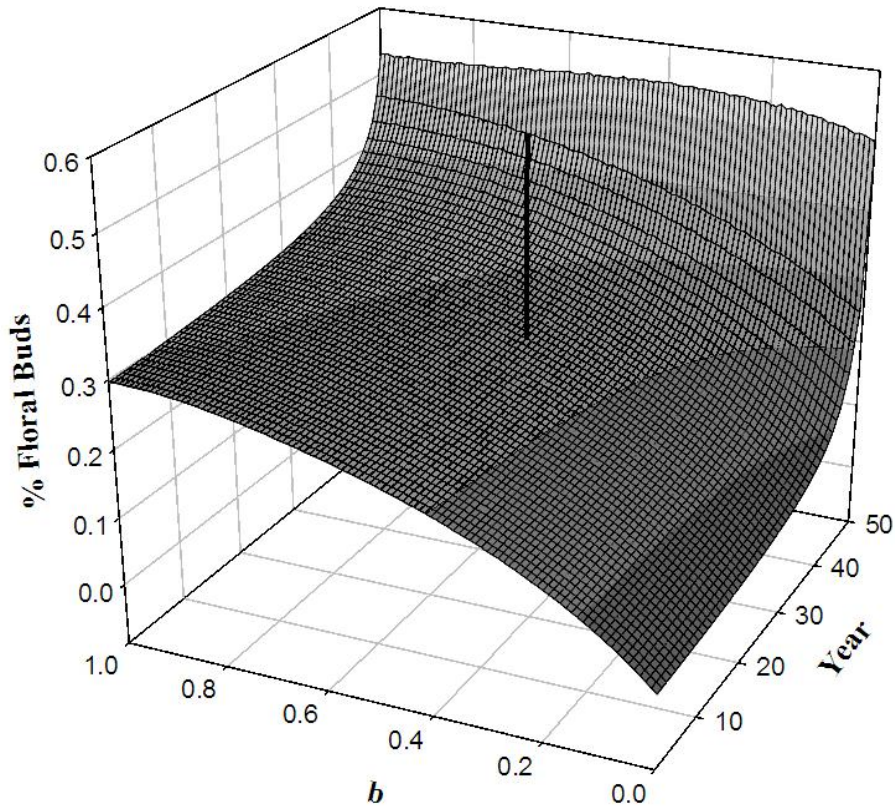
Fig. 4. Graph of the model results based on a fifty year simulation replicated 10,000 times. Parameter *b* is the percentage of floral buds removed in one year and transferred to the next as a result of thinning. The vertical line intersecting the graph indicates the location of year 30 and *b*=0.5 where the maximum repeatable percent floral buds is 26%.

Fig. 4 shows the simulation running for 50 years, clearly beyond the normal cropping life of an apple tree. The model suggests that the percentage of floral buds which should remain on a tree is dependent on the number of years into the future the tree is expected to be producing fruit. Running the model over shorter periods produces the same result; for example, if a 30 year production period were simulated, it would look

like years 21 to 50 shown in Fig. 4. The percentage of floral buds does not form a straight line from the first to final years of production. The strong increase predicted by the model in the percentage of floral buds that can be kept on the tree as the final years of the orchard approach results from the decreasing mathematical probability of a year of poor return bloom as the number of years remaining in the model calculation diminishes. This is not what is observed in reality but is because Eq. [1] uses data from future years to calculate the maximum repeatable flower number for any one year.

The parameter b is a measure of thinning effectiveness. The value b=1.0 represents 100% transfer of floral buds removed in one year to the next as a result of thinning before anthesis. The value b=0.0 represents 0% transfer of floral buds removed in one year to the next as a result of thinning after fruit set, which is ineffective at improving return bloom (Singh 1948; Dennis and Neilsen 1999).

The maximum annual percentage of floral buds is sensitive to values of b near b=0 as indicated by the steepening slope from b=1 to b=0 (Fig. 4). According to the model, if thinning were done when b=0.5 on trees intended to produce fruit for another 20 years, flower bud thinning should leave 26% bloom on the tree as indicated at the intersection of the vertical line with the graph in Fig. 4.

5.5: DISCUSSION

The theory may be useful for apple growers because it offers a conceptual framework on which to base a blossom management program to regulate cropping throughout the life of the tree. Simply stated, the flower bud number next year should always be at

least the flower bud number this year. Insuring that flower bud numbers are maintained allows for a regular annual cropping.

The equation presented in this paper can determine a maximum repeatable number of flower buds from real or simulated flower bud data and may be useful in managing biennial apple cultivars such as Honeycrisp. It may be possible to develop a management strategy to control biennial bearing based on this model.

