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First Things First: Matching an Alternate Bearing Model to Confirmed Field Phenotypes of Avocado (Persea americana, Mill.)

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# First Things First: Matching an Alternate Bearing Model to Confirmed Field Phenotypes of Avocado (*Persea americana*, Mill.)

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by

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June 2017

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# ABSTRACT OF THE DISSERTATION

## First Things First: Matching an Alternate Bearing Model to Confirmed Field Phenotypes of Avocado (*Persea americana*, Mill.)

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Doctor of Philosophy, Graduate Program in Plant Biology
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Dr. Mary Lu Arpaia, Chairperson

Avocado (*Persea americana,* Mill.) fruit production continues to be an expanding business worldwide. Larger amounts of quality fruit are needed to satisfy the demand but there seems to be an embedded tradeoff: after trees bear many fruits, they seem bound to produce too little the following season and vice versa. These yield fluctuations are horticulturally known as "alternate bearing" and is traditionally considered to be a consequence of negative perturbations rising from fruits themselves. But, is it? Are we approaching the issue with models that need fruits to be actively detrimental to the tree? Now that we are utilizing molecular tools in avocado research: do we properly distinguish the phenotypes when we sample? Future research could experience difficulties due to those scenarios and hence this investigation explored which were the actual vegetative and reproductive growth phenotypes between fruiting and a non-fruiting shoots as, due to "alternate bearing", they were expected to be divergent. Typically, fruiting shoots grow one vegetative flush and, by the end of the first growth season, do not release lateral branches. At their succeeding season, fruiting shoots display reduced release of axillary buds and, if they bloom, a low flowering complexity. Conversely, non-fruiting shoot initiate more consecutive vegetative flushes, undergo lateral branching and during the succeeding season release more axillary buds displaying a gradient of acropetally increasing reproductive growth presence and complexity. Although comprehensive and resilient in different production areas of the world, these divergent phenotypes can be practically swapped by sunlight exposure within the canopy environment. Avocado trees seem so dynamically responsive to sunlight that it can render fruit presence phenotypically unimportant and, with that, new working models for avocado “alternate bearing” need to consider contextual and dynamic research approaches. Phenotypically complying models that can accommodate to sunlight exposure may be achieved by assigning fruits with a more passive role of a strong sink organ that is necessary for the life cycle of the avocado tree, certainly not detrimental or powerful enough to be the single and ultimate explanation of the “alternate bearing” phenomenon.

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# 1 Chapter 1: Introduction to the “alternate bearing” phenomenon

## 1.1 General introduction

Maybe because there is only around a century of human involvement in their domestication, avocados (*Persea americana*, Mill.) continue to challenge growers and researchers alike while offering many opportunities for its improvement.

Literature is ample in efforts to understand and explain avocado tree plasticity but, at the same time, the horticultural business aspect of its cultivation generates a need to produce practical management tools that can be readily deployed in the orchards. This might have led to an unwilling preference for broad observations and research approaches together with subjective language and a preferential focus on the huge driver that is the tree “fruit yield”, leaving other more subtle but informative aspects of this specie somewhat relegated.

“Alternate bearing” is an agricultural problem that growers cope with and comprises both fruit production and tree growth aspects. In its simplest definition, “alternate bearing” is the yearly fluctuations in the fruit yield of the avocado trees and these variations become disruptive to the orchard management and planning.

The research below offer the avocado research community a reduction of the scale of observation of the “alternate bearing” phenomenon to the point that it remains pertinent to the avocado field phenotypes and at the same time tries to bridge it with molecular biology knowledge and tools. Figure 1-1 presents an overview and general structure of this research.

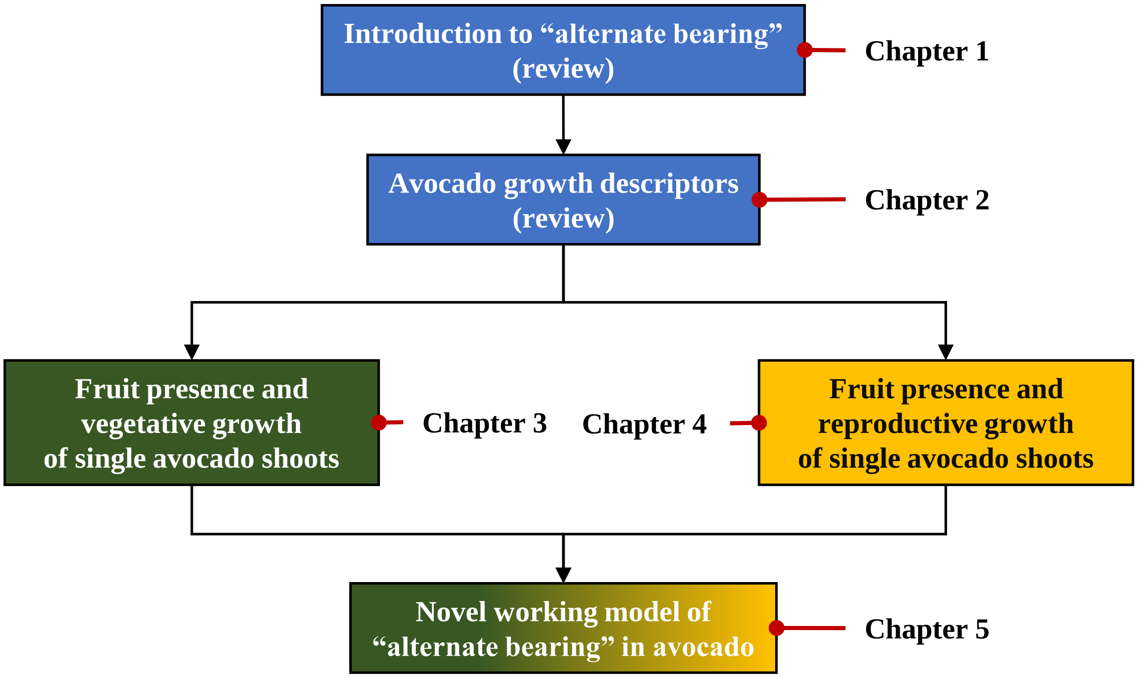

**Figure 1-1. Overview of the research structure.**

## 1.2 An agricultural problem

Fruit tree cultivation is a worldwide business and as in any other business, its ultimate goal is to reliably produce, distribute and sell its product. When a living organism (tree) is the "factory" and fruits are the final product then problems with consistency are bound to occur.

From the perspective of the growers , fruit trees exhibit what is called a "bearing behavior"[18, 22, 23] which can be described as the natural fluctuations in the amount of fruit supported by a single shoot, branch, tree, orchard or at even larger scales depending on the point of view and interest. Given the choice, growers would prefer consistent bearing behavior i.e. trees with low natural fluctuations in the amount of fruit produced per cycle, but in practice the opposite is prevalent which disrupts orchard management planning and ultimately affects economic returns.

"Alternate bearing" is the most commonly used descriptor of a bearing behavior that swings from high fruit yields to low fruit yield in two consecutive cycles. Figure 1-2 depicts the yield fluctuations per annual cycle ("seasons") for avocado production in California. A high yield season is often followed by a season of low fruit yield.

Despite the previous, Figure 1-2 also shows that, among other instances, 2 consecutive seasons (1982 to 1983 and 1983 to 1984) which had 2 seasons with increasing total yields were followed by 2 years of decreasing yields. Given that the data is generated by the avocado industry as a whole, normal agricultural decisions like new plantations and tree renewal could be affecting these yearly numbers. However, it does challenge the idea that "alternate bearing" behavior is "predictable" on itself.

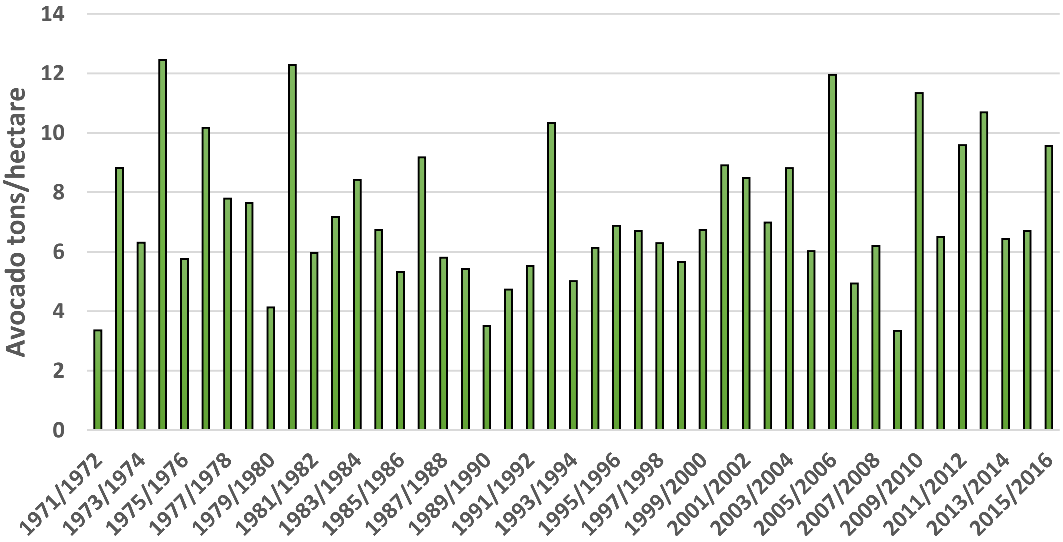

**Figure 1-2. Avocado bearing behavior and its crop value in California as reported by the California Avocado Commission.**

Source: California Avocado Commission (CAC)[4]

In 1946, when the ‘Fuerte’ avocado variety was still dominant[6], Professor Robert W. Hodgson, the Dean of Agriculture at UCLA and leading authority in research and cultivation of avocados was already highlighting this problem:

**Quote 1-1. Undisputed belief can be a problem.**

> “In addition, certain inaccurate beliefs founded on this known [“alternate bearing”] tendency have been accepted by many growers. Among these are the assertion that the "on" year crop [high yield] is always followed by the "off" year [low yield] and that all ‘Fuerte’ trees everywhere, without regard to planting dates, have the same "on" and "off" years.”[17]

The previous quote encompasses some aspects involved in the “alternate bearing” phenomenon, which are discussed below:

### 1.2.1 “Alternate bearing” is a growers, not a tree, problem

Growers prefer control and consistency in their fruiting trees cycles. Trees, on the other hand, are plastic in responding and adapting to their environments, searching for increased lifespan and fitness success. It was through agricultural history that humans created a demand for crops that consistently bear the marketable organ (fruits for example) while at the same time fit to production time frames and yield levels[41] in order to maintain markets and profitability.

### 1.2.2 We want to believe

It is important for growers and researchers to be able to predict or at least reach for higher levels of certainty, hence it is attractive to believe that regardless of local conditions and orchard management the trees you care for in terms of production or as research subjects are coordinated in the “alternate bearing” cycle, allowing prediction and planning.

## 1.3 Towards a definition

"Alternate bearing" is a widespread phenomenon on deciduous and evergreen polycarpic species[32]. Several fruit trees are prone to it, such as apple[9, 16, 32], pistachio[10, 32, 36, 44], pecan[32, 42], olive[11, 32, 34, 42, 52], citrus[32, 33, 48], avocado[6, 17, 22, 25, 32, 38, 49, 53] and mango[32].

The frame of reference used to discuss this horticultural phenomenon[19] of "alternate bearing" is ample and nonspecific, not due to the lack of knowledge but to its very nature of dealing with growth and reproduction. In the conclusions of a 2005 review of the subject, one of the most respected researchers of "alternate bearing" of fruit trees, Professor Eliezer E. Goldschmidt said:

**Quote 1-2. "Alternate bearing" as a homeostatic response.**

> "In a broader evolutionary sense, "alternate bearing" should perhaps be understood as a phenomenon of homeostasis. The alternating, homeostatic behavior of trees secures their survival as a long living fruiting organism."[13]

Certainly the fluctuations in fruit yield can be seen as a natural consequence of maintaining a dynamic equilibrium in spite of changes in the environment[40]. Using a population ecology perspective, the "Moran effect"[35, 43] states that climate plays a role in synchronizing large areas of a population into a similar behavior and in the case of "alternate bearing", it can be initiated by weather events like freezes and heat stress[25] which are more or less damaging depending on the phenological stage of the tree but given that weather events are not biennial[43], it is understood that the environment itself is not the cause of "alternate bearing"[41, 43].

The entrainment of this rhythmic production cycle[50] is thought to be the outcome of endogenous interacting factors[32, 41, 43] coded in the genome[50]. The fruit production swings caused by it are then perpetuated by those fruit loads themselves[25]. Once the rhythm is initiated, it remains until a new alteration of the cycle (a "change of phase" or "stride"[19, 22, 23]) is cause by the environment and/or by orchard management interventions[50].

Nonetheless, the concept of homeostasis is still broad. Furthermore, an organism invests energy to remain within their homeostatic limits so there should be an evolutionary advantage to “alternate bearing” (see Table 1-1).

The “principle of allocation” is closer to the agricultural time frame as it can be seen acting in shorter term succeeding (biennial) cycles (“ON” to “OFF”). On the other hand, the “predator satiation” perspective is more associated to forestry and non-cultivated perennials[43] in relation to the “masting” phenomenon, which occurs in cycles longer than two years[43].

Table 1-2 (adapted from Goldschmidt, 2013[12]) presents traits associated to the level of domestication of a specie. An intermediate genetic distance from their wild progenitors is still associated to a mixed and unpredictable behavior. Through selection and domestication we have and can continue to modify the phenotypic outcomes of fruit trees towards more reliable agricultural behavior[14].

**Table 1-1. Potential evolutionary advantages and agricultural problems due to "alternate bearing".**

| Name | Cause | Evolutionary advantage for the tree | Agricultural problem |
|---|---|---|---|
| *"Principle of allocation"*[43] | • Limited availability of resources<br>• Physiological constrains (allocation trade - offs) due to differential use of the limited resources<br>• Reproductive growth (flowering and fruiting) are resource demanding[49] | • Balances vegetative growth (tree survival) with reproductive events[43]<br>• Evolutionary pressure to improve tree physiology performance to better use the limited resources[43] | • Reduced allocation to vegetative growth[43]<br>• Reduced frequency of reproductive growth[43] |
| *"Predator satiation"*[43] | • Fruit/seed predator population | • Increased chances of seed germination (survival) by overproducing reproductive organs[43] | • Burden of abundant reproductive growth is detrimental for future reproductive cycles [43] |

**Table 1-2. Evolutionary scale of fruit tree domestication.**

| Factor | Wild | Intermediate | Domesticated |
|---|---|---|---|
| **Growing areas** | Native, forest habitat | Vicinity of human settlements, home gardens | Highly managed habitats |
| **Reproduction and propagation** | Seed reproduction, Dioecious | Seed and/or vegetative propagation, Mixed-type flowering | Vegetative propagation, grafting, Hermaphrodites, parthenocarpic fruiting |
| **Length of juvenile period (inability to be reproductive)** | Extended juvenility | Reduced juvenility | Juvenility short or absent |
| **Environmental constrains (stress)** | Environmental constrains, wild plant competition, biotic and abiotic stresses | Partial relief of environmental stresses | Management of nutritional and biotic stresses |
| **Fruit bearing behavior** | Irregular fruiting, masting | "Alternate bearing" | Regular fruiting |

Phenotypes and interactions with horticultural management in relation to an evolutionary distance from wild progenitors. Modified from Goldschmidt, 2013[12]

## 1.4 Actual definition

How is “alternate bearing” actually defined in the literature? As a way to give perspective to the definition below are three quotes potentially separated by more than 1,600 years:

**Quote 1-3. Searching for a specific definition.**

- 4th Century, on the *Talmud Yerushalmi*, *Sheviith* as cited by Monselise and Goldschmidt (1982): "all trees bear fruit one year and leave one year off, but the fig bears regularly every year”[32]
- Year 1847 as cited by Tukey (1922): “ It is well known that the Baldwin [apple] only bears every other year”[46]
- Year 2016 by Haberman, et al.: “This biennial cycle of fruiting, termed “alternate bearing” (…)”[16]

One of the most specific definitions encountered is that “alternate bearing” is a “biannual program of reproduction”[41] but most of the time, “alternate bearing” is assumed and simply spoken about without a more precise definition. To the best of my knowledge and as a compilation of what is commonly used across the literature, “alternate bearing” is a contextual definition that revolves around a grower’s perspective to which a “good” (usually meaning large) or “normal”[32] fruit yield is succeeded by an unsatisfying crop (usually meaning “low” yield). After that, the next season is expected to be “good” again.

## 1.5 “Alternate bearing” phenotype

Despite not having a straightforward definition, general tree phenotypes are expected (Table 1-3 and Table 1-4).

**Table 1-3. General phenotypes of the “alternate bearing” cycle (tree characteristics)**

| “Alternate bearing” cycle status | Fruit interpretation | Flower interpretation |
|---|---|---|
| “ON” | High fruit yield at harvest | Abundance of flowers at bloom |
| “OFF” | Low fruit yield at harvest | Reduced flower abundance at bloom |

The most common interpretation of the “alternate bearing” cycle status is the “fruit interpretation”[32, 42, 43]. Accordingly, a season is defined as “ON” when a high fruit load is observed during or at the end of a season. Also it is used as a projected concept when a high yielding season is expected in the future (“will be an “ON” season”). The opposite applies to an “OFF” season.

Sometimes a flower interpretation is used which is slightly different. An “ON” cycle would be the one that starts with an intense bloom[25] which after pollination set an “ON” crop (and vice-versa). Although it makes sense that and abundance of flowers has potential to lead to a high yield many months later, not only is there a chronological distance in between these two points of view (flowers are first along the phenological sequence) but also there are different sets of molecular events and many uncertainties between fruit set and final fruit yield such as tree variety, pollinizers, bee visitations, fruit drop, fruit retention and biotic and abiotic stresses. Independently of its likelihood, a tree with an abundance of flowers at the beginning of the season can potentially have a low fruit yield and at the same time, a tree with a reduced bloom could have a successful fruit crop on those few available flowers.

Adding to the confusion, there are also instances on which a reduced bloom that comes after an “ON” (large) fruit crop is itself called a “ON bloom” but it means that it is a bloom with a reduced intensity that is “caused” by the previous “ON” fruit crop. In this case also the vice-versa applies and an “OFF bloom” would then mean a “large”, abundant bloom at the succeeding season.

It is usual that “alternate bearing” research simplifies tree anthesis to a once a year event[42]. Accepting the previous and then more strictly relating the “alternate bearing” phenomenon to the overall fruit presence, the tree phenotypes in Table 1-4 are expected.

**Table 1-4. Expected “alternate bearing” tree phenotypes in reference to the fruit presence**

| **Season (n)** **Fruit yield at harvest** | **Season (n)** **Vegetative growth developed concurrently with the fruit** | **Season (n+1)** **Bloom intensity at beginning of the season (usually spring)** |
|---|---|---|
| High fruit yield | Reduced vegetative growth | Reduced bloom intensity |
| Low fruit yield | Increased vegetative growth | Increased bloom intensity |

Although for Table 1-4 the fruit presence is fixed as the initial criteria, it can still be difficult to grasp the fact that the amount of mature fruits that is expected, “high” or “low”, developed on the trees in the prior 6 to 12 months or more[5, 32] and the effects on tree size (vegetative growth) happened during that same period concurrently with the fruits development. In addition, the fluctuations in bloom intensity are only visible at the next flowering period.

### 1.5.1 Quantifying “alternate bearing”

In practice, as depicted in Figure 1-2, consistent alternation does not occur. Nonetheless, several parameters have been developed through time and were compiled in a review by Monselise and Goldschmidt (1982) [32].

One of them, the “I” ratio of intensity of deviation in yield through successive seasons [32] became the most accepted and the base for the current “Alternate Bearing Index” (ABI)[15, 24, 29] in which $y_i$ is the $i^{th}$ observed yield in an ordered series of size $n$, and $|y_i - y_{i-1}|$ is the absolute difference between successive yields. Ultimately, the ABI or other parameters are based on counting or weighing fruits and keeping track of total yields per unit of interest.

**Equation 1-1. Alternate bearing index (ABI).**

$$I = \left({}^{1}\!/_{n-1}\right) \times \left[\sum_{i=2}^{n} \frac{|y_i - y_{i-1}|}{(y_i + y_{i-1})}\right]$$

## 1.6 Mechanisms of “alternate bearing”

Beyond keeping track of fruit number or harvest weight fluctuations, and as a way to describe how ample the “alternate bearing” concept is, Table 1-5 shows mechanisms that have been associated to this phenomenon in different agricultural crops. As shown in the Table, the “alternate bearing” phenomenon is likely a multigenic trait[15] that is probably influenced by phytohormones[15] and other interacting factors.

Table 1-5 also highlights that not only several of the described mechanisms constitute reiterations of the same concepts but also that they can incorporate loose (or imprecise) language. Even contrasting phenotypes can be found, such as the described effects of high “ON” yields over the pistachio shoot length which is reduced for some authors but increased for others.

In general, the described mechanisms of “alternate bearing”, although abundant, are not straightforward. Many different points of view, language, approaches and phenological stages are employed in this research and that can be troublesome.

### Table 1-5. "Alternate bearing" mechanisms during "ON" cycle as described for different crops

| Fruit crop | Vegetative growth effects | Reproductive growth effects |
|---|---|---|
| Avocado | • Reduction in overall vegetative growth[31, 38, 47, 49]<br>• Reduction of fruiting shoots length[31, 51]<br>• Reduction of vegetative flushes[25, 38]<br>• Reduction of root growth[5]<br>• Potential bud break inhibition[38] | • Reduction in both intensity and spread of the bloom period[31, 38, 49]<br>• More indeterminate than determinate floral shoots (the latter being more "productive")[38]<br>• Does not cause flower nor fruit abscission[38]<br>• Reduced return bloom on fruiting shoots[25, 38] |
| Apple | • Repression of vegetative growth[41]<br>• Inhibition of bud break[49] | • Reduced floral intensity/flower formation/flower primordia formation[15, 16, 32, 49]<br>• Lack of flowering positions[32]<br>• Repression of floral induction[41]<br>• Inhibition of inflorescence formation on fruiting bourse shoots[16] |
| Citrus | • Reduced vegetative growth[38]<br>• Inhibition of bud break[49] | • Reduced floral intensity[33, 38, 49]<br>• Reduced "sheading of surplus fruits" ("self-thinning")[32]<br>• Reduced production of *"leafy"* inflorescences[32] |
| Olive | • Reduced vegetative growth[38, 41]<br>• Reduced production of new branches[32]<br>• Bud abscission[25] | • Reduced floral intensity / less flowers[32, 38]<br>• Reduced floral induction[11, 41]<br>• Limitation of floral initiation[37] |
| Pistachio | • Reduced shoot growth[32]<br>• Increased shoot growth[44] | • *"Unusual"* mechanism of "alternate bearing"[32]<br>• Abscission of partly developed flower buds (rather than their inhibition)[32, 44]<br>• Longer shoots retain less flower buds[32] |

### 1.6.1 Rootstock effects on “alternate bearing”

On avocados, there are older reports of no effects due to rootstocks on “alternate bearing”[20, 32] but this could have been due to high variation within the research trees[49] since these older studies used seedling rootstocks and avocado is known for its high level of heterozygosity. Later studies using ‘Hass’ avocado scions grafted onto clonal rootstocks were done as a way to standardize the scion and rootstock genotypes but still the “alternate bearing” indices of the research trees did not differ due to the rootstock influence alone[30, 31]. Despite the previous, the rootstocks used in those studies were actually a subset of a larger 10-year rootstock trial reported by Mickelbart et al.[29] which showed differences in “alternate bearing” tendencies due to rootstocks. Others have reported that rootstocks can influence “alternate bearing” in avocado[45, 49]. Rootstock effects on the “alternate bearing” phenotype can also be found for citrus[32, 49] and apple[32].

## 1.7 “Alternate bearing” models

There have been several models put forward to explain “alternate bearing” (Table 1-6). Earlier models try to explain the observed mechanisms of “alternate bearing” as “external” to the trees such as inadequate pollination and unfavorable weather conditions. The later models focus primarily on “internal” factors; some were thought as acting on specific moments in the phenology of the fruit tree (for example during the bloom period) while other span their influence over two seasons (the outcome of the first season would impact the outcome of the second season).

Although it is possible to distinguish certain limits within these models, in practice there is abundant crosstalk between them as fundamental knowledge progressed into new areas and previous ideas remained. This is certainly understandable as “alternate bearing” ultimately is a study of vegetative/reproductive growth and development which certainly involves complex interactions.

**Table 1-6. Models that explain the “alternate bearing” mechanism**

| Cause | Consequence | Status |
|---|---|---|
| Uncertain or inadequate pollination[21] | Lower fruit yields due to reduced synchronization between pollinizer flower behavior, pollinator behavior (bees) and also cross pollination opportunities | Was one of the first models but in early 1930’s it was discarded as a main cause[21, 23] |
| Weather conditions | For the ‘Fuerte’ avocado variety (dominant at the time which blooms during winter), unfavorable temperatures (lower than 13.3° Celsius[18]), frost events, overcast skies and wind would reduce fruit set | Also discarded as a cause and was only associated to changes in the change of “phase” or “stride” of the alternation (causing 2 consecutive low bearing seasons for example) |
| Amount of fruit | A large crop on the previous season causes “alternate bearing” by reducing “fruit-bud differentiation”, delays time of bloom, reduces its quantity and the yield the following season[23] | This point is basically the most general interpretation of “alternate bearing” up to this day. It became a pivotal point leading to focus on the existing fruits being the cause |
| Mineral depletion | Large fruit crop would deplete available mineral nutrients in their growth preventing future vegetative and reproductive growth[21, 28] | Research tended to demonstrate that the depleted materials were of organic nature instead of mineral[21] |
| Starch reserve depletion | (Related to the previous) A large crop would use the starch reserves during their growth and that depletion would prevent a large crop the succeeding season[21, 50] (“can’t recuperate”) | Due to variability at different scales of growth, time frames and location, it is considered unsatisfactory as an explanation of the alternation. Carbohydrates reserves are thought as useful “integrators” of the overall tree condition[26, 50] |
| Phytohormones produced and exported by growing fruits | Seeds inhibit flowering (succeeding season) by exporting hormones (focus is on gibberellins) which are thought to have “anti-flowering” effects[3, 38, 39, 43, 50] | • This model needs a transport system of GAs out of the fruits against bulk flow towards the fruit sink, which currently is unknown.<br>• An alternative explanation is that seeds compete with the shoot apex for flowering hormones[50].<br>• Also, there are reports of non-seeded fruits still being described as alternating[32, 43] |
|  | Dominant fruits export auxin which reduces the growth of the shoot that is bearing it[1, 2, 43] | Fruits are reported to import auxin[27] and to be poor auxin exporters[7, 8] |

|  |  |  |
|---|---|---|
| Fruit load affects gene expression | Fruit presence disrupts/alters flowering by modulating expression of flowering integrators and floral identity genes on leaves and meristems[16, 33, 53] | • Downregulation of FT expression is reported on leaves and bud meristems of citrus and avocado[33, 53] but on the latter, it is hypothesized that is due to the growing fruits being strong sugar sinks and that in turn, the sugar levels would be involved in the gene expression.<br>• Accumulation of *TFL1* transcripts is reported in bud meristems of apple[16]<br>• At this point, these are correlations and the actual mechanisms is still unknown although application of gibberellins had similar effects on apple *TFL1* accumulation. |

Interestingly, more recent Quantitative Trait Loci (QTL) analysis of “alternate bearing” in apple[15] and general reproductive growth in olives[37] are co-localizing with yield and fruit traits but less likely with flowering genes traits (or only as “year specific” events[37]) under their research conditions. Even further, more general traits like hormonal control (gibberellins and auxin)[15, 38], vegetative growth, branching[37] and tree architecture[15, 38] seem to be more pertinent to the relation between fruits, vegetative growth and reproductive growth. A genome wide identification of “alternate bearing” related microRNAs in olive concluded that nutritional status and hormonal control are involved in the “alternate bearing” phenomenon[52]. It is interesting that the same was previously reported in the seminal review from Monselise and Goldschmidt in 1982[32].

## 1.8 Research goals

From older to newer, many approaches that tried to mechanistically explain the “alternate bearing” phenomenon seem to reach broad conclusions or do not produce a general model that most researchers agree on, work with and cooperate. In contrast to the balance between molecular tools, phenotypical precision and consistency attained by the Arabidopsis research community, it is problematic that less precise, local or “commonly known” descriptions of the “alternate bearing” phenomenon are still present in the current literature leading to open interpretations and difficulty in the replication of the methods and sampling schemes deployed.

Despite the previous, an overarching concept across any “alternate bearing” research effort is the central role given to growing fruits as triggers (or cause) of the “alternate bearing” phenomenon but then again it is commonly addressed and measured at different scales (“branches”, “whole trees”, orchards) which introduces uncertainty to the results.

With a focus on a worldwide agricultural business that is the alternate bearer ´Hass´ avocado variety, our goal is to identify and confirm that the general “alternate bearing” phenotypes classically described at the whole tree level are truly and consistently observable at the smallest, relevant, more manageable and replicable scale. Then this consistency will be tested in different environmental conditions of several avocado producing areas. Finally, an “alternate bearing” working model that attempts to both fit the actual phenotypical patterns observed in the field and to explain the “alternate bearing” phenomenon will be introduced.

We want to contribute in the overall goal of improving our mechanical understanding of the avocado phenology and physiology by proposing a transition to a system that facilitates future exploration of the cascade of molecular and developmental events that are being overlooked by focusing on broad outcomes like tree size and yield. This transition is necessary to guide the use of molecular tools in the development of new elite avocado varieties less affected by the “alternate bearing” phenomenon.

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# 2 Chapter 2: From canopy to shoots: avocado growth descriptors and their relevance to "alternate bearing"

## 2.1 The avocado

The avocado (*Persea americana*, Mill.) is a basal angiosperm that emerged at the early development of flowers and fruits and sits among the oldest known flowering plants[6, 8, 9, 40]. It is a member of the Lauraceae family that originated in the tropical and subtropical areas of meso-america as an understory plant[10].

It is a diploid (2n = 24)[8] tree with a genome size of 883 Mb[7]. The flower's protogynous dichogamy[37, 51] together with hybridization among and within the different races (West Indian, Guatemalan and Mexican)[12, 51] makes it moderately to highly heterozygotic[8, 16] which is favorable in terms of preserving genetic diversity but that complicates the search for patterns and interactions[19, 41].

Appraised by humans for 10,000 years[1] it is only in the last ≈100 years that modern selection schemes and vegetative propagation has been used in this specie establishing it as a fairly new commercial crop[10]. Despite this short domestication period, it ranks among the top 25 produced fruits in the world[18] with an increasing consumption and space for market growth due to its nutritional value[7, 49]. The United States are the second largest consumer of avocados (after Mexico)[18] and at the same time the largest importer from top producing countries[18, 49].

## 2.2 Avocado tree form

Perhaps in part due to the absence of a publicly available avocado genome assembly and other fully developed molecular tools for this specie, the avocado research community has not made joint efforts to aid further gene discovery by developing basic consensus on phenotypical descriptors of structures, stages and growth patterns that are pertinent to their research goals. That is in opposition to the proven success of the *Arabidopsis* research community that has defined and embraced a common phenotypical language[5] that is valid and used throughout the world, hence maximizing objectivity, communication and collaboration among research groups.

Some important aspects of the avocado tree, like its floral behavior or that fruit expansion is mainly attained by cell division instead of cell expansion[6] just to name a few, denote that avocados are distinct from model plants and other commercially important fruit crops, not only through their considerable evolutionary distance as a basal angiosperm[8, 40] but in their practical field behavior. Caution should be taken when research intends to simply correlate avocados to previous knowledge and to models developed from the observation of more domesticated crops.

### 2.2.1 Descriptors to segregate

It is certainly possible to find published avocado descriptors with excellent reviews[47] that are rich in details and in visual aids but these publications[3, 4, 23] are clearly meant to be tools to characterize and/or differentiate avocado varieties for germplasm conservation, certification, plant breeders rights[4, 23] and patent applications.

To exemplify this point, verifying and comparing flower structures[4]; the characteristics of small sections within the leaf lamina[4]; the difference in cotyledon color[23] or the intensity of the anise scent[3] are without a doubt phenotypical traits that are useful to compare different accessions but in the context of research that attempts to study a set of developmental events that, through time, lead to a complex array of vegetative and reproductive phenotypical outcomes (such as the “alternate bearing” phenomenon), those traits are less useful and different descriptors at a more pertinent scale need to be developed.

### 2.2.2 General descriptors of avocado growth

At the whole tree level, the avocado tree structure matches the Rauh's architectural model[10, 43, 44], which is widely observed across plants[21]. It develops through the indefinite and rhythmic functioning of the apical meristems with axillary meristems also releasing into an equivalent growth "program"[15, 21]. Nonetheless, the overall tree form varies by cultivar and location[44].

For sections within a tree canopy, ontological terms such as "trunk", "branches" or "limbs" are commonly used despite their reduced precision. Hallé and Oldeman (1975)[21] described these concepts as "pre-scientific" and in need of definition. Those authors defined "trunk" as a vegetative axis with a mainly structural role (mechanical resistance, growth in height) while also giving "physiological coherence" to the tree. They defined "branch" as the vegetative axis that does what the "trunk" doesn't do anymore: photosynthesis and the development of sexual organs.

Agriculturally, these descriptors of vegetative growth are an outcome of accumulated working knowledge that compiles aspects like color and texture[44] , size, position and insertion angles, physical strength (lignification) and visual hierarchy of the vegetative structures which implies a degree of subjectivity. For example, lengths of 1 m[35] and 6 – 10 cm of basal diameter have been used as criteria for classification of vegetative growth as a "branch"[34]. Others, dealing more in depth with tree architecture, defined vegetative structures with basal diameters of 3.5 cm or larger as "major limbs" which themselves can have lateral "branches"[44] that range in length from 50 cm (short branch) to more than 1 meter (long branch). Placed together it could mean that what is a "branch" for one group is a "limb with branches in it" for others.

More detailed descriptions of the overall avocado vegetative growth dealt with aspects of tree height, tree diameter and specific features of "major limbs" (frequency, inclination) which together are an outcome of apical dominance and apical control interactions within the developing avocado canopy[44].

## 2.3 Avocado shoots (and their pertinence to "alternate bearing")

As a compilation of botanical definitions, a shoot is a collective term that includes the aerial parts of a plant[26] (or mainly above ground given the existence of corms and rhizomes[2]) which arose from the plumule[42, 46, 50]. Nonetheless, and as mentioned above (section 2.2.2), when discussing "branches" or "limbs", botanical definitions sometimes disagree with agronomical views which usually develop from accumulated practical knowledge. The agricultural concept of a shoot is better described by the quotes below:

**Quote 2-1. What is a "shoot"?**

- "A young growing branch or twig with its leaves"[14, 26]
- "Small branches that grow on the trees in spring and August" (translated from French)[25]
- "The part of the plant that bears buds, leaves and flowers"[2, 45]

From a practical point of view, these quoted phrases seem closer to what a grower or the lay person interested in fruit trees would think of as a "shoot". It is not precisely seen as the initial aerial growth of a germinating seed/plantlet but as the most tender and youngest vegetative growth that blooms, sets fruit and thrives throughout a growing cycle. This connection to fruits is what makes "shoots" pertinent to the "alternate bearing" of fruits phenomenon.

