

Performance and Impact of Strawberry (*Fragaria x ananassa*) Season Extension
in the United States Upper Midwest using Organic Practices

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ANDREW J PETRAN

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Emily Hoover, Adviser

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Dedication

This thesis is dedicated to my grandfather Julius "Pete" Petran, one of the hardest working people I know.

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CHAPTER 1

INTRODUCTION, OVERVIEW, RATIONALE AND OBJECTIVES

Andrew Petran

Sustainability and Strawberries in the United States Upper Midwest

Advocates of sustainable agriculture often find the word ‘sustainability’, in terms of farming, to be a complex idea with conflicting definitions and broad interpretations (Harwood, 1990; Hansen, 1996). Many authors focus on the term solely as an approach to improve the efficiency of farm-level resource consumption; others argue that such a narrow scope ignores the responsibility of sustainable agriculture to address global poverty and hunger issues (Harwood 1990; Allen et al., 1991; Gomiero et al., 2011). This discourse has led to a general consensus that sustainable agriculture, and sustainability in general, is an idea supported by three distinct pillars, representing environmental, economic and social philosophies (Larson et al., 2011; Morrison-Saunders and Pope, 2013). The three-pillar definition has become widely accepted, and is used by the EPA and USDA to define the word (EPA, 2012; Gold, 2012). Such a definition allows for multiple disciplines and ideals to work together under a common name.

The problem with such a definition is that these pillars, while often complementary, can sometimes come into conflict. For example, a successful conventional farmer who is able to comfortably support their family and provide jobs for their community and a smaller, organic farmer who works alone and focuses on biodynamic practices can both claim to be sustainable. Under the three pillars definition, both would be correct, and both would also be able to argue that the other doesn’t uphold sustainable principles due to deficits in other pillars. It becomes clear that for sustainable agriculture today, an ideal cultural practice would be one that maintains equitable sustainability across all three pillars; building soil health while reducing inputs, increasing and maintaining revenue,

and providing sustenance and work for the region (Rasul and Thapa, 2004).

The goal of equitable sustainability, however, can prove troublesome. Oftentimes a cultural practice that yields the most product or makes the most money- thus being economically sustainable- also has detrimental effects on the soil microecosystem, which damages environmental sustainability (Tilman et al., 2002). For organic farmers, NOP regulation § 205.203 requires cultural practices that build soil health and fertility.

Because these practices may not always be the most profitable, farmers are sometimes left with the dissonance of choosing among practices that uphold one facet of sustainability at the expense of another.

As mentioned before, an ideal cultural practice for sustainable agriculture would be one that maintains equitable sustainability: increasing revenue, building soil health, and filling a gap in consumer demand. The potential for such a practice exists for strawberry (*Fragaria x ananassa*) production in the Upper Midwestern United States. Though the market for fresh strawberries more than quadrupled from 1990-2012, the Upper Midwest only claims 5.3% of the total acreage for U.S. strawberry production, the vast majority of which only produces fruit from June to early July (Vilsack, 2007; Perez and Plattner, 2013). This reality is in direct conflict with an increased consumer demand for locally produced fruits and vegetables (Frith, 2007). In addition, traditional cultural practices for strawberry production in the United States- such as methyl bromide fumigation and many of its chemical alternatives- have been shown to reduce soil biodiversity and health both directly and indirectly (Wilhelm & Paulus, 1980; Klose & Adjwa, 2004). Some of these conventional techniques have even been shown to contribute to ozone depletion (Ristaino

& Thomas, 1997). If cultural practices could be modified to capture a larger market share while building soil health and extend the local season, strawberry production in the Upper Midwest would begin to embody the ideals of equitable sustainability. The topics covered in this document will thus lay the foundation for holistically sustainable strawberry research to be conducted in the Upper Midwest.

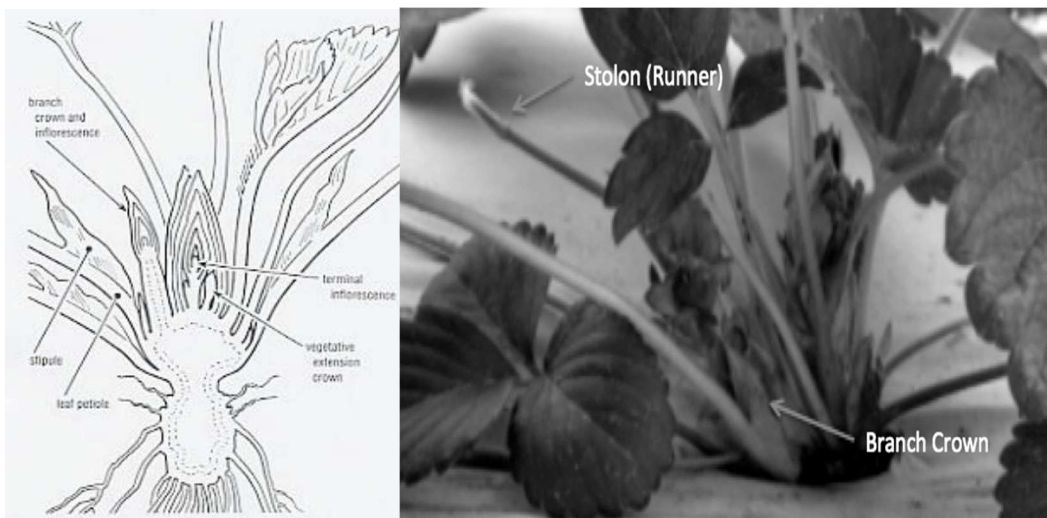
This review will first describe strawberry growth and reproduction habits as it pertains to production in the Upper Midwest, followed by a discussion on the potential for strawberry cultural practices in the region to become more equitably sustainable. Practices that contribute to environmental sustainability will be discussed first, followed by a connection of these practices to economic sustainability. Social sustainability will only be indirectly addressed because an increased social demand for local fruit has already been established, and the extended harvest season and construction time provided by these practices would likely require more labor, which would contribute to the community workforce (Frith, 2007; Lewers, 2012).

Strawberry Growth and Reproduction Habits

The strawberry plant is an herbaceous perennial with short internodes forming a modified stem rosette (Savini et al., 2005). This modified stem is commonly known as a crown, where long-petiole trifoliate leaves and axillary meristems converge spirally around its axis, ending in a terminal inflorescence (Figure 1.1) (White, 1927). Strawberry leaves present a typical dicot structure with long petioles and foliaceous basal stipules (Savini et al., 2005). Petiole length is often used as a measurement parameter for chilling requirement; petioles will be shorter when the dormant leaf bud is moved into growing

conditions before the plant's chilling requirement is met (Kronenberg & Wassenaar, 1972). Leaf lifespan can exceed 3 months in favorable conditions (Poling, 2012). Axillary meristems can differentiate into branch crowns, which stay near and are structurally identical to the original crown, or stolons (also called runners), which give rise to separate, daughter plants (Figure 1.2) (Demchak, 2011). Crowns typically produce 1-2 branch crowns in a season, but have been known to produce more than 5; from a production standpoint, 3-4 total crowns per plant is desirable, as more can result in decreased fruit size (Poling, 2012).

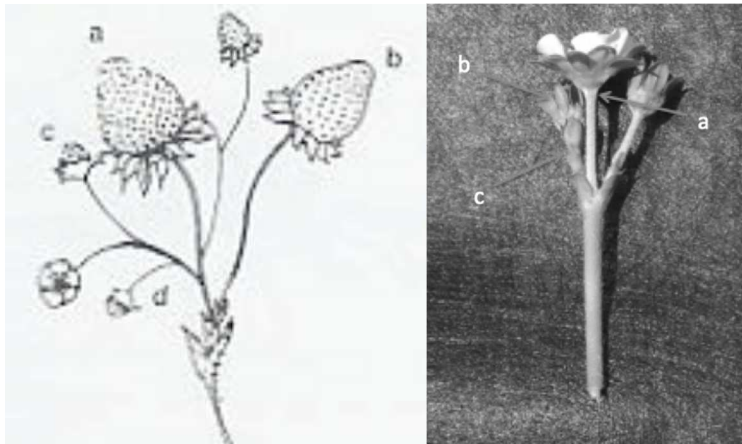
Figures 1.1 & 1.2. 1.1 (left): The crown of the strawberry plant (from Integrated Pest Management for Strawberries, Publication 3351, Univ. Calif., 1994). 1.2 (right): Axillary meristems of cultivar 'Evie-II'; photo taken July 10, 2014.



Inflorescences have two internodes and develop terminally on the crown or branch crown of the plant in a structure known as a dichasial cyme (Savini et al., 2005). Dichasial cymes have a terminal, primary flower branch with opposite secondary

branches beneath the terminal bud, leading to secondary flowers. In strawberry, the inflorescence is known as a flower cluster, and the primary flower, known as the “king flower”, typically bears the largest fruit (Thomson, 2008). Secondary branches begin at the juncture of the first and second internodes; some inflorescences have tertiary and quaternary branches and flowers as well (Figures 2.1 & 2.2).

Figures 2.1 & 2.2. 2.1 (left): Diagram of fully developed flower cluster with (a) primary flower, (b) secondary flower, (c) tertiary flower and (d) quaternary flower (from Poling, 2012). 2.2 (right): Picture of ‘Portola’ flower cluster, with (a) primary flower, (b) secondary flower bud and (c) tertiary flower bud.



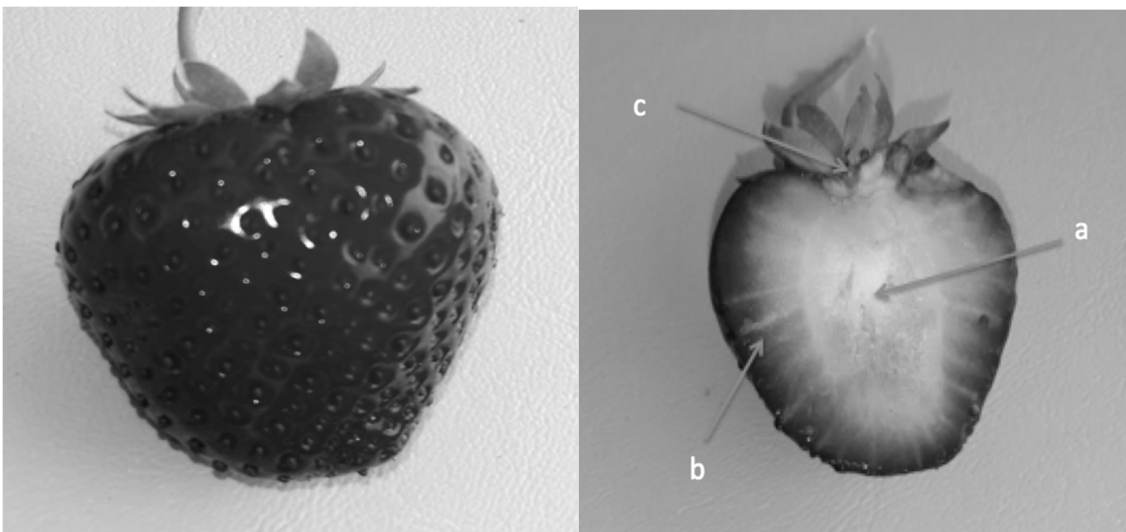
The principal parts of the flower itself are shown in Figure 3. Strawberry flowers have 5 sepals; fleshy green structures beneath the petals which enclose the flower at bud stage and eventually become the ‘calyx’, or cap of the berry. Stamens discharge pollen and fertilize the pistils, which are secured on a conical stem known as the receptacle. This receptacle becomes the full, fleshy “berry” at fruit maturity. Despite this plant’s common name, however, the fruit itself is not botanically classified as a berry- merely an enlarged receptacle. The seed-like organs embedded on the outside of the fruit are known as

achenes, tubular ovaries connected to the interior of the receptacle and hold the true seeds within their walls (Figures 4.1 & 4.2).

Figure 3. Principal flower parts of cultivar ‘Evie-II’, including (a) stamen, (b) pistil, (c) receptacle, (d) petal and (e) sepal. Photo taken July 10, 2014.



Figures 4.1 & 4.2. 4.1 (left): Profile of mature fruit ‘Amandine’, with embedded achenes. 4.2 (right): Cross section of ‘Amandine’, with (a) interior receptacle, (b) achene tube and (c) calyx.



Flower induction, initiation and development are highly variable by cultivar, and dependent on genotypic response to temperature and photoperiod (Savini et al., 2005; Stewart & Folta, 2010). These responses have been grouped into three flowering types: June-bearing, everbearing and day-neutral. Strawberry cultivars are typically classified under one of these three categorical groups based on their photoperiodic flowering habits, and it was originally assumed these habits remained constant over a scale of temperatures (Darrow, 1933). However, further research led to the discovery that the photoperiod response of many cultivars would be altered if temperatures encroached on sub- and supraoptimal levels (Guttridge, 1985; Nishiyama & Kanahama, 2000; Sonstebly & Heide, 2007). This interaction of temperature with photoperiod, known as thermo-photoperiod, adds a quantitative factor to the original categorical classifications. Thus, this review will discuss photoperiod response first assuming optimal temperature conditions, and then explore how the responses have been observed to change under different temperature ranges.

Natural flowering patterns of cultivated octoploid strawberry are of the June-bearing type (Darrow, 1966). June-bearing plants induce flowers under shortening daylengths, optimally from 9.5 to 13 hour days, depending on cultivar (Darrow, 1933). In the Upper Midwest this induction period would take place in early fall prior to dormancy. Figure 5 depicts the change in daylength over time in the Upper Midwest (specifically using Minneapolis, MN 44.9833° N as a representative point) compared to a more southern latitude where strawberries are grown (specifically using Santa Maria, CA 34.5914° N as a representative point). The figure implies that flower induction would

typically occur in mid-September for June-bearing cultivars in the Minneapolis area, until temperatures drive plants into dormancy. Savini (2005) notes that most June-bearers will also have flower initials before they enter dormancy. For many June-bearing cultivars the dormancy inducing temperature is a high of 10° C (Kronenberg et al., 1976). Figure 6 shows that the average high temperature in the Minneapolis area falls to 10° C in early November. Thus this would be the time that June-bearing cultivars transition to dormancy.