Early flower bud thinning of high-value cultivars is becoming more practical with new mechanical methods (Roche and Masseron 2002; Embree and Nichols 2005; Schupp et al. 2008). This model may offer a way to better manage biennial bearing; it may act as a catalyst for development of more real and useful tools to manage flower bud numbers and regulate cropping from year to year. Data are needed to refine the model and investigate major environmental and endogenous factors which affect bienniality. The model will be improved by iteration with more data sets and field testing in various management systems and growing conditions. We welcome input from other researchers and look forward to collaborating and refining this tool. As this work continues, growers may be able to access information like this for any cultivar and rootstock combination.

Chapter 6: LINKING SECTION

The first paper established the concept of maximum annual percent flowering; it assumed biennial bearing caused percent flowering to alternate between 0-50% in one year and between 50-100% in the following year. There are limitations to making such a general assumption of the mechanism of biennial bearing.

One limitation is that thinning should give a nonlinear response in return bloom. For example, Bailey (1929) removed 50, 75 and 100% of the blossoms by hand in the on-year but only 100% thinning consistently gave return bloom. Other hand thinning studies of the early twentieth century confirm this (McCormick 1933; Bobb and Blake 1938). Another limitation is that the model does not consider how hormones from seeds might inhibit flower bud inhibition. Current work suggests that endogenous gibberellins produced by seeds inhibit floral initiation in 'Red Delicious' and 'Golden Delicious' (Ramírez et al. 2001; Ramírez et al. 2004a, b). These limitations are reconsidered in the second paper.

Chapter 7: A NOVEL WAY TO CONSIDER CONTROL OF FLOWERING OF APPLE BY ENDOGENOUS HORMONES

Brian P. Pellerin, Deborah Buszard, Alex Georgallas, Richard J. Nowakowski

7.1: ABSTRACT

Inhibition of flower initiation by nearby developing fruits is one of the main causes of biennial bearing in apple trees. The theory that a critical ratio of inhibitor to promoter hormones could inhibit flowering of shoot apical meristems is modelled. The model shows that a critical ratio of hormones could be involved in floral inhibition and biennial bearing. Because floral inhibition is thought to be a localized effect, the spatial arrangement of shoot apical meristems (SAMs) on a limb is perhaps as critical as the number in determining whether or not SAMs initiate flower clusters. The assumptions behind the model follow current theory and test a plausible mechanism for floral inhibition and biennial bearing. This paper presents a novel way to view hormone hypotheses of biennial bearing in apple trees and how management strategies such as flower removal could be used to achieve regular numbers of flower clusters over consecutive years.

7.2: INTRODUCTION

Profitable apple production requires regular yields of marketable fruits. The challenge is that many commercially important cultivars are biennial and produce excessive numbers of flower clusters and fruits in one year which suppress flower initiation for the

following year. This leads to oscillation in crops between many small, poor quality fruits in one year and a few large fruits or no fruits at all in alternate years (Stover et al. 2001). Rootstock and environmental factors such as drought, spring frost, diseases, light interception and canopy architecture manipulation can also impact the bearing pattern of apple trees (Fulford 1965; Davis 2002; Willaume et al. 2004).

The distance between flower clusters in one year has long been known to influence the development of floral buds for the next year (Fulford 1966). 'Wealthy', a strongly biennial cultivar, requires a minimum distance of 15 to 25cm between individual flower clusters, approximately equivalent to 40 flower clusters per m^3 of canopy volume, to maintain regular yields (Bobb and Blake 1938). More recent work on 'Honeycrisp', a modern spur-bearing biennial cultivar, demonstrated that spur-pruning to reduce flower bud number to between 40 and 60 per m^3 of canopy volume is necessary to control biennial bearing (Nichols et al. in press).

These two studies seem to confirm the concept put forward by Chan and Cain (1967) that seeds can inhibit floral initiation on nearby shoot apical meristems (SAMs). The balance of hormone concentrations at SAMs has been hypothesized to control floral initiation (Luckwill 1970; Hoad 1984; Callejas and Bangerth 1997). Up to 2000, there had been many conflicting reports on the effects of endogenous hormones (Looney et al. 1985; Dennis and Neilsen 1999).