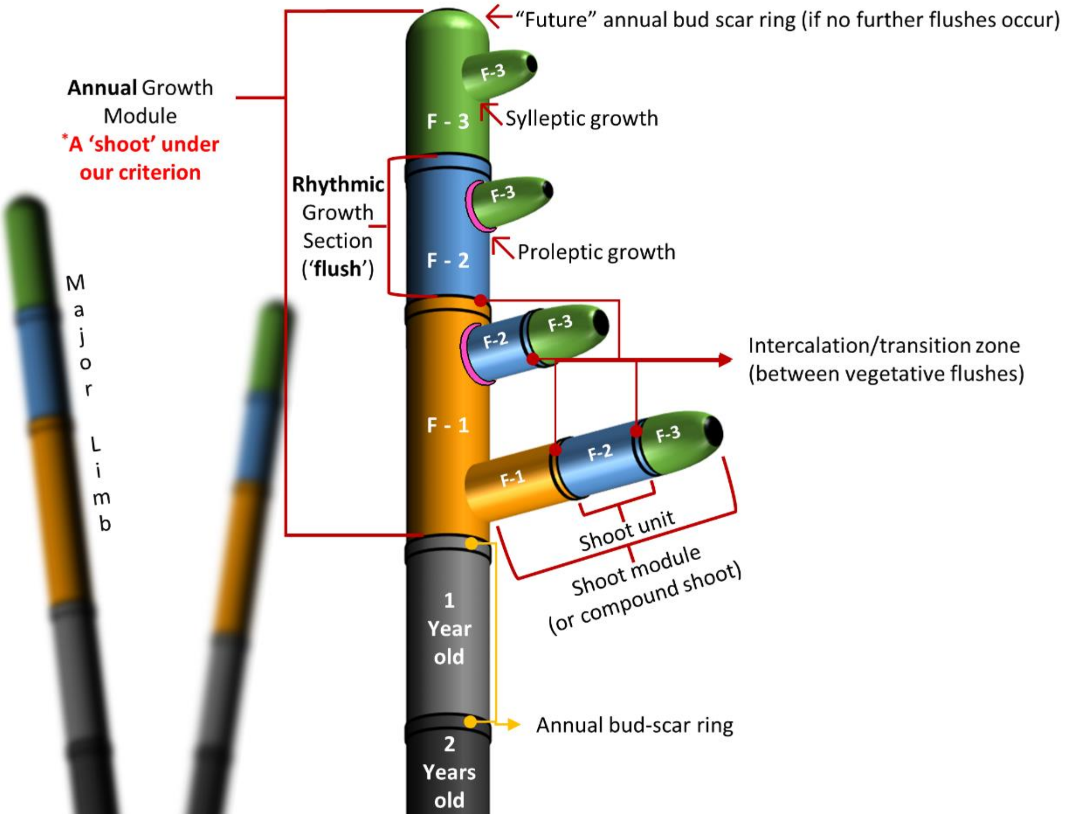

**Figure 2-1. General diagram of the avocado canopy construction.**

There can be several major limbs in an avocado canopy but there is a set of modular and repetitive sections that can be seen as the building units of the whole canopy. The formal naming of these sections is given as according to Thorp and Sedgley (1993)[44]. F-1, 2 or 3: indicates the vegetative growth "flush" according to their chronological occurrence. * Notice that under our research criterion which seeks for a relevant connection to the "alternate bearing" phenomenon, the avocado growth named "shoot" should be the whole "Annual Growth Module", despite the discrepancy with the authors of the original study. See section 2.3.4 for a more detailed explanation. Mainly based on Thorp and Sedgley (1993)[44] and Thorp et. al. (1994)[43].

Figure 2-1 depicts the organizational hierarchy of avocado as proposed by Thorp and Sedgley (1993)[44]. Their focus was on developing tools and language to describe tree form and to explain the observable differences between avocado varieties and their location. Their original set of 5 diagrams progresses from a simpler representation of the larger major structures of the canopy to the highest level of complexity at the intermediate level of "annual growth modules" to then decrease in complexity again towards the smaller and nested "compound shoots" and "shoot units".

### 2.3.1 Flushes, shoot units and rhythmic growth sections are equivalent

As depicted (Figure 2-1), “shoot units” are equivalent to growth flushes (F – 1, 2 or 3). A “growth flush” is commonly used as a vegetative descriptor and is defined as a rhythmic vegetative growth event[48], 1 to 6 per year[29], that can have different intensity, duration[10] and ends when the final length is reached[20]. Traditionally, they are thought to be one event per astronomical season (i.e. spring, summer and fall “flushes”[10, 29]) but given that several flushes can occur[20] in different growing locations around the world, it is probably best to simply rank them ordinally (i.e. first, second, third and so forth) unless the avocado research community wants to specify periods like “early” or “late” spring for example[11] which seem more precise but still are trying to link avocado vegetative growth and development to human chronology.

Because a single “flush” event can span over a period of several weeks[20], it is certainly possible to observe a main vegetative axis releasing axillary growth. When that happens, and as depicted in Figure 2-1, the “Rhythmic Growth Section” becomes also equivalent to the corresponding “flush” (or “shoot unit”) but with the distinction being that the latter is an axillary growth of the former that is comparatively older and hierarchically a “parent” structure.

Although it does represent a language that should have been widely adopted, avocado literature is mostly not taking into account tree architecture or a consensus language and freely uses the unit “shoots” in materials and methods without more specific details. This hurts replicability and can lead to confusion (see section 2.3.4).

### 2.3.2 Bud rings and “transition” zones

When the growth rate of a “flush” starts to decelerate by a reduction of the meristematic activity[48] (towards a period of quiescence[10] like the “end of a flush” or winter dormancy) the distance between axillary organs (buds) shortens and a visually noticeable section on which the latest axillary buds are in close proximity forms a distinct “band” around the growth circumference. This is horticulturally known as a “bud ring”.

The distance between the buds that belong to the “ring” remains short[48] compared to other sections of the vegetative axis, independently of the occurrence of a succeeding “flush”. Also, these clusters of buds are observable for several months[1] and are usually not abscised[10, 11]. Even further, they can undergo bud break under certain conditions such as a pruning stimulus[10].

Distinctions are made between different “bud rings” according to their location along the vegetative axis that has them. The distal most “bud ring” developed at the end of the latest “flush” and that includes what will be a dormant (overwintering) terminal bud[48] is named “annual bud scar ring”[44] (see Figure 2-1), with “annual” indicating that it represents the end of the yearly growth cycle and with “scar” making a reference to the indentations left by the shed protective bud scales[48]. The non-terminal “bud rings” that can be seen in-between “flushes” are simply called as such (a “bud scar ring”[44]), the “intercalation zone”[10] or the “transition zone”. (see Figure 2-1 and Figure 2-3)

#### *2.3.2.1 Vigorous growth*

Although “transition” zones are sometimes equated to “bud rings”, it is important to make a distinction because even though all growing axes can undergo a growth rate deceleration that indicates the “end of a flush”, if the axis is vigorously growing, the rate of growth will slow but not sufficiently to generate what is recognized as the tight “bud ring”. (see Figure 2-3 and compare the “transition” zone length between panel A (non-vigorous) and panel B (vigorous)).

When phenotyping avocado growth, it is best not to assume that there will always be a clear “bud ring” in between two distinct “flushes”. A second set of phenotypical descriptors is needed to distinguish that the section (or zone) being observed is in fact a transition between two flushes, instead of disregarding it as a continuous growth given that no “bud ring” is observable. These descriptors will be detailed in section 2.4.

### *2.3.2.2 Sylleptic and proleptic vegetative growth*

The presence or absence of an obvious “bud ring” at the basal most section (proximal to the point of attachment) of a vegetative axis is used as a phenotypical descriptor to differentiate two types of growth: sylleptic and proleptic.

In Figure 2-1, a pink colored basal “band” or “ring” represents an observable cluster of buds on proleptic type growth while the sylleptic type has no “ring” at the comparable location. The sylleptic and proleptic growth proportions within canopies have been used as indicators of avocado trees age, with older ones having proportionally more proleptic growth (although there is also a location interaction)[36]. It has also been linked to avocado productivity by declaring sylleptic growth as highly productive due to their comparatively higher bloom intensity than proleptic (although only observable the succeeding seasons)[27].

Ultimately, it can be difficult to grasp the fact that this distinction is only valid during a single growth season. From a whole tree perspective, and after most if not all vegetative flushes have stopped, all shoots will have an apical overwintering bud[44] that will develop protective scales and, with that, a basal “bud ring”, hence at the beginning of every season, all growth axes fall into the proleptic growth classification (Reuben Hofshi, personal communication).

**Table 2-1. Sylleptic and proleptic vegetative growth characteristics.**

| **Type of vegetative growth** | **Bud status** | **Presence of basal "bud ring"?** | **Simultaneous growth with parent axis?** | **Preformed nodes within the bud?** |
|---|---|---|---|---|
| **Sylleptic** | **Non-dormant/non resting**[10, 44] – Even the actual existence of the typical structure of a bud is questioned[48] | **No** –Only the presence of a long hypopodium (the distance between the base of the growth and the first axillary bud)[44, 48] | **Yes** – Growth is concurrent to the parent axis[10, 44] until the end of that "flush" | **No**[10] (organs are built *de novo*) |
| **Proleptic** | **Dormant / resting** for a period of time[10, 44] | **Yes** – Due to the resting period, scales do enclose the bud[1, 11, 15, 48] | **No** (given the bud resting period before outgrowth)[10, 44] | **Yes**[10] |

Another caveat of the classification of growth by the presence of the "bud scar ring" is that the length of the "rest period" (without bud break and elongation) has not been clearly defined. Syllepsis implies the occurrence of an almost "immediate" axillary branching without the real formation and release of an axillary bud[48] suggesting that any axillary growth that is not "immediate" should be of the proleptic category leaving a very open time frame of days, weeks or even months as plausible. Nonetheless, we have observed that the time frame for the appearance of a proleptic growth can be the length of the period of inactivity between two consecutive "flushes" but, then again, that period of time has been reported as ranging from practically immediate to up to 12 weeks of inactivity[20].

Under our perspective, the observation of syllepsis during a growth season within the avocado canopy can be useful in identifying and focusing on vigorous growth developed from apical meristem functioning at maximum levels of activity[48]. This type of completely vegetative *de novo* growth should be a focus for research on the environmental conditions that trigger flower induction. This is discussed in section 2.4.2.1.

### 2.3.3 Avocado phyllotaxis and orthostichy lines

Avocado spiraled phyllotaxis of the axillary organs (axillary bud and subtending leaf) is pentastichous, which implies a rotation angle of 144° between two consecutively developed organs and a divergence of 2/5 with the numerator indicating that 2 full turns around the vegetative axis will virtually align two axillary buds and the denominator indicating how many axillary buds are within those two full revolutions[24, 32]. The denominator also coincides with the 5 virtual parallel lines (orthostichy lines) of aligned organs along such vegetative axis[24]. This phyllotaxis arrangement is very common among angiosperms[13, 32]. Fruit trees like apple and cherry also display it[31] (Figure 2-2, left panel).

It is important for avocado research to be aware of this axillary organ arrangement. Experiments using avocado leaves labelled with $^{14}C$ photoassimilates demonstrated that their distribution is strongly influenced by the phyllotactic arrangement[17]. Also under field conditions, avocados are very responsive to the environmental input of sunlight exposure[20, 28, 48] and within the dynamic context of a canopy, all 5 orthostichy lines of axillary buds are not equally (or permanently) exposed to sunlight, nor respond equally to it.

Axillary bud outgrowth (bud break) is favored on the sunlit aspects of a growing axis and reduced on the comparatively more shaded sections of the same (facing the inner canopy of the tree), which leads to a distinctive “branching bias”, especially noticeable on vigorous growth (Figure 2-2, right panel). Also, there is a tendency of sunlit axillary buds to develop and undergo bud break sooner into their vegetative or reproductive fates.

In connection to a research focus, it is important to plan ahead and discuss, for example, what is the most appropriate sampling scheme: is it to collect only apical buds and claim it represents the overall growth behavior? Is it best to remove and flash freeze a vegetative section that contains 5 axillary nodes representing 2 revolutions around the growth axis? Should dissection and collection of axillary buds done in clusters of those that belong to the same orthostichy line given their differential responses?

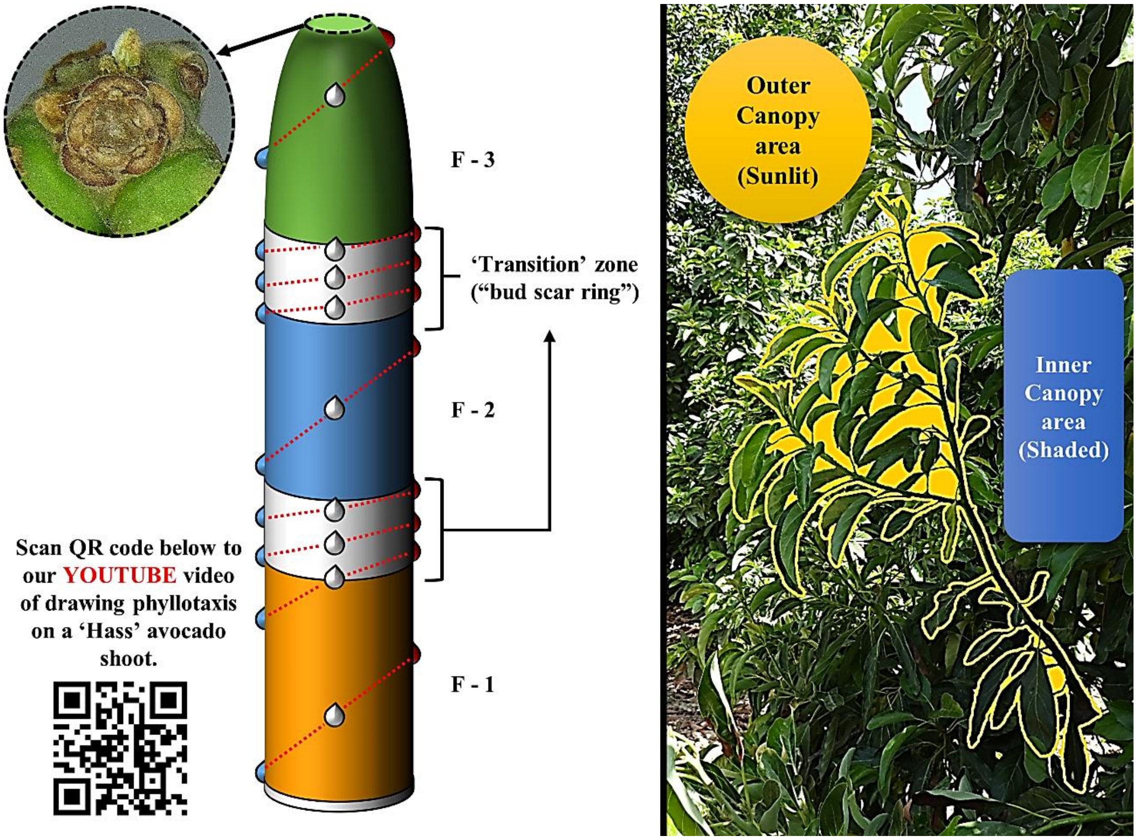

**Figure 2-2. Avocado phyllotaxis, orthostichy lines and branching bias.**

Left panel: F-1, 2 and 3 represent 3 consecutive flushes along a vegetative axis and the white zones in between them represent the "transition" zones. Although the spiraled phyllotaxis (denoted by the red dotted lines) usually does not change throughout the axis, the distance between buds is shortened by the growth rate reduction at the end of a flush. The teardrop shaped volumes represent axillary buds and the ones vertically aligned with the same color denotes their connection to the same orthostichy line. There are 5 orthostichy lines around the axis but only 3 are visible in this diagram. Please note that the right portion of the tridimensional diagram is more shaded than the left portion which is illuminated in a similar fashion to the situation in the right panel. Circled in black, a transverse plane of a shoot apex with the 2/5 phyllotactic organ disposition. Diagram is not at scale. Right panel: Example of the "branching bias" in 'Hass' avocado. Highlighted in yellow is the branching complexity of a single and vigorously growing avocado shoot within the context of a whole canopy. Bud outgrowth is favored at the sunlit sections of the growth axis and is remarkably reduced (despite the overall vigor) on orthostichy lines that face the inner canopy.

### 2.3.4 Finally, what is a "shoot" and why it pertains to the "alternate bearing" phenomenon?

The architectural classification presented by Thorp and Sedgley[43, 44] (see Figure 2-1) should have but was not widely adopted. If it prevailed, the language used in subsequent avocado research papers would have been more precise.

On the other hand, going back to the statements that authors in Quote 2-1 used while defining "What is a "shoot"?" there is a sense that the latest growth that bears flowers at the beginning of the yearly cycle fulfills the most general concept of what is a "shoot". Along with the previous, it is the "shoot" that is seen as the vegetative structure that experiences "flushes" (sensu Gregoriou and Kumar[20]).

Attempting to reconcile and integrate these higher and lower levels of precision while at the same time searching for <u>pertinence to the "alternate bearing" phenomenon due to its core connection to flowers and fruits</u>, the localization of the reproductive growth within the canopies and among the different yearly cycles should be used to discriminate where does "a shoot" begins and end. So, where is the potential to develop an avocado fruit until maturity? Where does the pollinator fly to? Where do the fruit(s) hang if pollination and set is successful? The answer is right above an "annual bud scar ring" at the basal section of the structure that Thorp and Sedgley[43, 44] (see Figure 2-1) defined as the "Annual Growth Module" and hence, for the remainder of this discussion, this will be referred to as equal to an "avocado shoot". When necessary and within this annual "shoot" structure, a parent or main axis will be simply described as the "main shoot" and any axillary growth release within it will be called "lateral shoot"[20]. The term "flush" will be used instead of "shoot module" (or compound shoot) in order to preserve the word "shoot" for higher order structures (main and lateral shoots) that can have several growth flushes within them.

## 2.4 Avocado shoot phenotyping: new approach

The discussion on avocado shoot phenotypes below focusses exclusively at the single shoot level while attempting to address both reproductive and vegetative growth behavior. Some novel points of view will be applied to traditional descriptors as a way to propose a language consensus for the avocado community.

### 2.4.1 Phenotypical traits at "transition" zones

One of the most important aspects of following avocado shoot growth is to be able to distinguish the occurrence of flushes, may that be within a season or after the fact while reconstructing the phenotypical history of an older shoot.

#### *2.4.1.1 Vegetative to Vegetative "transition" zone*

These are the most commonly referenced zones that one comes across in the literature. They are present in between two succeeding vegetative flushes. As commented above in section 2.3.2.1, a "bud ring" is usually expected but that is a reduction of the actual array of features that can occur in these areas which are detailed below.

##### 2.4.1.1.1 Distance between buds

The reduction in the growth rate leads to the increased closeness of the axillary buds but the distance between them (internode distance) fluctuates according to the overall vigor of the shoot. In a survey of 224 shoots of 'Hass' avocado trees in a commercial orchard in Fallbrook, CA., the most commonly observed length of the "transition" zone of vigorous shoots was 1 cm (41.4% of vigorous shoots). On the other hand, those in less vigorous shoots presented half that length (0.5 cm) as the most commonly observed one (29.7%). Both proportions are significantly different statistically

(P=0.000). The overall observed range fluctuated from 0.3 cm to 8 cm in “transition” zone length but no more than 5 cm were measured in non-vigorous trees.

In Figure 2-3, panel A depicts a “transition” zone that is fairly short (“bud ring”) with small distances between the buds that were laid down during that growth reduction. On the other hand, panel B shows an example of a longer “transition” zone, with its axillary buds being separated by a longer distance, similar to the observable between subtending leaves further above in the second flush.

2.4.1.1.2 “Transitional leaf”

The red circled leaves in both panel A and B (Figure 2-3) present a differently shaped leaf that can be found flanking the “transition” zone. Their leaf lamina is rounder and noticeably smaller than the ones developed at the previous or succeeding flushes. An average of 2.8 transitional leaves per shoot that displayed them (n=28 shoots) were observed in ‘Hass’ avocado trees (data gathered at the Agricultural Experimental Station of the University of California, Riverside).

Transition zones seem to be a consequence of the shoot apical meristem entering a quiescent period and that could cause internal changes leading to these differently shaped leaves. One of them could be the availability of auxins which would affect the development of normal leaf lamina[22]. Table 2-2 offers evidence of transitional leaf roundness being related to a reduced elongation of the lamina. This might be due to early arrest of cell proliferation and/or a shortened or altered ability to expand them[22], all developmental events on which phytohormones are involved.

Interestingly, cultivation of orchidea (*Spathoglottis plicata*, Blume) protocorms with increased availability of exogenous auxin or with polar auxin transport inhibitors affected their first leaf by either preventing its formation or by developing abnormal “short and fleshy” leaf[30], suggesting that future avocado studies should explore both auxin availability and polar transport.

**Table 2-2. Comparison of length and width between normal and transitional leaves**

| Leaf shape | Average length of lamina (cm) | Average width of lamina (cm) |
|---|---|---|
| Normal leaf (n=560) | 14.17 (a) | 6.07 (a) |
| Transitional leaf (n=77) | 9.23 (b) | 5.75 (a) |

Means that do not share a letter are significantly different after one way ANOVA and Tukey mean separation with a confidence level of 95% (P=0.000 for length and P=0.084 for width). Agricultural Experimental Station, University of California, Riverside

#### 2.4.1.1.3 Lateral branching

Axillary bud outgrowth and, as a consequence, lateral branching of the main shoots does not readily occur within the “transition” zone. On the other hand it occurs at the end of the previous vegetative flush, right before the “transition” zone. In practice, the same behavior can be described as occurring right “below” the “transition” zone. One or 2 branches are commonly found at these locations. Depending on the vigor, the lateral release can be proleptic as observed in Figure 2-3 (panel A) or sylleptic (panel B).

#### 2.4.1.1.4 Apparent change in phyllotaxis

Avocado shoots display spiral phyllotaxis during a flush (section 2.3.3 and Figure 2-2) but towards the end of a flush, the growth rate reduction of the shoot apex leads to the impression that the last axillary buds (usually the last two) are opposite to each other. This is due to the reduction of the internode distance between them (immediately below the “transition” zone) but without alteration of the 2/5 phyllotaxis and the related 144° degrees angle between two succeeding axillary organs.

It can be more obvious when lateral branching actually occurs. This concept is illustrated in the following:

- Figure 2-2 (left panel): the red dotted lines depicting the spiral phyllotaxis of the shoots becomes more horizontal than diagonal at “transition” zones and with that, also right below them;

- Figure 2-3 (panel A): To the left of the red circled transitional leaf, a proleptic lateral shoot was released during the second flush (F-2) from a bud developed during the first flush (F-1). If one compares the alignment of the lateral proleptic shoot with the closest axillary bud in the F-1 flush (yellow dotted line), it can be seen how close and opposite they seem to be although the spiral phyllotaxis did not change;
- Figure 2-3 (panel B): Similar to the previous. Two lateral shoots were released below the "transition" zone. The only reason why they don't seem to be "perfectly" opposite to each other (yellow dotted line) is the more vigorous growth rate of the main shoot. The apical meristem did not reduce the activity enough hence a "more spiral" phyllotaxis still remains. This also explain the longer "transition" zone.

#### 2.4.1.1.5 Other features

Other features that can be helpful in distinguishing the presence and limits of the "transition" zones are the absence of leaves within the transition zone and differences between succeeding vegetative flushes in aspects such as color, texture and suppleness.

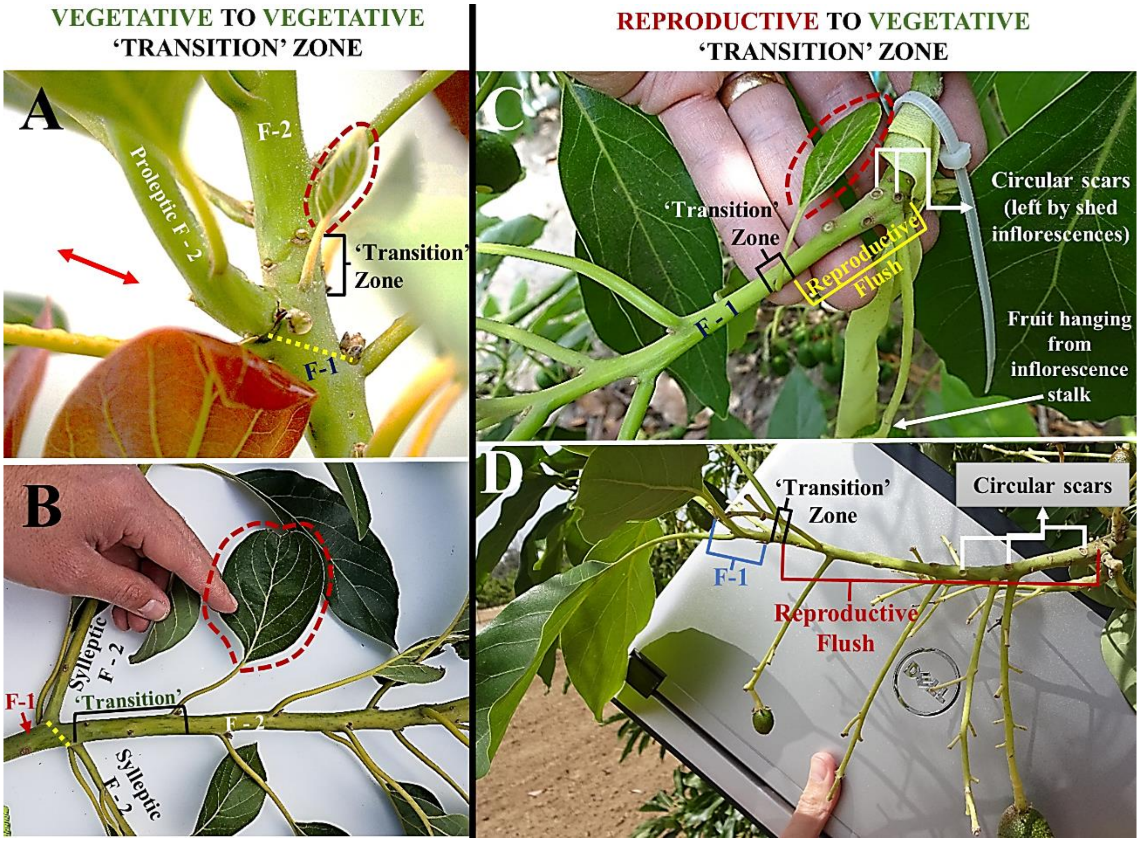

**Figure 2-3. Features of "transition" zones.**

Two types of "transition" zones are possible, the "vegetative to vegetative" (panels A and B) which occurs between two vegetative flushes and the "reproductive to vegetative" (panels C and D) which is present between the reproductive flush and the first vegetative flush (see section 2.4.1.2.1 for details on reproductive flushes). Leaves circled in red segmented lines are transitional leaves (see section 2.4.1.1.2). Yellow segmented line connects two succeeding axillary buds or lateral branches right below the transition zone. F-1 and F-2 represent first and second vegetative flush. See whole section 2.4.1 for more detailed explanations.

#### *2.4.1.2 Reproductive flush and the Reproductive to Vegetative "transition" zone*

Only once is there a reference in the literature to a reproductive related section within avocado shoots. It was published in 1958 by Venning and Lincoln[48] :

**Quote 2-2. A "band of flowers – branch scars"**

"These regions are readily recognizable on the bark as a series of closely-spaced scars (from the bud scales) which are distributed around the branch, but care must be taken not to mistake a band of flower-branch scars for bud scale scars."[48]

Most likely, a “flower-branch” refers to a single avocado inflorescences which in the strict sense is a single panicle or determinate thyrse[6] and the “band around the shoot” is the section where those inflorescences are present.

#### 2.4.1.2.1 Reproductive flush

It seems to be a highly overlooked aspect of avocado shoots that the truly first visible growth that occurs on a main shoot (that contains inflorescences) is an extension of the internode distances of the already differentiated inflorescences (making the quoted inflorescences “band around the shoot”). In that sense, the first growth is not vegetative but reproductive and should be recognized as the actual first flush of a shoot, hence the proposed name “reproductive flush” (Figure 2-3, panels C and D). The sequence of flushes is in chronological order from the first “reproductive flush” to the second growth flush overall. In this scenario the second growth flush is the first vegetative (first vegetative flush or F-1) and so forth if succeeding vegetative flushes occur (Figure 2-3, panels C and D).

A reason of why a “reproductive flush” concept is not widely mentioned in the literature might be that it can be short, both in length and duration, giving an observer the impression that inflorescences are arranged in a rosette fashion (similar to the leaf rosette in *Arabidopsis*) that soon gets obscured by the first vegetative flush. Nonetheless, Figure 2-3 (panels C and D) show that it can be of considerable length and deserving of more research attention. In a survey of 400 ‘Hass’ shoots growing (Saticoy, CA), the length of the reproductive flush ranged from 0.5 cm to 11.3 cm with an average length of 2.5 cm (±1.86 cm standard deviation).

Unanswered questions about the “reproductive flush” remain. Is a longer length is due to the differentiation of more inflorescences, longer distances between them or both? Does it challenge the widespread concept of an initial direct competition between the reproductive and the vegetative growth as the reproductive growth happens first as displayed in Figure 2-3 (panel D), where flowers are already shed and two fruitlets are clearly growing before the occurrence of the F-1 flush?

#### 2.4.1.2.2 Reproductive to Vegetative transition zone

Unless the growth is completely vegetative, without a reproductive flush, there will be a “transition” zone between the reproductive flush and the first vegetative flush. As with some of the features observed with “vegetative to vegetative” transitions, the “reproductive to vegetative” transition zones can also display the shortened distance between nodes and “transitional leaves” (circled in red in Figure 2-3, panel C). A distinctive difference is that “reproductive to vegetative” transition zones do not present lateral branching of shoots below them. This is due to all the axillary organs that belong to the reproductive flush develop into single inflorescences which, most likely, have no meristematic tissue left to initiate a completely vegetative axillary growth.

## 2.4.2 Two distinct avocado shoot classes

Avocado shoots can be clustered into two major classes: “spiral” shoots and “overwintering” shoots. Their proportions within the canopy can differ greatly within a single season but their vegetative volume and behavior can be equally important depending on the research goals or horticultural management points of view.

### *2.4.2.1 “Spiral” shoots*

Likely, Venning and Lincoln[48] were describing “spirals” shoots when they wrote about “fast-growing branches” that “may develop a diameter of 2” (5.08 cm) to 3” (7.62 cm) inches within the first year”. The “spiral” class of shoots has often been considered undesirable from a horticultural point of view. They are likely equivalent to the growth that is horticulturally referred to as "suckers" or "water shoots"[39] given their vigorous growth rate which is perceived as highly demanding of resources and "too vegetative" to contribute to the overall reproductive growth of the avocados.

They are usually eliminated[39] by pruning when they are recognized within the canopy but their true potential has not been fully explored.

The criteria to identify a “spiral” shoots is threefold:

- They grow from dormant vegetative buds in wood that is 2 years old or more. They may be released during the early parts of the season but can also be released into growth at different points within the season by the bending of older wood or the stimulation of axillary bud outgrowth due to pruning cuts or limb breakage.
- Within a yearly cycle, the main axis of the shoot grows up to a length of 50 cm or more. This measurement has both a practical length as it is the usual length of half a human arm but also because only 9.2% (n=458 shoots) of shoots that grew from 1 year old wood (“overwintering shots”) reached lengths longer than 50 cm, making it distinct.
- The main shoot displays a phenotype of releasing practically every single axillary bud along its axis into sylleptically grown lateral shoots. This phenotype in particular is what drives their name as all the lateral branching shows very clearly the spiral 2/5 phyllotaxis of the shoots.

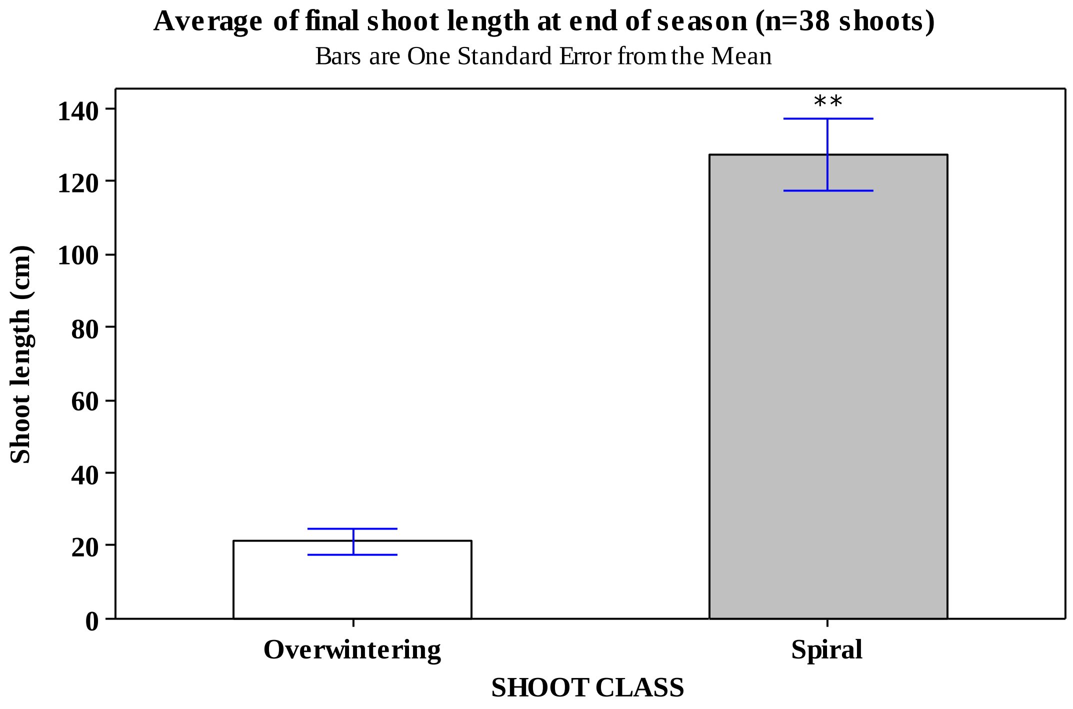

**Figure 2-4. Final length comparison of the two main classes of avocado shoots.** "Overwintering" data is from shoots that grew from resting buds but did not grow longer than 50 cm (as it is one of the criteria to belong to the "spiral" shoot class). Means are significantly different after ANOVA and Tukey method with a confidence level of 99% (P=0.000). Data collected at the Agricultural Experimental Station, University of California, Riverside.

"Spiral" shoots can be seen as "branches" as they are large and structural despite truly being the growth of just months from a single dormant bud released into vigorous, highly branched, growth. They belong to a category of their own simply by measuring the final length of the main shoots, as shown in Figure 2-4. This comparison was made with data of shoots that grew from resting buds in older wood but even when compared against the final length of all other shoots, the "spiral" shoots remain significantly longer (n=475. ANOVA and Tukey method with a confidence level of 99% (P=0.000)) and with a difference of 102 cm between the two averages. Nonetheless, "spiral" shoots are much more complex than that and to convey their real complexity, Table 2-3 contains a list of attributes typically observed in an individual "spiral shoot.

**Table 2-3. Detailed phenotype of a single "spiral" shoot**

| Feature | Measurement |
|---|---|
| Main shoot final length at the end of the season | 2.35 m |
| Sum of the lengths of all lateral shoots within it | 18.378 m |
| Total growth from the original resting bud until the end of the season (sum of previous two) | 20.728 m |
| Basal diameter | 4.5 cm |
| Amount of axillary organs along the main shoot | 69 axillary buds<br>• 57 % released into lateral shoots<br>• 33% lost (possibly bud shedding due to fast secondary growth rate [48])<br>• 9 % was reproductive growth directly attached to the main shoot (most distal axillary buds of the shoot)<br>• 1% remained as dormant bud (it was the first axillary bud at the base of the shoot) |
| Amount of vegetative flushes | 4 flushes |
| Fruits set and growth | • In mid-June (late spring): 151 fruits with an average diameter of 9.6 cm<br>• In late November (late fall): After fruit drops, heat waves and sunburn damage to the shoot and fruits: 26 fruits with an average diameter 17.3 cm. (83% lost) |
| Internode distances | • Diagonal distances:<br>○ Range: 0.7 cm to 10.5 cm<br>○ Average distance: 3.35 cm<br>• Linear distances:<br>○ Range: 0.3 cm to 9 cm<br>○ Average distance: 3.09 cm |

The "spiral" main shoot can display a bud release bias (see section 2.3.3). In fact, it is a "spiral" shoot that was used as an example of it in Figure 2-2 (right panel). In this sense, avocado research should further explore the avocado shade avoidance syndrome[33, 38] by focusing on the responses of "spiral" shoots within the canopy. Another very important reason of why "spiral" shoots should be given a proper place in avocado research is that they grow early in the season as a completely *de novo* and exclusively vegetative structure that can later develop abundant reproductive growth.