Figure 5. Average daylengths of Minneapolis, MN and Santa Maria, CA, taken on the 20th of each month. Raw data acquired from Time & Date AS

<http://www.timeanddate.com/worldclock/astronomy.html?n=3857&month=12&year=2014&obj=sun&afl=-1&day=1>

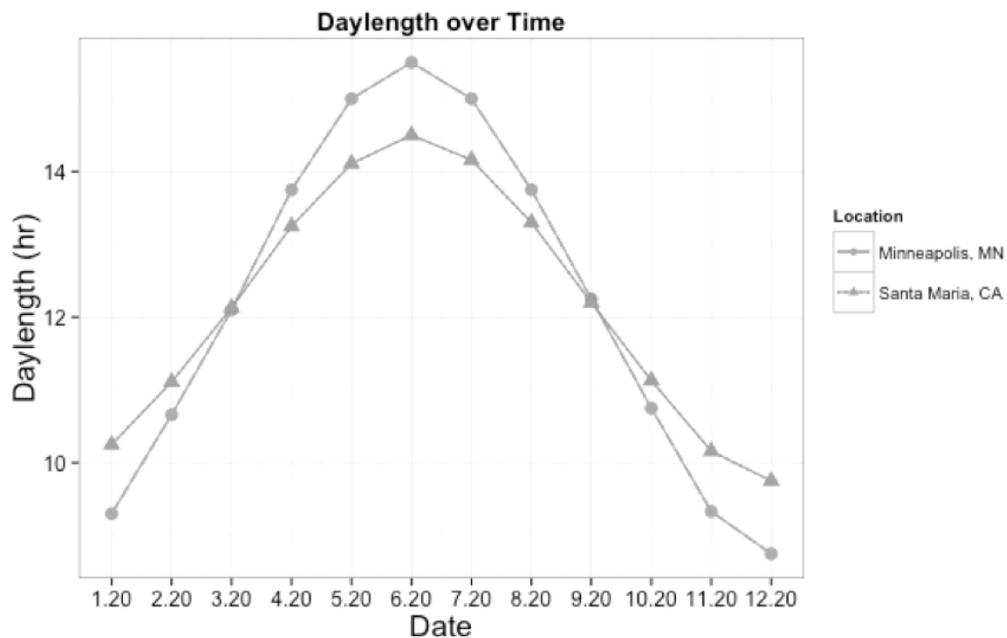
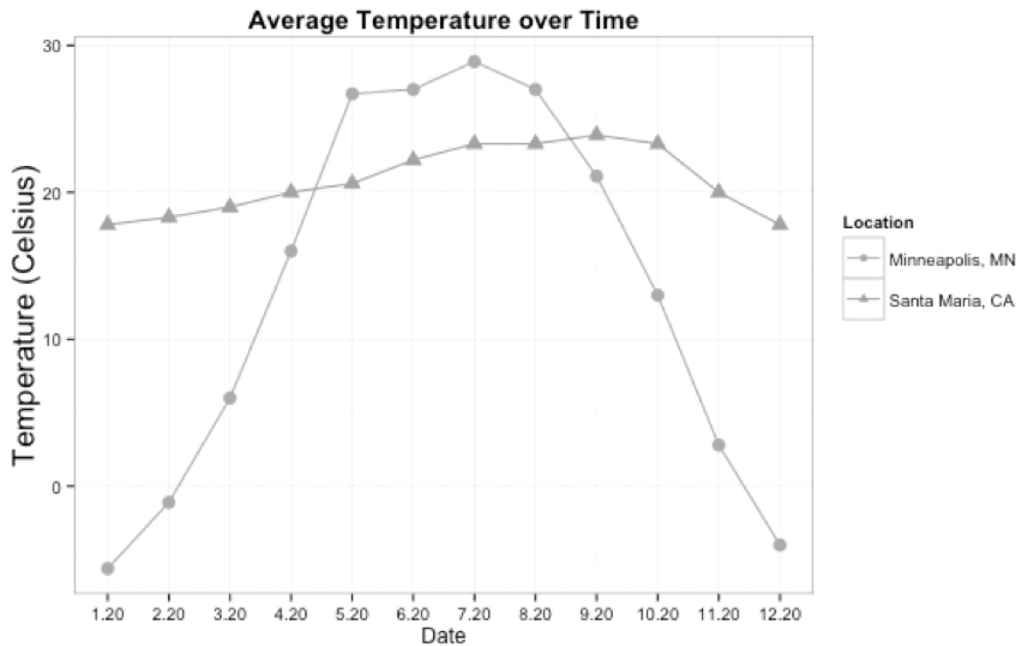


Figure 6. Average high temperatures Minneapolis, MN and Santa Maria, CA, taken on the 20th of each month. Raw data acquired from Intellicast <http://www.intellicast.com/>.



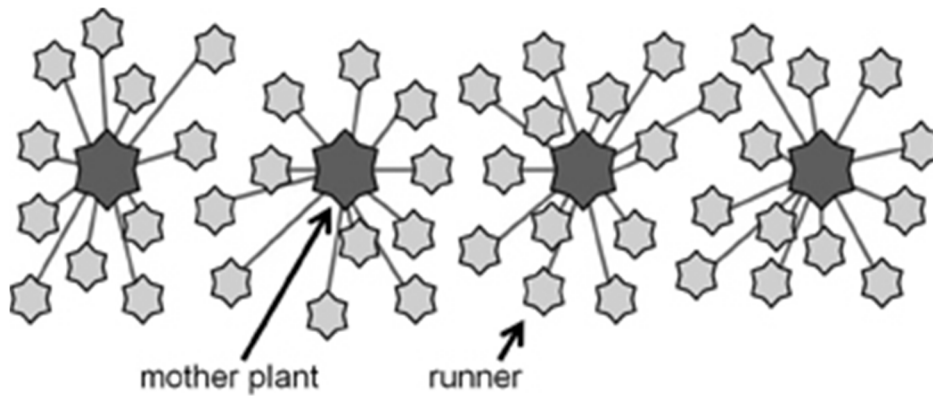
As daylength and temperatures increase the following spring, June-bearing plants stop flower induction and begin investing in flower development (Salisbury & Ross, 1992). This photoperiod mediated induction-to-development shift leads to June-bearing plants bearing high fruit yields until they run out of induced flower buds, typically in late June or early July for the Upper Midwest. Thus, June-bearing strawberry plants can be considered to have short day induction requirements and long day development requirements. Under high temperatures, $>30^{\circ}\text{C}$, June-bearing plants will experience severely reduced flower development even in optimal photoperiods (Serce & Hancock, 2005). Savini et al. (2005) also notes that the morphology and differentiation time of

inflorescences is based on the thermo-photoperiod the plant is exposed to; June-bearing plants growing in warmer, short day conditions tend to have faster and more prolific flower differentiation and shorter petiole length than plants exposed to long day, cooler conditions.

Common cultural practices treat June-bearing strawberries as a perennial crop, using what is called a ‘matted row’ system. Rooted plugs of the June-bearing crop are planted in the spring of the first year, known as the establishment year. Flower clusters are removed this entire first season, allowing the plant to invest more energy into crown/branch crown development, root development and runner production (Eames-Sheavly et al., 2003). Runners develop during the long photoperiods of summer and are arranged spatially from the crown to eventually root themselves, creating a thick, matted row of plants (Figure 7) (Archbold & MacKown, 1995). The plants then overwinter, and flower clusters are left on the plant the following spring for the first harvest. In this system, the number of leaves on each plant at the beginning of overwintering can be correlated with fruit production the following year (Poling, 2012).

Figure 7. Diagram of the matted-row system common to June-bearing cultivars. Figure taken from Colorado State Extension

<http://www.ext.colostate.edu/mg/Gardennotes/images/763-3.jpg> .



The second and third flowering types, everbearing and day-neutral, are often considered synonymous, although there are subtle differences. Everbearing cultivars include diploid alpine strawberry *Fragaria vesca*, along with various more common octoploids (Duchesne, 1766; Fletcher, 1917). Cultivars categorized as everbearers both induce and develop flowers under longer photoperiods, typically 12 hours or more. Sironval & El Tannir-Lomba (1960) found that flower induction and development of *F. vesca* var. *semperflorens* was inhibited when plants were exposed to short day treatments. Octoploid everbearers initiate most of their flowers on unrooted or recently rooted runners during the long days of summer, leading to fall harvests (Stewart & Folta, 2010). The origin of the everbearing trait appears to have occurred separately in North America and Europe, as little crossbreeding occurred between European everbearers *F. vesca* and North American everbearing *F. virginiana* cultivars (Stewart & Folta, 2010). The North American everbearing phenotype is due to a single, unstable locus within the typical June-bearing genome, while the origin of the European everbearing trait is older and more difficult to pinpoint (Darrow, 1966).

The first recorded instance of a day-neutral phenotype was *F. virginiana* sub.

glauca, and was used as a parent in commercial everbearing breeding efforts in the 1930s and 1940s (Darrow, 1966). Thus, many everbearing cultivars such as ‘Arapahoe’ and ‘Ogallala’ have day-neutral parents present in their pedigrees, and may be why everbearing and day neutral cultivars are sometimes thought to be the same (Hildreth & Powers, 1941). However, true day-neutral cultivars exhibit flowering habits that are phenotypically distinct from their everbearing relatives. The crowns of all day-neutral genotypes have a strong tendency to fruit prolifically in their first year, as oppose to most everbearers (Ahmadi & Bringham, 1991). Day-neutral runners can also have developed inflorescences before rooting occurs (Figure 8). Just as important, day-neutrals are historically documented as insensitive to changing photoperiods, fruiting at the same rate throughout a growing season of dynamic daylength (Durner et al., 1984). This distinguishes day-neutrals from everbearers, which display long day photoperiodism for flower induction and development. These traits, in addition to increased heat tolerance (Stewart & Folta, 2010), have contributed to abundant strawberry production in California, where day-neutrals performed well; other areas of the United States- such as the Upper Midwest- have not observed the same success, as day-neutrals yielded poorly in Midwestern climates and were difficult to propagate (Durner et al., 1984; Luby et al., 1987, Luby 1989). This day-neutral market advantage allows California to account for 44% of the total national strawberry acreage and almost 90% of total yields, leading to a total revenue of \$2.12 billion in 2012 (California Ag. Statistics Review, 2014; National Ag. Statistics Service, 2014). Similar to everbearing plants, the genetic basis for day-neutrality in commercial octoploid *Fragaria x ananassa* is poorly understood. Iwata et al.

(2012) discovered that diploid *F. vesca* plants homozygous for a 2-bp deletion in the *FvKSN* gene all displayed day-neutral flowering habits, though this phenomenon may not be directly applied to day-neutrality in the more complex octoploid *F. x ananassa* genome. Indeed, inconsistent inheritance ratios imply that day-neutrality is not controlled by a single locus in octoploid strawberries; two locus models offered a poor fit as well (Serce & Hancock, 2005).

Figure 8. Day-neutral ‘Monterey’ runner, with developed inflorescence.



The runnering habits of each flowering type respond to photoperiod as well. June-bearing cultivars typically do not establish runners in optimal short day photoperiods for flower development, followed by a dual flowering/runnering period as daylength increases, and finally develop runners alone in long day photoperiods (Stewart & Folta, 2010). Everbearing and day-neutral plants typically show the most prolific runnering under short day, higher temperature conditions, though there is cultivar variance (Sonsteby & Heide, 2008).

As mentioned before, there is still some debate regarding the photoperiodic nature of June-bearing, everbearing and day-neutral flower habits. While the common understanding is that June bearing cultivars display short day flower induction, everbearing cultivars display long day flower induction and day-neutral cultivars are truly photoperiod insensitive, additional research has led many to believe that the photoperiod tendencies of strawberry cultivars can be altered with temperature (Durner et al., 1984; Sonsteby & Heide, 2007). In many cases, cultivars classified under photoperiodic categories only display their classified flowering response in moderate temperature conditions; once a certain threshold temperature is breached, their photoperiodic nature changes. For example, Guttridge (1985) found that flower induction of certain June bearing cultivars can occur under any photoperiod if temperatures are $<15^{\circ}\text{C}$. Nishiyama & Kanahama (2000, 2002) demonstrated that everbearing cultivar ‘Summerberry’ and day-neutral cultivar ‘Hecker’ both experienced inhibited flowering at high temperatures ($30^{\circ}/26^{\circ}\text{C}$) when long day lengths were not present (>14 hr). This implies that some day-neutral cultivars may display long day flowering habits under heat stress. Indeed, Sonsteby & Heide (2007) found similar results when testing cultivar ‘Elan’, leading them to conclude that “...everbearing strawberry cultivars, in general, whether of the older European-type or the modern Californian-type originating from crosses with selections of *Fragaria virginiana* ssp. *glauca*, are qualitative (obligatory) LD plants at high temperature (27°C), and quantitative LD plants at intermediate temperatures. Only at temperatures below 10°C are these cultivars day-neutral.”

Such broad statements should be avoided, however, since there is considerable

variability in strawberry flowering and fruiting response to temperature, even within June-bearing, everbearing and day-neutral categories (Wagstaffe, 2009). For example, Bradford et al. (2010) discovered that day-neutral 'Tribute' plants required long day photoperiods for flowering after a threshold temperature of 26°C was breached, while day-neutral 'RH-30' plants required short day photoperiods for flowering after a threshold of temperature of 23° was breached. Thus thermo-photoperiod studies are merited on all cultivars of commercial significance, even if research has already been conducted on cultivars within their traditional photoperiod classification. Such findings would be of consequence for day-neutral production in the Upper Midwest, where summer temperatures are dynamic but near their highest in August, when daylengths dip below 14 hr (Figures 1 & 2).