In more recent years, endogenous gibberellins (possibly GA_1, GA_4 and iso-GA_7) produced by seeds have been shown to inhibit floral initiation in 'Red Delicious' and 'Golden

Delicious' (Ramírez et al. 2001; Ramírez et al. 2004a, b). This was confirmed with heavily cropping 'Fuji' trees having higher concentrations of endogenous GA_1 and GA_4 in apical buds at the time of floral initiation than was found on blossom thinned trees (Kittikorn et al. 2010). Other endogenous hormones (9,10-ketol-octadecadienoic acid (KODA) and jasmonic acid (JA)) have been found at high concentrations when GAs are low (blossom thinned trees) and at low concentrations when GAs are high (heavily bearing trees). KODA and JA are associated with flower development in other plants (Yokoyama et al. 2000; Suzuki et al. 2003; Kong et al. 2005; Yokoyama et al. 2005). They may promote floral initiation in apple but it is not known what tissue produces them (Kittikorn et al. 2010, 2011).

Although more than 70 years have passed since Bobb and Blake identified the effect of a heavy crop on return bloom, the underlying causes and mechanisms still remain unclear and conflicting hypotheses remain unconfirmed. Mathematical modelling may help researchers better understand the phenomenon and clarify the mechanisms involved. This paper models the hypothesis originally developed by Hoad (1984) that a balance of hormones controls flower initiation in apple trees with the inhibitor and promoter hormones recently identified by Kittikorn et al. (2010, 2011) and predicts the effect of flower thinning on maximum annual (MaxAnn) flowering. MaxAnn is defined as the number of flower clusters that a tree can carry in one year and produce an equal number of flower clusters in the following year.

7.3: THEORY AND MODEL

According to recent hormone studies in apple trees, seeds export GAs and some unknown tissue(s) exports KODA and JA (Kittikorn et al. 2011). For now we assume leaves export KODA and JA and SAMs flower unless the ratio of inhibitor to promoter hormones is greater than a critical value. In order to asses this possibility, the model first creates the canopy structure of a tree and randomly assigns flower clusters and vegetative growth to the tree tips. The ratio of inhibitor to promoter hormones coming from seeds and leaves are estimated at each tip (SAM).

The model generates hypothetical trees with 1000 SAMs. Using R, a standard programming language for computation and graphics (R Development Core Team 2011), trees were generated with the 'rtree' function within the Analyses of Phylogenetics and Evolution ('ape') package (see Appendix A; Paradis et al. 2004). This function assembles trees from branch lengths as pictured in Fig. 5. Branch lengths are selected from a random distribution between 0 and 20 cm. These branch lengths fit well within the range of branch lengths for 'Honeycrisp' on MM.106 and M.26 rootstocks in the Annapolis Valley, NS (personal observation). Further details about 'rtree' can be found by entering '?rtree' in R after installing the 'ape' package.
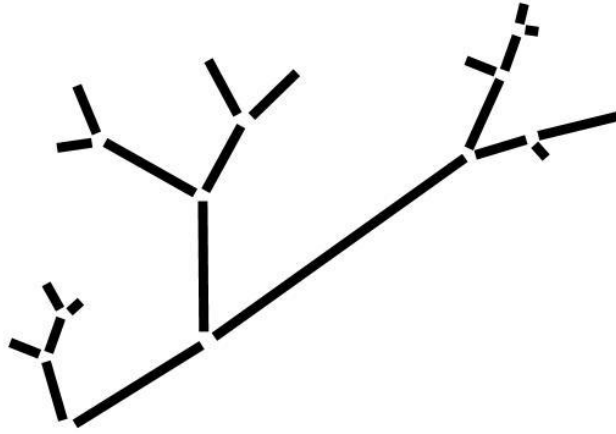
Fig. 5. A simulated branch assembled from 22 branch lengths.

The hormonal control of biennial bearing hypothesis assumes seeds and leaves produce hormones (Dennis and Neilsen 1999).The point source equation, common to physics, is a useful model where signal intensity at distance r can be demonstrated to be $I=\alpha/r^{(D-1)}$ where α ($0 \leq \alpha \leq 1$) is the signal strength at the point source and D is the fractal dimension (Griffith 2004).

Fractal dimension is the amount of space an object completely fills (Mandelbrot 1967; Vicsek 2001). The fractal dimension of a tree is greater than one (a straight line) and less than three (a cube); that is, the more branched the tree, the greater the fractal dimension. Trees have been successfully modelled as fractals because of their repeatedly branching architecture (Prusinkiewicz and Lindenmayer 1990; Holiday and Samal 1995; Samal et al. 2002). Fractals have the property that their length is indeterminate. For instance, measuring a coastline with a 1km scale will have a shorter length than measuring with a 1m scale and a still shorter length than measuring with a 1cm scale. This property, known as the Richardson effect, is used to estimate the fractal