This should be linked to research on the different environmental conditions that can trigger flower development.

#### *2.4.2.2 "Overwintering" shoots*

The concept of "overwintering" shoots comes from their origin as proleptic shoots that grew at the beginning of the season from buds (apical or laterals) that themselves developed the previous year, which implies that a winter or dormant season must had passed. Coincidentally, the concept of an "overwintering apical bud complex" has been presented before[44] and coincides with our distinction. The previous denotes the chronological age difference between the vegetative growth that generates "overwintering" and "spiral" shoots. The former usually grow from the shoot apices of 1 year old growth while the latter grows from older "wood" that is at least 2 years old (Figure 2-5). It is true that between both shoot classes, the "overwintering" represents the most abundant class within a canopy but is the "spiral" class that is larger and more structural on which future "overwintering" shoots will develop (Figure 2-5).

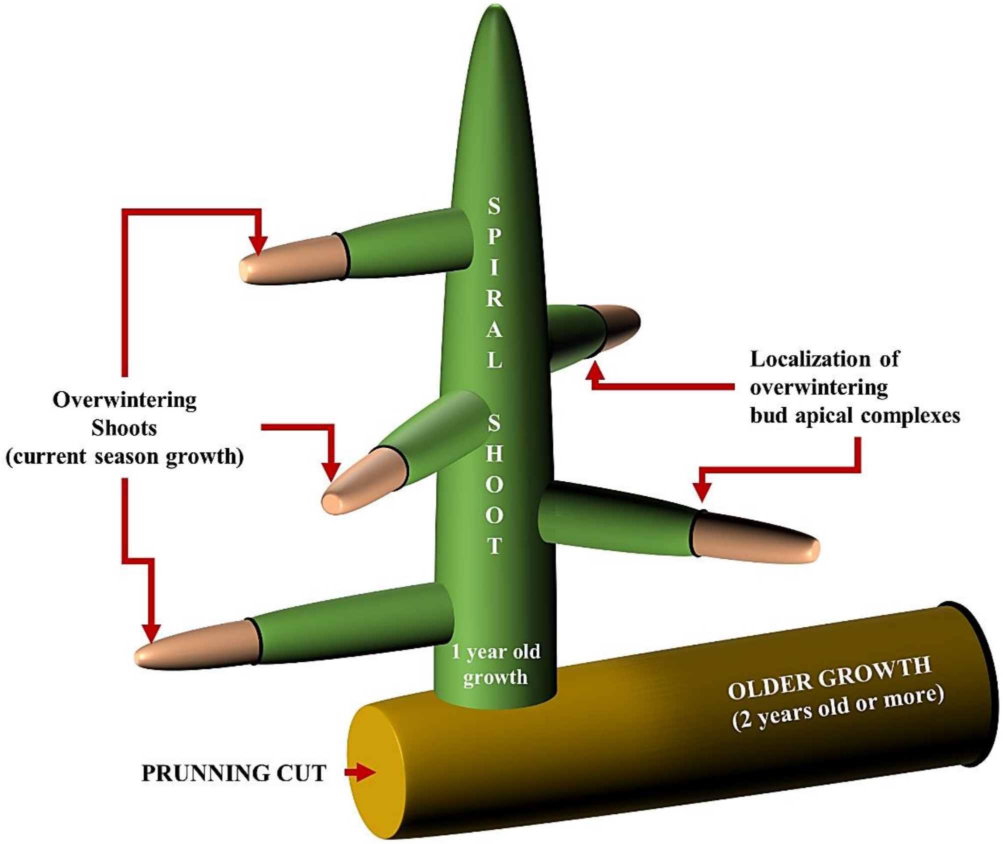

**Figure 2-5. Hierarchy of the "spiral" and "overwintering" classes.**
"Overwintering" shoots can be observed as coming from what once was a vigorous and branched growth ("spiral"). A pruning cut is a way to release resting buds near the cut into vigorous growth.

### 2.4.3 Overwintering shoots general phenotypes

Within the "overwintering" shoot class there are 2 general groups: vegetative and reproductive shoots. The former only include completely vegetative growth and the latter clusters all shoots that have at least one inflorescence within their growth axis. In relation to the previous, once again the language used in literature can be confusing[10]. In section 2.4.1.2 it was noted that the strict sense definition of an avocado inflorescence is a single panicle or determinate thyrse[6]. Nonetheless, concepts like "indeterminate inflorescences" are commonly used in the avocado literature despite all inflorescences being a determinate array of flowers[6, 10].

The actual growth structure that can have one or several inflorescences is the same growth structure that was defined as a shoot (section 2.3.4) and because of this reason the concept proposed by Chanderbali et. al.[10] that avocados can have "flowering shoots" and they can be either "functionally indeterminate" or "functionally determinate" is used for this discussion. From all the previous, there are three different shoot types arising from the "overwintering" shoot class: vegetative shoots, functionally indeterminate flowering shoots and functionally determinate flowering shoots.

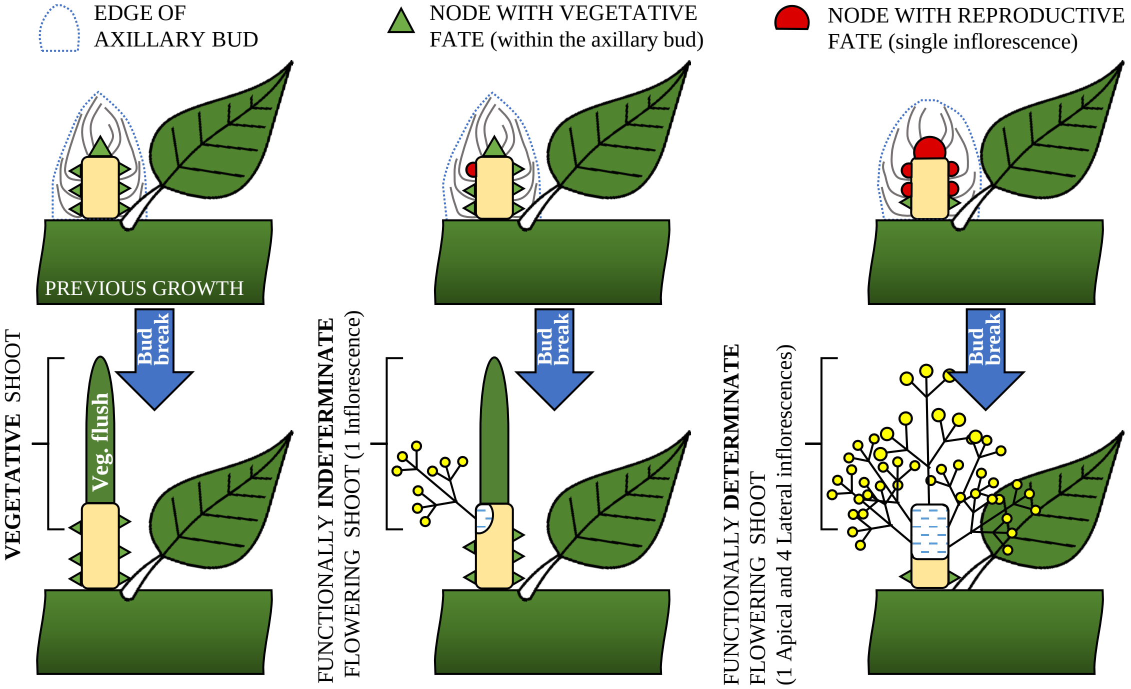

**Figure 2-6. Vegetative shoot and the reproductive group of shoots within the overwintering class.**

Upper row of the diagram proposes the internal structure of 3 different buds before their bud break (outgrowth) and the lower row depicts the observable phenotype after some growth and development. Functionally indeterminate flowering shoots have one (like in this figure) or many inflorescences excluding the apical position within the bud; if so, it becomes a determinate flowering shoot. Solid gray lines: bud scales; stippled areas: section were inflorescences can be observed. Developed with some of the concepts described at Buzgo, et. al[6].

The apical or axillary buds consist of undeveloped growth with second order nodes of their own at a minimal internode distance[43] (Figure 2-6). When the nodes within do not change their default vegetative fate then they have the potential to become observable **vegetative shoots** after bud break (Figure 2-6). Vegetative shoots should be the only shoot type observable during the long juvenile period[8] of the avocado tree.

"Mature" trees are capable of undergoing floral induction and differentiation. Under inductive conditions and inside apical or axillary buds, the meristematic tissues of a node can differentiate into a single inflorescence. When buds contain a single reproductive node (one "future inflorescence") while all the other nodes remain vegetative then the dormant bud still has a vegetative axis. That is the simplest flowering shoot possible and more specifically it is named "functionally indeterminate flowering shoot" due to the vegetative axis remaining and as such giving it potential "indeterminacy" (Figure 2-6).

Following along the same line of though, there can be an increasing amount of second order nodes within a single bud, each differentiating into single inflorescences but as long as the apical meristem remains vegetative the first order bud will "contain" an indeterminate flowering shoot with 2, 3 or more inflorescences within. The maximum reproductive potential for a flowering shoot should occur when most of the second order buds but *sine qua non* the most apical one differentiates into an inflorescence. When this occurs, that flowering shoot becomes a "functionally determinate flowering shoot", observable after bud outgrowth. It is "determinate" in the sense that even the apical meristem did not retained the default vegetative fate and only inflorescence stalks and flowers will be observable (Figure 2-6). It is possible to find simpler determinate flowering shoots of just one inflorescence but that must be in the apical position, otherwise what is being observed is a functionally indeterminate flowering shoot.

### 2.4.4 The “compressed” zone

In spite of the previous ideas which were meant to be a simplified explanation for the three distinct shoot phenotypes, the concept of “determinacy” or “indeterminacy” within flowering avocado shoots is not a sharply defined one because avocado shoots have more complexity to them. Every vegetative or reproductive avocado shoot will conserve a set of small nodes under default vegetative fate at their point of origin (shoot base; see lower triangles still present after bud break in Figure 2-6 and Figure 2-7). That area, which in Figure 2-1 was highlighted as one of the “bud rings” that visually defined the proleptic shoot category, can be called the “compressed zone” because despite the avocado shoot that has them could be already growing, this cluster of small vegetative nodes maintain their somewhat original short internode distance.

The number of buds within this zone is on average ≈15 (3 phyllotactic turns of 5 buds each, from field observations (Agricultural Experimental Station, University of California, Riverside). These buds usually do not undergo bud break unless driven to by natural conditions or human intervention (see section 2.3.2) after which they are capable of vegetative outgrowth and, in that sense, even a functionally determinate flowering shoot can still continue growth but only from their base.

Considering all the phenotypical traits described above, Figure 2-7 presents a more detailed diagram of the different zones and flushes that can be distinguished in “overwintering” avocado shoots.

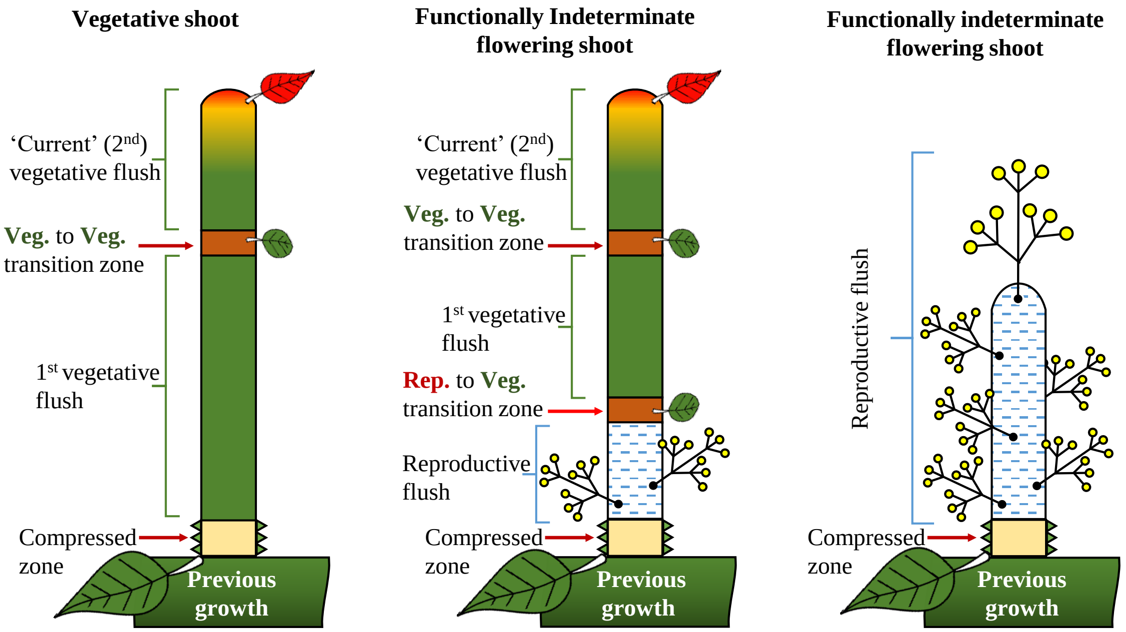

**Figure 2-7. Possible flushes and zones within the 3 overwintering shoot types.**
The number of vegetative flushes varies but is at least one unless functionally determinate. Notice that vegetative shoots do not have a reproductive flush and that in a functionally determinate flowering shoot only the reproductive flush can occur at the main axis. Round and green leaf represents a transitional leaf. Red leaf represents an immature leaf that still is a heterotrophic sink organ at the growing shoot apex. Veg.: vegetative; Rep.: reproductive. Diagrams are not to scale and do not consider the timing of these phenological events. More detailed descriptions of these different sections are within the text.

## 2.5 Shoot phenotyping after a growth season has passed

Until this point, most of the phenotyping concepts and distinctions are more applicable to the stages of shoot development as they occur and are visible through the annual growth season. It is also important to present a way to approach the description of final shoot phenotypes reached at the end of a season and also in reference to previous years.

### 2.5.1 General shoot type description

Figure 2-8 presents the simplified phenotype observable at the end of a season and points out to the fact that if a large enough shoot population is followed all categories can be found in fruiting and non-fruiting counterparts which has an important connection to research on the "alternate bearing" phenomenon. Up to four flushes were observed at the Agricultural Experimental Station, University of California, Riverside; however since the number of these is few (33% of 187 shoots), shoots with three or four flushes were clustered into the “type 3+” category (Figure 2-8). The intent here is to present a useful and fast way to differentiate shoots in the field but the decision to cluster shoots that are less common should be done depending on the research interest (for example “spiral” shoots are less common but important nonetheless), previous knowledge of the local conditions and the corresponding avocado growth behavior.

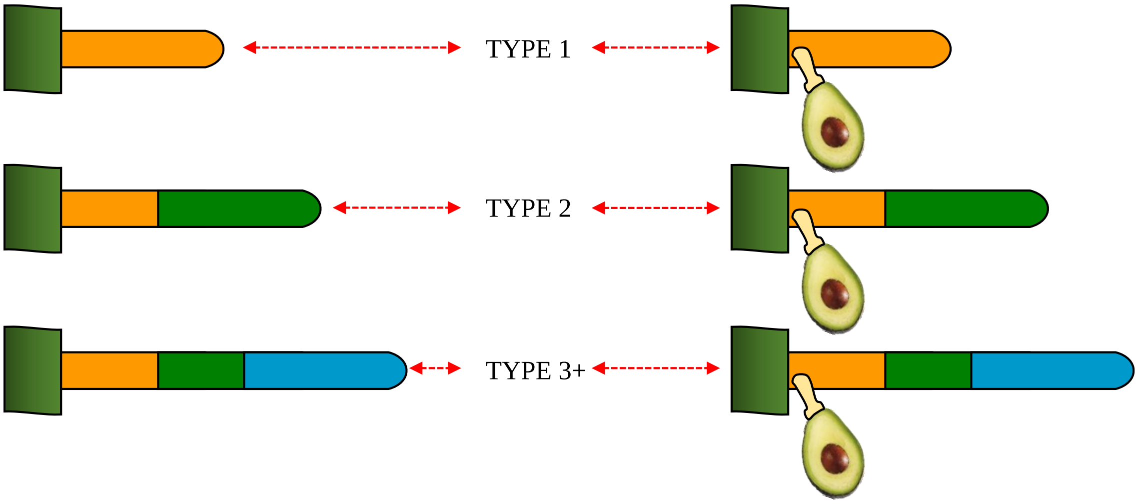

**Figure 2-8. General classification of avocado shoots by number of flushes.**
The type number indicates the amount of observed flushes at the end of a full season. Type 3+ can have 3 flushes or more. Fruit icon represent the fruiting version of the same shoot type which on the left side is non-fruiting.

### 2.5.2 Positional description of outgrown organs

In the avocado literature it is common that only the behavior of the shoot apical meristem is characterized, but there are many axillary bud along a single shoot that can potentially undergo bud break and grow into their default vegetative fate or onto either functionally indeterminate or determinate flowering shoots. It is therefore important to present an approach that allows placing any observed lateral growth (and its fate) into a fixed reliable position along a single shoot.

When the growth of a shoot ceases, sections such as the apex or any of the transition zones can be used as a fixed positional reference (i.e. “number of buds below the reference). The direction for numbering the outgrown events should be basipetal (from the shoot apex towards the shoot base).

Why designate the positions in “reverse” (i.e. increasing numbers basipetally) towards the shoot base instead of the order in which they originally were developed? The answer is practical. After primary growth cessation at the end of the season, axillary bud release events occur more often near and below the transition zones (see section 2.4.1.1.3), hence it is easier to count bud break events as they occur below transitions rather than counting the buds acropetally before reaching developmental events of interest (see Figure 2-9).

### 2.5.3 Time scale for phenotyping avocado shoots through different seasons

“Alternate bearing” research demands relating fruit presence to effects on vegetative and reproductive growth and this will be explored in the succeeding sections. It is important to place all those variables not only in terms of specific location but also in terms of chronological time and when to capture this information.

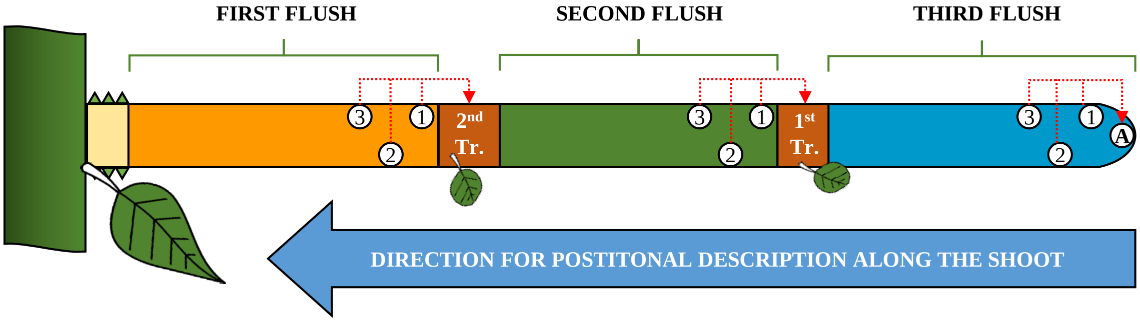

**Figure 2-9. Criteria and reference points to designate a specific position along a shoot.** At the end of a growth period (a flush or a whole season) the growth ceases and the shoot apex or the transition zones can be used to place organs grown along the observed main shoot axis. In this way, a lateral branch developed from an outgrown axillary bud can be recognized as located 1, 2, 3 or more "buds below" the reference. A: shoot apex; 1st and 2nd Tr.: first and second transition zone counted from the shoot apex downwards (basipetally); Circled numbers: represent axillary buds in ordinal numbers from the closest to the reference point.

For example, in the scenario presented at time point "A" in Figure 2-10, during harvest the following pertinent information can be documented:

- fruit presence itself (i.e. fruiting or non-fruiting shoots);
- general shoot type (and more specific measurements like length of reproductive and vegetative flushes);
- broad chronological age of the "wood" from which the shoot being followed developed;
- overall shoot location within the tree canopy.

After this, during the bloom period and along the same growth axis previously described, variables related to reproductive growth can be subsequently documented:

- type of flowering shoots (functionally indeterminate or determinate);
- complexity of the flowering shots (number of inflorescences per flowering shoot);
- position of the axillary buds that underwent bud break along the studied shoot.

At this point, it depends on the research design to either follow/sample tissues as they grow vegetatively or to allow a whole season to pass and evaluate growth at the succeeding season.

The effects that a growing fruit might have on the shoot's vegetative growth that is bearing it are contemporaneous in terms of a single season and the effects that the very same fruit could have on the new reproductive growth (bloom) are delayed and only visible at the beginning of the succeeding season. (Figure 2-10). These two aspects will be discussed in the succeeding sections.

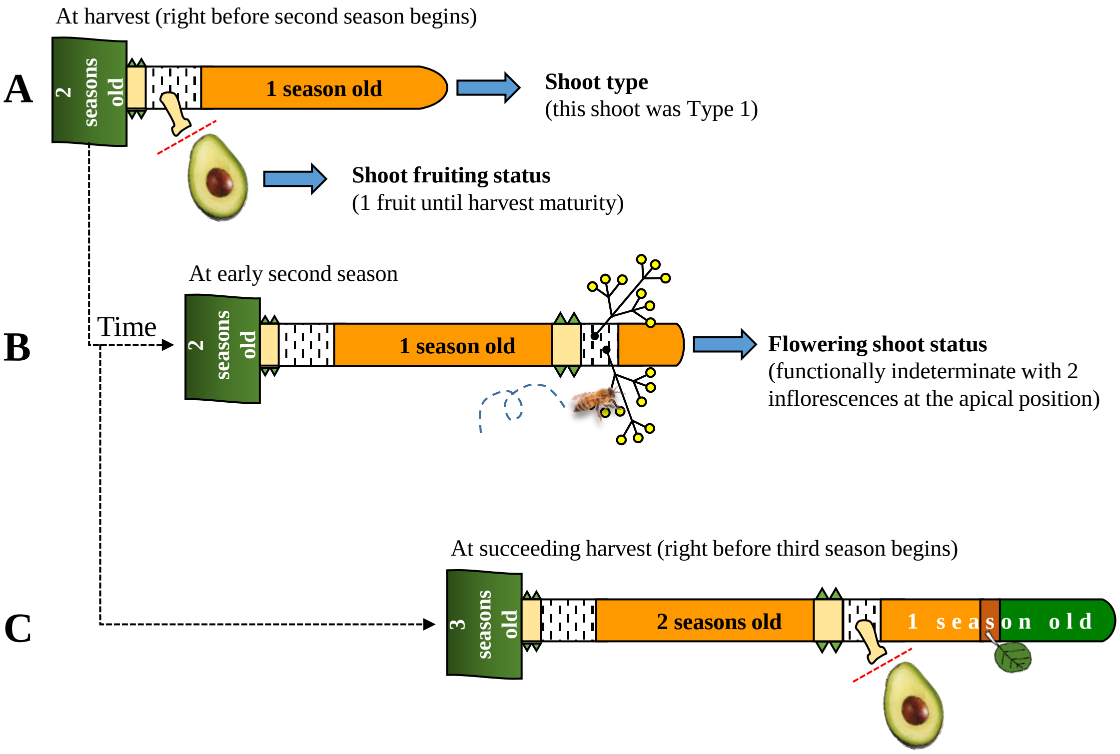

**Figure 2-10. Relative time scale of phenological events and the acquisition of phenotypic data through seasons.**

Time A: Measurements of fruit presence, shoot type and location of the fruit within the canopy can be done at harvest time. Time B: Early season, in which the outgrown vegetative or reproductive shoots can be identified and located at the apex but also laterally along the chosen shoot. At the same time bee visitations are happening potentially leading to new fruit sets. Time C: Equivalent to "Time A" but displaced in time by a whole season. Again, fruit presence, shoot type and location within canopy can be acquired. Notice the change of "wood" age of all vegetative axes at "Time C".

## 2.6 General conclusion and suggested phenotypical language

After reviewing and contrasting published and colloquial descriptors of avocado growth with phenotypical observations collected as part of this work, it seem necessary, especially for research purposes, to coordinate a common and consistent language. Although in this review some information of what is a branch or limb has been provided, the unit that is pertinent in terms of fruit presence is a “single shoot”. We propose that a “single shoot” should be defined as the whole structure (biomass) that is released into its own growth at the beginning of a bloom period from a resting bud.

Notice that to avoid human chronology into the description of phenological events, the reference that defines the beginning of a growing “single shoot” is when the whole tree canopy undergoes a period of anthesis (bloom), independently of it being a “main bloom” (intense and/or long) or a secondary bloom event within a year cycle. In most avocado producing areas this is an annual event, however that is not necessarily the case in all growing environments.

An actively growing shoot apical meristem (SAM) will lay down new leaves and corresponding axillary buds. Through time any of those apical or axillary buds can, if they are not shed or remain dormant, follow different routes depending on what is called their “fate”. The concepts of “vegetative” or “reproductive” fate are mutually exclusive and, simply put, the former refers to a bud which does not contain any developed inflorescences within, while the latter contains at least one inflorescence (Figure 2-11).

Within the buds that have “reproductive” fate, two types are possible: the functionally “determinate” and the functionally “indeterminate” flowering shoots (Figure 2-11). There is a bit of controversy or confusion when the language of “determinate” or “indeterminate” is used. Although botanically speaking it is more traditional to use the descriptors “determinate” and "indeterminate" as related to the characteristics of structures that are 100% floral, horticulturally in the case of avocados the concept is more loosely expanded to the bigger organ that is a single bud. This has created confusion in the literature that can be corrected by adhering to the concept proposed by Chanderbali et. al.[10] who suggested to formally refer to these structures as "functionally determinate" or "functionally indeterminate", meaning they behave in such way but botanically are not precisely that.

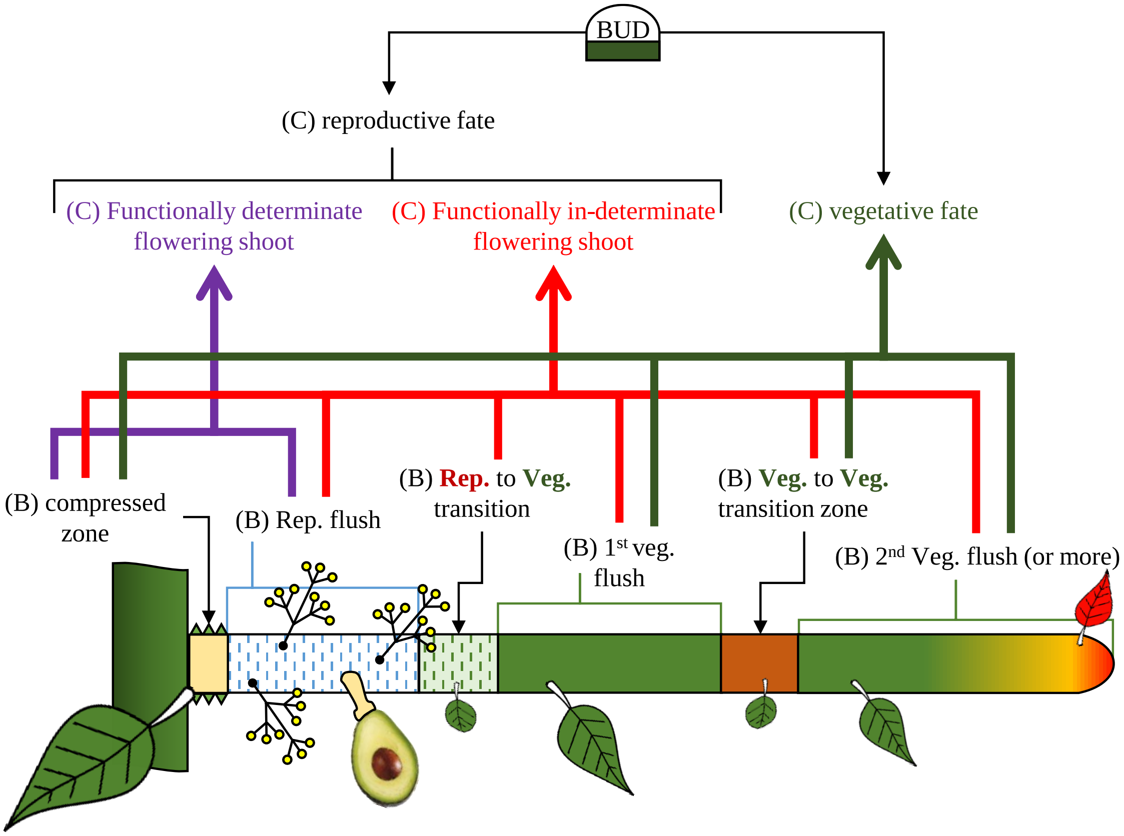

**Figure 2-11. The 3 distinctive avocado "main shoot" phenotypical features as related to their original bud fate.**
The different paths start with a dormant single bud right before the beginning of a bloom period. The "(C)" symbol indicates that the following words represent an abstract concept or descriptor while the "(B)" symbol indicates that the following is actually biomass, i.e. a vegetative or reproductive structure that is tangible and visible in the fields. Inflorescences and fruit presence is only possible within the length of the reproductive flush. Thick and color coded arrow related the main shoot phenotypical features to either the reproductive subcategories or the vegetative fate of the buds to which they point.

Embedded with the previous there is another source of potential confusion due to a colloquial lack of language: the use of the word “shoot” to classify structures that are grown from buds developed along the “main shoot”. It is simple to clarify if one uses a consistent language:

- what was described earlier as a “single shoot” refers to all the biomass developed from a resting bud between two blooming cycles. These structures can be quite simple or more branched and complex;
- the word “main shoot” refers to the first growth developed (and its continuation if it occurs) from the bud SAM that creates the primary axis. The “main shoots” is a structure subordinated but central to the “single shoot”;
- the word “lateral” (as in “lateral shoot”) should be understood as a secondary order growth that developed within a season from an axillary bud lay down at the previous vegetative flush. They are either proleptic or sylleptic but, nonetheless, subordinated and laterally attached to the “main shoot” axis;
- “flowering shoot” relate only to buds with reproductive fate. It simply means that the “main shoot” within this bud organ does contain inflorescences within. In contrast, vegetative shoots are exclusively developed from vegetative buds.

Finally, one of the goals of Figure 2-11 is to, in the most succinct manner, depict the different avenues and growth structures that develop into main avocado shoots depending on their original bud fate. Not every feature displayed in the diagram is possible in all cases. The thick and color coded arrows point towards those sometimes exclusive sections within the main shoots that together should become a new reliably way to describe shoots, their sections within and ultimately their usefulness as permanent reference points that can easily be used (basipetally) to described actual location within this small but incredibly rich growth that is a single avocado shoot.

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# 3 **Chapter 3: Fruit presence, sunlight and their interactions on the vegetative growth of ‘Hass’ avocado (*Persea americana*, Mill.) shoots**

## 3.1 Introduction

Intrinsic to the “alternate bearing” phenomenon is the concept of a negative correlation between reproductive growth and overall vegetative growth[23, 28, 36, 37]. As both are broad concepts, this can be interpreted in at least two ways, either by suggesting that a necessary tradeoff exists so in order to have flower development, vegetative growth must stop (and vice versa)[28] or that, later on in the chronology of the avocado phenology, a direct competition for the available resources occurs between developing fruits and the concurrent vegetative growth[20, 28]. The latter, which focusses on fruit presence, will be the focus of this chapter.

### 3.1.1 Fruit presence

In avocados, it is an unlikely circumstance that a single flower successfully progresses to a single mature fruit that can be harvested. Although the amount of flowers at bloom can be potentially a million in a mature tree[7], very few will actually successfully develop into a fruit and persist until commercial harvest. Some of the factors that influence the continuum from flowering to harvest are discussed below:

#### *3.1.1.1 Flower types*

Avocado flowers exhibit both protogynous dichogamy[3, 31, 38] and dianthesis[18]. Together it means that a single avocado flower will undergo anthesis twice (usually in two consecutive days[18]) and despite being perfect flowers, they will be functionally female first and functionally male the second time they open. Two general groups of avocado trees have been described[4-6, 18] in accordance to the time of day they undergo initial anthesis: flowers of the “A” group open first in the morning hours, then close around midday and open as functionally male their second day while flowers of the “B” group open first in the afternoon hours, close through the night and open as functionally male in the morning of their second day. Given that at the tree level these anthesis events are not synchronized, there is opportunity for pollination between first anthesis females and second anthesis males within and among different trees. These features are thought to be an outcrossing promoting strategy of avocados[5] and under an horticultural point of view, self-pollination (not within a single flower[18] but between flowers of a single tree) has been deemed as of limited influence in the overall production[6]. Altogether, the implication is that cross pollination is necessary and hence actual fruit presence is dependent on synchronized events between the pollinators (insects that carry pollen) and the pollinizer (tree that donates pollen). This topic however remains controversial.

#### *3.1.1.2 Agriculturally, honey bees (Apis mellifera) are needed*

As stated above, natural pollinators such as flies, beetles and wasps[18, 26] are necessary but in practice the agriculturally relevant and main pollinator of avocados is the European honey bee[6, 18, 20, 28] (*Apis mellifera*) which delivers pollen from functionally male flowers to the receptive stigmas of the flowers of the complementary group. Some of the practical events needed for a successful fruit setting are:

- strong bee colonies are available, healthy and its members are actively visiting flowers (high solar radiation increases their activity[17]) searching for food (nectar or pollen);
- a bee that collected pollen from a functionally male flower also visits a female flower for pollination and ultimately fertilization to occur. Although abundant, avocado flowers are

"poorly endowed"[29] and might not attract the bees as flowers from other nearby species[28], to which they may also visit;

- The stigma receptivity only lasts for 2 to 3 hours[4] so there is a short window of opportunity at a single flower level.

As it stands, bees are necessary to maximize pollination and with that the fruit setting potential but at the same time several events need to successfully concur. Ultimately this is compensated in the orchards with increased availability of bee hives in the fields.

#### *3.1.1.3 Flower, fruitlet and fruit drop*

Although it varies between studies, fruit set is between 0.001%[6] or at least less than 0.1%[28] due to the loss of hundreds of thousands of flowers[28] and thousands of developing fruits[28]. Naturally, avocados go through enormous events of flower formation and loss (if not pollinized), along with fruitlets and fruit abscission events[29], even under good cultivation conditions[2]. Going back to the general issue of understanding the peculiarities of fruit presence in avocado, one can comprehend why it can be considered an unlikely event. Even when more than 10,000 flowers of the 'Fuerte' avocado cultivar were carefully emasculated and hand pollinated only 4 fruits reached maturity[30].

#### *3.1.1.4 Research objective and hypothesis*

Although horticulturally counterintuitive and within the context of the "alternate bearing" phenomenon, fruits have a bad connotation. "Too many" of them seem to cause a detrimental perturbation to a normally functioning avocado tree that will have whole canopy consequences in subsequent flowering/fruiting cycles.

The objective of this research was to increase the precision and uncover what are the perturbations that a growing fruit causes under the hypothesis that they should be displayed by the smallest, hence most informative, growth unit that may or may not bear a fruit: single avocado shoots.