Temperatures can also affect fruit production in ways that are not related to photoperiod. Kumakira & Shishido (1995) discovered that strawberry flower buds of everbearing cultivars aborted during periods of high temperature, and Karapatzak et al. (2012) found that everbearers exposed to supraoptimal temperature conditions (30°C/20°C) experienced severely reduced pollen performance leading to significantly reduced yields. Similar supraoptimal temperature effects were observed on June bearing cultivars (Ito & Saito, 1962; Durner et al, 1984). The yield reductions likely manifest as a result of unviable pollen contributing to poor fertilization and misshapen fruit (Ariza et al., 2011). These reductions in pollen performance are dependent on high night temperatures, as supraoptimal day temperatures with cool night temperatures did not result in reduced performance (Wagstaffe, 2009). The effect of supraoptimal

temperatures on flowering and yield in day-neutral cultivars is less thoroughly researched, though day-neutrals have been regarded as more heat tolerant cultivars in the past (Stewart & Folta, 2010).

Suboptimal temperatures can affect fruit development as well. Ariza et al. (2015) conducted a thorough analysis of cold temperature on differentiating inflorescences and observed that chilling events (24h at 2°C) can reduce pollen grain production and viability as early as 20 days before anthesis, and increase ovule abortion 3-6 days before anthesis. These events would be especially deleterious for June-bearing plants, as all June-bearing flower buds are developing in the spring when chilling events are more likely to occur. A chilling event on day-neutral plants may inhibit fruit production on developing inflorescences as well, but since they tend to produce inflorescences throughout the growing season it likely would not have as large an effect on cumulative yields. In the diploid *Fragaria vesca*, Davik et al. (2013) observed the accumulation of alcohol dehydrogenase, dehydrins and galactinol as biomarkers associated with cold tolerance.

It is often considered good horticultural practice to remove flower clusters from June-bearing plants for the first 4-6 weeks after initial planting (Eames-Sheavly et al., 2003); this forces the plants to invest more energy in vegetative growth and runner production, making the perennial crop more productive in subsequent years. The practice of flower cluster removal is also practiced in day-neutral production, even though they are often only grown as annuals. Interestingly Lantz et al. (2009), conducting a study in Garrett County, Maryland (39.2833° N) demonstrated no significant difference in total

yield when day-neutral ‘Seascape’ plants did not have flower clusters removed, compared to treatments where flower clusters were removed 2 and 4 weeks after planting.

Inflorescence Architecture

Strawberry flower cluster anatomy has been thoroughly researched, as possible differences in inflorescence architecture have been hypothesized to correlate with differences in yield and berry weight among cultivars (Webb et al., 1978). Savini et al. (2005) documents the most common flower cluster and inflorescence anatomy in an ‘architectural model’, with primary, secondary and tertiary flowers, as mentioned above (Figure 9a). Inflorescences that follow this architectural pattern appear to display two primary internodes leading to the primary flower, secondary branch internodes that form opposite the primary node and lead to secondary flowers, and tertiary internodes that form at the node of secondary branches, leading to tertiary flowers (Figure 10). Unlike the Savini diagram (Figure 9a), tertiary internodes can grow much longer than secondary internodes, making tertiary flowers appear ‘ahead’ of secondary flowers (Figure 10). Thus, the best way to distinguish secondary flowers from tertiary flowers is to compare the differences in flower development; secondary flowers should be further along the development path to mature fruit than tertiaries (Figure 10). Interestingly, the formation of inflorescences from new buds after planting seem to follow the same architecture as flowers forming on individual inflorescences, with secondary inflorescences branching from primary buds, and tertiary inflorescences branching from secondary buds (Figure 9b).

Figure 9. From Savini et al. (2005): (a) diagram of a single, typical strawberry

inflorescence, and (b) model of ‘Seascape’, where 1°, 2° and 3° represent primary, secondary and tertiary inflorescences, which developed from buds after initial planting.

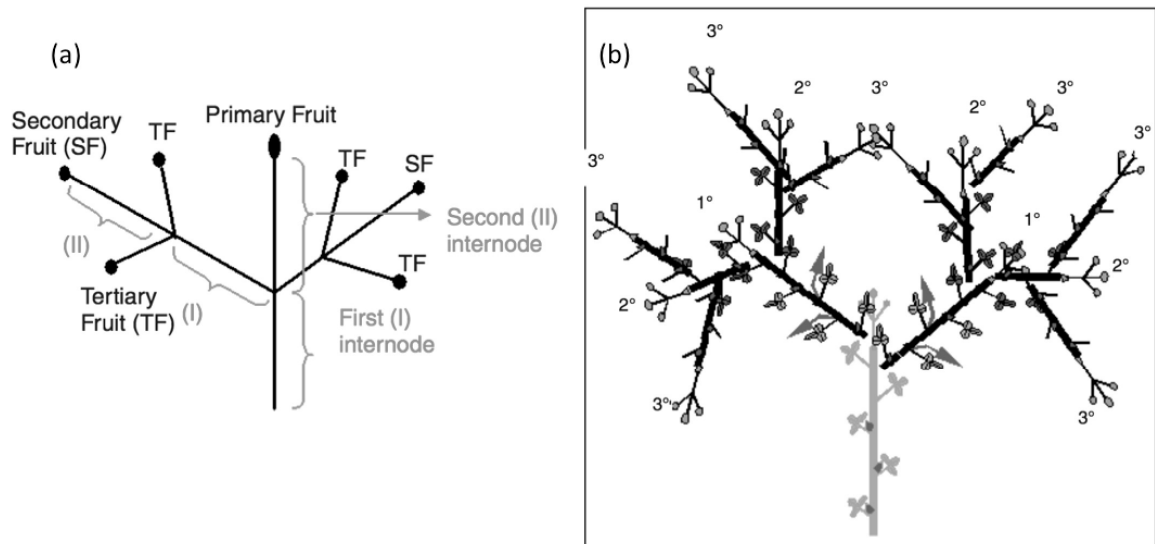
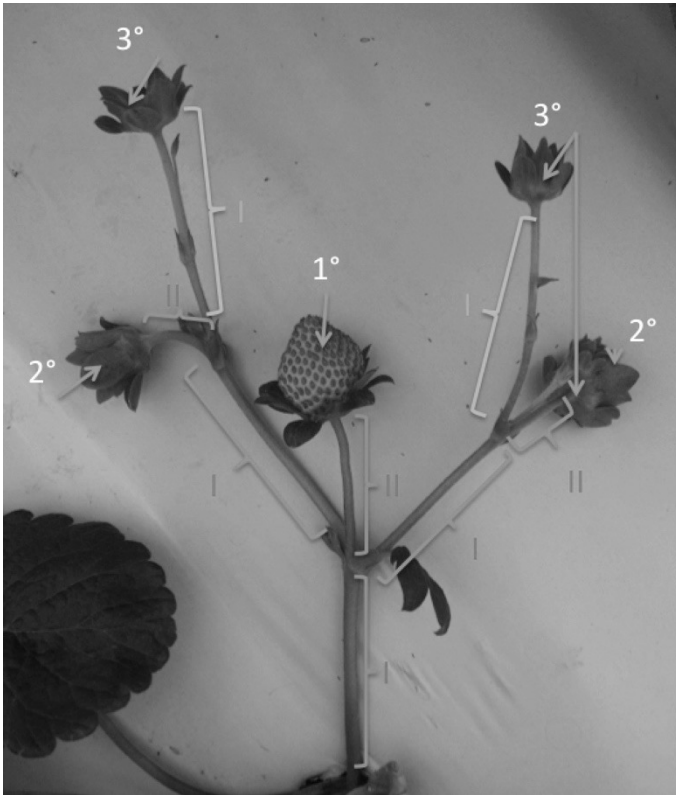


Figure 10. Photograph of ‘Portola’ inflorescence. 1°, 2° and 3° represent primary, secondary and tertiary flowers. Red brackets indicate primary internodes, purple brackets indicate secondary branch internodes, and orange brackets indicate tertiary internodes.



There is, however, observable variability from this typical inflorescence pattern, and of the day-neutral cultivars only ‘Seascape’ inflorescences have been formally documented (Hancock, 1999, Savini et al., 2005). Figure 11a shows June-bearing cultivar ‘Annapolis’ displaying the most typical inflorescence architecture, with day-neutral cultivars ‘Albion’ and ‘Seascape’ displaying similar habits (Figure 11b, 11g & 11h). ‘Monterey’ and ‘San Andreas’ inflorescences will sometimes only form a single secondary branch (Figure 11d & 11f). Occasionally, more developed inflorescences displaying this habit will create additional secondary branches, but these branches display an alternate growth habit, as opposed to the opposite secondary branching pattern of the more documented habit typical in ‘Seascape’. The inflorescences of ‘Evie-2’ and ‘Portola’ sometimes appear to form two separate primary branches, forking off the first

node (Figure 11c & 11e). Interestingly, ‘Monterey’ and ‘San Andreas’ whose inflorescences typically only form a single secondary branch, were also the two lowest yielding cultivars in University of Minnesota day-neutral trials, while ‘Evie-2’ and ‘Portola’, which seem to produce two primary branches, were the highest yielding (Petran et al., 2016).

Figure 11. Selected flower clusters of (a) ‘Annapolis’, (b) ‘Albion’, (c) ‘Evie-2’, (d) ‘Monterey’, (e) ‘Portola’, (f) ‘San Andreas’, (g,h) ‘Seascape’. Photos taken July 15, 2014.



Environmental Sustainability- Plant Nutrition

The successful production and performance of strawberry, or any food crop, is dependent upon its unique requirements for growth, development, and reproductive maturity (Reich, 2014). These requirements are complex and numerous, but can be broadly categorized into two main factors: environmental and nutritional. A plant's environmental requirements are a direct function of its physiological tolerances to abiotic factors such as temperature, photoperiod, water availability and biotic factors such as competition and pathogen stress (Koehler et al., 2012). The nutritional requirements of a plant are a function of the quantity of essential elements needed for proper growth, the presence of each element in the environment, and the ability of the plant to acquire elements from its environment (Roose et al., 2001). Because nutrition is ultimately acquired from the environment, these requirement categories interact. For example, plants whose optimal nutrient requirements are met can be more tolerant of environmental pressures and thus have expanded geographic distributions compared to undernourished plants of the same species (Schuphan, 1965; Ruiz-Lozano et al., 1995). Farmers need to be mindful of both of these categories, but are often more concerned with crop nutritional requirements, as they can be more easily accommodated and adjusted to enhance growth and production.

Accommodating the nutritional requirements of plants often manifests as altering soil nutrient content. While production crops can get a large portion of their nutrients from sources naturally present in the soil, farmers add supplemental fertility that enhances crop production. Supplemental fertility can increase the productivity of land;

however over fertilization has led to detrimental ecological side effects, such as nitrate-induced eutrophication of water, acidification of soil and release of nitrous oxide into the atmosphere (Smil, 1997). To avoid the deleterious environmental effect of over fertilizing a field, the nutrient requirement of any crop should be an amount of fertilizer that results in the potential for optimal plant growth and production while minimizing the risk of damaging other ecosystems. Such a practice would be considered environmentally sustainable. Indeed, Tagliavini et al. (2004) defines plant nutrient requirement as “those amounts that when yearly available in the soil for plant uptake maximise a desired performance of the plant (e.g. yields and fruit quality) but also minimise the environmental negative impact of cultivation”. In short, the nutrient requirement of a crop should consider a fertilizer rate that supports growth and production with minimal environmental impact.

In order to determine a fertilizer rate that upholds Tagliavini’s definition of nutrient requirement, one must obtain the specific nutritional demands of a crop in question, and also the plant available fertility naturally present in the soil. The supplemental fertilizer rate would then be the plant demand for a certain nutrient subtracted by the amount of available nutrient in the soil. This dynamic, multi-factorial equation is difficult to quantify for any crop, but should be determined nonetheless to optimize environmental sustainability (Olf et al., 2005). It is the goal of this section to first review nutrient demands of strawberry plants, followed by a discussion of soil health and fertility, and finally how to understand the interaction of these factors to determine environmentally sustainable fertility regimens for crop-soil combinations.

Strawberries, like all plants, need an array of essential nutrients for growth and survival, but from an agricultural perspective, nitrogen is usually the most important of these elements for optimal production (Smil, 1997). While other essential elements such as carbon, hydrogen and oxygen are required in higher quantities than nitrogen, they are also more readily available to the plant. The vast majority of terrestrial nitrogen is locked in the atmosphere, and only a minute fraction is naturally cycled into the soil microecosystem for plant absorption (Vitousek et al., 1997). Thus, for plants that cannot fix their own nitrogen from the atmosphere it often becomes the most limiting essential element for growth (Vitousek and Howarth, 1991). Farmers, then, concern themselves primarily with the nitrogen requirements of their crops, and often minimize plant nutrient requirement to this single essential element (Olfs et al., 2005; Frink et al., 1999). Though this minimalistic view can lead to nutritional oversight, environmentally sustainable nitrogen management practices can consequently increase the availability of other nutrients as well. For example, common cultural practices intending to increase soil nitrogen on organic farms, such as composting, manuring and reduced tillage typically add other essential elements to the soil in conjunction (Eghball & Power, 1999). These practices can also increase soil organic matter. Organic matter positively influences soils and plants alike: increasing plant residues returning to the soil, improving soil aggregation, and housing microbes responsible for transitioning soil nutrients into a plant-available form (Haynes & Naidu 1998; Kennedy, 1999). Thus comprehensive soil fertility can sometimes be attained by managing for nitrogen alone, and strawberry and soil nutrient requirement will often refer exclusively to nitrogen requirement for the

purposes of this review.