dimension by repeatedly measuring its length (L) with different size measuring scales (S) and solving for D in the regression equation Log[L] = (1-D)Log[S] + b (Mandelbrot 1967; Zhang et al. 2007). This calculation was done with two scale sizes (1cm and 60% of the maximum distance between tips) for the generated trees and used in the point source equation (Appendix A). The concentration of hormones as they spread from a point source through the tree would be distributed as shown in Fig. 6 where α =1 and D=1.5.
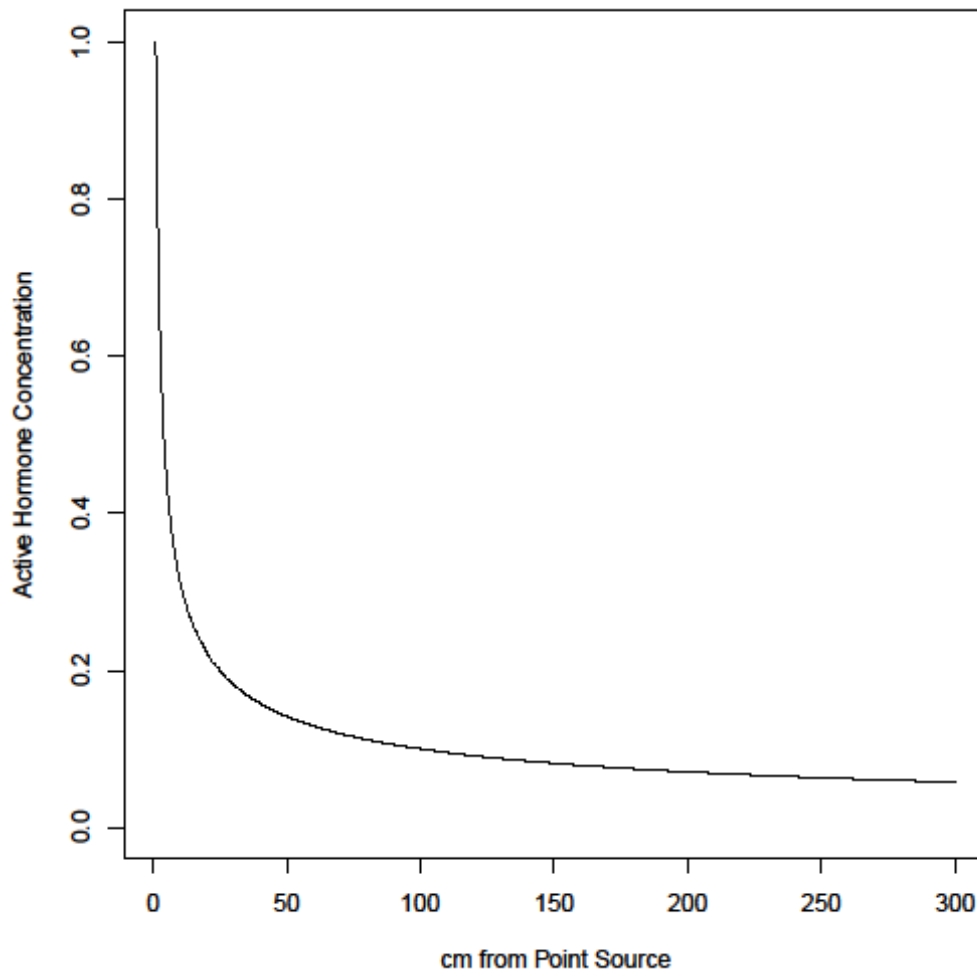
Fig. 6. Estimated hormone concentration along the branch using the point source equation $I=\alpha/r^{D-1}$ where $\alpha=1$ is the initial concentration, r is the distance along the limb and $D=1.5$ is the fractal dimension.

The model measures the branch distances between point sources (branch tips). In terms of R code, the function used is 'cophenetic'. The cophenetic distance is the branch distance between every SAM. Using the point source equation, these distances are converted to concentrations of hormone from seeds and leaves (see 'chs' and 'chl' in Appendix A). Floral inhibition is simulated by comparing the hypothetical hormone ratio at each SAM to a critical value beyond which flowering is inhibited (see 'crit' in Appendix A).

Seeds and leaves export equal quantities of hormone (between 0 and 1) or one exports $1/10^{th}$ (between 0 and 0.1) of the quantity of the other. Thinning t percent in the third year between 0 to 100% at 5% intervals was replicated 1000 times for the model (see 't' in Appendix A). Distances between individual flowering/fruiting SAMs were calculated for each t percent of thinning.