Nonetheless, the challenge is clear: we accept that is practically impossible to predict where, within a complex canopy, a fruiting shoot will be present to then compare the phenotype of a fruit bearing shoot with a non-fruiting one. Although detailed below, an approach of "tree caging" was borrowed from breeding programs and entomological research as it was the only way to "control" fruit presence but in the opposite sense: generate avocado trees that completely and naturally lacked fruits.

## 3.2 Materials and Methods

Twelve, 7-year-old, 'Hass' avocado trees grafted onto 'Duke 7' clonal rootstock where selected on flat land located at the Agricultural Experimental Station of the University of California, Riverside (lat.: 33.58° N; lon.: 117.20° W and 308 m above sea level). This southern California Inland Valley ecoregion (Level IV, 85k)[12] presents a soil temperature that is "thermic" (annual average between 15° to 22° C[24]) and a xeric moisture abundance which is the Mediterranean regime[32]. The experimental site has a sandy to coarse sandy loam soil classification[33].

**Table 3-1. Average air temperatures (° Celsius) at field site.**

| Time of day | First season | | | | Second season |
|---|---|---|---|---|---|
| | Winter | Spring | Summer | Fall | Winter |
| Day | 18.2 (d) | 21.0 (c) | 27.6 (a) | 25.3 (b) | 17.6 (d) |
| Night | 12.1 (d) | 14.8 (c) | 20.9 (a) | 18.0 (b) | 11.1 (e) |

Hourly data points obtained from the on-site CIMIS station (Station ID #44, UC Riverside[8]). Night time was defined by the hours in which the solar radiation sensor captured less than 100 W/m$^2$. Statistical significance valid by time of day. Within a row, means that do not share a letter are significantly different. Day n=4,281; P=0.000. Night n=6,637; P=0.000.

### 3.2.1 Fruit presence control

To purposely generate a set of 6 fruitless trees, the concept of "tree caging"[17] was adopted. Enclosing avocado trees with beehives is a classic breeding approach to maximize pollination events and minimize the potential pollen donors[2, 3]. In the opposite sense, having a screened cage surrounding the trees effectively and naturally prevents flower pollination. The latter was our approach using a "Polysac Crystal" net with 17% light scattering and a mesh size of 1.44 mm$^2$ [17].

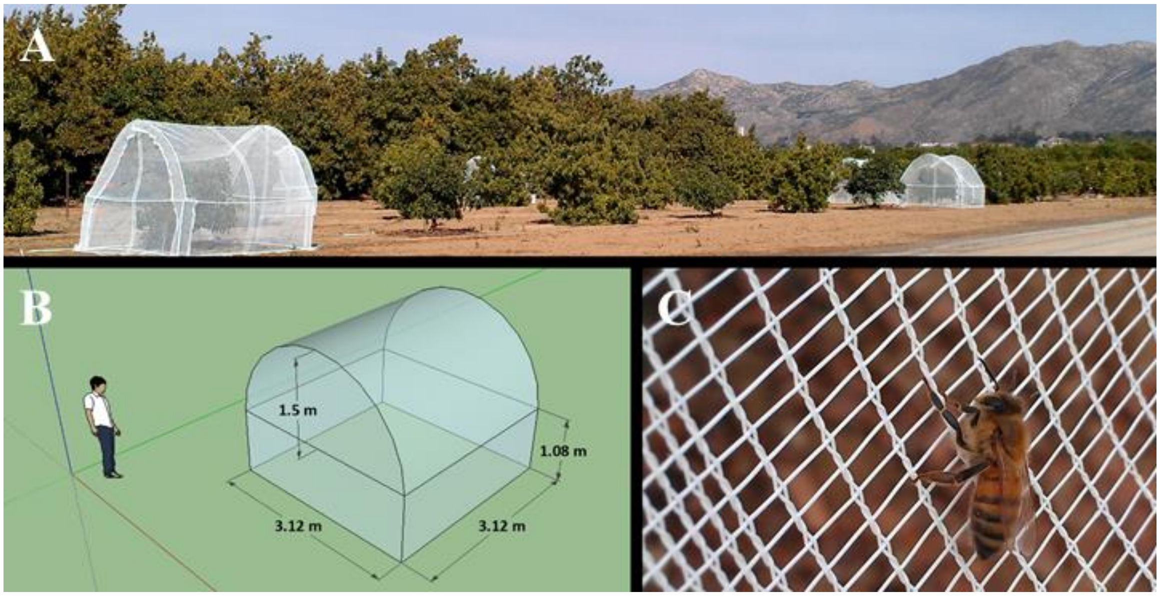

**Figure 3-1. General view of field site with "caged" trees and the PVC structure dimensions.**

A) Southwest corner of the experimental site during early spring. B) Design and dimensions of the netting structure. C) Bee exclusion by mesh size. Agricultural Experimental Station, UC, Riverside.

In order to standardize the initial tree canopy surface area, all twelve trees were pruned from an average canopy surface area of 3.3 m$^2$ down to 2.0 m$^2$, with the latter being a size that we could built the netting structure around. These cages naturally created the divergence in fruit presence intended as a treatment. The other 6 trees remained "open pollinated" and were allowed to receive bee visitations and freely set fruit. Three observable shoot combinations were followed during the season (Table 3-2).

**Table 3-2. Shoot conditions followed during the growth season.**

| **Shoots observable before "caging"** | **Tree group** | **Shoots observable after "caging" (3 combinations)** |
|---|---|---|
| Fruiting and non-fruiting shoots | "Caged" | Only non-fruiting shoots |
| | "Open pollinated" | Fruiting and non-fruiting shoots |

### 3.2.2 Tree canopy area and normalized fruit yield

The tree canopy surface area ($m^2$) was measured at the beginning of each season to evaluate the overall effects of fruits on the amount of vegetative growth during the previous season. Data was acquired by image analysis (Adobe Photoshop CS6, Extended version, 13.0.1) of scaled pictures taken on each cardinal quadrant (east-south-west-north) of their respective canopies. Mature fruit counts and harvest were also done by tree cardinal quadrant. Two diagonal lines that intersected at the main trunk defined the boundaries. To facilitate comparison, tree canopy area and the harvested fruits were used to normalize yield data as fruits/$m^2$ of canopy.

### 3.2.3 Fruit position within the canopy

Another aspect of overall fruit presence is the actual location of the growing fruits within the canopy. A coordinate system was used as a way to locate the position of a fruit in terms of height and depth. Tree height zones were defined as “lower” canopy when fruit was 100 cm from the group up while “middle” and “upper” canopy were between 100 cm to 170 cm and 170 cm to 250 cm, respectively. Depth was defined in respect to the canopy periphery. The “outer canopy” was defined as the distance from the outer layer of vegetative growth to a 50 cm depth. The “inner canopy” began and continued towards the trunk starting at 50 cm depth.

### 3.2.4 Shoot selection and phenotyping

Because trees naturally have different amounts of fruits there is an unbalanced amount of fruiting and non-fruiting shoots within a canopy. Our approach to shoot selection and tagging was simply based on fruit presence by which all fruiting shoots within a single tree would be selected and a similar amount of non-fruiting shoots will accompany. Nonetheless, there were practical limits when trees had large amounts of fruits in which case only around 30 fruiting and their non-fruiting counterparts were tagged. In accordance to this criteria, a total of 518 avocado shoots were followed. 43.2% of them on “caged trees” and 56.8% were on “open pollinated” trees. A total of 229 were fruiting shoots and 289 were non-fruiting shoots.

Although is the most specific research unit, the shoots database was simply based on annotating the observable features of the tagged shoots (all features are detailed in Chapter 2). A measuring tape was used for length measurements. For vegetative growth evaluations, all shoots that fell into the “spiral” avocado shoot class (Chapter 2) were filtered out since the length of the “spiral” shoot is much greater and would skew the average shoot length.

### 3.2.5 Length of the growth season

The growth season starts when an abundant outgrowth of resting buds (primarily apical buds) takes place. Because the first flush can be reproductive, the previous definition likely coincides with what is deemed as the main bloom period. On the other hand, the end of the growth season occurs when single shoots do not display any more primary growth elongation (i.e. no new vegetative flushes).

At the research site and for the period of data acquisition, the beginning of the season started in mid-January; all trees were actively flowering and producing vegetative flushes by March 5th (late winter) and measurable vegetative growth ceased between September 20th and October 12th (early fall).

### 3.2.6 Statistical analysis

When appropriate, for statistical analysis of continuous variables, one-way ANOVA, two way ANOVA or GLM were used for determining significance and interactions between factors. Means separation was obtained by Tukey post test with $\alpha=5\%$. For discrete variables and comparison of proportions, Pearson Chi-square test, binary logistic regression or confidence intervals of the difference between 2 proportions ($\alpha=5\%$) were conducted among possible combinations of factors involved. Software used was Minitab 16.2.4 (Minitab Incorporated).

## 3.3 Results

### 3.3.1 Canopy surface area

There were no statistically significant differences between the canopy areas of the trees when evaluated by cardinal quadrant (east, south, west, and north), either before the tree caging (n=12; P=0.631) or at the end of the succeeding season (n=12; P=0.587). When the statistical significance of having no fruits for a whole season was evaluated, the “caged” group was 1.2 $m^2$ larger than the “open pollinated” group (n=24; P=0.002), reaching an average canopy surface area of 3.9 $m^2$.

#### *3.3.1.1 Half spheroid volume*

Among other data captured by image analysis, the tree height and width was used to estimate the canopy volume as half of a spheroid with the formula $V = 0.5 \times (4/3\,\pi a^2 c)$ where $a$ is the equatorial radius and $c$ is the polar radius. There is a quadratic influence of the horizontal radius compared to the vertical one. ‘Hass’ trees tend to become wider through time and this is reflected in statistically significant larger volume in the second (“caged trees” were 1.6 $m^3$ larger. n=24; P=0.009) and third seasons (“caged trees” were 2.6 $m^3$ larger. n=24; P=0.022). Canopy area evaluations were not as informative as the half spheroid volume as no statistically significant differences were found at the third season (n=24; P=0.076).

### 3.3.2 Normalized fruit yield (fruits/$m^2$/tree)

Exclusively for the “open pollinated” group (n=18; P=0.050) where 3 seasons of yield data was collected, the east quadrant carried the largest amount of 20.1 fruits/$m^2$/quadrant; the north and south quadrants were not significantly different from each other (14.9 and 11.9 fruits/$m^2$/quadrant respectively) and the lowest was the west quadrant that yielded an average of 8.97 fruits/$m^2$/quadrant. There were no statistical differences in the normalized yield of the “open pollinated" group and the "caged" group either the first season before the tree "caging" (n=24; P=0.340) or the season after the "caging" (third season) (n=24; P=0.596).

The largest non-normalized yield registered was of 818 fruits/tree (average of 128 gr/fruit) and the largest amount on a single quadrant was 251 fruits/quadrant. These values were recorded on the largest tree. In terms of normalized yield, the largest value was 158.9 fruits/m$^2$/tree (average of 127.7 gr/fruit) and 53.5 fruits/m$^2$/quadrant. Although both normalized yield data points came from the same tree, the highest yield efficiency did not come from the largest experimental tree but a tree which had a 50.1% smaller canopy surface area than the largest tree in the study.

#### *3.3.2.1 Fruit location within the canopy*

Fruit presence is not homogeneous. Most fruits (59.8%) were located in the lower portion of the tree canopy, followed by the middle and upper canopy with 36.8% and 3.4% respectively (all P=0.000). At the same time, 75.1% of fruits were located at the inner canopy (all P=0.000).

**Table 3-3. Fruit location at harvest within 'Hass' avocado canopies.**

| **Fruit location coordinates** | | **Frequency of fruits (n=2882)** |
|---|---|---|
| **Height** | **Depth** | |
| Lower | Inner canopy | 47.0% (a) |
| | Periphery | 12.7% (c) |
| Middle | Inner canopy | 26.7% (b) |
| | Periphery | 10.2% (d) |
| Upper | Inner canopy | 1.4% (e) |
| | Periphery | 2.0% (e) |

All P values = 0.00 except the statistical difference of the proportion of fruits in the upper –periphery coordinate versus the upper – inner canopy coordinate which had a P value of 0.103. Data collected at Agricultural Experimental Station, UC, Riverside.

### 3.3.3 Observations at the single shoot level

From this point forward, broader variables that evaluated canopies as a whole will not be further addressed and the continuous or categorical variables analyzed are exclusively captured at the single avocado shoot level.

### *3.3.3.1 Shoot loss and fruit presence*

#### 3.3.3.1.1 Shoot loss under field conditions

Under the research field conditions and from the original population of healthy shoots, there was a natural loss during the season (no human involvement). One hundred and eight shoots (20.8%) of the total of 518 shoots were registered as fallen or completely dried out although still physically attached to older structures over the course of this study. It was even possible to find shoots with growing fruits that completely decayed. Within the shoots that were lost, 67.6% were originally fruiting shoots that had only one vegetative flush at the time of selection. Non-fruiting shoots with two vegetative flushes (10.2%) were the second largest grouping within the abscised shoots.

#### 3.3.3.1.2 Frequency of fruit bearing

The most common behavior for avocado shoots is not to bear fruits. Non-fruiting shoots dominated, accounting for 92.7% of the observed population (n=518; P=0.000). In the opposite sense (bearing fruits until maturity), the likelihood for a shoot that already developed a fruit, to grow and bear fruits again (two seasons in a row) is only 4.8% (n=229; P=0.000). For a shoot that was non-fruiting in the first year, the frequency of fruiting the second year, although higher than the previous, is still low with only 9.3% (n=289; P=0.000) of these shoots bearing fruit. The numbers of fruits borne on the selected shoots ranged from 1 to 6 fruits per shoot. However, the vast majority of shoot with fruits (86.82%; n=2283; P=0.000) will successfully bear only one fruit until its maturity. It is only distantly followed by the proportion of fruiting shoots with 2 fruits (12.09%).

#### *3.3.3.2 Shoot total length at end of season*

Independently of the season's number of vegetative flushes or more specifically the presence or absence of fruits, the lengths of the avocado shoots within the "caged" group (which had zero fruits for a complete season) averaged 29.27 cm. The shoots in "open pollinated" trees averaged 15.04 cm. The difference in length, 7.11 cm, was statistically significant (n=764; P=0.000).

**Table 3-4. Total shoot length (cm) by tree group, classification by number of flushes and fruit presence.**

| Tree group | Fruit presence on shoot | Shoot classification by number of vegetative flushes | | |
|---|---|---|---|---|
| | | 1 flush (n=368) | 2 flushes (n=185) | 3+ flushes (n=211) |
| **"Open pollinated"** | Fruiting | 7.38 (b) | 14.00 (b) | 23.35 (b) |
| | Non-fruiting | 8.36 (b) | 22.47 (a) | 39.77 (a) |
| **"Caged" trees** | Non-fruiting | 10.89 (a) | 22.76 (a) | 41.33 (a) |

Means that do not share a letter are significantly different. Grouping is valid within the amount of vegetative flushes (column). For 1 flush, 2 flushes and 3+ (3 or more flushes) P=0.000. Data collected at Agricultural Experimental Station, UC, Riverside.

Is noteworthy that fruiting shoots regardless of the number of vegetative flushes have a shorter final length than any non-fruiting shoot with the exception of the "open pollinated" non-fruiting/1 flush shoots which are statistically similar to the "open pollinated" fruiting shoots. For shoots with 2 or 3+ vegetative flushes, the non-fruiting condition has statistically similar final lengths independently of the tree group on which they developed (Table 3-4). Ultimately, there is a statistically significant interaction between fruit presence and the shoot length when single shoots are clustered by the amount of observed vegetative flushes (n=764; P=0.000 on GLM with $R^2$=60%; Figure 3-2).

Having more vegetative flushes usually relates to longer shoots. Nonetheless, for each of the shoot type clusters by their number of vegetative flushes, the non-fruiting shoots were significantly longer than their fruiting counterparts (Figure 3-2)

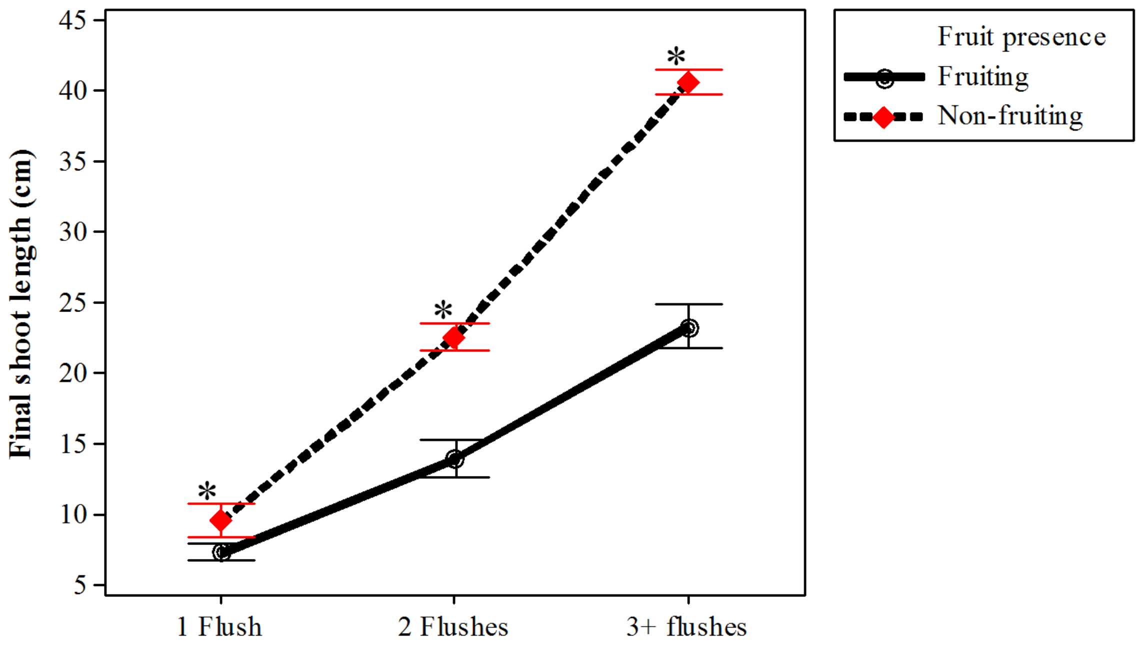

**Figure 3-2. Interaction plot and statistical significance of the fruit presence effect on final shoot length.**

Asterisks denote statistically significant differences in length of shoots by fruit presence within each flush cluster. For 1 flush, 2 flushes and 3+ flushes, n=368, 185 and 211, respectively; ANOVA and Tukey, α=5%; P=0.000

#### 3.3.3.2.1 Analysis of specific vegetative flush lengths

Due to the significant effects of fruit presence on the shoot final length, it is appropriate to also examine the more specific contribution of each of the vegetative flushes length to that final outcome (Table 3-5). Independently of any specific fruit presence, the first vegetative flush is longer if they belong to shoots that eventually developed an increasing amount of flushes (n=764; P=0.000). This is also true for the second vegetative flush length (n=396; P=0.000) which was 6.07 cm longer on shoots with 3+ flushes than on shoots with 2 flushes.

**Table 3-5. Reduction of specific vegetative flush length (cm) by fruit presence.**

| Shoots by amount of flushes | Fruit presence | Average of specific flush length | | | |
|---|---|---|---|---|---|
| | | 1st flush | 2nd flush | 3rd flush | 4th flush |
| **1 flush (n=368)** | Fruiting | 7.38 (b)<br>(23%) | - | - | - |
| | Non-fruiting | 9.59 (a) | - | - | - |
| **2 flushes (n=185)** | Fruiting | 10.14 (b)<br>(32.3%) | 3.87 (b)<br>(49.3%) | - | - |
| | Non-fruiting | 14.97 (a) | 7.64 (a) | - | - |
| **3+ flushes (n=211, except 4th flush with n=56)** | Fruiting | 10.90 (b)<br>(33.2%) | 6.60 (b)<br>(53.3%) | 5.45 (b)<br>(32.9%) | 2.31 (b)<br>(68.5%) |
| | Non-fruiting | 16.31 (a) | 14.13 (a) | 8.12 (a) | 7.34 (a) |

Percentages in parenthesis indicate the level of reduction from the non-fruiting to the fruiting shoot condition. Means that do not share a letter within a column are significantly different. Grouping is valid only within the specific combinations of shoots by final amount of flushes and flush within it. P=0.000 for all comparisons, except third and fourth flush with P values=0.004 and 0.032 respectively). Data collected at Agricultural Experimental Station, UC, Riverside.

After framing the analysis to the level of how the average length of any specific flush, within an individual shoot, is affected by the particular fruit presence, one can clearly see that not only in all cases is there a statistically significant effect but also that the effect is a reduction of their average length by the percentages stated in Table 3-5.

### *3.3.3.3 Axillary bud release into lateral shoots (branching)*

The outgrowth of axillary buds from the main shoot into lateral shoots (branching) is an essential aspect of the avocado shoot complexity. However is important to explain our criteria: a lateral shoot is the (mainly) proleptic growth that was released from axillary buds in the time frame within two consecutive flushes and not a proleptic release on a completely new yearly growth cycle. As described in Chapter 2, the presence of lateral shoots is a feature of the “vegetative to vegetative” transition zones.

There are fruit presence effects over the branching phenotypes. With branching being defined as 1 or more lateral shoots observed along the main shoot axis (non-branching meaning no lateral shoots), non-fruiting shoots displayed a lateral branching frequency of 44% (n=200; P=0.016) while fruiting shoots had only 8.5% branching (n=200; P=0.000), hence lateral branching is reduced on fruiting shoots (n=200; P=0.000; Figure 3-3).

If branching occurred, the amount of laterals observed ranged from 1 to 7 per main shoot. The most frequent amount of lateral shoots displayed is either 1 or 2 laterals per shoot as they are not significantly different from each other (n=105; P=0.651) and together account for 60% of the branching main shoots. They are followed by shoots with 3 lateral shoots (19.05%) and after that, there is a dramatic drop in the counts of shoots with 4, 5, 6 and 7 laterals which when summed only account for 20.95%.

Fruit presence reduces lateral branching as the frequencies of their observation on fruiting shoots is never higher than around 6% (Figure 3-3). Bear in mind that within a canopy, a non-fruiting shoot is a common shoot condition and fruiting shoots are basically rare. The most common location of a lateral shoot is one node below the first transition zone (Figure 3-3). Twenty five percent of the lateral shoots were observed in this position on branched non-fruiting shoots as compared to 5.9% on branched fruiting shoots. Overall, within the context of a whole tree canopy, the chances of finding a branched shoot with a lateral shoot in the most common position (1 node below transition 1) is 11% and 0.5% for non-fruiting and fruiting shoots respectively.

Figure 3-3 displays how there is a gradient of increased frequency of lateral shoots occurring right below the shoot tip at the end of the corresponding vegetative flush which, after the growth season ends, can be described as “right below” the transition zones.

Finally, independently of the final length of the vegetative flushes, if lateral branching occurs, then the shoot section where these lateral shoots can be found is the distal 7.8% portion of the main shoot axis (last 0.71 cm on average (n=212)) of any vegetative flush.

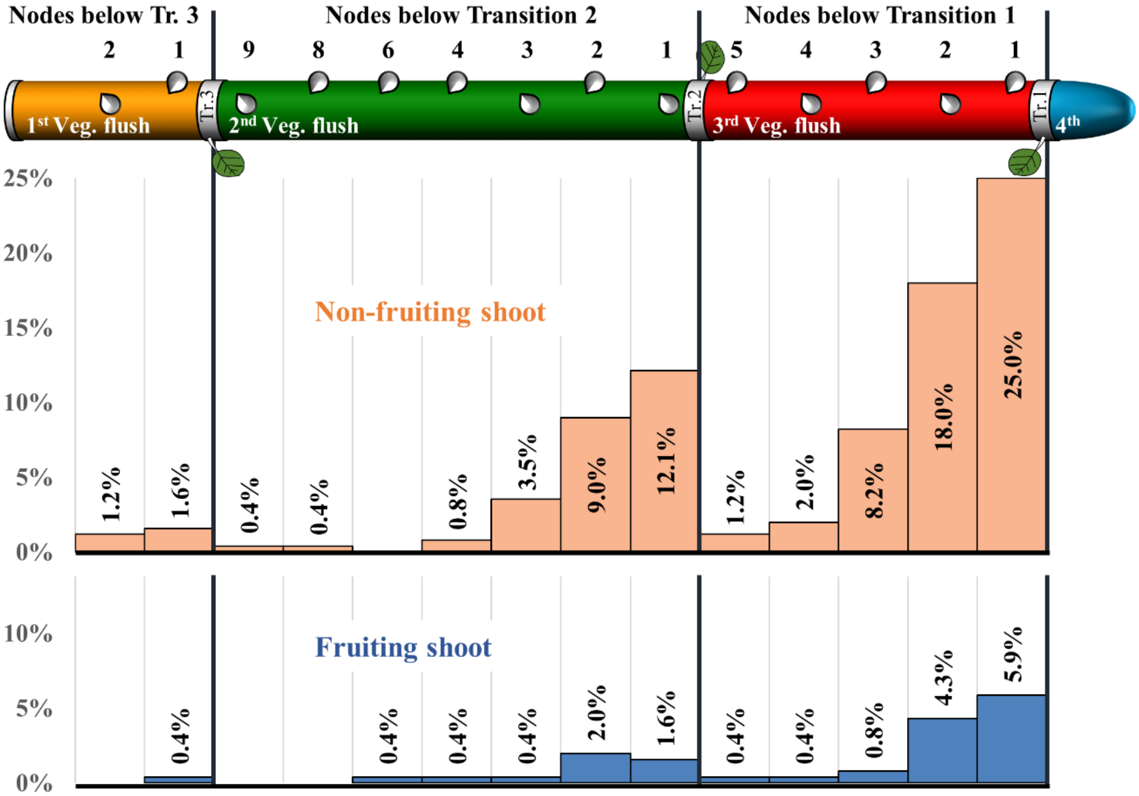

**Figure 3-3. On shoot that displayed lateral branching events: frequency of observation at particular locations along main avocado shoots.**

Collectively, all frequencies add up to 100% as they indicate the distribution of all 261 lateral shoots observed within 103 different branched ‘Hass’ avocado shoots. Upper section displays information for non-fruiting shoots while bottom is for fruiting shoots. As a positional reference, the center diagram represents an avocado shoot with 4 vegetative flushes and 3 “vegetative to vegetative” transition zones. Tr.: transition zone. White teardrop shape volumes represent single nodes (axillary buds). Round leaf represents a transitional leaf at transition zone. Note that the shoot diagram is not at scale and does not intend to represent the length of the different vegetative flushes (which should be longer). Note that nodes are not necessarily consecutive as it simply no observations of lateral branches were observed at that particular node position. Further details within the text.

### 3.3.4 Sunlight exposure on avocado shoot phenotypes

Sunlight exposure has a dramatic impact on the phenotypic outcome of the ‘Hass’ avocado shoot. Under our criterion, a ranking of “shaded” for a single shoots is given when it is observed 50 cm or more inwards from the canopy periphery towards the inner canopy (trunk area). This relates to Hadari (2004)[15, 37] who working with avocado canopies of multiple varieties detected a reduction of the relative radiation to around 55% (from 100% being full sun) when measured 50 cm into the canopy. This also coincides with the criterion for fruit location within a canopy (section 3.2.3, Table 3-3). Under field conditions and in the context of a ‘Hass’ avocado tree canopy, there is an interaction between fruit presence and sunlight exposure (n=764; P=0.000) to the point that fruit presence is a good predictor of shoot sunlight exposure. With an odd ratio (OR)[34] of 2.83, non-fruiting shoots are 73.9% more likely to be sunlit (instead of shaded) compared to fruiting shoots (n=764; P=0.000).

#### *3.3.4.1 Exposure to sunlight and shoot length*

In order to present the overall effects of the interaction between fruit presence and sunlight exposure, final shoot length was analyzed (Figure 3-4). Exclusively focusing on fruit presence, non-fruiting shoots were 17.17 cm longer that fruiting shoots (n=764; P=0.000). Correspondingly, focus on sunlight exposure reveals that sunlit shoots are 19.16 cm longer than shaded shoots (n=764; P=0.000). Interestingly, there is no statistically significant difference between the lengths of a fruiting shoot that is receiving sunlight with that of a non-fruiting shoots that grows under shaded conditions.

For each of the clusters presented in Figure 3-4, the non-fruiting were significantly longer than their fruiting counterparts. An interaction of fruit presence and shade leads to short final lengths while non-fruiting and sunlit shoots are the longest.

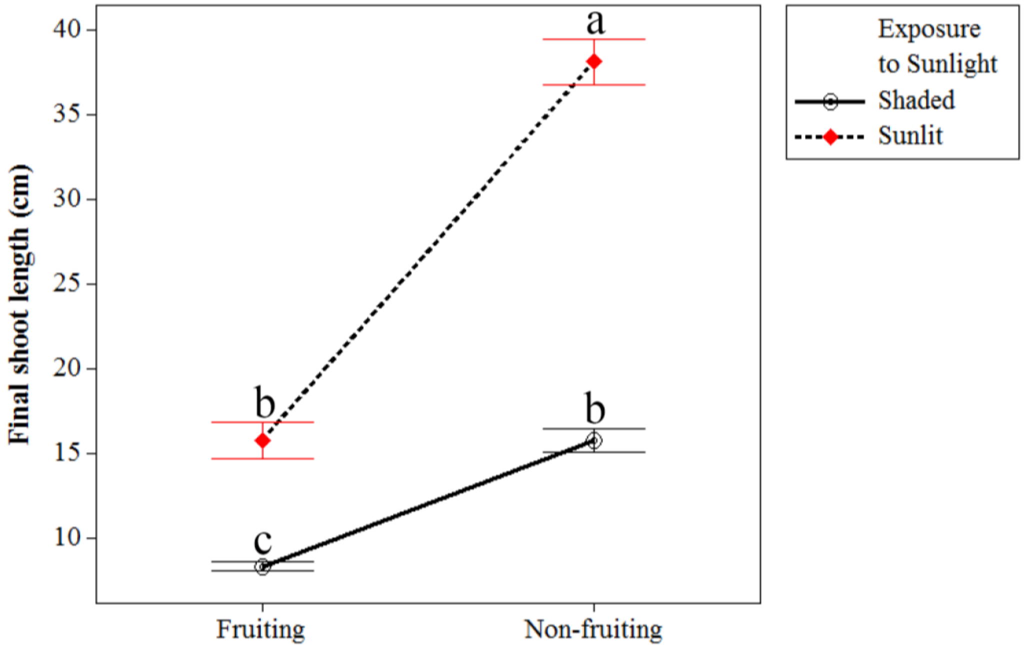

**Figure 3-4. Interaction plot and statistical significance of the fruit presence and exposure to sunlight on the final shoot length**

Means that do not share a letter are significantly different (P=0.000). For fruiting/sunlit; fruiting/shaded; non-fruiting/sunlit and non-fruiting shaded, n=106, 282, 197 and 179, respectively. Data collected at Agricultural Experimental Station, UC, Riverside.

### *3.3.4.2 Exposure to sunlight and lateral branching*

Once again for comparison, a focus on fruit presence showed that non-fruiting shoots develop lateral shoots 44% of the time (n=200; P=0.000) while fruiting shoots do so 8.5% (n=200; P=0.000). Similarly, a focus on sunlight exposure reveals that sunlit shoots develop lateral shoots 58.3% of the time (n=180; P=0.002) while 0% of 220 shaded shoots branched laterally (Table 3-6).

**Table 3-6. Interaction of fruit presence and sunlight exposure on main shoot branching.**

| Fruit presence | Sunlight exposure | Branching (observation of at least 1 lateral shoot on the main shoot) |
|---|---|---|
| Non-fruiting | Sunlit (for combination: n=100) | 88% (a) |
| Fruiting | Sunlit (for combination: n=80) | 21.3% (b) |
| Non-fruiting | Shaded (for combination: n=100) | 0% |
| Fruiting | Shaded (for combination: n=120) | 0% |

All P values = 0.000. Data collected at Agricultural Experimental Station, UC, Riverside.

### 3.3.5 Simplified phenotyping for field studies

All previous results were obtained using a more comprehensive phenotyping approach. Although informative, a potential caveat is the length of time involved in revisiting tagged shoots in larger tree populations. Having in mind practical aspects of increasing the ease and speed of data acquisition in actual orchard environments, a simplified phenotyping approach to avocado shoots in terms and fruit presence was designed based on the previous detailed analysis.

Total shoot and/or flush length was not measured since this is a time intensive activity. The approach was to only distinguish and count the following phenotypical instances listed below (Figure 3-5):

- **1 Flush – Simple**: applies to shoots that have 1 single vegetative flush and do not have lateral branching events (zero lateral shoots along the main shoot followed)
- **1 Flush – Branched**: applies to shoots that have 1 single vegetative flush and do have at least one lateral branching events (1 or more lateral shoots).
- **2+ Flush – Simple**: applies to shoots that have 2 or more vegetative flushes and do not have lateral branching events.
- **2+ Flush – Branched**: applies to shoots that have 2 or more vegetative flushes and do have lateral branching events.

This phenotyping approach was deployed in three settings, two within avocado producing areas with Mediterranean climate[35] (California, USA and Las Palmas, Chile) and one in the hot semi-arid climate of Limpopo[35], in South Africa (Figure 3-5). The comparison between the 4 different shoot phenotypes indicate that on fruiting shoots, the simplest shoots (1 flush – simple) dominate while on non-fruiting shoots, the most complex ones (2+ flush - branched) are predominant.

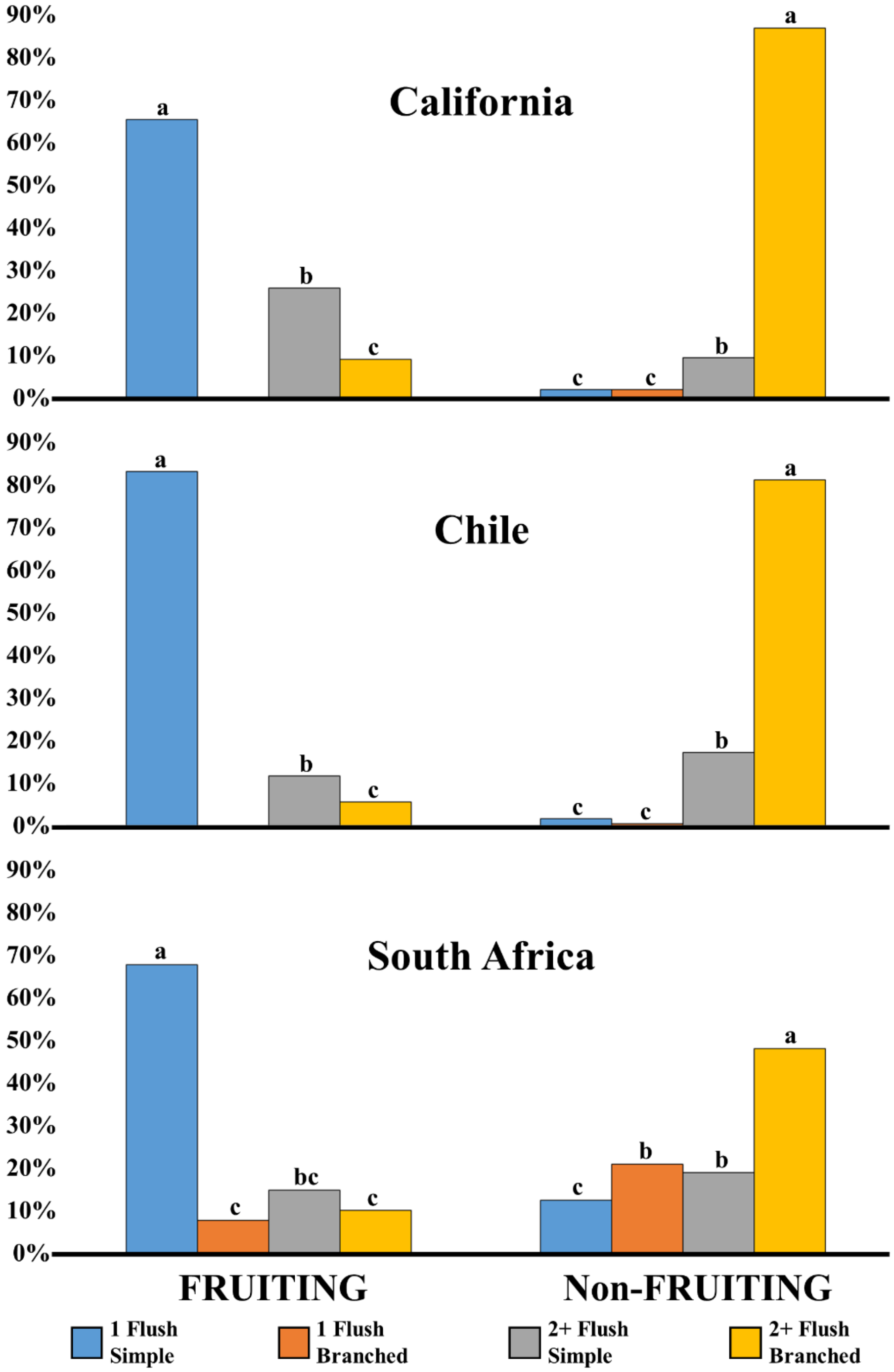

**Figure 3-5. Simplified phenotyping approach. Proportions of 'Hass' avocado shoots at 3 different avocado producing locations.**

Data from fruiting shoots population on the left and from non-fruiting shoots on the right section. Statistical significance is valid within country and fruit presence status only. All comparisons of proportions at 95% confidence interval. For sites in California, Chile and South Africa, n=315, 480 and 500 respectively.