The specific nitrogen demand of strawberry is spread unevenly throughout the plant. Close to half- 49.7%- of total absorbed N from plant tissue at the end of harvest season can be found in the fruit (Lieten & Misotten, 1993; Netsby et al., 2005). By discerning where nitrogen is proportioned within plant parts, one can also calculate how much nitrogen ends up in the fruit for human consumption. Lieten & Misotten (1993) calculated a total 62.3 kg of nitrogen in the fruit of a hectare that yielded 59,450 kg of ‘Elsanta’ strawberries. This reduces to 1.04 g of nitrogen in every kilogram of strawberries. When Albregts and Howard (1978) conducted a similar study on ‘Elsanta’, they found 31.3 kg of total nitrogen in a hectare that yielded 29898 kg of fruit. Interestingly, this relationship also reduces to 1.04 g N/kg strawberries. These figures agree with Tagliavini et al. (2004), stating “Average nitrogen present per unit of fruit yield are in the range of 1.0 – 1.1 kg N per ton”, regardless of cultivar. Using these relationships, Netsby et al. (2005) hypothesizes that nitrogen uptake by the strawberry plant is not a function of genotype, but rather soil type and cultivation system, and total N removed from the field can be calculated from the end of harvest yield figures. For example, day neutral strawberry research at the University of Minnesota, Saint Paul campus produced 726.92 kg of strawberries on a 2468 m² plot in 2013 (unpublished data). Using the figures provided above, this translates to 756 g nitrogen total in the fruit of the entire harvest. As fruit accounts for nearly 50% of the nitrogen taken by an entire plant (Lieten & Misotten, 1993; Netsby et al., 2005), we can estimate that 1.51 kg nitrogen was removed from the field over the course of the season. On a 2468 m² plot,

this equals a rate of 20.11 kg N removed per hectare using our production systems in 2013.

Environmental Sustainability- Soil Health & Fertility

As mentioned previously, strawberry plants acquire nitrogen from the soil, and available soil nitrogen can be considered as the sum of nitrogen added by farming practice + inorganic nitrogen naturally present in the soil at the beginning of the field season + nitrogen mineralized over the course of the growing season (Stanford & Smith, 1972; Stanford & Smith 1973). If these two factors combine to meet strawberry nitrogen requirement, then total yield will not be limited by nitrogen availability. This section will review supplemental fertility first, followed by natural soil health.

Hochmuth et al. (1996) compared the effect of different fertigation N rates on strawberry marketable yield over 2 growing seasons. A significant jump in yield when going from 0.28 to 0.56 kg N/hectare/day was found in the 1st year, but yield plateaued as the rate increased from there- 0.84, 1.12, and 1.40 kg N/hectare/day, respectively. Authors hypothesized that total nitrogen requirement of strawberry plants was met at 0.56 kg N/hectare/day, and further increases from that rate were superfluous to the system. In the second year of the study the biggest yield increase was also from 0.28-0.56 kg N/hectare/day. The rate of 0.56 kg N/hectare/day can be transformed to 1.59 kg N/acre/week, or 31.8 kg N/acre over an entire 20-week season. Frequency of fertigation may depend on soil type. Cook and Sanders (1991) found that frequency of fertigation had no effect on tomato yield and size on heavier soils but tomatoes became larger as frequency of fertigation increased on light, sandy soils low in organic matter. Conversely,

it has also been noted that an excess of nitrogen can inhibit strawberry yields. Poling (2012) notes that excess N will create a larger than necessary canopy, which potentially slows harvest times, increases fungal disease incidence, and reduces fruit quality due to shading.

While the rate found by Hochmuth et al. above is beneficial for practices and soil types similar to the one in the study, it is less useful for growers who produce strawberries in the wide variety of other soil types and locations that exist. An ideal fertility application considers current inorganic N availability in addition to the N mineralization rate of a particular soil. The amount of supplemental N that should be provided by growers is can be summarized by the equation:

$$N_{\text{sup}} = N_{\text{pl}} - (N_{\text{a}} + N_{\text{m}})$$

Where N_{sup} is the amount of supplemental N needed, N_{pl} is the amount of plant N needed for optimal yields, N_{a} is the amount of inorganic N available in the soil at the time of planting, and N_{m} is the amount of nitrogen that will be mineralized during the growing season, as determined by the soil's mineralization rate. However no studies investigating supplemental nitrogen rates on strawberry yield in the Upper Midwest exist. Such a study, along with the knowledge of general soil health and nitrogen availability would be highly beneficial for Midwestern growers interested in adding day-neutral strawberry production to their farms.

Soil health has an immense impact on crop quality; healthier soils often lead to higher yields and more nutritious crops (Borowicz, 2009; Moore-Kucera et al., 2008). Indeed, the National Organic Program has identified this correlation, and requires farmers

to actively build soil health in order to retain organic certification, as stated in NOP regulation § 205.203. However in order to quantify soil health it must first be succinctly defined, so as to clarify how it can be measured. Doran and Zeiss (2000) define soil health as "...the capacity of soil to function as a vital living system, within ecosystem and land-use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health." Not unlike sustainability, soil health is a broad concept, and thus could be measured many ways. Moore-Kucera et al. (2008) lists empirically measurable soil functions including "...the capacity to supply nutrients, retain optimal water content, support soil food webs, recycle nutrients, maintain microbial diversity, remediate pollutants, and suppress plant pathogens". However, since farmers are most concerned with how soil health impacts the yield and quality of their crops, we will focus on soil functions that directly impact those ends.

Of the soil functions that influence crop production, soil microbial ecosystems are one of the most important (Doran, 1980). The quantity of microbial life in soils is typically referred to as soil microbial biomass, SMB. An abundant SMB leads to increased availability of soil nutrients to plants; more than 90% of all nutrients in the soil food web are cycled through microbial biomass to higher trophic levels (Kennedy, 1999). Because of this microbial benefit to crops, organic farmers usually desire high levels of SMB in their soils and can adapt their cultural practices to optimize the SMB microenvironment (Abawi and Widmer, 2000; Peters et al., 2003). SMB levels can be affected by farm cultural practices such as tillage (Doran, 1980), cropping system (Acosta-Martinez et al., 2003) and fertilizer regimen (Kramer et al., 2006). Organisms

functioning within higher trophic levels of the soil microecosystem, such as nematodes, also exhibit similar responses to cultural practices (Forge et al., 2005; Neher and Olson, 1999). This relationship shows that cultural practices affect the makeup the soil microbial and faunal ecosystem, thus also affecting the availability of nutrients in the soil. A wise selection of soil health building cultural practices can prime intrinsic fertility for plants.

Despite its established role in soil fertility, there is a lack of research examining the effect of organic strawberry cultural practices on soil microbial communities (Goh et al., 2001; Yao et al., 2005). Indeed, studies that have shown correlations between strawberry yield and cultural practice have hypothesized soil health as a driving function in the relationship, but this has yet to be empirically quantified (Fan et al., 2011, Lewers, 2012). A study directly measuring possible differences in SMB between different strawberry production practices would fill this void.

The quantitative measuring of SMB is often accomplished through chloroform fumigation methodologies, as explained by Brookes et al. (1985) and Jenkinson et al. (2004) Soil cores (5 cm. diameter) are taken from random field locations, sieved at 4 mm and subsampled for water content. The core is then split into 2 samples, one for fumigation and one as control, and placed into beakers. Each beaker is then put into separate vacuum desiccators lined with moist filter paper. 40 ml of chloroform is placed into a glass vessel containing 3 boiling chips and placed into the desiccator designated for chloroform fumigation. Desiccator is closed, and fumigation proceeds for 24 hr. Control desiccator without chloroform is closed for this duration as well. After fumigation period, samples are placed into a fume hood and desiccators are washed. Samples are returned to

their desiccators, which are then evacuated with water pumps 5 times for 2 minutes and high-vacuum oil pumps 4 times for 5 minutes to remove chloroform. After evacuation, samples receive 1 g of unfumigated ‘inoculum’ soil from the original soil core, and enough distilled water to achieve 55% water holding capacity. Moist samples are then placed into 2-quart wide-mouth glass mason jars, closed with rubber septa and incubated for 10 days at 25° C. Organic Carbon analysis is performed after incubation using a Dohrmann analyzer.

To determine soil microbial biomass nitrogen, 3.5 grams of incubated soil is mixed with 150 mL 2M KCl and placed in a high speed reciprocal shaker at high speed for 30 minutes. After 10 minutes of settling, 30 mL of soil slurry is placed into centrifuge tubes and spun at 3000 rpm for 10 minutes. 15-20 mL of supernatant is transferred to clear, plastic scint vials and submitted for Nitrogen analysis. Unincubated soil is treated with these methods as a control to measure mineral N. If SMB levels are taken from each cultural practice at the beginning and end of the growing season, the temporal differences in SMB content can be compared among practices for statistical significance. Thus, we can empirically determine if a certain cultural practice has soil building or soil inhibiting tendencies relative to other practices.

While chloroform fumigation methodologies are still widely used in research, recently developed assays such as permanganate oxidizable carbon (POX-C) tests also reflect key soil processes. Compared to chloroform fumigation practices, POX-C tests are “...rapid, inexpensive and can be modified for use in the field, and in low-cost fee-for-service soil testing for commercial growers” (Culman et al., 2012). Thus POX-C testing

may be better equipped for farmers outside of research settings to test their soils for labile carbon in their soils.

POX-C testing utilizes the oxidizing potential of permanganate on easily decomposed organic material in the soil. Soil organic material (SOM) is heterogeneous, consisting of older, more recalcitrant humus, and younger pools of more easily decomposed plant material (Janssen, 1984). The younger pool disproportionately contributes to soil biological activity because its easily decomposable nature serves as the fuel for microbial processes; cultural practices can be modified to maximize this pool and contribute to farm-friendly soil health (Wander et al., 1994). Permanganate solutions are purple in color when in their natural, oxidized state. When combined with the highly reduced carbon young SOM, redox reactions occur that cause the permanganate to lose its color. This color loss can be measured with a spectrophotometer to quantify carbon present in young, labile SOM.

Economic Sustainability

As mentioned earlier, day-neutral strawberry performance in the Upper Midwest has been anemic, leading to primarily June-bearing production within the region (Luby, et al. 1987; Luby, 1989). USDA census data and yield projections based on 20 years of University of Minnesota research approximate June-bearers to average 6,160 kg/ha⁻¹/year 0.74 kg/m of row/year, after the initial establishment year. The most recent federal data estimates commercial strawberry area in Minnesota at 161.9 ha⁻¹, almost all of which can be assumed to be June-bearing (Natl. Agr. Stat. Serv., 2007). This translates to 997,304 kg of strawberries produced annually in Minnesota. Thinking in terms of consumption,

the above figure implies Minnesotans eat >0.1 kg of strawberries from local sources on average each year - a mere two servings. This reality is in direct conflict with the established demand for locally produced fruits (Frith, 2007).

The price of fresh strawberries averaged across selected major and minor production states was \$2.90/kg in 2012 (Natl. Agr. Stat. Serv., 2013). Using that price, the 997,304 kg of strawberries produced in Minnesota was worth \$2.89 million or \$17,864/ha⁻¹ gross. The incorporation of newer day-neutral cultivars in conjunction with best practices in the field shows great promise to improve these numbers, and thus improve economic sustainability (Lewers, 2012). Preliminary data from current University of Minnesota research calculated yield across cultivars averaging 19,054 kg/ha⁻¹/year for plasticulture without low tunnels and 23,538 kg/ha⁻¹/year for plasticulture with low tunnels. Again assuming \$2.90/kg, this would increase the average annual gross revenue for growers by \$37,392 or \$50,396 per hectare when using day neutral cultivars combined with plasticulture, without and with low tunnels, respectively. The increase in revenue becomes even more impressive when one considers the supplemental premium farmers can place on berries produced at a locally unconventional time (Rowley, 2010).

Rationale and Objectives

Although strawberry is a heavily researched crop in the United States, relatively little is known about the performance of new day-neutral cultivars in the Upper Midwest. There is also a lack of research regarding the effect of strawberry low tunnel production in the region, compared with other cultural practices. Thus a primary issue is to discover how new day-neutral cultivars perform under different cultural practices in the Upper

Midwest, using total yield, berry weight, total soluble solid (TSS) content, total phenolic content (TPC), environmental tolerance and soil health as performance parameters.

Despite a growing demand for access to locally produced foods, the growing season (and thus availability) of strawberries in the Upper Midwest is limited. This is due to commercial enterprises almost exclusively growing June-bearing cultivars in the region, which have a total harvest season of 6 weeks, on average. Though day-neutral cultivars with vastly extended seasons have been available for decades, they have historically not performed at commercially viable levels in the Upper Midwest due to poor yields, soft fruit and limited reproduction. The release of newer day-neutral cultivars combined with the adoption of more promising cultural practices merits a revisiting of day-neutral research in the region, to determine if the problems that affected day-neutrals in the past have been overcome.

Because day-neutral strawberry research has not been conducted in the Upper Midwest for a considerably long time, I was compelled to inquire: How well do 6 new day-neutral cultivars ('Albion', 'Seascape', 'Evie-2', 'Monterey', 'San Andreas', 'Portola') perform in terms of total yield, berry weight, TPC and TSS content in different cultural practices at sites with different environmental conditions? Is there a yield, weight, TPC or TSS difference among cultivars, and is there a consistently optimal cultivar/practice combination across different sites and years? How do the combinations perform in terms of yield and berry weight compared to June-bearing systems common to the region? The National Organic Program requires that farmers actively build soil health in order to become organically certified. Thus I also asked: what are the soil building

characteristics of each cultural practice, in terms of soil microbial biomass and permanganate oxidizable carbon?

Objective 1

To compare vegetative and reproductive parameters among day-neutral cultivars using three different organically certified cultural practices, at two different sites over the course of two growing seasons.

Rationale

‘Albion’, ‘Seascape’, ‘Evie-2’, ‘Monterey’, ‘San Andreas’, and ‘Portola’ are day-neutral strawberry cultivars that have yet to be studied in the Upper Midwest. Thus, we do not know how they perform in the region, or if there are any differences among cultivars. Since many of these cultivars have displayed high yields in other areas, a study of how the cultivars perform in the Upper Midwest is merited.