7.4: RESULTS

Tree canopies were generated 1,000 times. The fractal dimension of the canopy ranged between 1.69 and 1.85 with a mean of 1.77 and a standard deviation of 0.02. Changing the relative amounts of hormones exported by seeds and leaves from 1:10, 1:1 and 10:1 shifted the plot by a magnitude of 10 each time (Fig 7). Thinning in the on-year was plotted against return bloom using critical ratios of 1:1, 1:2, 1:4, 1:8 and 1:16 (Fig. 8).

The corresponding MaxAnn values for the plots in Fig. 8 are 50, 41, 30, 22 and 16%

bloom. An illustration of neighbouring SAMs inhibiting flower bud initiation is given in

Fig 9. Distances between individual flowering/fruiting SAMs was an average of 21.5cm

for all t percent thinning less than 100%.
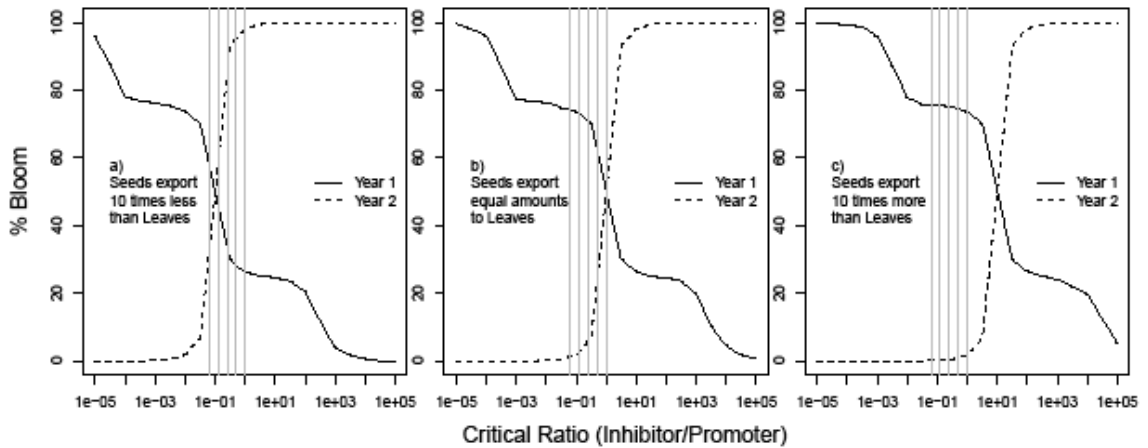
Fig. 7. Percent bloom and return bloom (dashed line) plotted against the critical ratio of

inhibitor to promoter hormones. The relative quantity of hormones exported by seeds

and leaves is a) 10:1, b) 1:1 and c) 1:10. Vertical lines indicate critical ratios of 1:1, 1:2,

1:4, 1:8 and 1:16.

Fig. 8. Percent fruiting SAMs remaining after thinning in the first year (solid line) and return bloom (dashed line). Seeds and leaves export equal amounts of hormone and flower inhibition occurs at the critical ratios of a) 1:1, b) 1:2, c) 1:4, d) 1:8 and e) 1:16 inhibitor to promoter hormones.