Interestingly, both areas with Mediterranean climate patterns (California and central Chile) displayed a remarkably similar distribution of the different shoot complexities while only the South African site exhibited a noticeable increase in the observation of branching shoots, either on fruiting and non-fruiting shoots.

## 3.4 Discussion

A mechanistic understanding of the "alternate bearing" phenomenon has been elusive in part because it conceptualizes fruit presence as causing detrimental consequences on vegetative growth and, although arguably true, that scope is a very broad one. After evaluating broad factors that were expected to be distinct at the whole tree level, the "caged" and the "open pollinated" groups revealed that having no fruits for a whole season is statistically linked to canopies that were on average 1.2 $m^2$ and 1.6 $m^3$ larger in area and volume respectively.

However, what can we do with that knowledge? Knowing that canopies are statistically larger just reiterates that there is a real effect but is not so informative in terms of how it occurred. Also, avocado canopies are very plastic and their overall shape is changed by field elements such as gusty winds, sunlight exposure (branching bias) and/or wood bending due to the combination of vegetative growth itself and wood strength so the particular moments on which canopy level factors are evaluated will lead to also particular numerical outcomes.

Furthermore, and under our conditions, an unexpected tree level result was that after a year of having no fruits, the "caged" trees produced a normalized fruit yield with no statistical differences with the "open pollinated" group. Additionally, reducing the scope of this observation to the quadrant level revealed that the east quadrant (and not the south) was consistently the largest producer; also, that the north quadrant was equally fruitful as the south quadrant despite the former traditionally expected to be the "worst" (as "it is the more shaded side") and the latter being the frequent choice of researchers as it is “better exposed to the sunlight during the season”. Preconceptions need to be abandoned and field aspects like dynamic canopies and even the changes of the sun height and angle throughout the season and in respect to the field sites need to be embraced and addressed for avocado “alternate bearing” research.

The realization of these problems is what led our research objective to be that of increasing precision and phenotypic knowledge of the vegetative growth unit that was both the smallest possible while relevant to fruit presence in order to reveal more consistent patterns. Our hypothesis that single avocado shoot would be highly informative and divergent by fruit presence alone was corroborated. Proven by their significant interaction (Figure 3-2) and in the simplest terms: at the end of a growth season, avocado fruiting shoots display a sole and smaller vegetative flush that is non-branched while their non-fruiting counterparts are longer and more complex, with added vegetative flushes and developing lateral shoots.

A comparison of commercial orchards outside California demonstrated that these patterns were consistent in different producing areas of the world and not only under our research conditions. Besides the information captured and presented within this discussion which shows these distinct patterns remaining constant in California, Chile and South Africa, anecdotally the patterns were also observed in two other avocado producing countries: Israel and Peru (data not presented).

Having stated the previous, there was another factor that was also interacting with fruit presence causing a perturbation of the already significant patterns driven solely by fruit presence: exposure of the shoots to sunlight. We learned that, independently of their particular fruit presence status, sunlit shoots tend to branch laterally while the shaded shoots do not. Also, either fruiting or non-fruiting shoots are proven longer if they are sunlit. Borrowing the language utilized in molecular studies, it could be seen as sunlight exposure “partially rescues” the “non-fruiting shoots phenotype" into the "fruiting shoots phenotype" by allowing them to grow longer and branch, to the point that after comparing 285 shoots that belonged to these combinations, the length of sunlit fruiting shoots was significantly the same as the length of shaded non-fruiting shoots with a numerical difference of only 0.02 cm (0.2 mm).

All previous factors considered, the detrimental vegetative effects of the "alternate bearing" phenomenon follow the patterns below:

- fruit bearing until maturity is a rare event which, of course, implies that non-fruiting shoots and their phenotypes are the most common within the avocado canopy;
- a single fruit bearing shoot will be shorter at the end of a season and this could be the consequence of a reduced growth rate at the early stages of fruit set;
- the non-fruiting shoot phenotype, which are much more common that the fruiting phenotype, will grow longer, flush more and branch laterally, most likely with a comparatively faster growth rate than the fruiting one;
- within the context of a whole canopy, a single fruiting shoot will likely be outgrown and outcompeted by the many non-fruiting ones that are growing around it. The many non-fruiting shoots remain with growing tips in the sunlit periphery of the tree, reinforcing their growth while at the same time leaving the fruiting shoots behind under an environment of increased shade;
- The majority (73.7%) of the fruit which persisted to maturity are located in the inner canopy at mid to lower height (section 3.3.2.1) but this observation is after the fact at the end of the season. All the shoots that together construct the canopy grew around the fruiting shoots, leaving them in what became the inner canopy over the growing season. From this point of view, it is certainly possible that early in the season most of the bloom is exposed in what once was the canopy periphery where it is visited by pollinating insects. As fruit set successfully

occurs, other shoots will remain non-fruiting (losing their fruit set early to mid-season) causing the conditions that lead to the overall canopy observations;

- A combination of the demanding sink that is the growing fruit and the shaded environment that developed around fruiting shoots cause them to be lost (probably starvation due to lack of light to drive enough photosynthesis) or to successfully carry fruits until maturity but remaining only with their first vegetative growth, shorter and non-branched.

With these patterns and combinations of fruit presence and sunlight exposure at the single shoot level one can understand that the overall reduction in growth caused by "alternate bearing" at the canopy level is explained by the collective and consistent responses of single avocado shoots.

## 3.5 Conclusions

It was important to finally dissect the perplexing "alternate bearing" phenomenon into a set of simpler and repeated phenotypes which gets us closer to how it mechanistically occurs. Furthermore, these patterns are consistent in different environments which allows collaboration among research groups using a common and simplified phenotyping approach.

Ultimately, avocado shoot phenotyping should be a tool for researchers and avocado breeders alike. Large scale observations within a mapping population requires phenotypical knowledge of the desired traits while being able to capture them fast and reliably (i.e. using our simplified yet informative phenotyping scheme) in the likes of Durand, et. al.[10], in apples, and Sadok, et. al. [27] in olives who combined canopy and shoot level observations to discriminate informative descriptors that related to QTLs related to "alternate bearing" but earlier in the productive life of the trees[10].

After this research, the "alternate bearing" phenomenon becomes dissected into two vegetative growth related events: shoot apical meristems reinitiating new vegetative flushes (more than one vegetative flush) and the control of bud outgrowth. Although these traits are more specific within current avocado knowledge, they are still broad physiological aspects that are most likely multigenic[27] in nature.

Is already know from research on the genetic control of fruit bearing of domesticated crops (apple and olives), that genes related to flowering are less involved in “alternate bearing” than plant hormone related genes[13, 27], hence the regulation of flowering genes by phytohormones was proposed. Along the same lines, apple bourse shoots with adjacent fruits or exogenous applications of gibberellins correlated with increased accumulations of the flower inhibitor *MdTFL1* (*Malus domestica* TERMINAL FLOWER 1) gene transcripts[14], both approaches pointing towards plant hormone involvement.

Nonetheless, in order to further explore the presented correlation between sunlight exposure and fruit presence over shoot growth and lateral branching we should not overlook branching integrators such as *BRANCHED 1* (*BRC1*)[1, 9] gene expression (and protein) at the axillary meristems as it controls their outgrowth (BRC1 protein is involved in prevention of axillary bud release). Also, along with fruit presence and sunlight exposure, a consideration of factors influencing lateral branching (and the overall avocado canopy dynamics, see Chapter 2) such as strigolactones, cytokinins and apical dominance[39], together with carbohydrate functions as developmental regulators and in relation to phloem content demand and sink strength[11, 16, 21], the role of the “shade avoidance syndrome”, light quality and quantity, light morphogenesis and brassinosteroids together with phytochromes[19, 25] should be consider potential constraints[22] to avocado fruit production and, with that, research interests of the now more phenotypically clear “alternate bearing” phenomenon.

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# 4 Chapter 4: Fruit presence and its influence on the reproductive growth of 'Hass' avocado (*Persea americana*, Mill.) shoots

## 4.1 Introduction

The characteristics of the avocado reproductive behavior can be deemed as distinct. Their floral behavior is protogynous and dichogamous[62, 71] (i.e. flowers are complete but male and female organs mature at different times and the female organs mature first), the fruits grow mainly by cell division instead of cell expansion[8] and they also acquire a high lipid content through time[30]. Adding to the previous, the "alternate bearing" behavior that avocados display seems to be the outcome of a complex mix of fruit numbers during the season and, only later on, a phenotypical response to their presence at the succeeding bloom season. If to this we add that fruit can remain on the tree from 8 to 12 months[77], then is almost a closed loop or a circular reference than needs more information to be resolved.

A continuing challenge is that despite up to four bloom events are reported in Michoacan, Mexico[54, 55] (i.e. with common names of "floracion loca" ("crazy bloom"), "aventajada" ("with an advantage"), normal and "marceña" ("of march")), and that several bloom events of different intensities can be observed through the year in our research plots in California, avocado research seems to only be approaching this specie as with a single main bloom that is the outcome of a single induction event, hence minimizing their actual phenotypical plasticity.

Even further, the fact that the same classification of "normal bloom" is given to the one observable during early winter in Mexico[54, 55] and to the spring one, hence after a winter period, in California indicates that our concepts are not aligned with the practical observations. From the perspective of fruit development, bud break events occur on vegetative growth (shoots) from previous seasons which are several months old. The apical and axillary buds are along them and they may undergo bud break into their vegetative or reproductive fate. When the latter is the case, flowering shoots will develop and their inflorescences will represent the potential of that season which is starting with that bloom.

It is our goal to contribute toward planning future avocado reproductive research by focusing on the more precise and relevant single shoot level to describe the actually observable effects of a growing fruit onto the reproductive behavior of the shoots that are bearing them. This should connect these phenotypes to possible avenues of molecular tools assisting avocado research.

## 4.2 Materials and Methods

Twelve, 7-year-old, ‘Hass’ avocado trees grafted onto ‘Duke 7’ clonal rootstock where selected on flat land located at the Agricultural Experimental Station of the University of California, Riverside (lat.: 33.58° N; lon.: 117.20° W and 308 m above sea level). This southern California Inland Valley ecoregion (Level IV, 85k)[22] presents a soil temperature that is “thermic” (annual average between 15° to 22° C[43]) and a xeric moisture abundance which is the Mediterranean regime[64]. The experimental site has a sandy to coarse sandy loam soil classification[65].

**Table 4-1. Average air temperatures (° Celsius) at field site.**

| **Time of day** | **First season** | | | | **Second season** |
|---|---|---|---|---|---|
| | **Winter** | **Spring** | **Summer** | **Fall** | **Winter** |
| Day | 18.2 (d) | 21.0 (c) | 27.6 (a) | 25.3 (b) | 17.6 (d) |
| Night | 12.1 (d) | 14.8 (c) | 20.9 (a) | 18.0 (b) | 11.1 (e) |

Hourly data points obtained from the on-site CIMIS station (Station ID #44, UC Riverside[11]). Night time was defined by the hours in which the solar radiation sensor captured less than 100 W/$m^2$. Statistical significance valid by time of day. Within a row, means that do not share a letter are significantly different. Day n=4,281; P=0.000. Night n=6,637; P=0.000.

### 4.2.1 Fruit presence control

To purposely generate a set of 6 fruitless trees, the concept of "tree caging"[29] was adopted. Enclosing avocado trees with beehives is a classic breeding approach to maximize pollination events and minimize the potential pollen donors[5, 6]. In the opposite sense, having a screened cage surrounding the trees effectively and naturally prevents flower pollination. The latter was our approach using a "Polysac Crystal" net with 17% light scattering and a mesh size of 1.44 mm$^2$ [29].

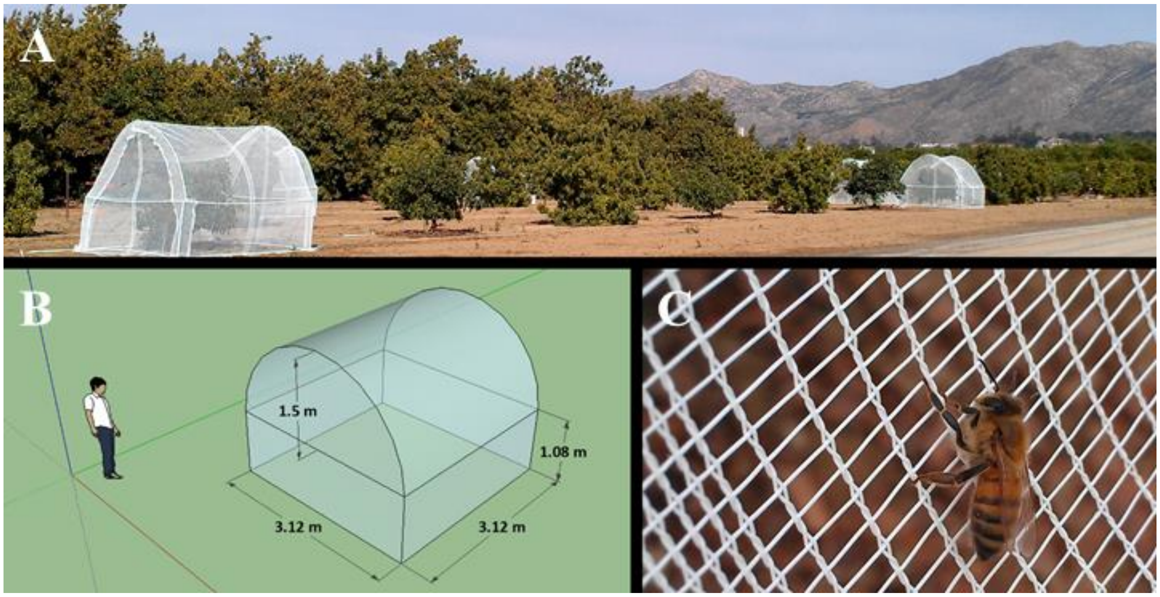

**Figure 4-1. General view of field site with "caged" trees and the PVC structure dimensions.**

A) Southwest corner of the experimental site during early spring. B) Design and dimensions of the netting structure. C) Bee exclusion by mesh size. Agricultural Experimental Station, UC, Riverside.

In order to standardize the initial tree canopy surface area, all twelve trees were pruned from an average canopy surface area of 3.3 m$^2$ down to 2.0 m$^2$, with the latter being a size that we could built the netting structure around. These cages naturally created the divergence in fruit presence intended as a treatment. The other 6 trees remained "open pollinated" and were allowed to receive bee visitations and freely set fruit. Three observable shoot combinations were followed during the season as stated in Table 4-2.

**Table 4-2. Shoot conditions followed during the growth season.**

| Shoots observable before “caging” | Tree group | Shoots observable after “caging” (3 combinations) |
|---|---|---|
| Fruiting and non-fruiting shoots | “Caged” | Only non-fruiting shoots |
| | “Open pollinated” | Fruiting and non-fruiting shoots |

### 4.2.2 Fruit position within the canopy

Another aspect of overall fruit presence is the actual location of the growing fruits within the canopy. Mature fruit depth was defined in respect to the canopy periphery. The “outer canopy” was defined as the distance from the outer layer of vegetative growth to a 50 cm depth. The “inner canopy” began and continued towards the trunk starting at 50 cm depth.

### 4.2.3 Shoot selection and phenotyping

Because trees naturally have different amounts of fruits, there was an unbalanced amount of fruiting and non-fruiting shoots within a canopy. Our approach to shoot selection and tagging was simply based on fruit presence by which all fruiting shoots within a single tree were selected and a similar number of non-fruiting shoots as control. Nonetheless, there were practical limits when trees had large amounts of fruits in which case only around 30 fruiting and their non-fruiting counterparts were tagged. Based on these criteria, a total of 518 avocado shoots were followed: 43.2% of them on “caged trees” and 56.8% on “open pollinated” trees. A total of 229 were fruiting shoots and 289 were non-fruiting shoots.

The acquisition of the phenotypical data was done by recurrent observation of the selected shoots and the annotation of their shoot reproductive features (detailed in Chapter 2) as they occurred throughout the growing season.

### 4.2.4 Tree bloom intensity

Tree bloom intensity is a categorical variable measured at the canopy level. It is a visual rating of the balance between the vegetative and reproductive growth. At a distance of ≈ 3 m from the trunk a visual evaluation of the southern quadrant of the canopy was done and one of the 4 levels detailed in Table 4-3 was assigned to the canopy under evaluation. Because similar initial canopy bloom intensities were needed, only 4 trees of the "caged" group and 4 on the "open pollinated" group had their bloom evaluated in this manner.

**Table 4-3. Description of the canopy bloom intensity rating**

| Rating | Approximate flower/leaf ratio | Defoliation (mid-season) | Notes |
|---|---|---|---|
| Predominantly vegetative | 0 – 25% | None | Few, if any, blooms are visible |
| Light bloom | 26 – 50% | Little | Blooming is apparent but tree appears to have more leaves than flowers |
| Normal bloom | 51 – 75% | Moderate | Individual branches may have heavy bloom with defoliation |
| Heavy bloom | 76 – 100% | Heavy | Heavy bloom, upper part of tree may be completely defoliated |

A "predominantly vegetative" bloom intensity rating was given to trees that, at the time of evaluation, were essentially more vegetative than reproductive and regarded as "reproductively off" under the flower interpretation of the "alternate bearing" cycle (see Chapter 1). When the bloom rating reached the "normal bloom" category, the evaluated tree was deemed has having a balanced amount of vegetative growth along with a commercially important bloom. A "heavy bloom" level was given to a tree whose vegetative cover was visibly reduced to a point that is commercially undesirable due to the increased probability of fruit being exposed to the sun heat (sunburn damage) hence reducing the commercial value.

### 4.2.5 Apical and axillary bud release

Without human intervention to trigger bud release (break or outgrowth), the shoot apical and axillary buds were followed during the growth season. Four aspects were investigated:

- comparatively, presence or absence of released buds and counts of number of buds that underwent bud break between fruiting and non-fruiting shoots;
- the relative location of those natural bud break events along the fruiting and non-fruiting shoots (classification as apical or axillary buds and location along the whole shoot);
- a distinction of the two mutually exclusive fates possible: buds containing an entirely vegetative growth or buds containing reproductive structures in the form of flowering shoots. The latter are broken into 2 subcategories, functionally “indeterminate flowering shoots” (grown from buds whose inflorescence meristem (IM) retained their shoot apical meristem (SAM) undifferentiated and hence are able to generate a vegetative flush at their shoot apex) and the functionally “determinate flowering shoots”, which are completely reproductive structures;
- survival of those outgrown vegetative or reproductive structures until the end of the season.

### 4.2.6 Flowering shoot complexity

Flowering shoot complexity refers to the relative number of inflorescences per flowering shoot. Using a functionally indeterminate flowering shoot as an example, its reproductive complexity (and potential) was measured by counting the inflorescences that developed and released at the beginning of the season around the circumference of the flowering shoot. The simplest is an indeterminate flowering shoot of 1 inflorescence but other buds can contain flowering shoots with many more, hence increasing their complexity level.

### 4.2.7 Length of the growth season

The growth season starts when an abundant outgrowth of resting buds (primarily apical buds) takes place. Because the first flush can be reproductive, the previous definition likely coincides with what is deemed as the main bloom period. On the other hand, the end of the growth season occurs when single shoots do not display any more primary growth elongation (i.e. no new vegetative flushes).

At the research site and for the period of data acquisition, the beginning of the season started in mid-January; all trees were actively flowering and producing vegetative flushes by March 5th (late winter) and measurable vegetative growth ceased between September 20th and October 12th (early fall).

### 4.2.8 Statistical analysis

When appropriate, for statistical analysis of continuous variables, one-way ANOVA, two way ANOVA or GLM were used for determining significance and interactions between factors. Means separation was obtained by Tukey post test with α=5%. For discrete variables and comparison of proportions, Pearson Chi-square test, binary logistic regression or confidence intervals of the difference between 2 proportions (α=5%) were conducted among possible combinations of factors involved. Software used was Minitab 16.2.4 (Minitab Incorporated).

## 4.3 Results

### 4.3.1 Canopy bloom intensity

The first season was non-informative because of the preconceived criteria of trees having the same “normal” bloom intensity rating and this is reflected in Table 4-4 by all trees having the exact same mode for the total n number. After a complete season without bearing fruits, the “caged” group of trees displayed a return bloom equal to the one they had a year before the “caging” treatment, which was the “normal” rating, indicating that the lack of fruits allowed a generally normal reproductive development observable the succeeding season. On the other hand, 3 out of 4 “open pollinated” trees exhibited the “predominantly vegetative” rating indicating an almost exclusively vegetative second season after bearing fruits during the first one.

During the third season, trees in the group that was originally “caged” group were essentially equivalent to the “open pollinated” trees in their second year (just chronologically separated by a whole season) because they exhibited a “predominantly vegetative” mode after a previous season of “normal bloom”. Interestingly, the “open pollinated” group showed no mode at their third season meaning that each one of the 4 trees exhibited a different bloom intensity rating.

**Table 4-4. Mode and respective n numbers for the canopy bloom intensity of evaluated trees**

| Tree group | Season | Mode | N for Mode |
|---|---|---|---|
| “Open pollinated” | First | Normal bloom | 4/4 |
| | Second | Predominantly vegetative | 3/4 |
| | Third | None | 0/4 |
| Caged | First | Normal bloom | 4/4 |
| | Second | Normal bloom | 4/4 |
| | Third | Predominantly vegetative | 3/4 |

Due to the categorical nature of the variable, the most commonly observed rating (mode) is reported. Data collected at Agricultural Experimental Station, UC, Riverside.

### 4.3.2 Patterns of dormant (overwintering) bud release

Patterns linked to bud release into growth at the beginning of the season (bud outgrowth or bud break) and the vegetative or reproductive fate of the growth within them changed depending on the fruiting status of the shoot being observed. These patterns were not only observed in the apical buds of every shoot but also along the shoots axis with the axillary buds also behaving differently.

### *4.3.2.1 Release of the shoot apical bud and shoot survival*

At the beginning of the growth season, the majority of the apical buds located at the shoot tip underwent natural bud break and then grew into their vegetative or reproductive fate (Table 4-5). In all non-fruiting shoots (n=265), 100% of the apical buds grew out during the spring bloom while 9.01% (n=222) of fruiting shoots displayed apical buds that remained dormant. Only half of the latter shoots survived and later in the season progressed to exclusively vegetative apical growth while the other half eventually decayed.

#### 4.3.2.1.1 Vegetative or reproductive fate of apical buds

As shown in Table 4-5, a striking and statistically significantly difference between fruiting and non-fruiting shoots (n=487; P=0.000) is that the fate of the shoot apical meristem (apical bud) is to most likely be completely vegetative (60.81%) in the former and reproductive ("flowering") (63.77%) in the latter. Furthermore, if their proportions within their respective groups are compared, they are statistically equivalent but with opposite fates. Conversely, but second in numerical weight, the proportions of apical buds being of reproductive fate on fruiting shoots and those having the default vegetative fate in non-fruiting shoots is also statistically similar.

About half of the apical vegetative growth on fruiting shoots senesced through the season (50.37%) whereas on non-fruiting shoots, senescence only occurred 4.17% of the time. Interestingly, the percentage of reproductive (mainly indeterminate flowering shoots) growth lost through the season, which was 17.91% for fruiting and 13.02% for non-fruiting shoots, is statistically similar.

**Table 4-5. Fate of the growth from apical buds released at early season and its survival until the end of the season.**

| Shoot fruiting status | Apical bud growth type | Comparison of proportions | Shoot loss percentage |
|---|---|---|---|
| Fruiting shoot (n=222) | Vegetative growth | 60.81% a | 50.37% a |
| | Reproductive growth | 30.18% b | 17.91% b |
| | Dormant bud | 9.01% c | 50% a |
| Non-fruiting shoot (n=265) | Vegetative growth | 36.23% b | 4.17% c |
| | Reproductive growth | 63.77% a | 13.02% b |
| | Dormant bud | 0% d | Not applicable |

Proportions that do not share a letter are significantly different ($P<0.05$). Grouping is valid within columns. P=0.502 and P=0.157 for the comparison between fruiting/vegetative apical growth frequency *versus* non-fruiting/reproductive apical growth and between fruiting/reproductive apical growth *vs* non-fruiting/vegetative apical growth, respectively. In the "Shoot loss percentage" column, P=0.975 and P=0.361 for the comparison between fruiting/vegetative apical growth loss *vs* loss of shoots with dormant apical bud and between fruiting/reproductive apical growth loss *vs* non-fruiting/reproductive apical growth loss, respectively. Data collected at Agricultural Experimental Station, UC, Riverside.

#### *4.3.2.2 Release of shoot axillary buds*

Although it was already established above that the common observation that when shoot apical buds released into growth, the release of axillary buds into their vegetative or reproductive fate is significantly reduced in fruiting shoots (n=202; P=0.000) to the point that a massive ≈85% of the fruiting shoots did not present even a single axillary bud released into growth at the beginning of the growth season (Table 4-6). In non-fruiting shoots, one or more axillary bud was released into their vegetative or reproductive fate ≈54% of the time. This value is ≈2.5 times more frequent than in the equivalent condition in fruiting shoots.

**Table 4-6. Frequency of axillary buds release at early season.**

| Shoot fruiting status | Axillary bud release events | Percentages |
|---|---|---|
| Fruiting shoots (n=202) | With axillary bud release | 15.35% d |
| | No axillary release | *84.65% a |
| Non-fruiting shoots (n=265) | With axillary bud release | *54.34% b |
| | No axillary release | 45.66% c |

Proportions that do not share a letter are significantly different. Grouping is valid within the percentages column. Asterisk denotes statistically significant difference between the proportion of shoots with and without axillary bud release by their corresponding shoot fruiting status. All P=0.000 except the comparison within the non-fruiting shoots with a P=0.045. Data collected at Agricultural Experimental Station, UC, Riverside.

#### 4.3.2.2.1 Vegetative or reproductive fate of axillary buds

Keeping in mind that, according to the data in Table 4-6, the release of axillary buds was much more frequent in non-fruiting that in fruiting shoots, it was desired to capture and uncover differences in the frequencies of vegetative and reproductive growth released from axillary buds within these fruiting and non-fruiting shoots. The comparison of those proportions is presented in Table 4-7. Similar to the patterns observed on the apical bud outgrowth, the axillary growth released on fruiting shoots was more likely to be of vegetative fate than reproductive fate whereas the reciprocal proportions were observed in non-fruiting shoots.

Also, similar to the observations on apical bud behavior, the proportion of axillary vegetative growth on fruiting shoots was statistically similar to that of axillary reproductive growth in non-fruiting shoots. Further, the frequency of axillary buds having a reproductive fate on fruiting shoots and those having the default vegetative fate on non-fruiting shoots was also statistically similar, ultimately achieving a replication of the statistical significance patterns of the proportions.

**Table 4-7. Fate of the growth from axillary buds released at early season and its survival until the end of the season.**

| Axillary buds release by shoot fruiting status | Axillary bud growth type | Comparison of proportions | Percentage of axillary growth loss |
|---|---|---|---|
| From fruiting shoots (n=92) | Vegetative growth | *58.70% a | 55.56% a |
| | Reproductive growth | 41.30% b | 57.89% a |
| From non-fruiting shoot (n=574) | Vegetative growth | 45.82% b | 48.29%a |
| | Reproductive growth | *54.18% a | 45.66% a |

Proportions that do not share a letter are significantly different (P<0.05). Grouping is valid within columns. Asterisk denotes statistically significant differences between the proportion of vegetative and reproductive axillary growth within their corresponding shoot fruiting status. Data collected at Agricultural Experimental Station, UC, Riverside.

Despite the previous similarities, a notable difference with the growth developing from apical buds was in relation to the loss of the tissues at the end of a season. The percentage of axillary growth lost was statistically equal in proportion, independent of the axillary outgrowth occurring on fruiting or non-fruiting shoots or themselves being of vegetative or reproductive growth fate. Approximately 52% of the axillary growth died by the end of the season while a similar tissue loss was only attained by the apical vegetative growth developed on fruiting shoots (Table 4-5).

#### *4.3.2.3 Positional information on axillary bud release*

Above, the effect of fruit presence on the frequency of axillary bud release into their mutually exclusive vegetative or reproductive growth was presented. Nonetheless, this information does not convey a potentially important phenotypical aspect of these events: their physical location along the avocado shoots. Firstly and with the intention to give context, Table 4-8 presents the frequencies of the combinations used to build Figure 4-2 as it is important to understand that the selected combinations of fruiting status and shoot flushes are not equally distributed in an avocado field setting. In general, the two most common while at the same time divergent shoot phenotypes were fruiting shoots with only 1 vegetative flush at the end of the season and non-fruiting shoots with 3 flushes at the end of the season.

More specifically in relation to the outgrowth of axillary buds, the 1 flush/fruiting shoot cluster most commonly did not have any axillary buds released into growth (68%). For the 2-flush cluster, the most common phenotype was non-fruiting with no release of axillary growth (41%), followed by a 33% proportion that did display axillary bud release. Finally, the majority of the 3-flush cluster were non-fruiting and underwent release of axillary buds at the beginning of the new succeeding season.

In addition, a comparison of the proportions of all possible combinations weighed against each other indicate that the most likely phenotype to encounter in a mature tree canopy (30%) is a fruiting shoot, with a single vegetative flush that does not release any axillary buds into growth. This phenotype is followed (16%) by a completely divergent one that is non-fruiting shoots with 3 flushes and with axillary bud release a year later.

**Table 4-8. Counts of shoots according to axillary bud release events, fruit presence status and number of vegetative flushes.**

| **Shoot arranged by number of vegetative flushes** | **Shoot fruiting status** | **Axillary bud release status for each shoot** (Yes=1 or more released along the shoot; No=none released) | **Percentage within number of flushes** | **Percentage of grand total** |
|---|---|---|---|---|
| 1 Flush (n=206) | Fruiting (n=152) | Yes (n=12) | 6% c | 3% e |
| | | No (n=140) | 68% a | 30% a |
| | Non-fruiting (n=54) | Yes (n=14) | 7% c | 3% de |
| | | No (n=40) | 19% b | 9% cd |
| 2 Flushes (n=120) | Fruiting (n=32) | Yes (n=6) | 5% c | 1% e |
| | | No (n=26) | 22% ab | 6% d |
| | Non-fruiting (n=88) | Yes (n=39) | 32% ab | 8% cd |
| | | No (n=49) | 41% a | 11% c |
| 3 Flushes (n=136) | Fruiting (n=16) | Yes (n=9) | 7% c | 2% e |
| | | No (n=7) | 5% c | 2% e |
| | Non-fruiting (n=120) | Yes (n=76) | 56% a | 16% b |
| | | No (n=44) | 32% b | 10% c |

In the "Percentage within number of flushes" column: the embedded green, red and blue proportional bar charts (and their percentages) relate to shoots with 1, 2 or 3 flushes respectively. On the same grouping, proportions that do not share a letter are significantly different ($P<0.05$). In the "Percentage of grand total" column, yellow proportional bars visually display the frequencies across all factors combinations. The longest bar represents the highest frequency (30%) and the others are proportionally shorter. Proportions that do not share a letter are significantly different ($P<0.05$) within this column and across all combinations. Data collected at Agricultural Experimental Station, UC, Riverside.

Using the axillary nodes as a way to define a physical location along the main shoots, Figure 4-2 depicts axillary buds being naturally released into their either vegetative or reproductive growth. At the same time, this figure separates the information into a combination of the shoot fruiting status (fruiting or non-fruiting) together with the amount of vegetative flushes they developed. The fruit presence relates to already mature fruit that developed during the previous season but the axillary bud release events occur at the beginning of a new growth season from dormant axillary buds that are approximately 1 year old.

There is a general pattern of axillary buds being released in a basipetal gradient fashion with the ones closer to what became the shoot apex (at the end of the previous season) more likely to undergo bud outgrowth at the beginning of the new season (Figure 4-2). Nonetheless, it is not exclusively basipetal in reference to the shoot apex, but also at each time there was a reduction in the growth rate of the whole shoot. Using a practical language, after a year in the history of a shoot, there is an increased chance of axillary bud release “right below” the apex but also below transition zones which, at some phenological stage, were also (transiently) the shoot tip.

Another pattern within the previous one is that the development of axillary buds with reproductive fate is more likely in the non-fruiting shoots than in the comparable fruiting shoots. Having stated the previous, a common observation in non-fruiting shoots with 3 flushes was that they more frequently had axillary buds with a completely vegetative fate near the shoot tip.

However, one has to keep in mind that for the sake of having enough resolution to see these bud release patterns, Figure 4-2 was exclusively built from data of shoots that in fact displayed axillary bud release. Therefore this does not suggest that buds with reproductive fate are unlikely in a non-fruiting shoot with 3 flushes. In the avocado fields, the latter combination is the most common on shoots with axillary bud outgrowth (second in frequency overall), hence the importance of Table 4-8 for the interpretation of what is presented in Figure 4-2.