Three organically approved cultural practices (straw mulch, plastic mulch and plastic mulch with low tunnels) will be used to determine if vegetative and reproductive parameters are influenced by these practices. The practices were selected due to their commercial popularity and history of study within the literature (Fan et al., 2011; Gast and Pollard, 1991; Lewers, 2012). Determining the influence of cultural practice on vegetative and reproductive parameters will provide valuable insight for growers considering day-neutral production in the Upper Midwest.

Objective 2

To determine if the selected cultural practices (straw mulch, plastic mulch and

plastic mulch with low tunnels) differ in the ways they influence soil health.

Rationale

Because organic growers are required to actively build soil health over time to remain certified (NOP regulation § 205.203), a study of how cultural practice may affect soil systems is important. To measure soil health, we will perform assays determining SMB-C, SMB-N and labile carbon. If SMB readings are taken from the soil of each practice at the beginning and end of the field season, one can determine if practices have different effects on soil building capabilities. Similarly, the permanganate oxidizable carbon assay (POX-C), which quantifies labile soil carbon, can be used to infer soil microbial activity (Culman et al., 2012). Differences in POX levels of each cultural practice over time can demonstrate possible differences in the soil building characteristics of each practice.

CHAPTER 2

**THE FLOWERS OF *Fragaria x ananassa*: MORPHOLOGY, RESPONSE TO
PHOTOPERIOD AND GENETICS OF INDUCTION**

(submitted to *Horticultural Reviews*)

Andrew Petran

Summary

The common cultivated strawberry (*Fragaria x ananassa*) is a healthy and popular fruit throughout the world, but its octoploid genetic structure poses difficulties to breeders and growers who wish to optimize production in diverse environmental settings. In particular, the plants flowering response to temperature and photoperiod has been challenging to predict, resulting in multiple flowering phenotypes throughout the commercial germplasm. This chapter reviews the morphology and physiology of these phenotypes, the cultural practices which are common to each flowering response, and focuses on recent efforts to map the genetic basis of day-neutrality within *F. x ananassa* and its progenitor *Fragaria vesca*.

Introduction

The strawberry (*Fragaria* spp.) is one of the most widely distributed fruit crops in the world. Production of the fruit is present in almost every continent and has exceeded 4 million tonnes per year since 2007 (Wu et al. 2012). There is considerable genetic diversity within strawberry germplasm; wild diploid through decaploid plants have been discovered (Stewart and Folta 2010). This diversity leads to genotypic and phenotypic variance even within the same strawberry species. Perhaps the most commercially important variance is that of flowering habit within the commercially cultivated strawberry *Fragaria x ananassa*.

Because of its commercial value and popularity, the strawberry is a thoroughly documented fruit crop. The purpose of this review is to compile and contrast the morphologic and physiologic traits of *Fragaria x ananassa* flowering types and review the most recent efforts to identify the underlying genetics behind flowering habit.

Strawberry growth, reproduction and commercial management

A. Vegetative Growth

The strawberry plant is an herbaceous perennial with short internodes forming a modified stem rosette (Savini et al. 2005). This modified stem is commonly known as a crown, where long-petiole trifoliate leaves and axillary meristems converge spirally around its axis, ending in a terminal inflorescence (White 1927). Strawberry leaves present a typical dicotyledonous structure with long petioles and foliaceous basal stipules (Savini et al. 2005). Leaf lifespan can exceed 3 months in favorable conditions (Poling 2012). Axillary meristems can differentiate into branch crowns, which stay near and are structurally identical to the original crown, or stolons (also called runners), which give rise to separate daughter plants (Fig. 1) (Demchak 2011). Crowns typically produce one to two branch crowns in a season, but have been known to produce more than five; from a production standpoint, three to four total crowns per plant is desirable, as more can result in decreased fruit size (Poling 2012).

B. Flower Structure

Inflorescences have two internodes and develop terminally on the crown or branch crown of the plant in a structure known as a dichasial cyme (Savini et al. 2005). Dichasial cymes have a terminal, primary flower branch with opposite secondary branches beneath

the terminal bud, leading to secondary flowers. In strawberry, the inflorescence is commonly known as a flower cluster, and the primary flower, known as the “king flower”, typically bears the largest fruit. (Thomson 2008). Secondary branches begin at the juncture of the first and second internodes; some inflorescences have tertiary and quaternary branches and flowers as well (Figs. 2.1 and 2.2).

The principal parts of the flower itself are shown in Figs. 3.1 and 3.2. Strawberry flowers have five sepals; fleshy green structures beneath the petals which enclose the flower at bud stage and eventually become the ‘calyx’, or cap of the berry. Stamens discharge pollen and fertilize the pistils, which are secured on a conical stem known as the receptacle. This receptacle becomes the full, fleshy “berry” at fruit maturity. Despite this plant’s common name, the fruit itself is not botanically classified as a berry. The seed-like organs embedded on the epidermis of the receptacle are actually modified dry fruits known as achenes. Each achene is connected to the interior of the receptacle by fibrovascular strands, and hold the true seed within their pericarp (Fait et al. 2008) (Figs. 4.1 and 4.2). In *Fragaria vesca* auxin and gibberellin biosynthesis occurs in the endosperm and seed coat of the developing achenes, which in turn triggers the maturity of the surrounding receptacle (Kang et al., 2013). Because the strawberry fruit contains multiple achenes and is comprised of a receptacle in addition to its ovaries it can be classified as both an aggregate and accessory fruit.

C. Flower Induction, Initiation and Development

Flower induction, initiation and development are highly variable by cultivar, and dependent on genotypic responses to temperature and photoperiod (Savini et al. 2005;

Stewart and Folta 2010). These responses are commonly grouped into three flowering categories: June-bearing, everbearing and day-neutral. Strawberry cultivars are typically classified under one of these three categories based on their photoperiodic flowering habits, and it was originally assumed these habits remained constant over a wide range of temperatures (Darrow and Waldo 1933). However, further research led to the discovery that the photoperiod response of many cultivars would be altered if temperatures were either sub- or supraoptimal (Guttridge 1985; Nishiyama and Kanahama 2000; Sonstebly and Heide 2007). This interaction of temperature with photoperiod, known as thermo-photoperiod, adds a quantitative factor to the original categorical classifications. Indeed some believe it improper to assign broad flower habit categories to strawberry at all, as photoperiod responses appear to be cultivar specific (Durner, 2015). However as the vast majority of strawberry publications use these classifications, this review will utilize them as well with the implicit understanding of variance and interaction even within each flowering type. This section will discuss photoperiod response and common cultural practices of the three groups assuming optimal temperature conditions, and then explore how the responses have been observed to change under different temperature ranges.

1. June-bearing

Natural flowering patterns of cultivated octoploid strawberry, *F. x ananassa*, are of the June-bearing type (Darrow 1966). June-bearing cultivars are predominantly grown for commercial purposes in the Upper Midwestern United States, where other flowering types have historically performed poorly (Durner et al. 1984; Luby et al. 1987, Luby 1989). June-bearing cultivars induce flowers under shortening daylengths, optimally from

9.5 to 13 hour days, depending on cultivar (Darrow 1933). Fig. 5 depicts the change in daylength over time in the United States Upper Midwest (specifically using Minneapolis, MN 44.9833° N as a representative point) compared to a more southern latitude where strawberries are also grown (specifically using Santa Maria, CA 34.5914° N as a representative point). The figure implies that flower induction would typically occur in mid-September for June-bearing cultivars in the Minneapolis area, until temperatures drive plants into dormancy. Savini et al. (2005) notes that June-bearing cultivars will also have flower initials before they enter dormancy. For many June-bearing cultivars the dormancy inducing temperature is a high of 10° C (Kronenberg et al. 1976). On average this threshold temperature will be reached in early November in the United States Upper Midwest (Fig. 6).

As daylength and temperatures increase the following spring, June-bearing plants stop flower induction and divert resources into flower development (Salisbury and Ross 1992; Nishizawa & Shishido, 1998). This induction-to-development shift leads to June-bearing plants bearing high fruit yields until the induced flower buds are depleted, typically in late June or early July. Thus, June-bearing strawberry plants can be considered to have short day induction requirements and long day development requirements. Under high temperatures (>30°C), June-bearing plants will experience severely reduced flower development even in optimal photoperiods (Serce and Hancock 2005). Savini et al. (2005) also noted that the morphology and differentiation time of inflorescences is based on the thermo-photoperiod the plant is exposed to; June-bearing plants growing in warmer, short day conditions tend to have faster and more prolific

flower differentiation and shorter petiole lengths than plants exposed to long day, cooler conditions.

Common cultural practices treat June-bearing strawberries as a perennial crop, typically using a ‘matted row’ system. Rooted plugs of the June-bearing crop are planted in the spring of the first year (the “establishment” year). Flower clusters are typically removed during this entire first season, allowing the plant to divert more reserves into crown/branch crown development, root development and runner production (Eames-Sheavly et al. 2003). June-bearing cultivars rarely establish runners during early season flower development. However, both flowering and runnering period take place as daylength increases, and finally runners alone are developed during the hottest, longest photoperiods of the summer (Stewart and Folta 2010). Growers often arrange runners spatially from the crown to eventually root themselves, creating a thick, matted row of plants (Fig. 7) (Archbold and MacKown 1995). The plants then overwinter, and flower clusters induced during the short daylengths of fall are left on the plant the following spring for the first harvest. In this system, the number of leaves on each plant at the beginning of overwintering can be correlated with fruit production the following year (Poling 2012).

2. Everbearing and Day-neutral

The second and third flowering types, everbearing and day-neutral, are sometimes considered synonymous, likely due to crossover in pedigrees. Everbearing cultivars include the diploid alpine strawberry *Fragaria vesca*, along with various more common octoploids (Duchesne 1766; Fletcher 1917). Cultivars categorized as everbearing

cultivars both induce and develop flowers under longer photoperiods, typically 12 hours or more. Sironval and El Tannir-Lomba (1960) found that flower induction and development of *F. vesca* var. *semperflorens* was inhibited when plants were exposed to short day treatments. Octoploid everbearing cultivars initiate most of their flowers on unrooted or recently rooted runners during the long days of summer, leading to fall harvests (Stewart and Folta 2010). The origin of the everbearing trait appears to have occurred separately in North America and Europe, as little crossbreeding occurred between European everbearing *F. vesca* and North American everbearing *F. virginiana* cultivars (Stewart and Folta 2010). The North American everbearing phenotype is due to a single, unstable locus within the typical June-bearing genome, while the origin of the European everbearing trait is older and more difficult to identify (Darrow 1966).

The first recorded instance of a day-neutral phenotype was *F. virginiana* sub. *glauca*, and was used as a parent in commercial everbearing breeding programs in the 1930s and 1940s (Darrow 1966). *F. vesca* may display day-neutrality as well (Iwata et al. 2012). Many everbearing cultivars such as ‘Arapahoe’ and ‘Ogallala’ have day-neutral parents present in their pedigrees, which may contribute to why everbearing and day neutral cultivars are sometimes thought to be the same (Hildreth and Powers 1941). However, true day-neutral cultivars often exhibit flowering habits that are phenotypically distinct from their everbearing relatives. The crowns of all day-neutral genotypes have a strong tendency to fruit prolifically in their first year, as opposed to most everbearing genotypes (Ahmadi and Bringham 1991). Day-neutral runners can also develop inflorescences before rooting occurs (Fig. 8). Just as important, day-neutral cultivars are

historically documented as insensitive to changing photoperiods, fruiting at the same rate throughout a growing season of dynamic daylength (Durner et al. 1984). This distinguishes day-neutral cultivars from everbearing cultivars, which display long day photoperiodism for flower induction and development. These traits, in addition to increased heat tolerance (Stewart and Folta 2010), have contributed to abundant strawberry production in California, where day-neutral cultivars performed well. Other areas of the United States, such as the Upper Midwest, did not observe the same success, as day-neutrals yielded poorly in Midwestern climates and were difficult to propagate (Durner et al. 1984; Luby et al. 1987, Luby 1989). This day-neutral market advantage allows California to account for 44% of the total national strawberry acreage and almost 90% of total yields, leading to a total revenue of \$2.12 billion in 2012 (California Agric. Statistics Review 2014; National Ag. Statistics Service 2014).

In environments where they are commercially viable, day-neutral phenotypes are typically managed as annual plants in raised-bed systems with drip-tape irrigation and plastic mulch. There has been an abundance of research conducted on cultivar/plastic combinations, with the consensus being that yearly and environmental variances complicate the development of a single, optimal cultural practice for day-neutral production (Himelrick et al. 1992; Hughes et al. 2013). Recently, high tunnels structures that increase air and soil temperatures offer season extension potential and have been shown to increase total and marketable yields in day-neutral strawberry cultivars without inhibiting pollination (Kadir et al. 2006). However there has been a documented increase in fungal disease incidence in high tunnel systems due to reduced air circulation

(Kennedy et al. 2013).

It is often considered good horticultural practice to remove flower clusters from June-bearing plants for the first 4-6 weeks after initial planting (Eames-Sheavly et al. 2003); this forces the plants to partition more metabolites into vegetative growth and runner production, making the perennial crop more productive in subsequent years. The practice of flower cluster removal is also practiced in day-neutral production, even though day-neutral cultivars are often only grown as annuals. Interestingly Lantz et al. (2009), conducting a study in Garrett County, Maryland (39.2833° N) demonstrated no significant difference in total yield when day-neutral 'Seascape' plants did not have flower clusters removed compared to treatments where flower clusters were removed two and four weeks after planting.