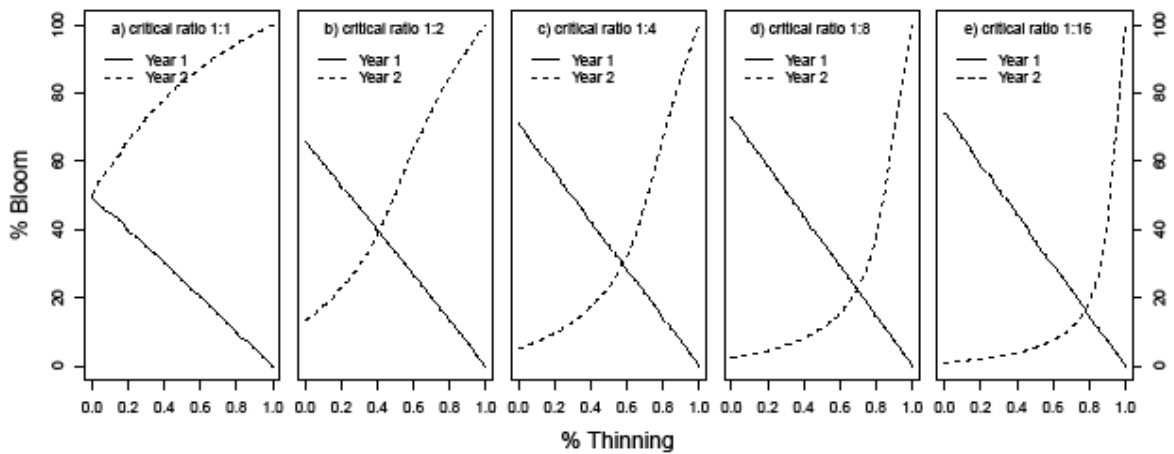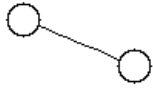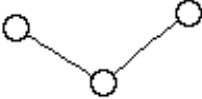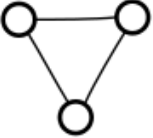| Neighbouring SAMs	All Flowering Possibilities	Year 2	Year 3	Year 4	Year 5
(two SAMs connected by a line)	○-○	●-●	○-○	●-●	○-○
	○-●	○-○	●-●	○-○	●-●
	●-○	○-○	●-●	○-○	●-●
	●-●	○-○	●-●	○-○	●-●
(three SAMs in a path)	○-○-○	●-●-●	○-○-○	●-●-●	○-○-○
	○-○-●	●-○-○	○-○-●	●-○-○	○-○-●
	○-●-○	○-○-○	●-●-●	○-○-○	●-●-●
	●-○-○	○-○-●	●-○-○	○-○-●	●-○-○
	○-●-●	○-○-○	●-●-●	○-○-○	●-●-●
	●-○-●	○-○-○	●-●-●	○-○-○	●-●-●
	●-●-○	○-○-○	●-●-○	○-○-○	●-●-●
	●-●-●	○-○-○	●-●-●	○-○-○	●-●-●
(three SAMs in a triangle)	○-○-○	●-●-●	○-○-○	●-●-●	○-○-○
	○-○-●	○-○-●	●-●-●	○-○-○	●-●-●
	○-●-○	○-○-○	●-●-●	○-○-○	●-●-●
	●-○-○	○-○-○	●-●-●	○-○-○	●-●-●
	○-●-●	○-○-○	●-●-●	○-○-○	●-●-●
	●-○-●	○-○-○	●-●-●	○-○-○	●-●-●
	●-●-○	○-○-○	●-●-●	○-○-○	●-●-●
	●-●-●	○-○-○	●-●-●	○-○-○	●-●-●

Fig. 9. A representation of how small groups of neighbouring vegetative SAMs (open circles) and reproductive SAMs (closed circles) can interact over time. Lines indicate where reproductive SAMs are close enough to neighbouring SAMs to inhibit flowering in the next year.

7.5: DISCUSSION

Many apple cultivars exhibit biennial bearing. Bienniality may be due to the relative concentrations of floral inhibiting and promoting hormones (Luckwill 1970; Kittikorn et al. 2010). This model represents this hypothesis mathematically with simulated trees. Although the hormone ratio hypothesis may be a simplistic representation of floral

initiation, it may be a reasonable estimate that captures the essence of inhibition from seeds and promotion from leaves.

The hormone balance hypothesis used in this model was suggested by Hoad in 1984. Other horticulturalists suggested only key inhibitor hormones (GAs) inhibited flower bud initiation; however, studies considering only inhibitor hormones did not give consistent results suggesting a combination of both inhibitor and promoter activity (Dennis and Neilsen 1999). In light of this, Dennis and Neilsen hypothesized a single leaf promoter hormone accomplishes both inhibition and promotion (1999). Seeds may be a strong sink for a 'florigen' compound which induces flowering. This would inhibit flower bud initiation if seeds accumulate most of the compound or if leaves are unable to export enough.

Mature apple trees on semi-dwarfing rootstocks typically have approximately ten main branches each with 50 to 200 SAMs. Such trees would have between 500 and 2000 SAMs. The model builds a canopy with 1000 SAMs using functions in the 'ape' package of R. This and similar packages of R ('ade4', 'adephylo' and 'seqinr') are standard tools for analyzing phylogenetic trees (Freckleton and Jetz 2009). For the agricultural modeller, the functions also offer powerful measurement tools to simulate fruit trees and other vegetation. Creating models with branching structures could improve our understanding of observed phenomena such as canopy structure and architecture (Prusinkiewicz and Lindenmayer 1990; Lauri et al. 2006).

The point source equation may be a suitable representation of hormone concentration over distance from the source. This equation supports the concept of distance-dependant floral inhibition as seen from the results of Bobb and Blake (1938). Because floral inhibition is thought to be a localized effect, the spatial arrangement of SAMs on a limb is perhaps as critical as the number in determining whether or not SAMs initiate flower clusters.