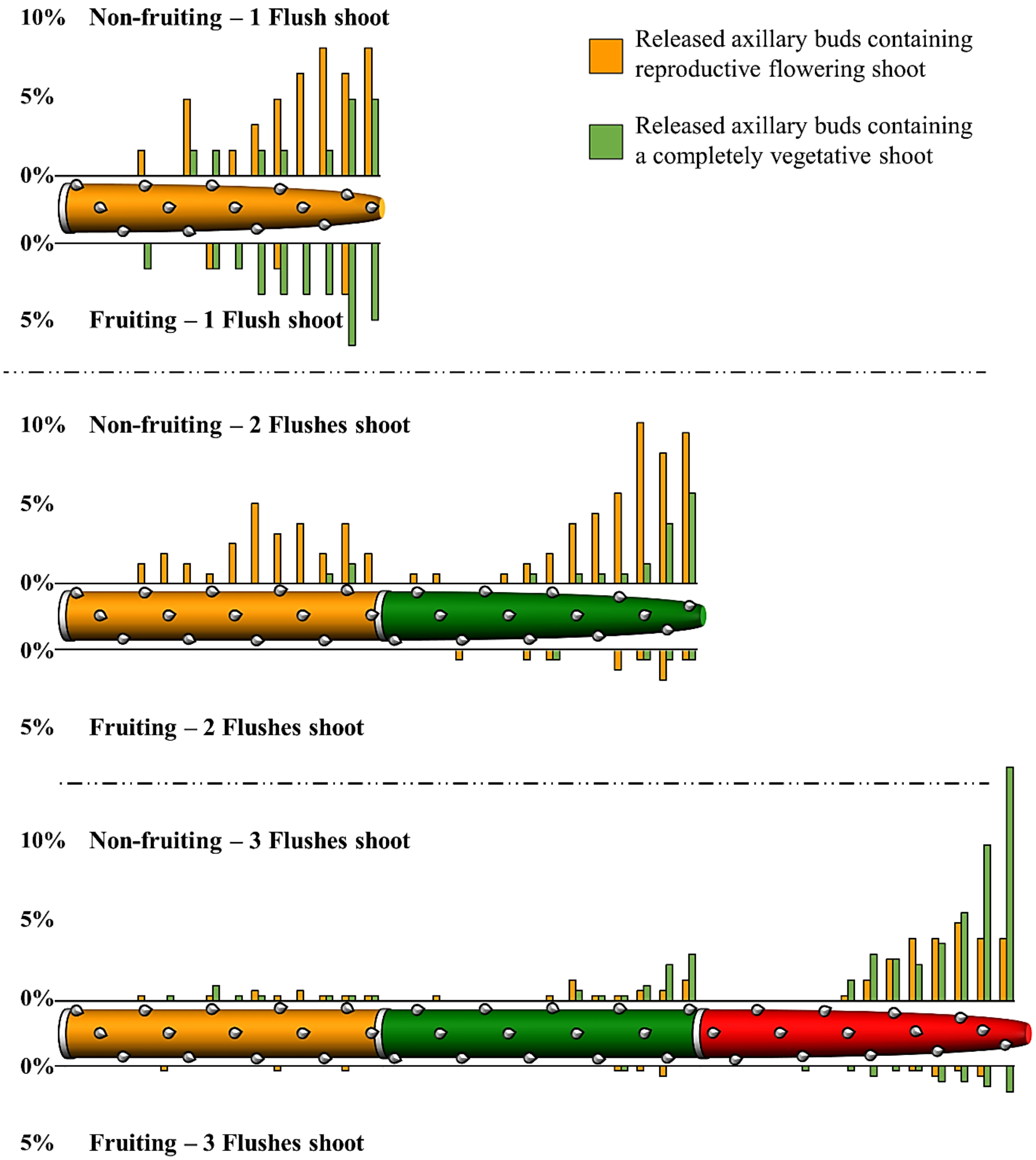

**Figure 4-2. On 'Hass' shoots that displayed natural axillary bud outgrowth: compilation of observed bud fate and frequency by location along the main shoot.**

The three sections presented in the figure are a classification of the shoots by their amount of vegetative flushes developed the previous season. The proportions of released axillary buds add up to 100% when the fruiting and non-fruiting shoot frequencies are added together within the upper, middle or lower panels. White teardrop shape volumes represent single axillary buds. White bands represents transition zones between consecutive vegetative flushes. Please consider that the shoot diagram is not at scale and does not intend to represent length of the different vegetative flushes nor the actual number or distance between axillary nodes. Data collected at Agricultural Experimental Station, UC, Riverside.

### 4.3.3 Flowering shoot complexity

Flowering shoots can grow from the shoot apex and/or develop laterally from axillary buds. As shown in Figure 4-3, the complexity of indeterminate flowering shoots located at the shoot apex is higher than the ones that developed laterally. This difference is statistically significant (n=576; P=0.000) for the “caged” tree group which had a complete season without any fruits and were observed the succeeding season. In this “caged” group, the apical flowering shoot displayed an average of 5.6 inflorescences while the laterally grown had 3.7 inflorescences per flowering shoot. Interestingly, for the “open pollinated” group, there was no statistically significant difference between the flowering complexity of the indeterminate flowering shoots at the apical position (2.9 inflorescences per flowering shoot) and the ones located in axillary positions (2.6 inflorescences per flowering shoots).

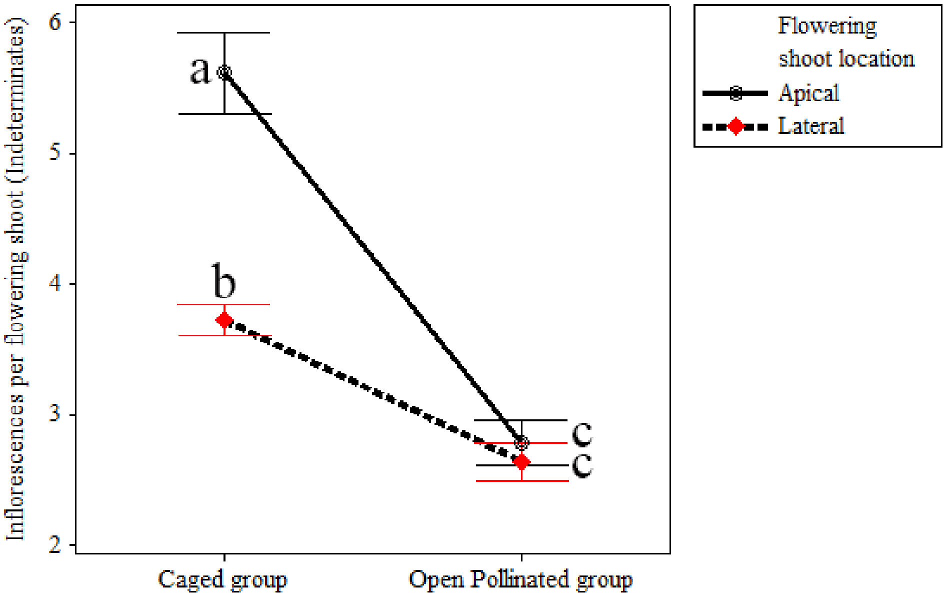

**Figure 4-3. Flowering complexity comparison of indeterminate flowering shoots by group and location along the avocado shoots**

Values that do not share a letter are significantly different (n=576; P=0.000). Data collected at Agricultural Experimental Station, UC, Riverside.

#### *4.3.3.1 Positional information of flowering shoot complexity*

Above, it was mentioned that the apical flowering shoots were more complex than the ones developed in axillary positions when fruits were absent. Furthermore, Figure 4-4 depicts another pattern of flowering complexity that relates to the more specific location of the axillary flowering shoots along the main axis. This figure was built using the information of fruiting and non-fruiting shoots that developed 2 flushes until the end of the season (n=152). There was a reduction in the indeterminate flowering shoot complexity following a basipetal gradient. For the non-fruiting shoots, that reduction was more gradual while for their fruiting counterparts, it was much sharper. It is also possible to observe the increased complexity “below” the transition zone in the area that once was the shoot tip at the end of the first vegetative flush.

### 4.3.4 Fruiting dynamics at the single shoot level

As reported above, fruit presence from the previous season perturbs the observable basipetal gradients of axillary bud outgrowth (release) and of the flowering complexity within those axillary buds along the several months old avocado shoots. Together these combinations represent the potential of a future fruit set and growth that could be fulfilled over the starting new season. Aspects of actual mature fruit presence and location along older shoots is reported below

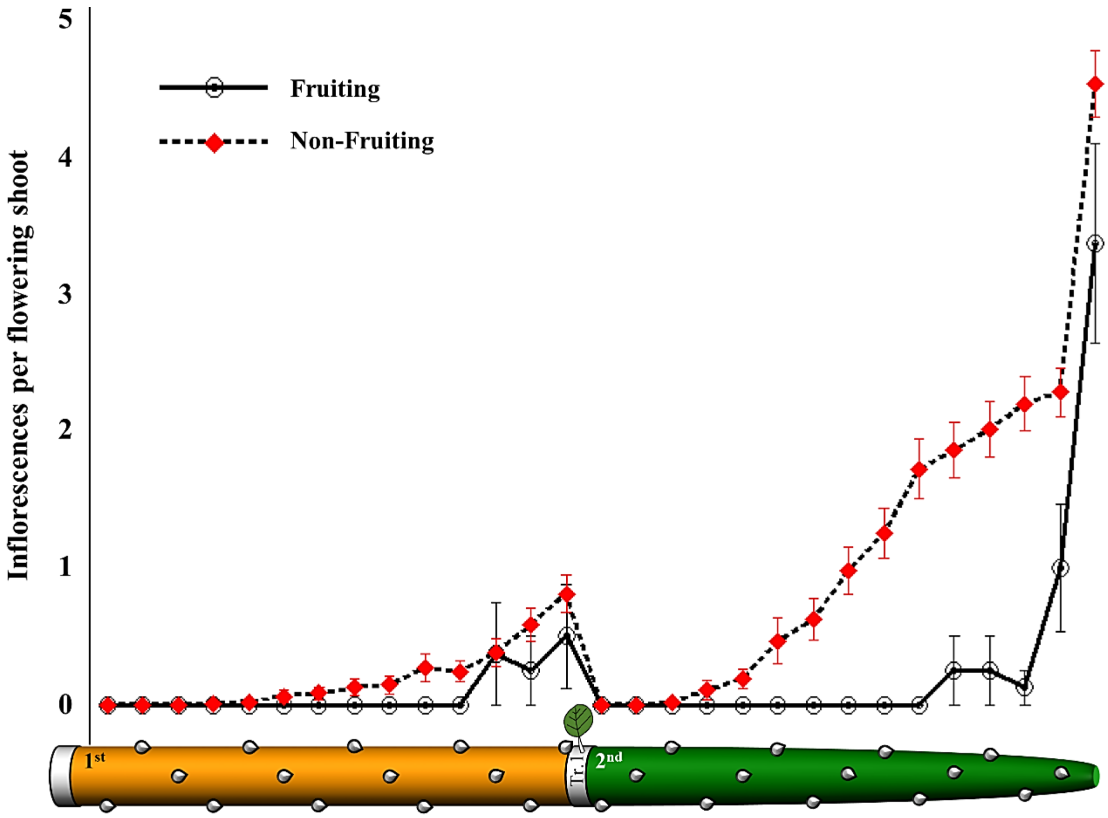

**Figure 4-4. Flowering complexity of indeterminate flowering shoots by fruit presence and location along the avocado shoots.**

Bars indicate one standard error from the mean values (n=152 shoots). Data collected at Agricultural Experimental Station, UC, Riverside.

#### *4.3.4.1 Successful mature fruit development is more frequent in shoot apices*

In general, 66.2% of the fruits that fully developed and matured until harvest were located apically at the previous year shoot tips while significantly less (33.8%; n=2204; P=0.000) developed in lateral flowering shoots within axillary buds. More specifically, the “caged” and “open pollinated” groups displayed this pattern with 60.5% (n=1155; P=0.000) and 72.5% (n=1049; P=0.000) of the actually fruiting shoots having apical positions, respectively. Nonetheless, fruiting shoots that successfully grew mature fruits in axillary positions is not a negligible amount and is part of the avocado shoot reproductive dynamics.

#### *4.3.4.2 Fruit presence in determinate or indeterminate flowering shoots*

The series of events that need to come together in order to allow an avocado fruit to reach harvest maturity are many (bloom intensity, pollination, bee behavior and fruit drops, among others) and, in practice, successful fruiting is a rare event. Because of this, the proportions of the type of flowering shoot (determinate or indeterminate) that transitioned (respectively) to determinate or indeterminate fruiting shoots (when they were successful in developing a mature fruit), could be considered a proxy to the actual abundance of both types of flowering shoots within the canopies. The distribution of mature fruits as they developed on either determinate or indeterminate fruiting shoots yielded the ratios presented in Table 4-9.

**Table 4-9. By tree group, proportions of mature fruits that successfully developed from determinate or indeterminate flowering shoots.**

| Tree group | Fruiting shoot type | Comparisons of proportions |
|---|---|---|
| "Caged" trees (n=1327) | Determinate | *85.38% a |
| | Indeterminate | 14.62% d |
| "Open pollinated" (n=1190) | Determinate | *63.78% b |
| | Indeterminate | 36.22% c |

Asterisks indicate statistical difference of the proportions within the tree groups. Proportions that do not share a letter are significantly different across tree groups. All P=0.000. Data collected at Agricultural Experimental Station, UC, Riverside.

Overall and in the year of this evaluation, both tree groups displayed their mature fruits as growing more frequently on determinate than indeterminate fruiting shoots. Nonetheless and across all combinations, there was a significantly distinct frequency of mature fruits being developed in higher numbers on determinate fruiting shoots within the "caged" tree group. This is the tree group that bloomed and set these fruits after going through the previous season without any fruit bearing due to the "caging" treatment.

#### 4.3.4.2.1 Exposure to sunlight

Table 4-10 presents a combination of tree group and exposure to sunlight in relation to the proportions of mature fruit that developed from either determinate or indeterminate flowering shoots. By simply focusing on the general "n numbers", it is clear that in both the "caged" and "open pollinated" groups, mature fruit were mostly located in the inner canopy, under more shaded conditions. Along with the previous, and in all the possible combinations, the most abundant fruiting shoots were determinate (compared to indeterminate). This statistically significant pattern was consistent and independent of the fruit being harvested from trees that belonged to the "caged" or the "open pollinated" groups.

When compared directly, all the proportions in Table 4-10 are significantly different from one another. We already established that the combinations leading to determinate fruiting shoots are dominant but, interestingly, if the focus is set to the indeterminate fruiting shoots combinations, one can see that within both the "caged" and the "open pollinated" groups, it was more frequent to encounter indeterminate fruiting shoots in shaded areas of the inner canopy of the trees when compared to the sunlit periphery.

**Table 4-10. Proportions of mature fruits as they successfully developed from determinate or indeterminate flowering shoots. Data clustered by tree group and sunlight exposure.**

| Tree group | Canopy sunlight exposure | Fruiting shoot type | Proportions |
|---|---|---|---|
| "Caged" trees (n=1327) | Inner canopy (Shaded) (n=1034) | Determinate | *84.24% b |
| | | Indeterminate | 15.76% g |
| | Periphery (Sunlit) (n=293) | Determinate | *89.42% a |
| | | Indeterminate | 10.58% h |
| "Open pollinated" (n=1190) | Inner canopy (Shaded) (n=860) | Determinate | *60.12% d |
| | | Indeterminate | 39.88% e |
| | Periphery (Sunlit) (n=330) | Determinate | *73.33% c |
| | | Indeterminate | 26.67% f |

Asterisks indicate statistical difference of the proportions within the combinations of tree group/sunlight exposure. Proportions that do not share a letter are significantly different across tree groups. All P=0.000. Data collected at Agricultural Experimental Station, UC, Riverside.

### 4.3.5 "Flora Loca" observations

The "flora loca" concept (from the Spanish: "crazy flower") represents any bloom event that does not occur during the main (most intense and/or longer) bloom. It is an aspect of the avocado reproductive growth that remains unexplained and overlooked. If understood it could be an agriculturally manageable phenotype with the potential of being a commercially important bloom behavior, depending on the orchard environmental conditions. Our field survey shows that shoots that belong to the "spiral" class (concept introduced in Chapter 2) are the ones that, in a statistically significant manner, more frequently display these bloom events (see Figure 4-5).

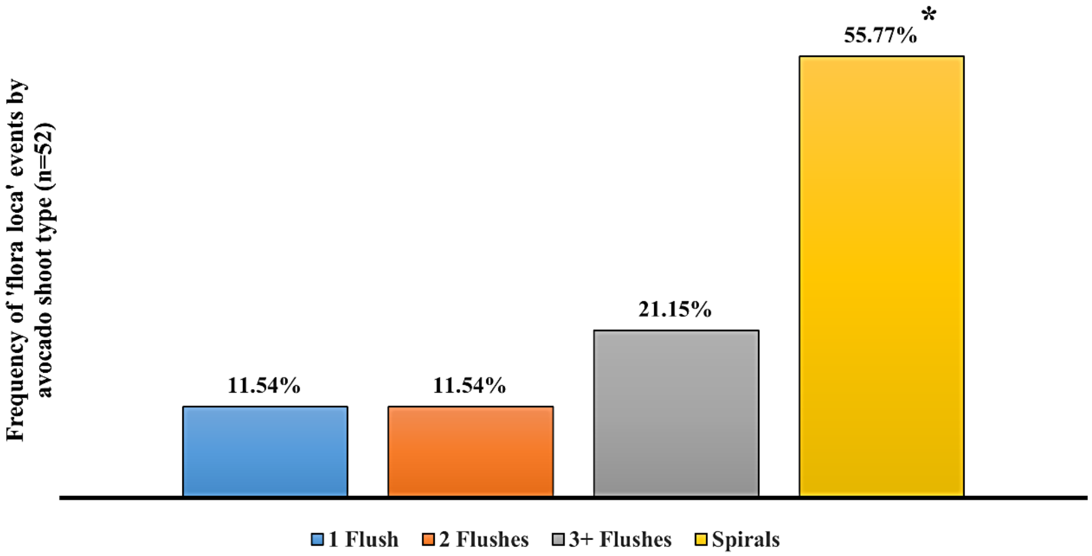

**Figure 4-5. Frequency of "flora loca" observations by shoot types in 'Hass' avocados.** Asterisk indicates statistical difference of the proportions of the spiral shoots over the frequencies of "flora loca" observations on shoots of the overwintering class (whose proportions are not significantly different from each other). Data collected at Agricultural Experimental Station, UC, Riverside.

Figure 4-6 shows an example of the "flora loca" bloom as it occurred on a single "spiral" class on an avocado tree. It is not one of the research 'Hass' trees but an ungrafted 'Duke 7' rootstock tree adjacent to them. This was preferred given that for creating this visual follow up, a full defoliation of the spiral shoot was necessary. It is possible to observe the "spiral" highly branched vegetative growth that leads to an abundance of reproductive growth along its vegetative scaffold.

At our research site and under those environmental conditions, the earliest moment these "flora loca" blooms were observed occurred on November 16th, hence during mid to late fall. To convey perspective, this implies that the vegetative scaffold itself along with reproductive growth processes like induction, differentiation, growth and development of buds with that reproductive fate and then apical and/or axillary bud release (outgrowth) had all occurred by then within vegetative growth that developed during the past spring and was approximately 6 months old.

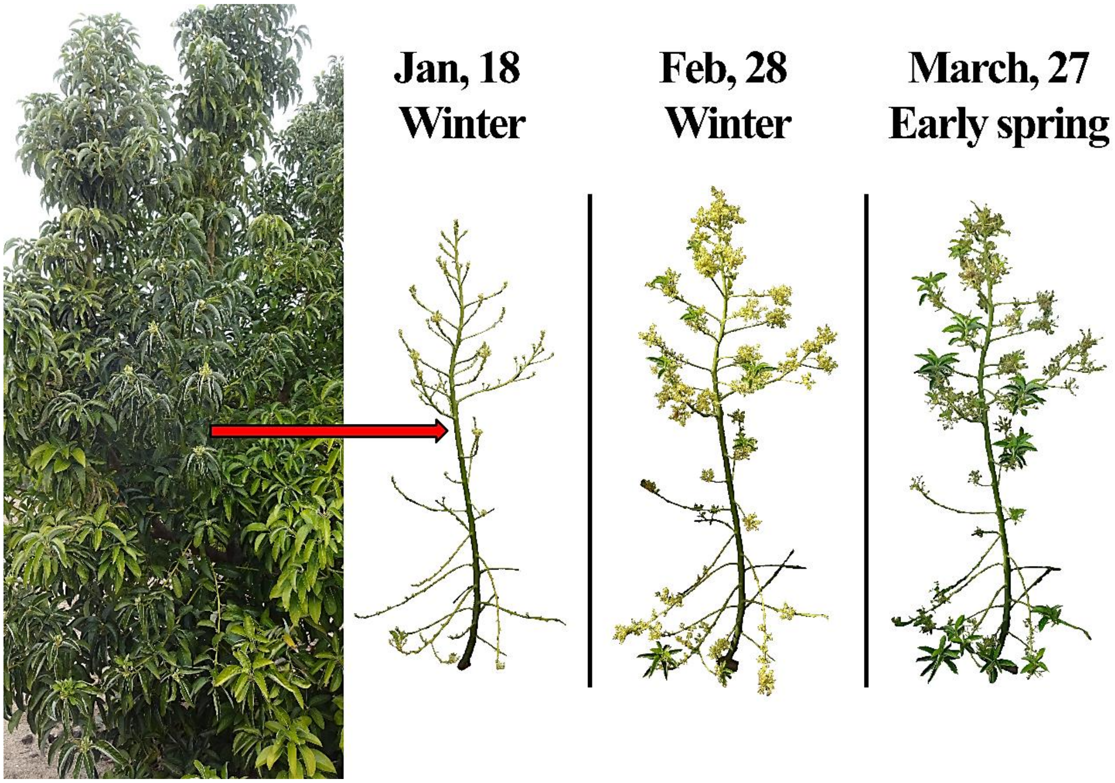

**Figure 4-6. "Spiral" class shoot displaying "flora loca" bloom through time.** This large and laterally branched vegetative structure grew at the canopy periphery from a single resting bud that underwent bud break the previous spring. It displays the importance of branching in the reproductive potential. It is possible to notice that there is a gradient of bud release with the shoot tips outgrowing first and then axillary buds follow. As displayed at the early spring panel (on the right), this complex growth already had several fruitlets set and most of the other not pollinized flowers shed, while other buds within the same canopy were just initiating their new growth of the season. Avocado variety in this picture is 'Duke 7'. Data collected at Agricultural Experimental Station, UC, Riverside.

## 4.4 Discussion

It can be difficult to capture the series of circumstances that lead to the reproductive behavior of avocado trees. Furthermore, reproductive growth itself is a gradient that goes from flower induction within buds to their release as buds with reproductive fate (determinate or indeterminate flowering shoots), later on to successful fruit set and fruit development (determinate or indeterminate fruiting shoots) and finally to those fruits themselves affecting the development of the future reproductive potential of the following season. Similar to the analysis of fruit presence effects on the avocado vegetative growth (Chapter 3), whole canopy measurements of the effects of fruit presence over the reproductive growth present a caveat: they attempt to explain smaller events with overarching measurements. For example, the evaluation of the canopy bloom intensity is both practical and informative on breeding programs as a tool to capture reproductive precocity and lengths of the bloom period of new and established avocado varieties but when the same approach was used in our “caged” and “open pollinated” groups as a way to connect overall fruit presence and overall bloom intensity, the outcomes were also general and in that sense did not contribute to a more in depth phenotypical and mechanistic understanding of those interactions. In this study, focusing the research to the single shoot level proved appropriate to find significant connections between the growing fruit and the perturbations to the reproductive growth of those shoots that are bearing them. Three aspects became evident at this level: bloom reproductive potential, mature fruit presence/location and, in a self-standing manner, “flora loca” bloom.

### 4.4.1 Bloom reproductive potential

This concept should be understood as the phenotypic outcome of several nested events that lead to the definite amount of inflorescences presented to the environment and the avocado pollinators at the beginning of a growth season. If one thinks of a single indeterminate flowering shoot that successfully became a fruiting shoot, it will display a reduced reproductive potential at the new season as compared to a non-fruiting counterpart due to the following:

- (Apical bud) There will be a ≈9% chance that the apical dormant bud will remain dormant for the whole new season;
- (Apical bud) If released, there is a ≈61% chance that the apical bud is of the default vegetative fate instead of blossoming reproductively (as non-fruiting ones would do with a ≈64% chance);
- (Apical growth) The chances for that new apical growth to be lost (dead) are significantly higher (≈50%) than the non-fruiting counterpart (≈4%);
- (Axillary buds) Independently of their vegetative or reproductive fate, there are significantly higher chances (≈85%) for all axillary buds along the fruiting shoot to <u>not be released</u> into growth, remaining mainly unreleased for the rest of the season;
- (Axillary growth) If the axillary buds previously mentioned are actually released into growth, there is again a higher chance (≈59%) for them to be of the default vegetative fate instead of blossoming reproductively (as non-fruiting ones would do with a ≈54% chance);
- (Basipetal axillary release gradient) The noticeable basipetal gradient of bud outgrowth (Figure 4-2) will remain in the fruiting shoot but because bud outgrowth is an unlikely event in this case (only ≈15% occurrence), that shoot would display only few growth events (mostly vegetative) near the shoot tip and not abundantly through the whole shoot axis;
- (Basipetal flowering complexity gradient) As above, the flowering complexity gradient (number of inflorescences per flowering shoot) is higher at and below the shoot tip than towards the base of the vegetative flush, but given that we are thinking of a fruiting shoot, the unlikely phenotypic outcome would be that the apical flowering shoot develops only 1 to 3 inflorescences with the whole length of the shoot itself being quite dormant and simple.

All the points above lead a fruiting shoot to be a poor contributor to the succeeding season bloom potential.

### 4.4.2 Fruit presence/location until harvest

As part of the fruiting dynamics of the avocado shoots, we learned that fruit which grows and develops until harvest is more frequently found (≈66%) at the apical position of the 1 year old shoot. This natural bias might be due to more ample opportunities of pollinator visitations that apical buds have by being both the first ones to be released into growth (basipetal axillary release gradient) while also having higher flowering complexity (flowering complexity gradient). Despite the previous, the amount of mature fruit that develops laterally along 1 year old shoots is still ≈34% of the total harvest.

#### *4.4.2.1 Determinate and indeterminate fruiting shoots*

Some interesting patterns also became apparent while separating successfully fruiting shoots into determinate (completely reproductive structure) or indeterminate (grown from buds with shoot apical meristems (SAM) that remained undifferentiated producing a vegetative shoot).

##### 4.4.2.1.1 More determinate reproductive structures when fruits are absent

There was a statistically significant and numerically large presence of determinate flowering/fruiting shoots in the “caged” group of trees when fruit set was prevented the previous season (Table 4-9). This implies that avocados are not only capable of producing determinate flowering structures but, at the same time, it suggests that the cascade of molecular events involved in the development of flowers and their scaffolds (inflorescences) occur abundantly when fruits are absent.

#### 4.4.2.1.2 More indeterminate flowering shoots at the inner canopy (shaded areas)

Although observed in both the "open pollinated" and "caged" groups, it was more obvious in the latter that indeterminate fruiting shoots were mostly located in more "persistently shaded" inner canopy areas, while determinate flowering shoots were mainly found on the canopy periphery.

#### 4.4.2.1.3 Presence and outgrowth of buds with reproductive fate is "pushed acropetally"

The non-fruiting shoot's basipetal gradient of axillary bud outgrowth (bud break) and flowering complexity seems to be "pushed acropetally" on fruiting shoots, making these events only visible at and/or near the shoot apex instead of spread throughout the length of the shoots (Figure 4-2, Figure 4-3 and Figure 4-4). There seems to be a necessary distance from a growing fruit to actually develop axillary buds with reproductive fate and for them to have higher flowering complexity. A similar observation was interpreted in the reverse sense by Ziv et al.[77]: that the "rate of flowering" decreased and that the percentage of dormant buds increased with increasing distance from the shoot apical meristem. This was described as a "classical effect of apical dominance"[77].

Why would a whole canopy and/or a single shoot system that is currently growing avocado fruits need to reduce its own future reproductive potential for the succeeding season, which is several months away? Current and future fruit development is, of course, a necessary event in the life cycle of the avocado trees and assigning to fruits an active and purposeful detrimental role to the tree own reproduction might not be the simplest answer. What unifies all reproductive growth[15, 21] but especially growing fruits[25, 40, 72, 73] together is that they are autotrophic sinks organs with a strong ability to demand the photosynthates translocated in the phloem bulk flow. It is plausible then that our previous field observations are predominantly related to nutritional imbalances and their unintended molecular consequences. (see section 4.4.4 for more details)

### 4.4.3 “Flora loca” bloom

The “flora loca” bloom is a feature of avocado trees that tends to be seen as undesirable as it creates the potential for more than one cohort of developing fruits which can complicate the future labor of harvesting the orchards. Nonetheless, it is not truly a managed aspect of avocado cultivation and if possible it is just preferred to acquire avocado varieties that have a naturally reduced tendency (or intensity) to develop this fall or winter “crazy” bloom. This pragmatic approach had left us without research driven to understand how and/or under which environmental conditions this early bloom comes to occur.

Our research indicates that, at the single shoot level, “flora loca” blooms on ‘Hass’ were biased towards shoots that grew vigorously through the spring and summer (Figure 4-5). The maximum expression of vigorous growth are the shoots that belong to the “spiral” class and correspondingly the observation of “flora loca” was significantly higher in this class than in shoots of the “overwintering” class. Another aspect is that, under the experimental conditions, “flora loca” occurred on shoots that initiated their growth in the early spring and displayed abundant “flora loca” bloom by mid-fall, hence was not exposed to the conditions of a true winter period.

This observation does not readily fit previous models that describe avocados as having an absolute need of a period of “low” air temperatures to occur in order to promote reproductive growth otherwise it would be severely inhibited [7, 46, 58, 59, 61]. It has been stated based on a field study of ‘Hass’ avocados in Mexico, that the accumulation of an equivalent of 27.5 days at temperatures ≤ 19° Celsius (counted from the beginning of the vegetative growth of interest) results in the flowering of apical buds [58, 59] and, furthermore, that this amount of “chilling” is equivalent to 4 weeks of continuous exposure of ‘Hass’ trees to 10 °C/7 °C day/night (with a 10 hours photoperiod) while being studied in growth chambers[46, 58, 61].

Utilizing air temperature data from our research site, a simple count of hours ≤ 19° C (registered either day or night) indicate that in the succession of spring, summer and fall that end up with the followed shoots displaying “flora loca” bloom, 88.5 “chilling days” were accumulated. At the same time, a total of 138.8 days that were “not chilling days” also occurred during the same period.

It would be of no purpose to accumulate “chilling days” right at the beginning of the vegetative growth because the apical and axillary buds that actually attained reproductive fate and displayed the “flora loca” inflorescences were non-existent at that point in time and only developed at different points through the spring and summer. Still, if calculated, under our conditions, 74% (n=231; P=0.000) of the days that led to the observation of “flora loca” were “non-promoting” of flowering. Broken down into the conditions per astronomical season, Table 4-11 presents the proportions of days as they have been linked to be “promoting” or “non-promoting” of flowering.

**Table 4-11. Classification of days leading to “flora loca” reproductive growth.**

| **Astronomical season** | **Classification of days defined as “promoting” or “non-promoting” of flowering[7]** | **Percentage** |
|---|---|---|
| Spring (n=87) | Promoting | 46% a |
| | Non-promoting | 54% a |
| Summer (n=92) | Promoting | 0% |
| | Non-promoting | 100% |
| Fall (n=52) | Promoting | 40% b |
| | Non-promoting | 60% a |

Classification of days based on Buttrose and Alexander (1978)[7]. Proportions that do not share a letter are significantly different within the astronomical season. P=0.287 for spring cluster and P=0.046 for fall cluster. Data collected at Agricultural Experimental Station, UC, Riverside.

An abundance of flowering “promoting days” occurred during the spring but were no significantly different from the “non-promoting” days. The summer was completely “non-promoting” and the fall period also had significantly more days that were “non-promoting” to avocado reproductive growth. Nonetheless, at the end of this period, ‘Hass’ “flora loca” bloom was observed in our research field.

Figure 4-7 is intended to convey another aspect of cold accumulation: under natural conditions, days with average air temperatures that are "flower promoting" occur interrupted by "non-promoting" days. This suggests that the 'Hass' avocado is more plastic than previously envisioned and can quickly[77] respond to conditions which promote reproductive events.

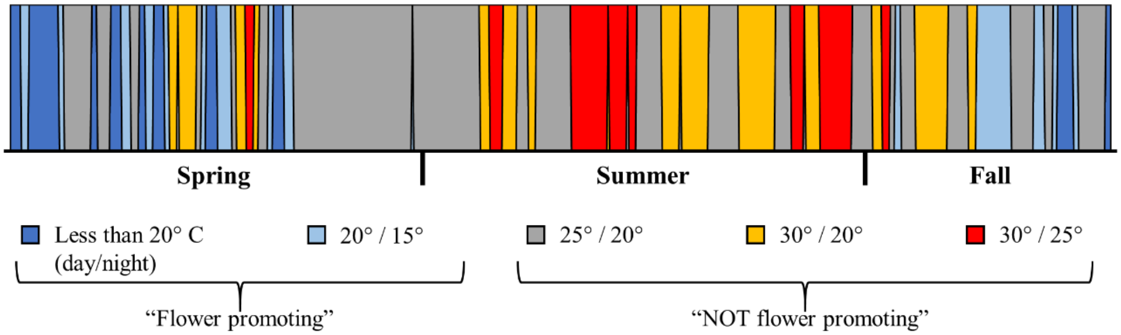

**Figure 4-7. Daily classification of air temperature day/night ranges previous to the observation of "flora loca" bloom.**

Day/night temperature ranges and their "flowering promotive" influence is according to the results of Buttrose and Alexander (1978)[7] and cited by others[46, 57-61]. Data collected at Agricultural Experimental Station, UC, Riverside.

The previous also call for an improvement of avocado phenological modeling. Similar approaches that track the accumulation of chilling hours needed for the break of bud dormancy in temperate fruit and nut species evolved from simply adding hours within a threshold to consider the negation of chilling accumulation by warmer temperatures to now embrace continuously adjusting dynamic models[39].

### 4.4.4 Potential consequences of strongly demanding the phloem bulk flow content

Leaving the "flora loca" analysis aside for the moment, the divergence of the observable reproductive phenotypes of fruiting *versus* non-fruiting avocado shoots could be potentially explained by research focused to two questions: where is the FLOWERING LOCUS T (FT) protein located and how does a growing fruit perturbing FT localization? Current models explain that the phloem mobile FT[12] protein travels to and reaches the apical meristems where it interact with the transcription factor FLOWERING LOCUS D (FD) to form the FT-FD complex[1, 34, 63, 70] (and potentially a ternary complex with the 14-3-3 protein[1, 25, 42, 52]), triggering the expression of floral identity genes[37] that promote reproductive fate within buds.

#### *4.4.4.1 More reproductive complexity when fruits are absent*

Non-fruiting shoots tend to display higher flowering shoot complexity with the maximum level being the "determinate flowering shoot" (buds on which even the shoot apical meristem (SAM) of the inflorescence meristem (IM) completely differentiated into reproductive growth[42, 68]). On the other hand, fruiting shoots, if they develop buds with reproductive fate, are more likely to contain less complex "indeterminate flowering shoots" (which retain their SAM undifferentiated[42, 68]).

The previous bias could be a simple consequence of reproductive growth[15, 21] and especially growing fruits[25, 40, 72, 73] being stronger sinks for the phloem content than a vegetatively growing shoot apex. Consider the following: once the FT-INTERTACTING PROTEIN 1 (FTIP1) (and potentially the FE[1, 52] protein) loads the FT protein from the companion cells of mature leaves into the sieve elements [1, 25, 38, 75], then FT should be transported in the phloem bulk flow stream[42, 52], rendering this protein less available to the comparatively weaker vegetative sinks.

Ultimately, the observed increased presence of complex or determinate reproductive growth in either the tree ("caged" group) or at the more specific non-fruiting shoot level might be an indication of when the strongest fruit sink is absent from the observed system, the second strongest (a growing shoot tip) could have an increased FT protein accumulate at the post-phloem level[66, 75] that subsequently might reach a threshold after which the flower transition repressor *TERMINAL FLOWER 1* (*TFL1*)[24, 37] cannot maintain the SAM undifferentiated as the floral development pathway FT-FD protein dimer[1, 63, 70] is forming more abundantly[75] instead of the flower repressing TFL1-FD[4, 20, 26] complex, even within the SAM of the developing inflorescence meristem (IM).