3. Thermoperiod and temperature effects

There is still some uncertainty regarding the photoperiodic nature of June-bearing, everbearing and day-neutral flowering habits. While the common consensus is that June-bearing cultivars display short day flower induction, everbearing cultivars display long day flower induction and day-neutral cultivars are truly photoperiod insensitive, additional research has led many to believe that the photoperiodic tendencies of strawberry cultivars can be altered with temperature (Durner et al. 1984; Sonstebly and Heide 2007). In many cases, cultivars classified under photoperiodic categories only display their classified flowering response in moderate temperature conditions; once a certain threshold temperature is exceeded, their photoperiodic nature changes. For example, Guttridge (1985) found that flower induction of certain June bearing cultivars

can occur under any photoperiod if temperatures are $<15^{\circ}\text{C}$. Nishiyama and Kanahama (2000) demonstrated that day-neutral cultivar ‘Hecker’ had inhibited flowering at high temperatures ($30^{\circ}/26^{\circ}\text{C}$) when long day lengths were not present (>14 hr). This implies that some day-neutral cultivars may display long day flowering habits under high temperature conditions. Indeed, Sonstebj and Heide (2007) found similar results when testing the cultivar ‘Elan’, leading them to conclude that “...everbearing strawberry cultivars, in general, whether of the older European-type or the modern Californian-type originating from crosses with selections of *Fragaria virginiana* ssp. *glauca*, are qualitative (obligatory) LD plants at high temperature (27°C), and quantitative LD plants at intermediate temperatures. Only at temperatures below 10°C are these cultivars day-neutral.”

Such general statements should be avoided, however, since there is considerable variability in strawberry flowering and fruiting response to temperature, even within the June-bearing, everbearing and day-neutral categories (Wagstaffe 2009). For example, Bradford et al. (2010) discovered that plants of the day-neutral cultivar ‘Tribute’ required long photoperiods for flowering after a threshold temperature of 26°C was exceeded, while plants of the day-neutral cultivar ‘RH-30’ required short photoperiods for flowering once temperature exceeded 23° . This variance of thermo-photoperiod within a flowering category suggests that study is merited on all cultivars of commercial significance, even if research has already been conducted on similar cultivars within their traditional photoperiod classification.

Temperatures can also affect fruit production in ways that are not related to

photoperiod. Kumakira and Shishido (1995) observed that strawberry flower buds of everbearing cultivars aborted during periods of high temperature (30°C), and Karapatzak et al. (2012) found that everbearing cultivars exposed to supraoptimal temperatures (30°C/20°C) experienced severely reduced pollen viability leading to significantly reduced yields. Similar supraoptimal temperature effects were observed with June bearing cultivars (Ito and Saito 1962; Durner et al. 1984). Yield reductions likely manifest as a result of unviable pollen contributing to poor fertilization and misshapen fruit (Ariza et al. 2011). These reductions in pollen viability appear to be dependent on high night temperatures, as supraoptimal day temperatures with cool night temperatures did not result in reduced viability (Wagstaffe 2009). The effect of supraoptimal temperatures on flowering and yield in day-neutral cultivars is less thoroughly researched, though day-neutral cultivars have previously been regarded as being more heat tolerant (Stewart and Folta 2010).

Suboptimal temperatures can affect fruit development as well. Ariza et al. (2015) conducted a thorough analysis of cold temperature on differentiating inflorescences and observed that chilling events (24h at 2°C) can reduce pollen grain production and viability as early as 20 days before anthesis, and increase ovule abortion 3-6 days before anthesis. These events would be especially deleterious for June-bearing plants, as all June-bearing flower buds are developing in the spring when chilling events are more likely to occur. A chilling event on day-neutral plants may inhibit fruit production on developing inflorescences as well, but since they tend to produce inflorescences throughout the growing season it likely would not have as large an effect on

cumulative yields. In the diploid *Fragaria vesca*, Davik et al. (2013) observed the accumulation of alcohol dehydrogenase, dehydrins and galactinol as biomarkers associated with cold tolerance.

Inflorescence architecture

Strawberry flower cluster anatomy has been thoroughly researched, as possible differences in inflorescence architecture have been hypothesized to correlate with differences in yield and berry weight among cultivars (Webb et al. 1978). Savini et al. (2005) documents the most common flower cluster and inflorescence anatomy in an architectural model, with primary, secondary and tertiary flowers (Fig. 9a). Inflorescences that follow this architectural pattern appear to display two primary internodes leading to the primary flower, secondary branch internodes that form opposite the primary node and lead to secondary flowers, and tertiary internodes that form at the node of secondary branches, leading to tertiary flowers (Fig. 11). Unlike the Savini diagram (Fig. 9a), tertiary internodes can grow much longer than secondary internodes, making tertiary flowers appear “ahead” of secondary flowers (Fig. 11). Thus, the best way to distinguish secondary flowers from tertiary flowers is to compare differences in flower development; secondary flowers should be further advanced along the development path to mature fruit than tertiary flowers (Fig. 11). Interestingly, the formation of inflorescences from new branch crowns after planting follows the same architecture as flowers forming on individual inflorescences, with secondary branch crowns branching from the primary crown, and tertiary branch crowns branching from secondary branch crowns (Fig. 9b).

There is, however, observable variability from this typical inflorescence pattern, and of the day-neutral cultivars only ‘Seascape’ inflorescences have been formally documented (Hancock 1999; Savini et al. 2005). Fig. 10a shows June-bearing cultivar ‘Annapolis’ displaying the most typical inflorescence architecture, with day-neutral cultivars ‘Albion’ and ‘Seascape’ displaying similar habits (Figs. 10b, 10g and 10h). ‘Monterey’ and ‘San Andreas’ inflorescences will sometimes only form a single secondary branch (Figs. 10d and 10f). Occasionally, more developed inflorescences displaying this habit will create additional secondary branches, but these branches display an alternate growth habit, as opposed to the opposite secondary branching pattern of the more documented habit typical in ‘Seascape’. The inflorescences of ‘Evie-2’ and ‘Portola’ sometimes appear to form two separate primary branches, forking off the first node (Figs. 10c and 10e). Interestingly, ‘Monterey’ and ‘San Andreas’, whose inflorescences typically only form a single secondary branch, were also the two lowest yielding cultivars in 2013 University of Minnesota trials of day-neutral cultivars, while ‘Evie-2’ and ‘Portola’, which seem to produce two primary branches, were the highest yielding (Petran et al. 2015). While causation cannot be applied, these findings do raise the question of inflorescence architecture/yield relationships for further research.

Genetics of flower induction

The underlying genetics that promote or inhibit flowering is complex and debated, and in order to appreciate that complexity in strawberry, the history of genetic flowering research in general can provide some background. The idea of florigen, a plant hormone (or family of hormones) responsible for flower initiation and development in all

flowering plant species was proposed by Chailakhyan (1936) after a series of grafting experiments. A quest to isolate and identify the florigen hormone took place thereafter, spanning the rest of the 20th century (Zeevaart, 2006). The existence of florigen as a universal floral initiator was doubted after genetic research discovered multiple distinct flowering pathways in different species, but this dissonance was resolved after it was seen that the each separate pathway converged to a shared set of flower promoting genes, the most well known being *FLOWERING LOCUS T* (*FT*, Koornneef et al. 1991; Samach et al. 2000; Simpson and Dean 2002; Putterill et al. 2004). Thus florigens are indeed understood to be universal flowering inducers, but the production of florigen hormones are regulated by single genes in certain species and are polygenic in others. The protein produced by *FT*, now considered to be a florigen hormone, travels through the phloem to the shoot apical meristem and interacts with other proteins already present in the meristem to induce flower differentiation (Abe et al. 2005; Notaguchi et al. 2008).

Lifschitz et al. (2014) emphasizes that, in addition to florigen, there are agents that act antagonistically to this pathway, known as anti-florigens. The protein of *TERMINATION FLOWER 1* (*TFL1*) in the model plant *Arabidopsis thaliana* is an anti-florigen that suppresses termination of the inflorescence and maintains vegetative growth in shoot meristems (Bradley et al. 1997). Lifschitz et al. (2014) propose that not just florigens but the florigen:anti-florigen ratio determines flowering times in short day, long day and day-neutral flowering plants. Gene pathways that regulate florigen and anti-florigen production in strawberry must be discovered and understood before breeding efforts can be refined to accurately select for day-neutral habits.

While previous reviews have stated that no genes involved in strawberry flowering have been identified (Darnell et al. 2003), subsequent research has proposed specific floral promoters and suppressors within the strawberry genome. These discoveries are first reviewed within the diploid *F. vesca* genome, followed by the octoploid genome of commercial *F. x ananassa*.

A. *Fragaria vesca*

Fragaria vesca, commonly known as the woodland strawberry, shares morphologic and genotypic similarities with the more commercially significant, *F. x ananassa*. Despite its smaller size the petal and sepal number, inflorescence architecture and early fruit development of *F. vesca* are notably similar to *F. x ananassa*, and the two species share a high degree of colinearity between their genomes (Rousseau-Gueutin et al. 2008; Hollender et al. 2012). Hollender et al. (2012) performed a thorough analysis of *F. vesca* floral development (Fig. 12), linking the genetic similarities to *F. x ananassa* with a documentation of morphologic similarities as well.

To date, most strawberry genetic research has focused on *F. vesca* because of its relatively simple diploid genome compared to the octoploid *F. x ananassa* (Slovin and Michael 2011). *F. vesca* has an appealingly small genome size (~240 MB; $x = 7$) with a recently published reference genome (Shulaev et al. 2011) and several cultivars that have had success with *Agrobacterium*-mediated transformation (Folta and Dhingra 2006). Research within *F. vesca* has been undertaken with the belief that any genetic discoveries within *F. vesca* could be used as model for subsequent research within *F. x ananassa* (Weebadde et al. 2007; Hollender et al. 2012). Among the most commercially desired

discoveries is that of flowering habit. Since *F. vesca* also contains multiple lines and cultivars with day-neutral flowering habits, it has been long hoped that understanding the genetic precursor to day-neutrality in *F. vesca* would serve as a springboard for similar discoveries in *F. x ananassa*; such a discovery would be highly beneficial to breeders looking to ensure a stable day-neutral habit in potential cultivar releases.

A genetic model for flowering habit in *F. vesca* was proposed by Brown and Wareing (1965) when F₁ and backcrossed progeny of seasonal x perpetual flowering parents segregated into 9:3:3:1 and 1:1:1:1 ratios, respectively. Such results indicated that a single gene controlled *F. vesca* flowering habit, with seasonal flowering as the dominant phenotype. Cekic et al. (2001) went further, using a type of microsatellite analysis (ISSR-PCR primer pair combinations) to identify two markers specific to *F. vesca* located near a single seasonal flowering locus. Finally Iwata et al. (2012) discovered a specific anti-florigen *TFL1* homologue, *KOUSHIN* (*KSN*) within the genome of *Fragaria vesca*, naming it *FvKSN*. A 2-bp deletion in the first exon of the *FvKSN* allele causes a frame shift, leading to a non-functional mutant *ksn*. All *F. vesca* strawberries displaying a continuous flowering habit were homozygous *ksn/ksn*, while once-seasonal flowering habits were either *KSN/ksn* or *KSN/KSN*. These results coincided with findings of Koskela et al. (2012) that a *TFL1* homologue, which they named *FvTFL1*, served as an anti-florigen, expressing itself in long-day photoperiods. Transgenically silenced *FvTFL1* lines and lines overexpressing a mutated *FvTFL1* with a 2-bp deletion both displayed day-neutrality, supporting the findings of Iwata et al. (2012) (Fig. 13). It was later found that *FvSOC1* activates the *FvTFL1* anti-florigen gene in the

shoot apex during long day conditions; thus *FvSOC1* is currently alleged as the photoperiod control center of floral differentiation in *Fragaria vesca* (Mouhu et al., 2013).

B. *Fragaria x ananassa*

F. x ananassa contains a complex octoploid genome, yet progress has been made in identifying specific florigens and anti-florigens within the species. Examining the June-bearing cultivar 'Nyoho', Nakano et al. (2015) observed mRNA accumulation of the gene *FaFT3* in the shoot tip under short day (8h), cool temperature (13°C) conditions just prior to induction of a floral meristem identity gene, *FaAPI*. The protein of *FaFT2* was found in the flower bud shortly thereafter, and both of these appeared to act antagonistically to *FaFT1*, which accumulated in plant leaves under long day (16h), conditions. *FaFT1* was also associated with the upregulation of *FaTFL1* in shoot tips in warmer temperatures (27°C). From these observations, Nakano et al. (2015) propose that *FaFT1*, *FaFT2* and *FaFT3*, all of which contain amino acid residues similar to the florigen FT in *Arabidopsis thaliana*, work to regulate flowering in June-bearing *F. x ananassa*, with *FaFT2* and *FaFT3* functioning as florigens in short day, cooler temperatures and *FaFT1* being a precursor to *FaTFL1* anti-florigen production in long day, warmer temperatures. Further work should be conducted to analyze how these genes and proteins interact in day-neutral *F. x ananassa*.

While Nakano et al. (2015) proposes homologs of the *FaFT* proteins in *Fragaria vesca*, the work does little to elucidate the cause of flower habit differentiation in *F. x ananassa* and its relation to flower habit control in *Fragaria vesca* because it only

focused on June-bearing cultivar 'Nyoho'. In fact despite its morphologic and genetic similarities, a preponderance of research has indicated that the single-gene models of flowering habit in *F. vesca* cannot be directly applied to day-neutrality in the more complex octoploid *F. x ananassa* genome. Inconsistent inheritance ratios imply that day-neutrality may not be controlled by a single locus in octoploid strawberries; two-locus models offered a poor fit as well (Serce and Hancock 2005). While this complexity certainly makes breeding for specific flowering habits more difficult, it is not a new phenomenon, as flowering is a polygenic trait in many other plant species (Hayama and Coupland 2004; Esumi et al. 2005).

In an effort to map the polygenetic sources of day-neutrality in *F. x ananassa*, Weebadde et al. (2007) conducted an AFLP marker analysis of the progeny of day-neutral 'Tribute' and June-bearing 'Honeoye' in five states throughout the US. Weebadde et al. (2007) found several (>5) QTL that were correlated with phenotypic variation in flower habit. Interestingly, no single QTL explained more than 36% of this variation, and the ability of a QTL to explain variation changed based on the location in which it was grown. For example, a QTL on LG 28 was a significant predictor of flower variation on plants grown in the hotter climates of every central and eastern state (Minnesota, Michigan and Maryland) but the same QTL could not predict variation in California, where average temperatures are milder. Such a finding implied that not only is flower habit a polygenic trait in *F. x ananassa*, but the genetic requirements for day-neutrality change based on other environmental factors. Weebadde et al. (2007) postulated that a minimum threshold of genes favoring day-neutrality must be present in order to achieve

the habit, and the strength of a gene to contribute to reaching that threshold - i.e. the necessity of a gene that confers heat tolerance in hotter climates - is dependent on its location. This genetic x environmental (GxE) theory helps understand previous research that found the photoperiodic nature of *F. x ananassa* to change based on temperature and light conditions (Guttridge 1985; Sonstebj and Heide 2007). Research investigating whether markers associated with flowering habit in *F. vesca* co-localize with flowering habit markers in *F. x ananassa* has yet to be conducted.