Calculating the inhibition and promotion hormone concentrations at every SAM provides the information needed to test the hormone balance hypothesis of floral initiation. If flowering is inhibited when the ratio of inhibitor to promoter hormones reaches a critical value (Hoad 1984), we would expect to find a range of possible critical values that could cause biennial bearing. This appears to be the case, as shown in Fig. 7. The plot shifts depending on the how much hormone reproductive and vegetative point sources export. A large range of critical ratios could cause biennial bearing (Fig. 7); thus it is possible flowering is inhibited when the ratio of inhibitor to promoter hormones exceeds a critical ratio. A critical hormone ratio is very common in many plant processes such as germination (Kucera et al. 2005), fruit growth (Cowan et al. 2001) and cell growth (Müller and Sheen 2008). In fact, GA is one of the main hormones involved in germination (Kucera et al. 2005).

Flower cluster or fruitlet thinning are common measures to control crop load and bienniality but the effect of such thinning on return bloom is frequently disappointing (Dennis 2000). Bailey (1929) recorded return bloom of two biennial cultivars in their off-

year after removing 50, 75 and 100% of the blossoms by hand in the on-year. Only 100% thinning consistently gave return bloom suggesting a nonlinear response to thinning as shown in Fig. 8e which represents a ratio of 1:16 inhibitor to promoter hormones. The model may help explain how SAM proximity limits the effectiveness of such treatments and may help us better understand biennial bearing. It is interesting that the minimum distance between fruiting SAMs did not change with thinning but remains at an average distance of 21.5cm. This suggests that the distances among flower clusters are like spokes on a tire. Many fruiting SAMs are almost the same distance apart so removing several does not leave remaining fruits further apart. This may be an artefact of the way canopy architecture was generated in the model but it does support studies that suggest tree architecture is an important component of biennial bearing (Lespinasse 1980; Lauri et al. 2006).

The aim of thinning on apple trees is to optimize the ratio of fruiting to vegetative growth. Modelling could help predict the effect of thinning on return bloom and predict maximum annual flowering SAMs. MaxAnn is achieved when the number of flowers on the tree is equal from year to year; it is the best possible annual bloom and difficult to achieve in reality, particularly on cultivars prone to bienniality. Where reproductive and vegetative point sources export equal amounts of hormone, as shown in Fig. 8b, MaxAnn values for critical hormone ratios of 1:1, 1:2, 1:4, 1:8 and 1:16 are 50, 41, 30, 22 and 16% bloom. The amount of thinning required to achieve MaxAnn for these was 0, 40, 60, 70 and 78%, respectively. These five graphics may represent real situations of the level of bienniality in a cultivar. They show levels of thinning that are more or less

effective in terms of increasing return bloom for MaxAnn flowering SAMs. This model outcome is similar to results in a recent apple orchard model by Hester and Cacho which predicts optimal thinning strategies for a 15-year period (2003).

This model presents a logic framework which shows how easily biennial bearing can be established (Fig 9). This may be useful to growers, fruit breeders and others seeking to understand the impact of flower bud proximity and branch architecture on crop yield. The model was designed to help researchers understand biennial bearing and is not ready to be used by growers. Field experiments are needed to validate the model and determine its applicability to specific cultivars, management systems and environments. It could be a powerful tool to manage biennial bearing. We encourage critique of the model and look forward to collaborating with other researchers to refine and field test the model for different cultivars and environments.

Chapter 8: CONCLUSION

This thesis presents two models to describe biennial bearing. The first model used an empirical approach and estimated biennial bearing patterns as alternations between 0 and 50% bloom followed by 50 to 100% bloom. Based on the current understanding of bienniality, this is a reasonable if simplistic approach but it fails to describe underlying reasons for the pattern.

This led to the second model of bienniality where hormonal inhibition by flower clusters was considered to be distance dependant. This provided a possible basis for bienniality and addressed hormone inhibition in a way not yet considered in the apple tree literature. The model is focused on the hypothesis that a balance of hormones produced by reproductive and vegetative tissues controls floral initiation; it does not take account of factors such as cultivar, rootstock, environment and the timing of thinning, which are also known to influence biennial bearing.

The concept of maximum annual flowering (MaxAnn) is useful in setting goals for thinning to ensure equal numbers of flower clusters are borne annually. In the first model, MaxAnn is calculated for a mature tree right up to the final year of the orchard. In the second model, MaxAnn is calculated over only a two year period which provides insight on year-to-year blossom fluctuation due to the previous crop. It does not take account of more complex phenomena such as annual growth, or pruning regimes or the very complex and confounding long term interactions of annual management treatments with climatic variations.