### *4.4.4.2 Reduced reproductive complexity at inner canopy (shaded areas)*

As presented in Table 4-10 and as observed among the trees of the "caged" group, it was more likely to find indeterminate fruiting shoots (less complex than determinate flowering shoots) in more "persistently shaded" inner canopy areas. The normal ability of shoots to demand the phloem content (carrying FT[12]) could be affected by the shaded environment not allowing them to grow and be sufficiently strong enough sinks or even produce enough photosynthates to thrive which ultimately should bring less FT proteins into their meristematic tissues. As a consequence, fruiting shoots (which are usually shaded as described in Chapter 3), would be less prone to develop buds with reproductive fate and, if so, more prone to develop "indeterminate" than "determinate"[33, 63] flowering shoots.

### *4.4.4.3 Fruit presence and the reproductive fate acropetal "displacement (push)"*

As discussed previously, released buds with reproductive fate and increased reproductive complexity seem to require a certain distance from a growing fruit. (Figure 4-2, Figure 4-3 and Figure 4-4). If one considers that the fruit is a strong sink, any synthesized FT protein moving in the phloem bulk flow would be transported towards and/or into the reproductive organs where it may have either a currently unknown function or none at all, while leaving weaker sinks in its vicinity without a threshold abundance of FT protein to trigger the reproductive process. If these fruiting shoots happen to be exposed to sunlight (naturally unlikely), new vegetative flushes (shoot elongation) can occur, leading to new mature leaves and respective axillary buds at sufficient distance from the growing fruit that newly synthesized FT protein would be again available to the "local" strongest sink in the distal portion of those fruiting shoots (the shoot tip and nearby axillary buds).

## 4.5 Conclusions

Similar to the influence of fruit presence on the vegetative growth of the shoots that bear them, this research focused in finding if the avocado shoot reproductive aspects are perturbed when a fruit successfully sets and grows until harvest maturity. This was especially interesting given that quantitative trait locus (QTL) mapping analyses in apple and olives lean towards the conclusion that their "alternate bearing" phenotypes correlate better to plant hormone related genes and how hormones could regulate flowering related genes instead of a direct connection with the latter[23, 56].

This research indicates that the natural reproductive growth perturbations that fruit presence exerts onto what would have been the phenotype of a non-fruiting shoot are primarily:

- a reduction in the number of apical and axillary buds that develop reproductive fate through the current season;
- a reduction in the number of apical and axillary buds that naturally release into growth (either vegetative or reproductive fate) at the beginning of the succeeding season

Although the naturally outgrown apical and axillary buds already show statistical significance for reduced chances of reproductive fate, a more precise study of axillary bud fate along the length and flushes of different shoots should be attempted with the aid of molecular tools to corroborate where, more precisely, the limits in reference to the fruit location are. Within excised axillary buds, the expression of a potential avocado ortholog of the flower transition repressor *TERMINAL FLOWER 1* (*TFL1*)[24, 37] could be used as denoting vegetative fate and the expression of orthologs of *LEAFY* (*LFY*) or *APETALA1* (*AP1*) as transcription factors involved in specifying floral meristem identity[63, 77] could be indicators of a developing reproductive fate within buds. Nonetheless, a comparable approach already used and published by Ziv et al.[77] in Israel concluded that for bud fate map studies, it is best to use *LFY* and not *AP1* as the latter was equally expressed in buds and leaves of trees with or without fruits while *LFY* had significantly higher relative expression levels in buds than leaves and also was significantly different when trees with and without fruits were contrasted[77].

Our hypothesis of why QTL analysis does not directly correlate fruiting trees phenotypes to flowering genes expression could be that it is not necessary to disrupt the cascade of molecular events that lead to reproductive fate directly, but indirectly by reducing the chance of it from initiation at all. Presence of a growing fruit could simply be adding a sink strength driven perturbation to the usual distribution of the phloem bulk flow carrying the mobile FT protein, and this is not directly linked to gene expression *per se*. A similar idea was recently published by Putterill and Varkonyi-Gasic (2016)[52] that in itself is indicative that the research and evidence of the FT protein movement in perennials is poorly understood and not fully explored[52] despite its potential usefulness in accelerating perennial trees breeding programs with transformed rootstocks[52] or Virus Induced Flowering (VIF)[41, 42] techniques.

To test the hypothesis of FT protein availability as modified by avocado fruit presence the following studies could be undertaken:

- explore Western Blot protocols and already available anti-FT antibodies[2, 32] to determine the presence of the avocado FT protein in tissues not usually thought to be related to the FT protein path, like reproductive flush sections, inflorescences stalks (that are holding fruits), fruit peduncles and junctions with the avocado fruits themselves;
- further explore the previously tested proof of concept (Maria Antonieta Cardemil, Chilean entomologist invited to UCR) of stylectomy[19, 50], which allows the acquisition of small

droplets from the severed stylets of the avocado black scale (*Saissetia oleae*) which naturally taps into the phloem vessels to obtain their nutrients from the phloem stream content. These samples could be digested and studied with Western blots[75] and mass spectrometry[21] for comparisons with the biologically active *Persea americana* FT (*Pa*FT) protein from the transformed *Arabidopsis* line developed by avocado researchers at the Volcani Center in Israel[77];

- explore the utilization of gall (cecidia) forming insects. One of the phenotypic modifications that these insects introduce to affected leaves is that the galled sections will display increased sink strength (status of the affected tissues changes from autotrophic to heterotrophic)[44, 45] and, if developed, it could be a system to test the movement of the FT protein to the nearest sink sections within the affected leaf. There is an exclusively monophagous (specialist) avocado pest, the *Trioza* psyllids[28, 69] that develops galls in young and mature avocado leaves[69]. However, none of the 4 *Trioza* species are found within main avocado producing areas of the United States[28], hence collaborations with researchers in Mexico, central America and/or Peru would be necessary to test this concept.

Besides the bud vegetative or reproductive fate discussed above, fruiting shoots displaying a reduced apical and axillary bud release (bud break or outgrowth) is certainly another complex aspect with potentially several cross talking research avenues. Nonetheless, as a bud outgrowth repressor and branching integrator, orthologs of the transcription factor BRANCHED 1 (BRC1)[3, 13] should not be overlooked in avocado research along with potential connections to auxin, cytokinins and strigolactones[76] whose availability could also be perturbed by the strong fruit sink depending on their phloem or xylem movement.

Interestingly, BRC1 directly interacts with the FT protein such that it is described as a negative regulator to the reproductive transition in axillary buds[47], but taken from the opposite perspective, it could be possible that, when the strong fruit sink is absent, an abundance of the FT protein could not only make it available to trigger reproductive development in axillary buds (FT-FD complex) but also the interaction of FT with BRC1[47] might reduce the latter's outgrowth repressing function[53].

### 4.5.1 "Flora loca" bloom

In relation to the reproductive induction of "flora loca" inflorescences, bloom predicting models that count "chilling days" accumulations have practical agronomic implications related to preparedness for main bloom events, but those blooms normally occur either during the winter period[54, 55] or during the spring, hence after a winter period (like in California). A good correlation between those main blooms and the accumulation of hours of cold air temperatures is not strange as either part or a full winter period occurs before the expected bloom. There is no reason to reject that avocados evolved a vernalization pathway for FT upregulation, nonetheless, "flora loca" events do not seem to fit with a model of accumulation of "days" with low air temperatures leading to flowering unless shorter induction time frames are considered[77]. Given that many pathways converge into floral integrator genes[17] other models should be considered[77] that can offer explanations to the richer reproductive plasticity that avocados have at different locations, for example the up to 4 blooming events reported in Michoacan, Mexico[54, 55].

"Flora loca" could be the final outcome of the direct binding to the *FT* promoter region[35] and the upregulation of the *FT* gene transcription (at leaf vasculature[35]) by an avocado ortholog of the transcription factor PHYTOCHROME INTERACTIVE FACTOR 4 (PIF4)[14, 35, 36, 67]. PIF4 is a photo-labile protein under continuous light[35, 48, 67] and unstable at low temperatures[35] but conversely is phosphorylated (active form) and accumulates in shade or darkness[16, 27] under warm air conditions (which facilitates chromatin access and binding to the *FT* promoter)[9, 16, 18, 35, 74]. This set of conditions could trigger upregulation of FT during warmer air temperatures, either completely at night or in shaded areas within the canopy, which are a common condition during the spring and summer growth seasons.

PIF4 is starting to be considered a multiple signal integrator in plants[10] and is connected, along with brassinosteroids, to phototropism and shade avoidance responses[10, 31, 49, 51] that involve fast elongation growth[16, 27, 31, 49]. At the same time, and from their observed behavior in the avocado fields, the "spiral" shoot class, which significantly displayed more "flora loca" blooms in the field (Figure 4-5 and Figure 4-6) are fast elongating vegetative structures and could be a good candidate to explore and explain "flora loca" despite some recent controversy about the previously mentioned direct connection with the upregulation of *FT* by PIF4[10].

The overall message in relation to avocado reproductive growth and the perturbations that a growing fruit generates is that we need to expand and enrich the pool of pathways that we associate to avocados and not limit ourselves to a single traditional pathway.

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# 5 Chapter 5: Phenotypical joint probabilities and future directions in ‘Hass’ avocado (*Persea americana*, Mill.) “alternate bearing” research

## 5.1 Introduction

Reviewing the literature on this commercially important problem of “alternate bearing” (Chapter 1) and the related avocado growth in particular (Chapter 2) together with the patterns observed and described at the single shoot level within this text (Chapters 3 and 4), reveal that the lack of consensus and working models on how “alternate bearing” occurs can lead to research that pivots on different visions, hypotheses, research trends and chosen scales of observation of the phenomenon.

During the 1930’s and 40’s, when the ‘Fuerte’ avocado variety was the dominant standard[10, 50], the tree alternating yield behavior was understood as a consequence of a reduced synchronization between flower’s behavior and pollen availability[25] (other pollinizer trees and the pollinators) along with unfavorable or freezing cold air temperatures (lower than 13.3° Celsius[24]), which would damage the flowers since the ‘Fuerte’ variety bloomed primarily in late winter under California conditions, hence reducing the next season reproductive potential.

Later in the 1950’s, the ability to measure mineral content of the trees led researchers to think of tree “alternate bearing” as the consequence of a mineral depletion but at the same time research was tending to show that the depletion was more of an organic nature[25]. This began connections between yield alternation and carbohydrates[25], ultimately leading to the starch reserves depletion concept.

At the same time, techniques for precisely measuring carbohydrate content started to conflict with the scale of observations, growth, time frames and environmental conditions chosen during those sample's collections. The technical specificity of the measurements was not necessarily congruent with the "whole tree" agricultural point of view and, ultimately, carbohydrate reserves were thought of as "useful integrators" of the whole tree condition[32, 57].

Phytohormone research made its way into the forefront of "alternate bearing" research and offered two main hypotheses: a) that the avocado seeds inhibit the next season flowering through hormone (focus on gibberellins) exports that have "anti-flowering" properties[9, 47, 48, 51, 57] and b) that growing fruits are likely rich auxin exporters and, with that, would themselves become the apical dominant growing organ instead of the apex of the shoot that is bearing it[7, 8, 51].

With these new ideas, conflicting views appeared, such as an explanation of how gibberellins are transported against the bulk flow and how to reconcile this idea with the fact that non-seeded fruit also display "alternate bearing" patterns[37, 51]. Also, and in relation to auxin export, the polar auxin transport (PAT) system is understood and is likely to be present in avocados, however auxin also move longer distances by the phloem bulk flow[2, 18], which possibly explains why strong sinks like growing fruits have been reported as poor auxin exporters[16, 17] and at the same time auxin importers[33].

More recently, the molecular era brought novel approaches offering connections between fruit presence and potential detrimental disruptions/alterations of the normal expression of floral integrators and floral identity genes, hence reducing the next season's bloom potential. Nonetheless, more recent QTL analyses in apple[21] and olives[46] suggest weaker links between "alternate bearing" phenotypes and flower related genes while connections with tree architecture, nutritional status and hormonal control were stronger[21, 46], consistent with the earlier conclusions reviewed by Monselise and Goldschmidt in 1982[37]

Despite the conflicting views, the general concept of a negative correlation between reproductive growth and vegetative growth[36, 47, 54, 55] stands. However, there seems to be a tendency to assign an “actively negative role” to growing fruits that would cause detrimental effects over the current vegetative and future reproductive growth. It seems that the only approach that research has not fully explored is to attribute fruits with a more passive role of a nutrient demanding growing organ that has a necessary role in the tree life cycle.

Choosing the scale of observation to be the smallest but pertinent single avocado shoot level while also adhering to an approach to fruit presence as mainly heterotrophic organs demanding phloem bulk content set the research stage to more precisely capture the dynamic interactions of growing fruits, growing shoots, reproductive growth and sunlight exposure that led to the observed phenotypes of ‘Hass’ avocado trees (Chapters 3 and 4). Collectively, these small shoot scale interactions shape the overall canopy dynamic but at the same time not all the shoot phenotypes are encountered equally within the canopy hence an analysis of the probability of the occurrence of specific phenotypes and how these combinations become nested within previous phenotypic outcomes resulted in an analysis of joint probabilities presented below in this chapter.

Finally, a set of suggested approaches to research on “alternate bearing” is presented and ultimately leads to the introduction of a working model of the “alternate bearing” phenomenon in ‘Hass’ avocado that hypothesizes about how the related phenotypes develop through time and attempts to be a guide for future research in the subject.

## 5.2 Understanding growth to understand “alternate bearing”

In 2009, Dr. Nigel Wolstenholme proposed a hierarchy of factors affecting “alternate bearing” in avocado[56, 57] that, in an organized manner, show the many levels of this phenomenon. This figure (Figure 5-1) is, at the same time, indicative of a cross talking network of possible interactions between environmental cues, carbohydrates, plant hormones and genetic control[15, 43, 44].

Attaining a deeper mechanistic understanding of avocado growth and physiology is needed to guide the use of molecular tools in the development of new elite avocado varieties/rootstocks[14] less prone to the “alternate bearing” phenomenon. With the previous, there is also a need to transition to research systems that allow exploration of the cascade of molecular and developmental events being overlooked by focusing on broader aspects and final observable outcomes such as tree size and yield.

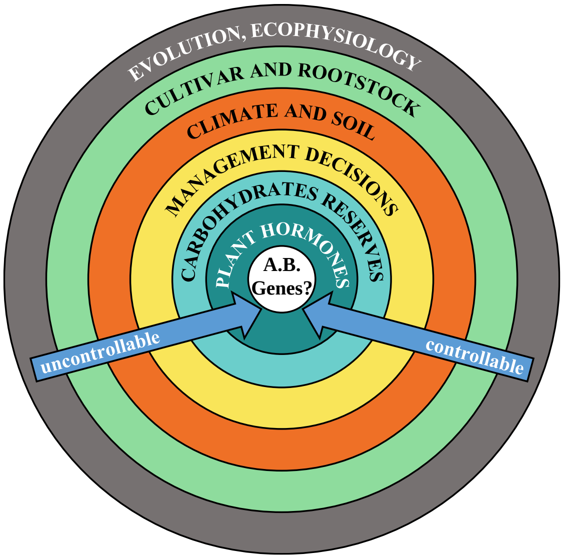

**Figure 5-1. Hierarchy of factors involved in avocado “alternate bearing”.**
Notice the different scales of the problem; that some variables are more controllable than others (agricultural management) and the question mark the author used in relation to possible “alternate bearing related genes”, as an indication of our search and their importance. Transcribed from Wolstenholme, 2009[56]

The approach to tackle and penetrate into the hierarchy of factors was to both minimize our scale of observation to the smallest relevant unit (single shoot level) (Chapter 2) and then maximize our phenotypic knowledge as it was perturbed by the developing fruit presence. The search was for specific and robust phenotypical patterns that could offer an explanation to what is inherently recognized as the whole canopy "alternate bearing" behavior.

### 5.2.1 Shoot growth patterns as they interact with fruit presence and sunlight

Even though it is a simplification of the actual dynamic plasticity observed in the avocado field, Figure 5-2 presents hierarchical and temporal connections among the most distinct and consistent patterns of avocado shoot growth (Chapter 3). It is difficult to convey that these patterns ultimately are a combination of a developing vegetative growth, future reproductive growth and an essential repeat of the "cycle" not on the "initial" growth but on the latest vegetative growth. In the previous sense, Figure 5-2 is not a true cycle but a constant flow of growing events through several seasons with the main factors being vegetative growth, reproductive growth and sunlight exposure.

As part of our road map for future avocado growth research, each binary event within the diamond shapes in Figure 5-2 is likely to be a complete research area unto itself:

- bud damage: even though the vegetative or reproductive potential resides within the buds, their survival is not secured unless some counter measurements are deployed. Agricultural management like proper irrigation and freeze protection is necessary for field deployed research;
- bud break (outgrowth): in avocados, the ratios and/or interactions between apical dominance, strigolactones and cytokinins[58], along with a potential ortholog of the BRANCHED 1 (BRC1)[3, 13] bud outgrowth repressor may be important. Given that BRC1 interaction with the FLOWERING LOCUS T (FT) protein disrupts the FT floral transition role in *Arabidopsis*[38],

is the BRC1 repressing function also disrupted due to the same interaction?[41] How does a growing fruit perturb these interactions? In non-fruiting vigorous avocado shoot growth, is the sylleptic release of buds an almost immediate release of a previously formed bud or alternatively, lateral bifurcation of the shoot apical meristem (SAM) occur[54] that continues growth without undergoing bud break *per se*?

- fruit set: although for Figure 5-2 “fruit set” means that an avocado fruit successfully developed until harvest on the observed shoot, on a holistic sense the concept of fruit set is as broad and imprecise as saying “alternate bearing”: the arrival of the necessary pollen grains to receptive stigmas is, to the least, driven by interactions between the environment and its effects on the flowers and the pollinator’s behavior. Furthermore, assuming the flower quality is nutritionally good[4, 5] and that the pollen tubes successfully grow and fertilization is attained (fruit set at its minimal expression), then what causes the several fruitlet abscission events that avocados undergo? A mechanistic understanding of this is essential.
- sunlight exposure: sunlight exposure has the power to “rescue” the non-fruiting shoot phenotypes on fruiting shoots (sunlit fruiting shoots can grow like non-fruiting shoots). It raises questions about how avocado researchers have been approaching shoot selection and studying “alternate bearing”, especially when linked to gene expression on tissue obtained in the fields. What is more at hand and at human height can be more preferentially sampled leaving inner and lower canopy sections (more shaded areas) less represented. Avocados seem to be very responsive to sunlight exposure, in terms of reinitiating new vegetative flushes and also to preferentially release axillary growth towards the sunlit sections (“branching bias”, Chapter 3), which might be an evolutionary advantage as avocados evolved in the tropical and subtropical areas of meso-America as understory plants[11, 49]. Nonetheless, what elements are driving these responses in avocado: is it the quantity and/or the quality of the light reaching the shoots? Are

these responses triggered by interactions among brassinosteroids, photoreceptors (phytochromes)[27, 39] and avocado orthologs of the PHYTOCHROME INTERACTING FACTORS (PIFs) protein family[45]?. Is it also possible that there is an avocado response to sunlight's infrared radiation by differentially reaching exposed sections and generating temperature gradients?

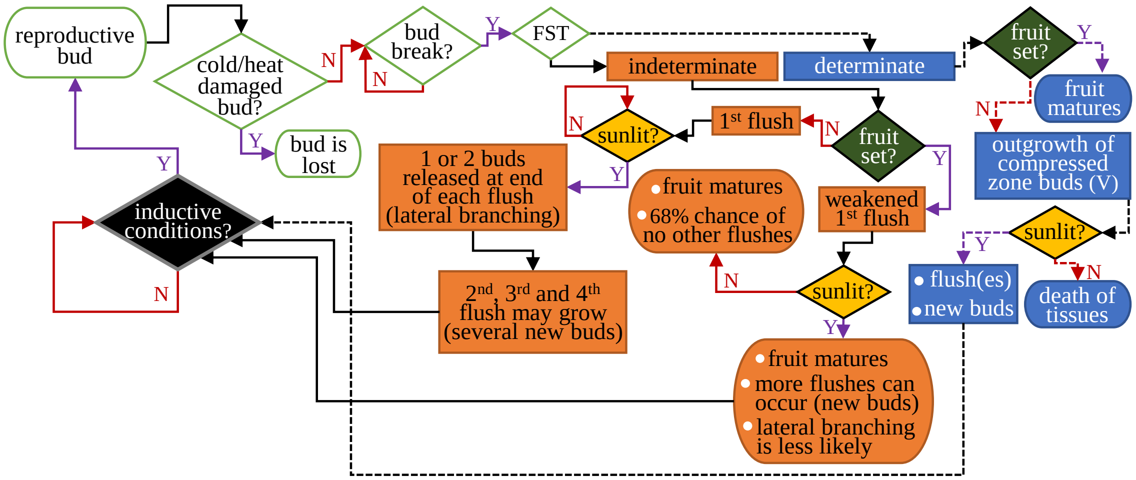

**Figure 5-2. Flow diagram of factors and events involved in 'Hass' avocado "alternate bearing" growth phenotypes.**

The flow starts in the upper-left corner with a single bud with reproductive fate. When this point is reached again, it would be a different (new) bud. Flow chart symbols are: ⬭ = start/end point, ◇ = binary event, ▭ = outcome or phenotypes. "Y": Yes (purple); "N": No (dark red). Connecting arrows are purple for "Y" option paths and dark red for "N" option paths. Black arrows are connections to common events. FST= flowering shoot type (see Chapter 2). Once the FST binary event is reached, solid arrows are exclusively related to the "indeterminate" flowering shoot path and dashed arrows are exclusively related to the "determinate" flowering shoot path. Flush: when used, the word "flush" refers to a period of active vegetative growth (vegetative shoot elongation) that develop new leaves and axillary buds. "Bud break?": relates to the outgrowth (release) of a resting bud into growth. "Fruit set?": a reference a complex set of events that include flower quality, pollinizer (pollen donor) abundance and distance, pollinator (bees, commercially) availability, pollen load and activity on receptive female stage flowers. "Sunlit?": relates to the exposure to sunlight during all or most of the day (conversely, an "N" option paths refers to shaded conditions). "Inductive conditions?": a reference to another complex cluster of environmental, physiological and molecular events that lead to the induction and differentiation of reproductive organs within a bud that, when successful, leads to a reproductive bud fate status. "V": although only used in the "determinate" FST path, it represent the cluster of small and resting vegetative buds present at the base of any avocado shoot (unless sylleptic) (see Chapter 2). Diagram does not include references to the at least 2 developing fruit droppings events that avocados undergo under the local conditions. Based on the 'Hass' avocado phenotypical database built at the Agricultural Experimental Station, UC, Riverside.

## 5.3 Joint probabilities of occurrence for different avocado growth descriptors

Although Figure 5-2 presents several factors likely involved in the avocado field's vegetative and reproductive dynamics, it was only a subset of those that were quantified. While mathematical models of fruit presence and avocado growth ("alternate bearing") should be develop to help bridge the field behavior with molecular approaches and quantitative variables of the factors truly involved in the "alternate bearing" phenomenon, at this point the data acquired from the related factors identified and described below were of qualitative nature and hence of the discrete type.

The following tables display the probabilities of occurrence of nested factors as they relate to fruit presence and vegetative growth outcomes, from one season to the next. To explore this set of conditional probabilities, two inputs are needed: 1) a choice of the tree group where the data were gathered ("caged" or "open pollinated") and 2) a choice of fruit presence status (fruiting or non-fruiting shoot data) at the end of the first season. These choices lead to 4 different pools of data: "caged" group/fruiting shoots (n=78), "open pollinated"/fruiting shoots (n=128), "caged" group/non-fruiting shoots (n=156) and "open pollinated"/non-fruiting shoots (n=183).

In Equation 5-1: P= probability, $ST_1$ (shoot type 1)= shoot type by number of flushes at end of season 1 (1, 2 or 3+ flushes), Su=survival of the shoot at the end of season 1, $FP_2$ (fruit presence 2)= fruit presence at the end of season 2, $ST_2$ (shoot type 2)= shoot type by number of flushes at end of season 2 (1, 2 or 3+ flushes) and $SL_2$ (sunlight 2)= sunlight exposure during season 2.

**Equation 5-1. Joint probability of a shoot type at end of season 2.**

$$ST_2 \text{ JOINT } P\,(ST_1,Su,FP_2,SL_2)=P(ST_1)\times P(Su|ST_1)\times P(FP_2|ST_1)\times P(SL_2|FP_2)\times P(ST_2|SL_2)$$

Although $ST_2$ joint probability is dependent of $ST_1$, Su, $FP_2$ and $SL_2$, they actually are nested conditional probabilities:

- $P(ST_1)$ is the probability of a shoot belonging to one of the 3 different shoot types (classified by number of flushes) depending on the initial inputs of tree group (“caged” or “open pollinated”) and fruit presence status (fruiting or non-fruiting shoots) at the end of season 1.
- $P(Su|ST_1)$ is the probability of survival (or not) of a shoot at the end of season 2 given the previous probability of shoot type ($ST_1$).
- $P(FP_2|ST_1)$ is the probability of a shoot having (or not) a growing fruit at the end of season 2 given that it is a surviving (Su) shoot of 1 of the 3 shoot types ($ST_1$).
- $P(SL_2|FP_2)$ is the probability of a shoot growing in sunlit conditions (or shaded) during season 2 given its fruiting status ($FP_2$: fruiting or non-fruiting)
- $P(ST_2|SL_2)$ is the probability of a shoot being one of the 3 different shoot types at the end of season 2 given that the shoot grew sunlit (or shaded).

The five tables below are the specific conditional probabilities that are part of the $ST_2$ joint probability (Equation 5-1).

**Table 5-1. Conditional probability of shoot type (by amount of flushes).**

| **Tree group and fruiting status** | **$P(ST_1)$ or shoot type at end of season 1** | | |
|---|---|---|---|
| | **1 flush** | **2 flushes** | **3+ flushes** |
| **“caged”/fruiting** | 0.782 | 0.115 | 0.103 |
| **“O P “/fruiting** | 0.742 | 0.195 | 0.063 |
| **“caged”/non-fruiting** | 0.218 | 0.359 | 0.423 |
| **“O P”/non-fruiting** | 0.301 | 0.333 | 0.366 |

Color has been added as a visual aid of the probability values as a gradient from white to dark green for the lowest to the highest value, respectively. Please notice that data by row adds to 100%. Based on our ‘Hass’ avocado phenotypical database built at the Agricultural Experimental Station, UC, Riverside.

Table 5-1 probabilities represent the basal conditions. It simply indicates the probability that at the end of the first season the shoot being followed would have 1, 2 or 3+ flushes depending on them bearing a fruit.

At this point the classification of the shoots as belonging to trees within the "caged" or the "open pollinated" group is just for preserving the database organization as it was during the next season (season 2) that the trees on the "caged" group completely lacked fruits within their canopy.

Aided by the color gradient within the table, it is possible to notice how heavily the fruiting shoot status relates to shoots with only 1 vegetative flush at the end of the season. On the other hand, non-fruiting shoots have a more balanced distribution between the three shoot types.

The set of conditional probabilities in Table 5-2 are clearly binary except in the 1 flush/fruiting shoot combination, indicating that at this level of specificity and like in no other combination, the survival of those shoots is essentially random.

**Table 5-2. Conditional probability of shoot survival at the end of season 2 given shoot type at season 1.**

| Tree group and fruiting status | $ST_1$ | $P(Su\|ST_1)$ or survival given $ST_1$ | |
|---|---|---|---|
| | | Yes | No (shoot died) |
| "caged"/fruiting | 1 flush | 0.574 | 0.426 |
| | 2 flushes | 0.778 | 0.222 |
| | 3+ flushes | 0.875 | 0.125 |
| "O P"/fruiting | 1 flush | 0.505 | 0.495 |
| | 2 flushes | 0.800 | 0.200 |
| | 3+ flushes | 0.875 | 0.125 |
| "caged"/non-fruiting | 1 flush | 0.912 | 0.088 |
| | 2 flushes | 0.893 | 0.107 |
| | 3+ flushes | 0.939 | 0.061 |
| "O P"/non-fruiting | 1 flush | 0.873 | 0.127 |
| | 2 flushes | 0.918 | 0.082 |
| | 3+ flushes | 0.985 | 0.015 |

Color has been added as a visual aid of the probability values as a gradient from white to dark green for the lowest to the highest value, respectively. Please notice that data by row adds to 100%. Based on our 'Hass' avocado phenotypical database built at the Agricultural Experimental Station, UC, Riverside.

Table 5-3 explores the conditional probabilities of single shoots to successfully develop a fruit until harvest for 2 consecutive seasons. Nonetheless, bear in mind that fruit presence was completely avoided in the "caged" group hence those proportions, although shown, are absolute because of human intervention.

**Table 5-3. Conditional probability of fruit presence on season 2 on surviving shoots given their shoot type (by number of flushes).**

| **Tree group and fruiting status** | **ST$_1$** | **P(FP$_2$\|ST$_1$) or fruit presence given surviving ST$_1$** | |
|---|---|---|---|
| | | **Yes (fruiting shoot)** | **No (non-fruiting)** |
| **"caged"/fruiting** | **1 flush** | 0.000 | 1.000 |
| | **2 flushes** | 0.000 | 1.000 |
| | **3+ flushes** | 0.000 | 1.000 |
| **"O P"/fruiting** | **1 flush** | 0.125 | 0.875 |
| | **2 flushes** | 0.250 | 0.750 |
| | **3+ flushes** | 0.143 | 0.857 |
| **"caged"/non-fruiting** | **1 flush** | 0.000 | 1.000 |
| | **2 flushes** | 0.000 | 1.000 |
| | **3+ flushes** | 0.000 | 1.000 |
| **"O P"/non-fruiting** | **1 flush** | 0.563 | 0.438 |
| | **2 flushes** | 0.518 | 0.482 |
| | **3+ flushes** | 0.167 | 0.833 |

Color has been added as a visual aid of the probability values as a gradient from white to dark green for the lowest to the highest value, respectively. Please notice that data by row adds to 100%. Based on our 'Hass' avocado phenotypical database built at the Agricultural Experimental Station, UC, Riverside.

Exclusively contrasting the data from the "open pollinated" group, it is possible to notice that the probability of bearing fruits two years in a row (i.e. the original shoot on season 1and its new apical growth on season 2) is low and that is more likely that a vegetative axis that bears fruit once, will not do it again the next season (if, in fact, it survived).

On the other hand, shoots going from a non-fruiting to a fruiting status are more likely (again nearly random), except from the 3+ flush type, which is more rare and, as shown in Table 5-5, driven by sunlight exposure more than by fruit presence.

**Table 5-4. Conditional probability of shoots growing exposed to sunlight at season 2 given their fruiting status at season 2.**

| Tree group and fruiting status | $FP_2$ | $P(SL_2 \mid FP_2)$ or sunlit conditions given fruiting status at season 2 ($FP_2$) | |
|---|---|---|---|
| | | **Yes (sunlit)** | **No (shaded)** |
| **"caged"/fruiting** | **Yes (fruiting)** | 0.000 | 0.000 |
| | **No (non-fruiting)** | 0.224 | 0.776 |
| **"O P"/fruiting** | **Yes (fruiting)** | 0.000 | 1.000 |
| | **No (non-fruiting)** | 0.317 | 0.683 |
| **"caged"/non-fruiting** | **Yes (fruiting)** | 0.000 | 0.000 |
| | **No (non-fruiting)** | 0.678 | 0.322 |
| **"O P"/non-fruiting** | **Yes (fruiting)** | 0.403 | 0.597 |
| | **No (non-fruiting)** | 0.583 | 0.417 |

Color has been added as a visual aid of the probability values as a gradient from white to dark green for the lowest to the highest value, respectively. Please notice that data by row adds to 100%, except for the "caged"/fruiting combination due to the caging treatment. Based on our 'Hass' avocado phenotypical database built at the Agricultural Experimental Station, UC, Riverside.

Table 5-4 is the first assessment of the connection between fruit presence and sunlight exposure. Nonetheless, the "caged" group has a man-made lack of fruits and is indicated in 2 rows by having only zeroes.

The general trend is that, independently of the tree group, shoots that already were fruiting once during the previous season 1 are more likely to grow shaded the succeeding season 2 while non-fruiting shoots from season 1 have more chances of growing sunlit during season 2.

**Table 5-5. Conditional probability of becoming a specific shoot type (at end of season 2) given their exposure to sunlight.**

| Tree group and fruiting status | $SL_2$ | $P(ST_2\|SL_2)$ or shoot type at end of season 2 given sunlight exposure ($SL_2$) | | |
|---|---|---|---|---|
| | | **1 flush** | **2 flushes** | **3+ flushes** |
| **"caged"/fruiting** | **Yes (sunlit)** | 0.000 | 0.182 | 0.818 |
| | **No (shaded)** | 0.500 | 0.447 | 0.053 |
| **"O P"/fruiting** | **Yes (sunlit)** | 0.050 | 0.400 | 0.550 |
| | **No (shaded)** | 0.691 | 0.273 | 0.036 |
| **"caged"/non-fruiting** | **Yes (sunlit)** | 0.010 | 0.196 | 0.794 |
| | **No (shaded)** | 0.413 | 0.413 | 0.174 |
| **"O P"/non-fruiting** | **Yes (sunlit)** | 0.034 | 0.172 | 0.793 |
| | **No (shaded)** | 0.566 | 0.398 | 0.036 |

Color has been added as a visual aid of the probability values as a gradient from white to dark green for the lowest to the highest value, respectively. Please notice that data by row adds to 100%. Based on our 'Hass' avocado phenotypical database built at the Agricultural Experimental Station, UC, Riverside.

Table 5-5 explores the connection between sunlight exposure and the shoot type at the end of that season. Independently of the tree group on which the shoots grew, the conspicuous pattern is the absolute reversal of the shoot type probabilities when the environmental conditions change from sunlit to shade. As mentioned in Chapter 4 and Chapter 5, if sunlight exposure is not taken into account, it can be a confounding factor on the effects of fruit presence on the shoots that bear them.

Finally and as a summary, Table 5-6 presents the conditional probabilities gathered during our work that lead to the six more probable combinations that occurred in the research field.

Independently that for this data to be in Table 5-6 it had to belong to shoots that survived the second season (second colored column), it was interesting to see how those survival chances are increased for non-fruiting shoots when compared to the fruiting ones.

The data from shoots in the "caged" group had no fruits during the second season (man-made intervention), but is important to reiterate (Chapter 3) that even for the last two rows of data from the "open pollinated" group (in Table 5-6), the probabilities of not bearing fruit again the succeeding season are high (third colored column).

**Table 5-6. The conditional probabilities leading to the six largest joint probabilities.**

| Tree group and fruiting status | Shoot type (season 1) | Conditional probabilities | | | | Joint probability |
|---|---|---|---|---|---|---|
| | | Survives season 2? | At season 2: fruiting again? | Is it sunlit? | Shoot type (season 2) | |
| "caged"/ non-fruiting | 3+ flushes (0.423) | Yes (0.939) | No (1.000) | Yes (0.678) | 3+ flushes (0.794) | 0.214 |
| "caged"/ fruiting | 1 flush (0.782) | Yes (0.574) | No (1.000) | No (0.776) | 1 flush (0.500) | 0.174 |
| "caged"/ non-fruiting | 2 flushes (0.359) | Yes (0.893) | No (1.000) | Yes (0.678) | 3+ flushes (0.794) | 0.173 |
| "caged"/ fruiting | 1 flush (0.782) | Yes (0.574) | No (1.000) | No (0.776) | 2 flushes (0.447) | 0.156 |
| "O P"/ fruiting | 1 flush (0.742) | Yes (0.505) | No (0.875) | No (0.683) | 1 flush (0.691) | 0.155 |
| "O P"/ non-fruiting | 3+ flushes (0.366) | Yes (0.985) | No (0.833) | Yes (0.583) | 3+ flushes (0.793) | 0.134 |

Color has been added as a visual aid for the contributions of the conditional proportions to the final joint probability. The red to green color gradient is valid per row with the lowest within the row in red and the highest value within the row in green. Based on our 'Hass' avocado phenotypical database built at the Agricultural Experimental Station, UC, Riverside.