There is still some debate regarding the polygenic control of flowering habit in *F. x ananassa*. In a recent study, Gaston et al. (2013) used marker analysis to find a QTL named *FaPFRU* that they identify as the major controlling locus of day-neutrality and runner production in *F. x ananassa*. The study found day-neutrality to be dominant over seasonal flowering, suggesting that the genetic basis for flowering in *F. x ananassa* was not inherited from *F. vesca*, where seasonal flowering is dominant over day-neutrality. *FaPFRU* also appeared to control vegetative development, a discovery different than the locus controlling flower development in *F. vesca*, which does not control vegetative growth (Koskela et al. 2012). While acknowledging the polygenic evidence provided by previous research (Hancock et al. 2002; Weebadde et al. 2007), Gaston et al. (2013) still proposed day-neutrality in *F. x ananassa* to be under single-locus control by *FaPFRU*. Indeed, *FaPFRU*, located on the IVb-f linkage group explained up to 59.3% of phenotypic variation in flower habit in that study (Table 1).

The dissonance regarding genetic control of *F. x ananassa* flower habit in current literature may be due to the GxE interaction effects described previously. The research by

Gaston et al. (2013) was conducted in only one location (Villenave d'Ornon, France, 44.7806° N, 0.5658° W) and, while *FaPFRU* explained a majority of the phenotypic variation in that study, the effect of this gene may be lessened in regions with different environmental conditions, as was observed with the LG 28 QTL by Weebadde et al. (2007). Before *FaPFRU* can be universally accepted as the single-control locus of flowering habit in *F. x ananassa*, its predictive strength would also have to be tested in areas such as Maryland and coastal California, where environmental pressures against day-neutrality appear to be high and low, respectively (Hancock et al., 2002, Weebadde et al., 2007). If *FaPFRU* remained a strong predictor of phenotypic variation in flower habit in each environment the claim of Gaston et al. (2013) that *FaPFRU* is the major controlling locus of flower habit in *F. x ananassa* would be strengthened.

Indeed, such a project was conducted by Castro et al. (2015). Crosses of day-neutral 'Tribute' and June-bearing 'Honeye' were qualitatively and quantitatively scored for day-neutrality in five different states in the US with contrasting temperature conditions: Maryland (MD), Michigan (MI) and Minnesota (MN), where average maximum summer temperature is at least 28°C, and California (CA) and Oregon (OR), where maximum temperatures are at 21°C and 26°C, respectively. The authors also used 267 molecular markers in an attempt to construct a map of linkage groups associated with flower habit in each environment. As expected, even with the same parents, a higher proportion of progeny scored as day-neutral in cooler CA than in hotter MD, reinforcing the role of GxE interaction in flower habit (Fig. 14). Similar to the findings of Gaston et al. (2013), the day-neutral: June-bearing inheritance ratio was 1:1 in the hotter MD, MI

and MN environments, suggesting single locus control. However ratios were 3:1 in CA and 5:3 in OR, implying the presence of multiple alleles at one or more loci that only express themselves in cooler temperatures.

Through their marker analysis, Castro et al. (2015) found that day-neutrality could be mapped to a specific genetic region on linkage group (LG) IV-T-1 of the ‘Tribute’ map - a region with multiple QTLs – regardless of environment (Fig. 15). In MD, a QTL on LG IV-T-1 explained between 63.9 and 63.6 % of the phenotypic variation, while in CA a separate QTL on the same LG explained between 51.2 and 50.1 % of the phenotypic variation. These results imply that while day-neutrality may be under single-locus control in certain environments, multiple loci within the same genetic region may be expressed in cooler environments, and “the distortion toward day-neutral progeny found in OR and CA presents a slight challenge to the single-locus theory and should be remembered in future research” (Castro et al. 2015). The single-locus control apparent in warmer regions seems to mirror the genetic findings of Gaston et al. (2013), but the QTL found on LG IV-T-1 could not be confirmed as *FaPFRU* because the two studies did not use common markers to identify QTLs.

Taken together, research on the genetics of strawberry flower induction has shown *F. x ananassa* to contain more complexity than *F. vesca*. Control of day-neutrality in *F. vesca* is almost certainly controlled by a single locus (Cekic et al. 2001; Iwata et al. 2012; Koskela et al. 2012), and while the inheritance of flower habit in *F. x ananassa* is more uncertain, it appears that GxE interaction leads to single locus control in hotter regions and potential multi-locus control (albeit on the same linkage group) in cooler

regions (Weebadde et al. 2007; Gaston et al. 2013; Castro et al. 2015). While the understanding of induction in *F. vesca* was proposed as a springboard for induction research and consequent breeding efforts in *F. x ananassa* (Hollender et al. 2012), the support for this proposal remains unrealized; in fact, recent evidence suggests the genetics of flower induction in *F. vesca* and *F. x ananassa* are not orthologous (Gaston et al. 2013). Regardless of this relation, continued research into the inheritance of day-neutrality in commercial *F. x ananassa* will assist breeders in releasing cultivars with extended yields in diverse environments throughout the world.

Conclusions

The strawberry is a thoroughly documented horticultural crop. There appears to be adequate information regarding the growth and reproduction habits of commercial *F. x ananassa* and its progenitors *F. vesca*, *F. virginiana* and *F. chiloensis*. Within the literature there is a focus on *F. x ananassa* flowering habit, as this response represents the aspect of growth most commercially significant to strawberry growers. The three categories of flowering habit, June-bearing, everbearing and day-neutral, each respond differently to photoperiod and have separate cultural production practices endemic to the environments they are most easily grown. The day-neutral phenotype is the most desired because it offers the potential for extended seasons and increased yields, but until recently commercial day-neutral production has been limited to only a few regions such as Mexico, and California and Florida within the United States. This is apparently due to older cultivars having a narrow set of environmental tolerances, but newer cultivars are

showing promise to increase the commercial range of day-neutral production (Petran et al, 2016).

Despite its value and popularity there has been little progress in mapping the genetic basis of day-neutrality in *Fragaria x ananassa* until recently. This is due to its complex octoploid genome and a history of inconsistent inheritance ratios even within traditional breeding efforts. Because of these difficulties, most strawberry genetic research has focused on *Fragaria vesca*, which features a simpler diploid genome, inheritance ratios that implied single-gene control of flower habit, and an assumption that any discoveries made within this species could be used as a springboard for genetic research in *F. x ananassa*. Indeed it was discovered that a 2-bp deletion in a homologue of the anti-florigen gene *TFL1* resulted in day-neutrality in *F. vesca* (Iwata et al, 2012; Koskela et al, 2012).

Recently there has been progress made in the mapping of day-neutrality in *F. x ananassa*, though it has little to do any discoveries made within *F. vesca*; in fact it has been proposed that genes controlling flower habit in the two species are not orthologous (Gaston et al, 2013). It appears that day-neutrality in *F. x ananassa* is influenced by GxE interactions, with single-locus control taking effect in regions where average maximum temperatures are above 28 C, and potential multi-locus control in cooler regions (Castro et al, 2015). This may explain observed higher proportions of progeny displaying day-neutrality in milder vs. hotter climates, even when parents are the same (Weebadde et al. 2007). While no project has discovered common markers between the flowering loci of *F. vesca* and *F. x ananassa*, the results of the induction research covered in this review

imply that such work may not be as useful as previously believed. Instead efforts may be better focused on investing a higher density of markers in linkage group IV-T-1 of the *F. x ananassa* genome, where QTLs determining day-neutrality appear to be focused.

Designing markers for targeted genome regions will be made easier now that a reference genome for octoploid *F. x ananassa* has been released (Hirakawa et al, 2014), and will allow researchers to determine if markers highly correlated with day-neutrality in ‘Tribute’ will explain the habit in other parental lines as well. Despite its complexities, recent advancements in understanding *F. x ananassa* flower habit have been promising, and increase the potential for production of this valuable crop to be commercially viable throughout the world.

Fig. 1. Axillary meristems of cultivar ‘Evie-II’; photo taken July 10, 2014 in Minnesota.



Figs. 2.1 and 2.2. 2.1 (left), from Poling 2012: Diagram of fully developed flower cluster with (a) primary flower, (b) secondary flower, (c) tertiary flower, and (d) quaternary flower <http://www.hort.cornell.edu/grower/nybga/pdfs/2012berryproceedings.pdf> 2.2 (right): Picture of a flower cluster of the cultivar ‘Portola’, with (a) primary flower, (b) secondary flower bud, and (c) tertiary flower bud. Photo taken July 10, 2014 in Minnesota.

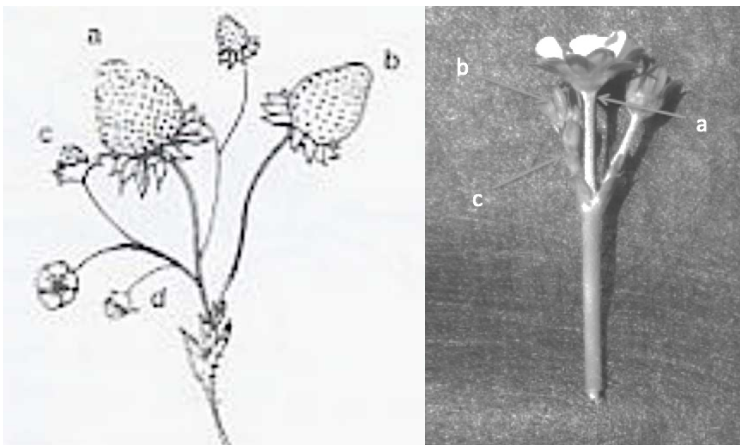


Fig. 3.1 and 3.2. 3.1 (top) Principal flower parts of cultivar ‘Evie-II’, including (a) stamen, (b) pistil, (c) receptacle, (d) petal, and (e) sepal. Photo taken July 10, 2014 in Minnesota.

3.2 (bottom) Cross-section of *F. x ananassa* showing (a) pistil and (b) receptacle. Photo obtained with permission from G. D. Carr, December 9, 2015, http://www.botany.hawaii.edu/faculty/carr/images/fra_sp.jpg



Figs. 4.1 and 4.2. 4.1 (left): Profile of mature fruit of the cultivar ‘Amandine’, with embedded achenes. 4.2 (right): Cross section of an ‘Amandine’ fruit, with (a) interior receptacle, (b) fibrovascular tube and (c) calyx. Photo taken July 10, 2014 in Minnesota.

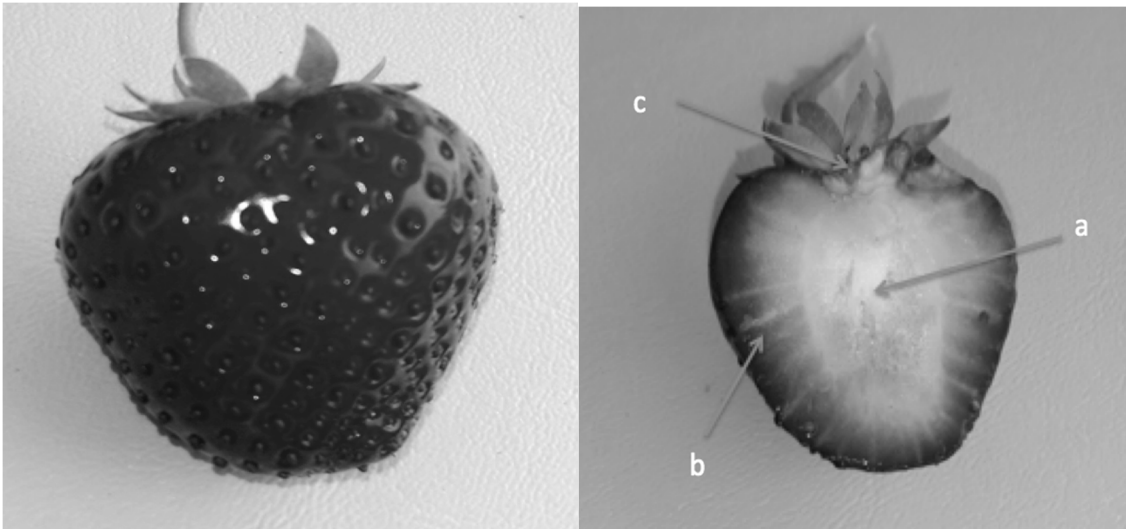


Fig. 5. Average daylengths of Minneapolis, MN and Santa Maria, CA, taken on the 20th of each month. Raw data acquired from Time & Date AS:

<http://www.timeanddate.com/worldclock/astronomy.html?n=3857&month=12&year=2014&obj=sun&afl=-1&day=1>

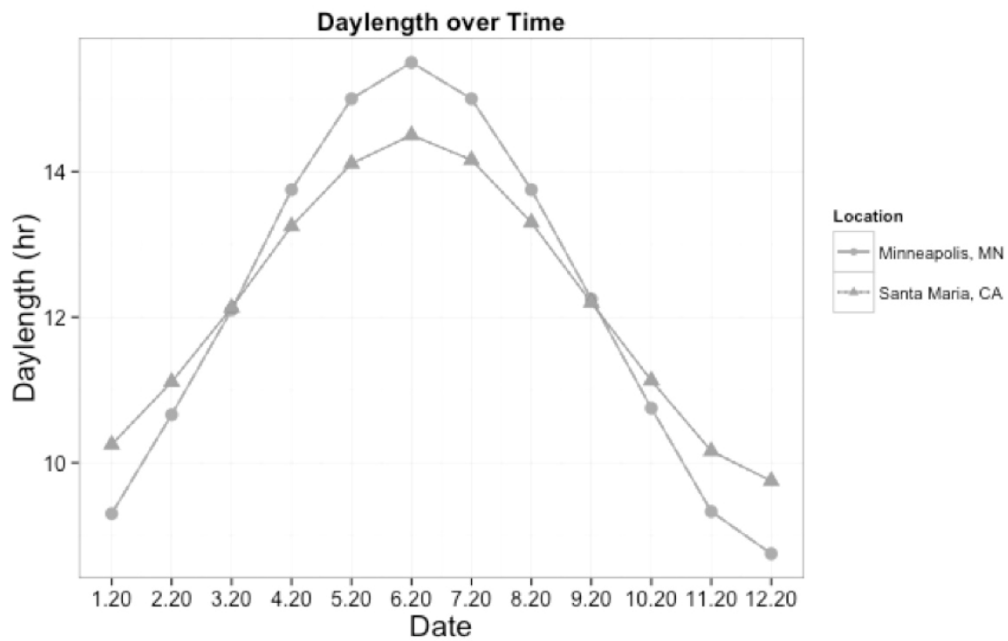


Fig. 6. Average high temperatures Minneapolis, MN and Santa Maria, CA, taken on the 20th of each month. Raw data acquired from Intellicast: <http://www.intellicast.com/>.