Our understanding of apple tree bienniality remains limited. This second paper demonstrates how the proximity of flower clusters could cause biennial bearing. At this stage, the model is not ready to be applied directly to field work because it considers only one factor affecting biennial bearing, flowering and does not take account of other influences such as leaf area (Lakso et al. 2008), climate and pruning regime. Regional differences may make particular solutions for biennial bearing ineffective. For instance, Honeycrisp is a biennial cultivar in the Annapolis valley region of Nova Scotia but an annual cultivar in Michigan (personal correspondence with Dr. Frank Dennis). However we hope this model contributes to helping researchers and fruit growers better understand the hormonal mechanism behind biennial bearing and make more informed decisions about chemical thinners.

As modelling biennial bearing progresses we should be able to incorporate herbivory and abiotic factors that affect crop growth and yield. For example, Dr. Lakso developed a carbon balance model for apple trees with light and temperature as inputs (Lakso et al. 2008) and Allen et al. (1993) developed a plant-herbivore model of grape vine insect pests. Recently a bio-economical approach to apple orchard management was developed (Hester and Cacho 2003) helping to bring different interdisciplinary approaches together.

This model may have applications to other perennial fruit crops. It could also be applied to other situations where causal or spatial relationships exist such as regional

economics, stratospheric contaminants, military deployments, ecosystem management and molecular structures.

Aside from systematically measuring the entire canopy and locating individual flower clusters as done in the second model, a crop load prediction for the apple industry should include canopy architecture or volume (Wright et al. 2006). Fractal dimension of canopies as discussed in the thesis should be explored as well because it is a powerful way to simplify complex tree architecture into a dimension. It would be useful to implement spur wood pruning (Nichols et al. in press) because it encourages new growth which bears fruit after two or three years.

Having modelled hormone concentration to predict flower bud initiation leads naturally into measuring promoter and inhibitor hormone concentrations at SAMs. Considering how complex flower initiation pathways are discovered in Arabidopsis (Glover 2007), studies of hormone pathways in apple flowering would be very beneficial and expand our understanding of flowering plants.

APPENDIX A

Essential R code to create the flowering branch model.

```
library(ape)                          #Analyses of Phylogenetics and Evolution package

crit<-1e-02                           #Critical value beyond which floral inhibition occurs

t<-0                                  #Thinning 0% of the flower clusters

ntip<-1000                            #Number of SAMs on a tree

max.yr<-4                             #Maximum year iterated

br<-sample(0:20,2*(ntip-1),replace=T)#Branch lengths for crown

crown<-rtree(ntip,br=br)              #Tree crown

r<-cophenetic(crown)                  #Distances between SAMs

r[r<1]<-1                             #Setting minimum values to 1cm

S<-c(max(r)*.6,1)                     #Scale size to estimate fractal dimension

L<-S*c(1,sum(br))                     #Length to estimate fractal dimension

D<-1-diff(log(L))/diff(log(S))        #Fractal dimension based on Richardson effect

y<-matrix(1,ncol= ntip,nrow=max.yr)  #flower matrix

colnames(y)<-crown$tip.label          #Each column represents a unique SAM

y[1,]<-sample(0:1, ntip,replace=T)    #Initial flower data
```

```
for(yr in 2:max.yr){                      #Years 2 to max.yr

        hs<-runif(ntip)                    #Hormones exported from seeds

        hl<-runif(ntip)                    #Hormones exported from leaves

        seeds<-y[yr-1,]                    #Location of seeds

        leaves<-abs(y[yr-1,]-1)            #Location of leaves

        chs<- (hs/r^(D-1))%*%seeds #Concentration of seed hormones

        chl<-(hl/r^(D-1))%*%leaves #Concentration of leaf hormones

        not.f<-chs/chl>crit                #Floral inhibition if greater than a critical value

        y[yr,][not.f==T]<-0                #Which SAMs don't flower?

        if(yr==3){                         #Thinning flower clusters in year 3

                y[3,]<-y[3,]*sample(0:1, ntip,prob=c(t,1-t),replace=T)

        }

}

z<-apply(y,1,sum)                          #Number of flower clusters each year
```

APPENDIX B

January 24, 2011

ASHS Executive Director
1018 Duke Street
Alexandria, VA 22314

Dear Michael Neff,

I am preparing my MSc thesis for submission to the Faculty of Graduate Studies at Dalhousie University, Halifax, Nova Scotia, Canada. I am seeking your permission to include a manuscript version of the following paper as a chapter in the thesis:

A theory of blossom thinning to consider maximum annual flower bud numbers on biennial apples trees. Pellerin, B.P., Buszard, D., Iron, D., Embree, C.G., Marini, R.P., Nichols, D.S., Neilsen, G.H., and D. Neilsen. HortScience 46(1):40–42. 2011

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