The general outcome of this analysis is that, as presented in Table 5-6, completely avoiding fruit presence ("caging") was not as important at the single shoot level as merely having or not a fruit and then if those single shoots grew while sunlit or not. Simply put, non-fruiting shoots will have similar probabilities of developing 2 or 3+ flushes while fruiting shoots of just being 1 flush independently of the general presence/absence of fruits in the canopy. Also, if vegetative growth occurs in the shade, is likely to generate 1 flush and while sunlit growth tends to display 3+ vegetative flushes.

## 5.4 Surveying shoot growth and fruit growth interactions

Due to our current inability to reliably capture and ultimately associate them with an appropriate metric, the events presented below only qualify as anecdotal evidence supporting the consequences of having reproductive growth developing on a vegetative shoot.

### 5.4.1 Fruit sink growing under shaded conditions

Figure 5-3 shows a simple but sometimes overlooked visual example of how the growing fruits demand photoassimilates in their condition of strong sink to the point that the allocation of resources leads to the death of the tissues after it. This seems to be exacerbated under shaded conditions where photosynthetic levels are likely reduced. Similar fruiting shoots but under sunlit conditions would likely survive and reinitiate new vegetative flushes (Chapter 3).

**Figure 5-3. Single shoot level consequences of the fruit sink strength under shaded conditions.**

These images are presented to depict how a growing fruit seems able to sustain its own development and the previous vegetative growth up to its physical location along the shoots. The panel on the left is a lateral (axillary) determinate fruiting shoot affecting the original main shoot axis from which it developed. The central and right panel are showing apical determinate flowering shoots with the inflorescences stalks decayed up to the growing fruit location. Agricultural Experimental Station, UC, Riverside.

### 5.4.2 Buds delayed release (break) when no growing sink is present

We refer as delayed bud release (break or outgrowth) to the observation of ≈1 year old axillary buds outgrowing and displaying their vegetative or reproductive fate sometime after the main bud release period (main bloom) at early spring (in California).

During this period, apical and axillary buds with reproductive fate are released. They grow many individual flowers within the flowering shoots structures, hence several active sinks (single flowers) demanding phloem content are present at the time[19].

In the case of determinate flowering shoots, there are only two possible options after all the flowers are shed: either none of them successfully set fruit or some of them did so. As depicted in Figure 5-4, determinate structures that did not set fruits (left panel) will undergo a “delayed release” of other available nearby growing points that are close to the structure that once was a demanding sink area (i.e. the compressed zone resting buds at the base of the flowering shoots) (Chapter 2). On the other hand, if some growing sink organs remain (growing fruits), there won’t be any other “delayed release” of buds (Figure 5-4, right panel).

This “delayed released” is not only circumscribed to the absolute nearest available growing points at the compressed zone but can also go further back into the ≈1 year old main shoot and release other axillary buds with their vegetative or reproductive fates (not displayed in Figure 5-4).

Finally, if the flowering shoot being observed is functionally indeterminate, even if no fruit set is achieved, the shoot apical meristem (SAM) will eventually initiate its first vegetative flush and the heterotrophic sink that is that growing shoot tip will reduce the chances of any “delayed axillary bud release”, similarly to the influence that a growing fruit sink has over a functionally determinate flowering shoot.

These clues of the influence of a growing sink at the single shoot scale provide visual insights onto the internal transport of nutrients, hormones and proteins that, due to the dead of the tissues previously demanding them, might accumulate in their vicinity and then trigger other responses and perturbations like bud release.

**Figure 5-4. “Delayed release” of vegetative growth depending on active sink presence.** All fruiting shoots depicted are of the functionally determinate kind (Chapter 2). Within their structures, and after all flowers are shed, their determinacy reduces the chances of presence of a growing sink to only a successful fruit set. Red arrows point to a similar combination of factors with the same responses but earlier in the time scale of a season. Please notice that the newly released buds that generate the visible vegetative growth belong to the shoots compressed zone (Chapter 2). Agricultural Experimental Station, UC, Riverside.

## 5.5 Thoughts on bridging future field and molecular avocado research

### 5.5.1 “Alternate bearing” shoot phenotype

After all is said and done, the general “alternate bearing” phenomenon can be boiled down to a much more succinct set of observable phenotypes at the single shoot level. Figure 5-5 depicts the divergent fruiting and non-fruiting shoot phenotypes that research needs to explain in order to move towards a mechanistic understanding of avocado “alternate bearing”.

Focusing on what is shown in Figure 5-5 while at the same time considering the more detailed discussions in chapters 3 and 4, it is imperative that any working model attempting to explain the “alternate bearing” phenomenon in avocado relates back to these consistent and divergent shoot level phenotypic patterns.

However, the real experimental challenge for avocado research is to actually refine the hypotheses and design research approaches and molecular tools to explain how these depicted “snapshots” in the phenology of the fruiting and non-fruiting shoots are dynamically and gradually built through the season.

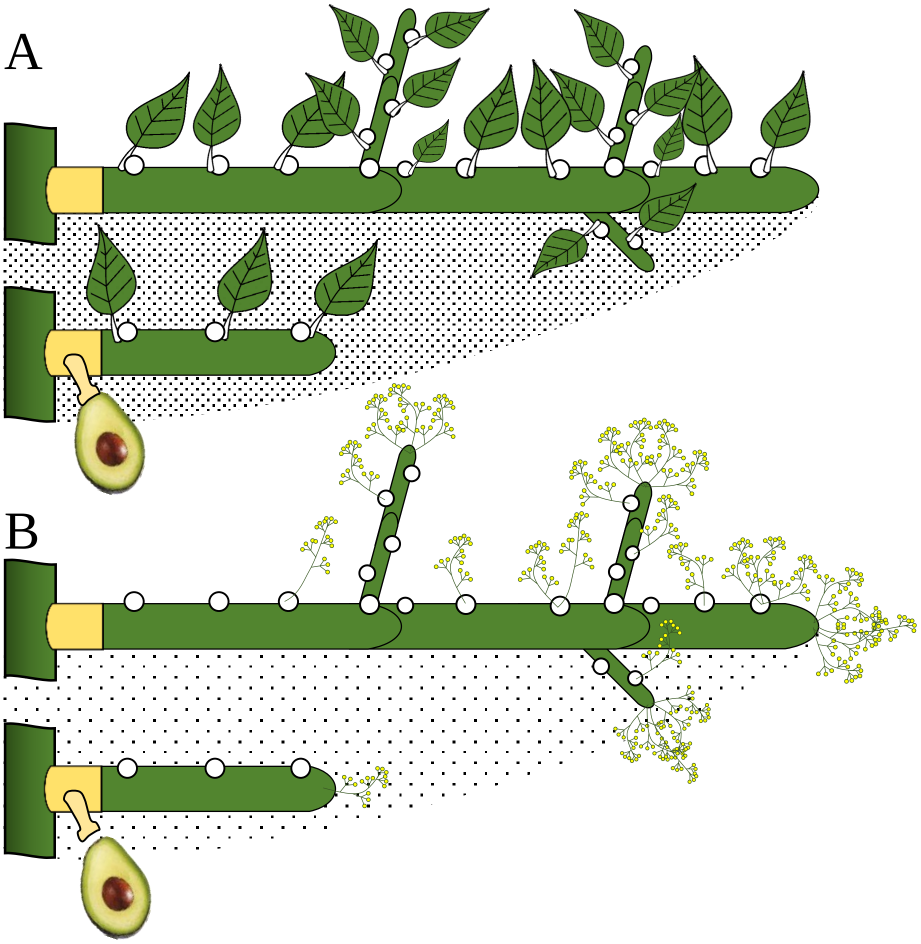

**Figure 5-5. Differences in the phenotypes of fruiting and non-fruiting shoots.** Panel A and B depict the same shoots, but the former represent a moment at the end of their first season and the latter represent a moment at the beginning of their second season (main bloom). ○ = axillary buds. Dotted area in panel A shows how the vegetative complexity of the non-fruiting shoot can cast shade over nearby fruiting shoots. Panel B has the same area less densely dotted as by that phenological moment, several leaves are likely shed; nonetheless by then those same dotted areas (canopy locations) will be part of the inner canopy (shaded). Detached fruit in panel B represents the fruit as harvested. Note the flowering complexity gradient that is higher at the shoot tip of the main and lateral axes as described in Chapter 4.

### 5.5.2 Developing connections leading to the observed shoots reproductive patterns

Considering the previous analysis of how the presence of a growing fruit can interact and influence shoot growth (section 5.4) it is possible to start developing research connections between the observed divergent reproductive phenotypes of fruiting and non-fruiting ‘Hass’ avocado shoots and the current literature on the cascade of events that lead to the development of reproductive growth in model plants.

#### *5.5.2.1 Sink strength and FT phloem translocation*

Current models explain that the phloem mobile FT[12] protein travels to and reaches the apical meristems where it interact with the transcription factor FLOWERING LOCUS D (FD) to form the FT-FD complex[1, 28, 52, 53] (and potentially a ternary complex with the 14-3-3 protein[1, 22, 34, 40]), triggering the expression of floral identity genes[30] that develop reproductive fate within buds.

Despite the previous, a mostly unexplored area within the general reproductive induction process is the long-distance transport (translocation) of the FLOWERING LOCUS T (FT) protein from its site of synthesis (leaf companion cells) to the competent meristematic tissues, mainly the shoot apical meristem (SAM). Current literature indicates that the FT INTERACTING PROTEIN 1 (PIFT1) and the FE[1, 40] protein are involved in loading of FT into the phloem sieve elements (Chapter 4), but the translocation itself seems to be a mainly unknown path. It has been hypothesized that FT transport is simply subject to the phloem bulk stream dynamics[34, 40], basically going to where the carbohydrates are being most strongly demanded[19]. This should render the FT protein less available for weaker sinks and offering potential explanations to the phenotypical reproductive perturbations that fruit presence causes to fruiting shoots (Chapter 4).

This concept crosstalk with a whole area of research that is sink and source status and the sink to source transition of organs. A new avocado leaf starts to develop every 2 to 3 days (Mickelbart and

Heath, unpublished[35]) and around 30 days later it reaches full size[11, 35, 49]. Slightly earlier, at about 80% of the final leaf area (20-24 days after bud break), the ‘Hass’ leaf would transition from sink to source[11, 49]. Within that time frame, the very first leaves of the first vegetative flush should become mature and possibly competent to transcribe, translate and load FT to the phloem bulk flow. This could open a research avenue that links *PaFT* expression[59] to the leaf plastochron index (making these molecular events more precisely associated to developmental leaf age). All together this has the potential to explain why is unlikely to observe buds with reproductive fate at the basal portion of the first vegetative flush on non-fruiting avocado shoot (Chapter 4), as older leaves are shed already and some leaf developmental and chronological time is required to even have a chance to develop a growing inflorescence meristem.

Furthermore, the acropetally increased flowering shoot complexity (Chapter 4) might be due to an increased abundance of the FT protein towards the distal portion of the shoots when the rapidly growing flush is ending. This means that the very first vegetative flush will have (if any) a short window of opportunity for reproductive induction, but the succeeding second, third and even fourth flush of the same shoot will gather an increasing array of fully mature set of leaves with a potentially exponential increase of the FT protein concentrations reaching the sink sections within the shoot.

These internal dynamics may also offer an explanation to the observed “acropetal push” (Chapter 4) of the reproductive bud fates on fruiting shoots as more distance is needed from the growing fruit in order to have the needed phloem content “dragging” ability of what is overall the second strongest sink within the fruiting shoot system (i.e. the fruiting shoot SAM). The physical distance from a strong sink organ could be an area of importance in fruit tree research (and potentially breeding) but it has to be approached dynamically by choosing factors and variables that can capture growing fruits, growing shoots that bear them, leaf developmental age and sink to source transitions within the single shoot study systems.

As an overarching summary, Figure 5-6 attempts to organize some of the several steps that can be hypothesized as involved in generating the observed reproductive phenotypical patterns of the 'Hass' avocado shoots.

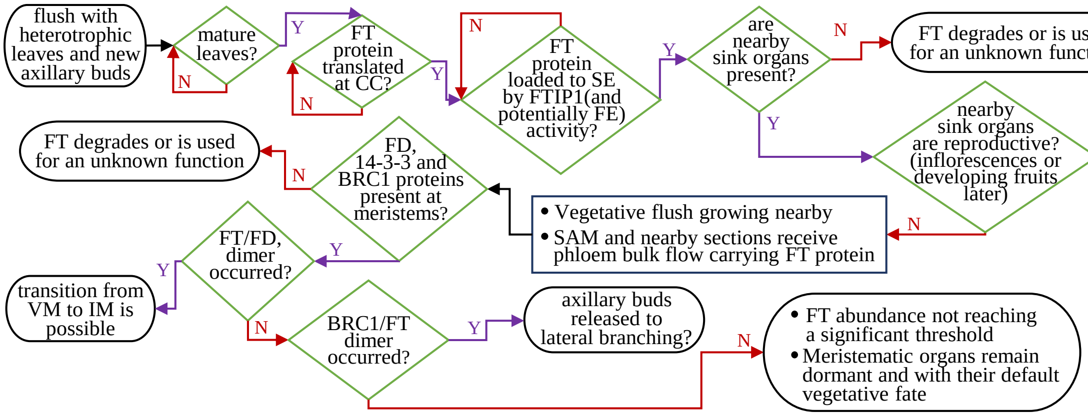

**Figure 5-6. Flow diagram of factors, events and hypothetical outcomes related to 'Hass' avocado "alternate bearing" reproductive growth phenotypes.**

The flow starts in the upper-left corner with a growing vegetative flush generating immature new leaves and axillary buds. Flow chart symbols are: ▢ = start/end point, ◇ = binary event, ▭ = outcome or phenotypes. "Y": Yes (purple); "N": No (dark red). Connecting arrows are purple for "Y" option paths and dark red for "N" option paths. FT= FLOWERING LOCUS T. FD= FLOWERING LOCUS D. CC= phloem companion cells. SE= phloem sieve elements. FTIP1= FT INTERACTING PROTEIN 1. FE= FE protein. 14-3-3= 14-3-3 proteins. SAM= shoot apical meristem. BRC1= BRANCHED 1. VM= vegetative meristem. IM= inflorescence meristem. Flush: when used, the word "flush" refers to a period of active vegetative growth (vegetative shoot elongation) that develop new leaves and axillary buds. Based on the 'Hass' avocado phenotypical database built at the Agricultural Experimental Station, UC, Riverside.

### *5.5.2.2 Speed of developing buds with reproductive fate*

Besides the need for expansion of the prevalent model of avocados being a species that requires 3 to 4 months of exposure to low air temperatures in order to undergo "transition to flower" (Chapter 4), another aspect of the avocado reproductive behavior that also should be addressed is the speed of the reproductive development process.

The "flora loca" bloom (Chapter 4) challenges the notion of a slow pace reproductive process in avocados. When a vegetative axis goes from growth initiation to visibly displaying inflorescences in 2.5 months[42], it demonstrates how rapidly macro and microscopic events can occur.

At this point, avocado research is entering into more precise evaluations of these transient events by using molecular tools[59]. This approach has already put forward possible modifications to the reproductive time frame for flower induction from mid to late summer to the time frame between October and November (where a peak of *PaFT* mRNA was observed[59]). Considering that these inflorescences are expected to be released during the succeeding spring, then going from a start of the process at mid-summer to one at early winter already reduces the time frame of the reproductive development.

Nonetheless, new molecular approaches in avocado will still be only as informative as our field samples allow. Embracing more informed and complex sampling schemes[59] instead of minimizing our samples to, for example, the resting shoot apical meristem of the last flush, is the only way we can attempt to fully explain the observed reproductive phenotypes. If not, how at the end of each vegetative flush, reproductive development can occur? (leading to the bud fate map detailed in Chapter 4 and depicted in Figure 5-5).

### 5.5.3 Novel “alternate bearing” working model for ‘Hass’ avocado

With the intention to bridge the avocado shoot phenotypes in the field with future research efforts, a working model for the “alternate bearing” phenomenon in ‘Hass” avocado was developed.

To convey the set of dynamic events in a static diagram form, Figure 5-7 and then Figure 5-8 depict two moments in the phenology of a fruiting and a non-fruiting shoot while together they constitute the avocado “alternate bearing” working model that has the potential to explain (hypotheses) how the observable divergent vegetative and reproductive phenotypes develop through the season.

Although, like any other one, this working model reduces the environmental and biological factors associated with the phenomenon, it was important to select proteins of interest among the ones that have receive the status of signal integrators, such as the FT protein for reproductive growth and

BRC1 for vegetative architecture and shoot branching along with other proteins located upstream of main pathways as FD for flower (reproductive) development and TFL1 for vegetative fate maintenance.

Finally, as part of this novel working model, the focus on the fruit role was switched from the need of a very actively exporting molecular signals (usually needed to be disruptive in their actions) outwards and into the bearing shoot to the “simplest” passive role, that is of a mainly heterotrophic organ just growing through the season, hence a strong consumer of the phloem content (strong sink organ).

#### *5.5.3.1 Early stages of the “alternate bearing” phenotype*

The moment represented in Figure 5-7 falls into the avocado phenology after the initial reproductive flush (bloom) (discussed in Chapter 2) but before the end of the first vegetative flush, hence an actively growing shoot apex is present in both cases. If fruit set is successful (Figure 5-7, panel A), several fruitlets should be developing in the “soon to be” fruiting shoot despite being represented by a single and large fruit in Figure 5-7.

As mentioned earlier (section 5.5.2), a new avocado leaf develops approximately every 2 to 3 days and takes 20 to 24 days to transition to a source organ status. It is unlikely that within this time frame leaves are exporting the FT protein into the sieve elements. Nonetheless, our phenotypical observations do indicate that reproductive axillary bud fate can develop towards the end of the first vegetative growth (Chapter 4) and is more likely to occur in the non-fruiting shoots. It is then necessary to accept that the synthesis and load of the FT protein does occur, faster than previously thought (Chapter 4) and that growing fruitlets can make a difference in those reproductive fate chances towards the end of that first vegetative growth period.

The differential chances could be due to the non-fruiting shoots having a physically longer vegetative flush (Chapter 3) and hence two different conditions: more leaves that eventually will contribute to the FT protein abundance and a shoot apex that continues to grow for a longer period of time while demanding that phloem content.

In panel A of Figure 5-7, the red dotted circles near the growing shoot apex display how on the fruiting shoot the unlikely presence of the FT protein (△) in that apical area sets the stage for the pool of FD (●) to be used in the TFL1/FD dimer (●⇦), preventing floral differentiation and keeping the shoot apical meristem indeterminate and the axillary meristems in a vegetative fate. At the same time the BRC1 protein monomer (▭) fulfills its function as bud outgrowth repressor.

On the other hand, the non-fruiting shoots (panel B) have increased chances for the occurrence of the FT/FD protein dimer (●△) that triggers the cascade of molecular events that lead to bud reproductive fate while leaving the floral repressor TFL1 protein (⇦) mostly as a monomer, hence not able to efficiently exclude the floral identity proteins from the meristematic areas[6, 20, 23].

Finally, it is also plausible that the FT/BRC1 protein dimer (△▭) can be formed but that discussion is more appropriate with Figure 5-8.

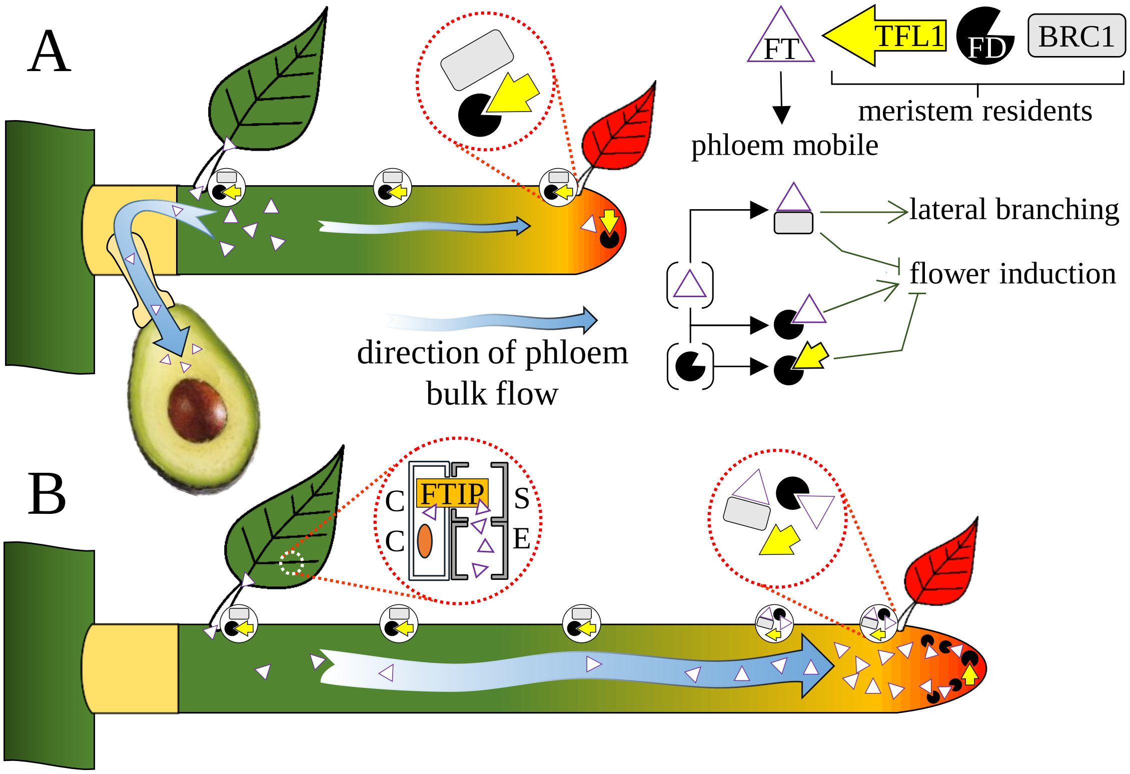

**Figure 5-7. Model of the early stages of the "alternate bearing" phenotype.**

For this figure: △ =FLOWERING LOCUS T (FT) protein; ⬅ =TERMINAL FLOWER 1 (TFL1) protein, ●=FD protein, ▭=BRANCHED 1 (BRC1) protein. ○=axillary buds along the main shoot axis (consider that there is a developing leaf for each axillary bud although not all depicted). (△)=FT protein pool (concentration) at a particular moment reaching the meristems, (●)=FD protein pool (concentration) at a particular moment within the meristems, △▭ =FT/BRC1 protein dimer which disrupts their monomer functions as flower differentiation promoter (FT) and bud outgrowth repressor (BRC1)[38, 41] respectively, ●△ =FT/FD protein dimer which triggers meristem reproductive status, ●⬅ =TFL1/FD protein dimer which prevents reproductive differentiation at the central area of the SAM[6, 20, 23]. <u>Panel A - Fruiting shoot</u>: a successful fruit set leads to the development of a fruit which becomes the strongest sink of phloem content on which the FT protein travels hence most of the protein goes towards the fruit and less goes to the second strongest sink, the growing shoot tip. <u>Panel B - Non-fruiting shoot</u>: either by unsuccessful fruit set or lack of inflorescences, the strongest sink section within the shoot should solely be the growing shoot tip, hence a higher comparative abundance of the FT protein is available to interact at the shoot apical meristem. Along the shoot axis there is a color gradient from dark green to red: dark green represent sections with mature autotrophic leaves and red areas represent heterotrophic sections with immature leaves and an actively growing shoot apex. "CC": phloem companion cells, "SE": phloem sieve elements, FT INTERACTING PROTEIN 1 (FTIP1): involved in loading the FT protein into the sieve elements[31]. Arrow indicates the main direction of the phloem bulk flow. Notice that the ●⬅ =TFL1/FD protein dimer is still present at the shoot apical meristem keeping the shoot axis indeterminate. Agricultural Experimental Station, UC, Riverside.

### *5.5.3.2 Later stages of the "alternate bearing" phenotype*

As depicted, Figure 5-8 occurs at a later moment on the same shoots from Figure 5-7, between the first and second vegetative flush (Chapter 3). By then is likely that most of the previously developed leaves during the first flush became autotrophic source organs.

This is a mayor divergence point for these shoot conditions: the fruiting shoot (Figure 5-8, panel A) continues to have a growing fruit and 68% of the time will not initiate a second flush during the season. It is likely the end of the road in terms of vegetative growth for these shoots, and only the fruit will continue to grow. It is also likely that the chance to reinitiate flushes is driven by sunlight exposure due to random canopy dynamics (sunflecks, shoot bending and breakage, gusty winds and other along with pruning practices that also can make a difference). For this fruiting shoot (Figure 5-8, panel A), the conditions described in the previous Figure 5-7 analysis remain similar due to the non-growing shoot tip.

On the other hand, the shoot in panel B of Figure 5-8 remains dynamic. Not only is able to reinitiate apical growth with its second vegetative flush, but also two major differences can occur:

- the longer first flush and its mature leaves are active (also likely to be more exposed to sunlight) and potentially able to contribute to the FT (△) protein pool in the phloem and hence reaching the again active shoot apical meristem;
- lateral branching is more likely to occur in 1 or 2 axillary buds below the transition zone (Chapter 3) which increases the shoot complexity and reinforces it by increasing the number of actively growing shoot apical meristems that laterally repeat the pattern of the main shoot axis.

The former bullet point falls into a similar explanation of the increased reproductive chances mentioned in the discussion for Figure 5-7 but the lateral branching point was not mentioned before.

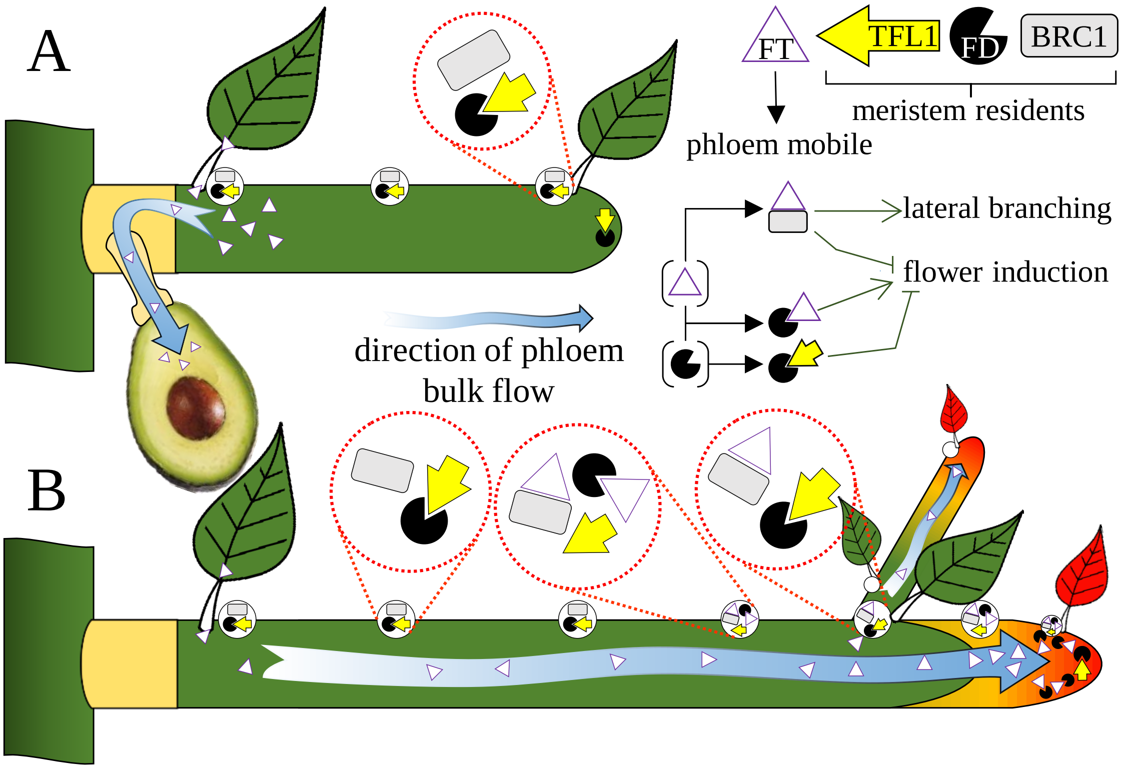

**Figure 5-8. Model of the later stages of the "alternate bearing" phenotype (period between the first and second vegetative flushes is depicted).**

For this figure: △ =FLOWERING LOCUS T (FT) protein; ⇦ =TERMINAL FLOWER 1 (TFL1) protein, ◕ =FD protein, ▭ =BRANCHED 1 (BRC1) protein. ○ =axillary buds along the main shoot axis (consider that there is a developing leaf for each axillary bud although not all depicted). (△) =FT protein pool (concentration) at a particular moment reaching the meristems, (◕) =FD protein pool (concentration) at a particular moment within the meristems, ⏏ =FT/BRC1 protein dimer which disrupts their monomer functions as flower differentiation promoter (FT) and bud outgrowth repressor (BRC1)[38, 41] respectively, ◕△ =FT/FD protein dimer which triggers meristem reproductive status, ◕⇦ =TFL1/FD protein dimer which prevents reproductive differentiation at the central area of the SAM[6, 20, 23]. Panel A - Fruiting shoot: the growth of the fruit continues as the shoot apex ceases to grow at the end of the first vegetative flush wave. In more than 68% of the time, fruiting shoots will not initiate any new flush period and if FT is produced and loaded to the sieve elements, the fruit remains the sole strongest sink of phloem content including the protein and, with that, reducing the chances of protein interactions with it at the meristems. Panel B - Non-fruiting shoot: the shoot can initiate a second vegetative flush (or more later on) not only at the shoot tip but also as a newly released axillary bud growing laterally. The mature autotrophic leaves can continue synthesizing and loading the FT protein (under inductive conditions) and its higher abundance increases the chances of protein interactions within the axillary apical and axillary meristems. Along the shoot axis there is a color gradient from dark green to red: dark green represent sections with mature autotrophic leaves and red areas represent heterotrophic sections with immature leaves and an actively growing shoot apex. Arrow indicates the main direction of the phloem bulk flow. Notice that the ◕⇦ = TFL1/FD protein dimer is still present at the shoot apical meristem keeping the shoot axis indeterminate. Agricultural Experimental Station, UC, Riverside.

Why at the end of each vegetative flush is there an increased chance for 1 or 2 lateral shoots to be released? It can be hypothesized that is a combination of leaf maturity abundance "behind" (basipetally) the "recently stopped" shoot apex and at the same time an accumulation of their produced FT (△) as an available pool in that vicinity.

It is plausible that, under similar conditions, the mentioned FT/BRC1 protein dimer (⏏) can be preferentially formed over the FT/FD (●△) dimer (right most red dotted circle in panel B) given that the former FT/BRC1 forms directly[26, 38] while the latter FT/FD dimer is mediated by the action of 14-3-3 proteins[38].

Simultaneously, other factors that promote axillary bud outgrowth and at the same time are down regulators of *BRC1* (in its pathways integrator role) could be coincidentally converging 1 or 2 buds below a non-fruiting shoot apex that is ending an active vegetative growth period:

- higher levels of sucrose[41] due to a transient sucrose maxima shuttled from the source leaves behind that is not readily being used by a growing shoot apex anymore;
- lower auxin:cytokinin ratios[3, 41], which could be a transient consequence of the shoot apical meristem reducing its activity and generating no new auxin producing young leaves[2, 18];
- lower levels of strigolactones[41] which are upstream and promoters of *BRC1* but are transported through the xylem vessels[29], hence a non-growing shoot tip should be reducing its transpiration pull towards itself (no new immature leaves are being developed);
- high ratios of R:FR ratios[41], (i.e. sunlit conditions) which is much more likely for non-fruiting shoots as they are longer and their shoot apices are probably part of the canopy periphery at the time.

Finally, as represented by the central red dotted circle and the "newly developed" axillary buds on the second vegetative flush of Figure 5-8 - panel B, why the reproductive complexity of the axillary and apical bud increase towards the end of flushes and shoot tips (Chapter 4)? It can be hypothesized that, similarly to the previous mechanistic explanation of the observable shoot phenotypes, it is simply a consequence of an increasing number of mature source leaves “behind” (basipetally) the growing shoot tip together with the location of the strongest sink within the shoot (growing shoot apex).

If, for the sake of argument, all mature source leaves are capable of producing a similar amount of FT protein (while environmental inductive conditions are present), then as time and vegetative growth progresses, new leaves go into FT synthesis setting the stage for an acropetal “drag” (by the growing shoot tip sink) of a gradually increased FT protein pool available to participate in generating higher reproductive complexity within the new axillary buds near the growing shot tip, to the point that the absolute maximum complexity belongs to the very apical reproductive bud (Chapter 4) at the tip of the last vegetative flush at the end of the whole season.

## 5.6 General Conclusion

It is safe to say that the “alternate bearing” phenomenon is too broad and complex to be researched at less informative (broad) scales.

Going back to the 2009 publication, Dr. Wolstenholme added a question mark at the center of his proposed hierarchy of factors involved in avocado “alternate bearing”[56, 57] (Figure 5-1), as there was a search for molecular connections (“alternate bearing genes”) to the broad phenomenon of avocado “alternate bearing”.

Researchers would have been lucky, by now, to discover a monogenic cause of the whole “alternate bearing” phenotype but a set of cross talking networks and interactions between environmental cues, carbohydrates, plant hormones and genetic control[15, 43, 44] is more likely to be the cause.

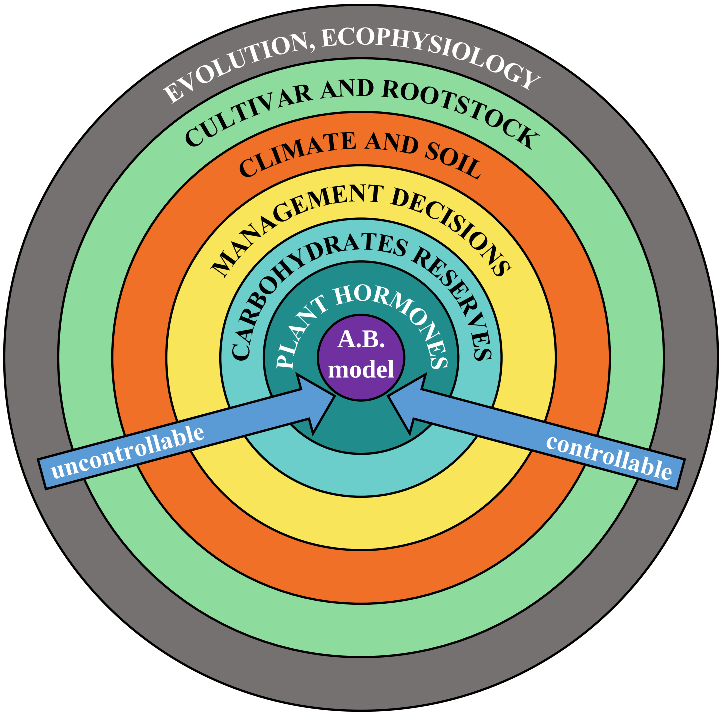

**Figure 5-9. Full circle: Hierarchy of factors involved in avocado "alternate bearing".**
Modified from Wolstenholme, 2009[56] by replacing the words "A. B. genes?" by A.B. model at the center.

Properly choosing the pertinent scale of single shoots and being heavily based on long term field observations and measurements done throughout the complex and dynamic context of the 'Hass' avocado canopy within this research's experimental site but also confirmed in other avocado producing areas within California, Chile, Israel, South Africa and Peru, allow us to offer a novel "alternate bearing" working model (Chapter 5) as a potential road map to design research and test hypothesis that fit the actual phenotypical divergence between fruiting and non-fruiting avocado shoots. In that sense we can start our work in doing a full circle to the original goal: what genes and factors are actually involved in avocado "alternate bearing"? (Figure 5-9).

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