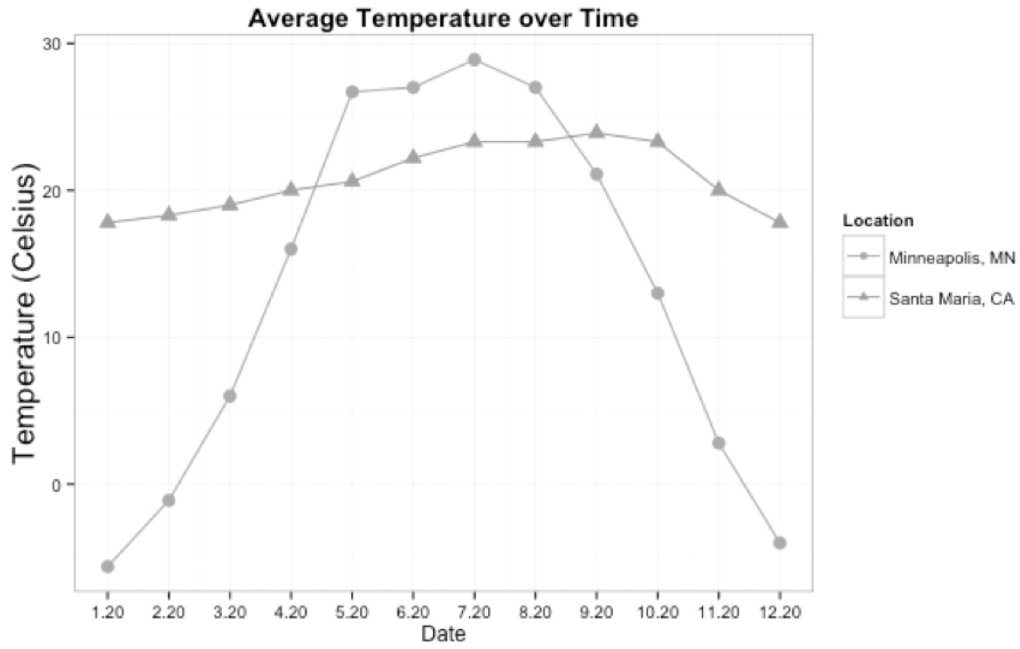


Fig. 7. Diagram of the matted-row system common to June-bearing cultivars.

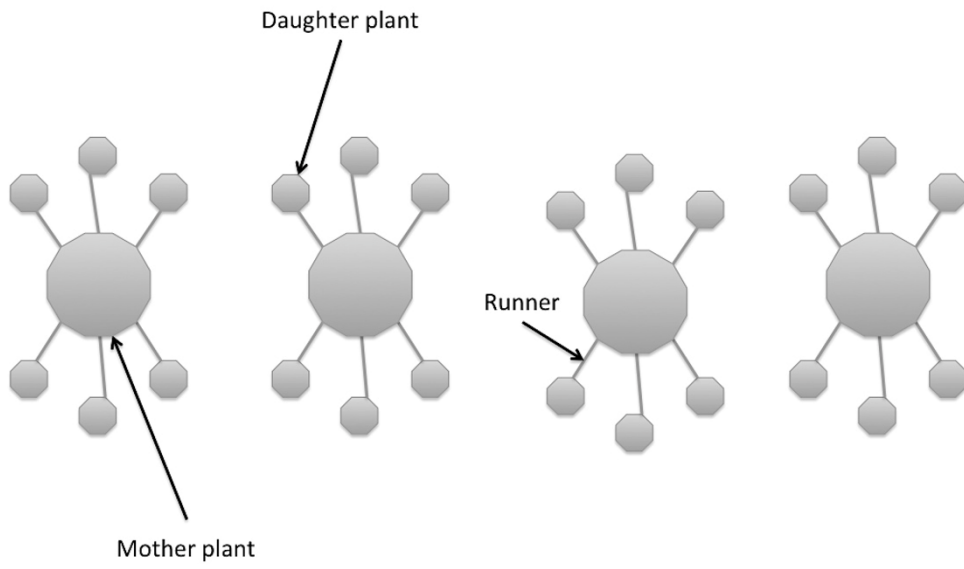


Fig. 8. Day-neutral 'Monterey' runner, with developed inflorescence. Photo taken July 10, 2014 in Minnesota.



Fig. 9. From Savini et al. (2005): (a) diagram of a single, typical strawberry inflorescence, and (b) model of ‘Seascape’, where 1°, 2° and 3° represent primary, secondary and tertiary inflorescences, which developed from buds after initial planting. Reprinted by permission of Taylor & Francis Ltd.

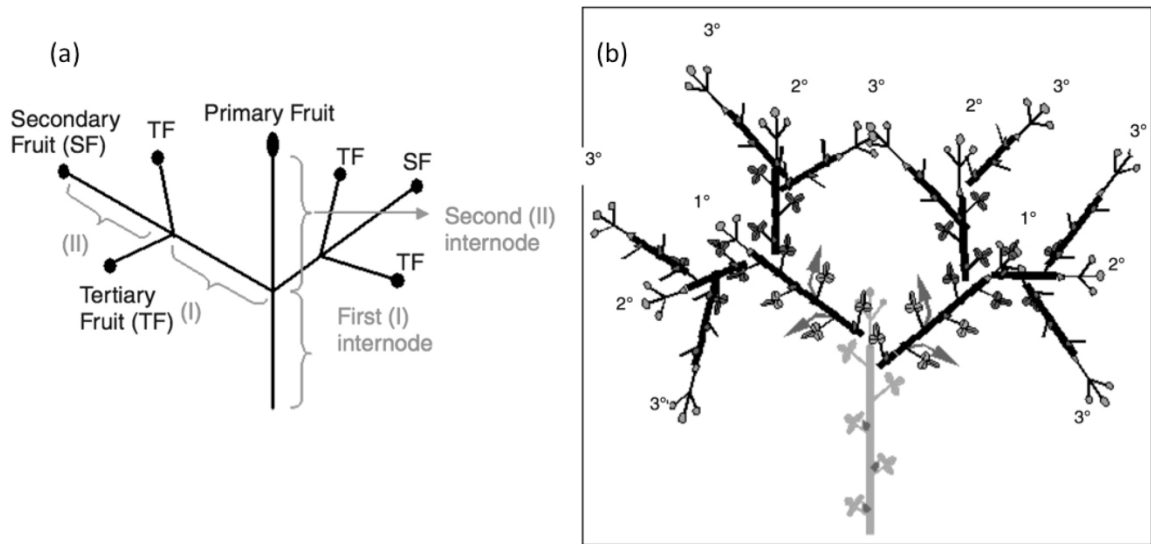


Fig. 10. Selected flower clusters of (a) 'Annapolis', (b) 'Albion', (c) 'Evie-2', (d) 'Monterey', (e) 'Portola', (f) 'San Andreas', (g, h) 'Seascape'. Photos taken July 15, 2014.



Fig. 11. Photograph of 'Portola' inflorescence. 1° , 2° and 3° represent primary, secondary and tertiary flowers. Labeled brackets indicate primary (I) and secondary (II) internodes.

Photo taken July 15, 2014.



Fig. 12. From Hollender et al. (2012): *Fragaria vesca* shoot and flower development: **a.** *F. vesca* YW5AF7 grown in a 10.2 cm pot. **b.** YW5AF7 dichasial cyme bearing yellow berries. **c.** Inflorescence with primary flower (1) and two developing secondary flowers (2). Young tertiary buds (*arrows*) are present beneath the secondary flower buds. **d.** Diagram of shoot architecture. *Numbers* indicate primary, secondary and tertiary flower buds. **e.** Diagram illustrating floral organ arrangement. The two outer whorls are concentric rings of five bracts (*b*) alternating with five sepals (*s*). The third whorl consists of five white petals (*p*). Interior to the petals are two whorls of stamens. Stamens are arranged in a repeating pattern of five tall (*T*) and five short (*S*) in the inner whorl and 10 medium length (*M*) in the outer whorl. The *center circle* indicates a receptacle topped with numerous, spirally arranged carpels. **f.** Scanning electron micrograph (SEM) of a developing floral bud, illustrating spirally arranged carpel primordia. **g.** Abaxial view of a typical *F. vesca* flower with five narrow bracts (*b*) alternating with five wider sepals (*s*). **h.** Adaxial view of typical *F. vesca* flower illustrating a whorl of five white petals, two whorls of ten stamens each, and an apocarpous gynoecium with ~160 pistils. **i.** Dissected flower illustrating the “S, M, T, M, S” stamen pattern. *Scale bars a:* 2 cm; **g–i:** 1 mm.

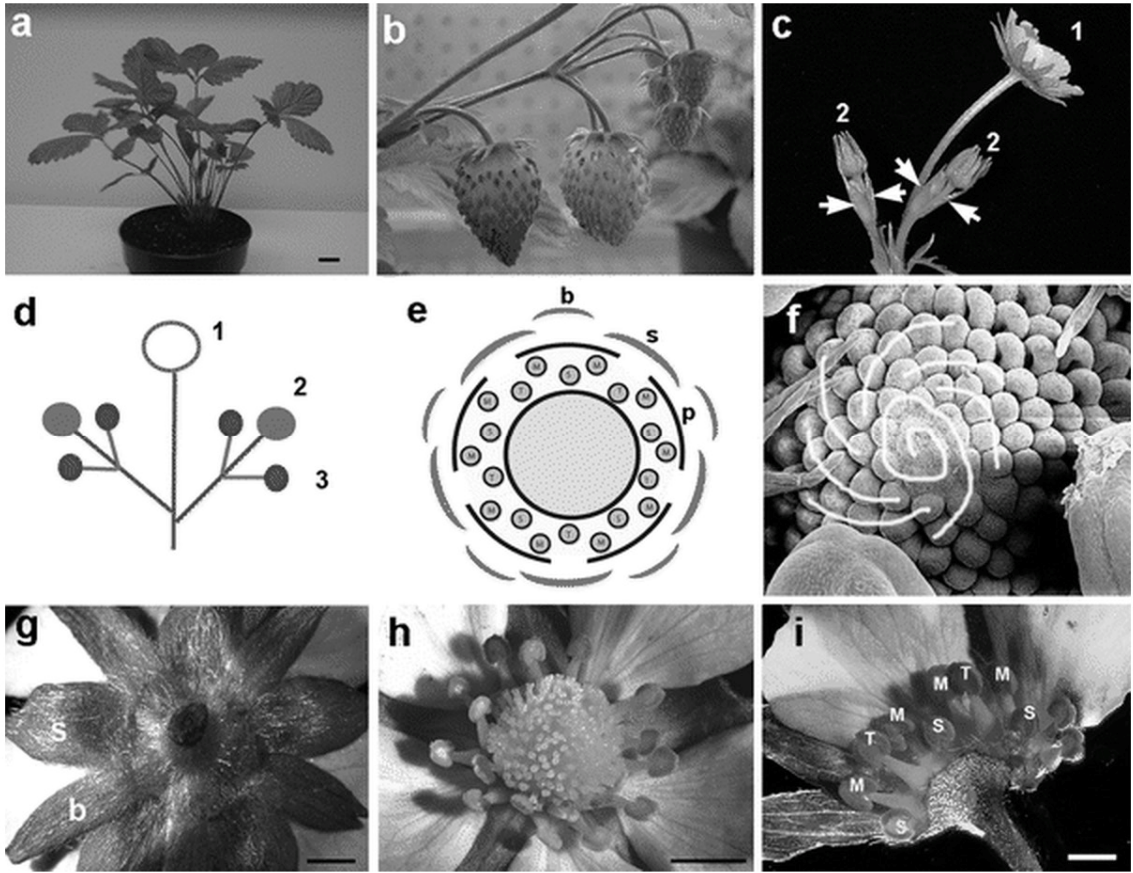


Fig. 13. From Koskela et al. (2012): Silencing of *FvTFL1* leads to daylength-independent flowering. A, Phenotypes of *FvTFL1* RNAi silencing and overexpression lines in the SD *F. vesca* background. Clonally propagated plants (runner cuttings) of SD *F. vesca* and *P35S:FvTFL1-RNAi-1* and *P35S:FvTFL1-1* lines were subjected to SD induction treatment for four weeks followed by LDs (left) or grown continuously under LDs (right). B, Flowering time of SD *F. vesca* and *P35S:FvTFL1-RNAi* and *P35S:FvTFL1* plants (RNAi and OX, respectively) in SDs and LDs. Flowering time is indicated as days to anthesis from the beginning of the treatments. Treatments and plant materials were as described in A. Values indicate means \pm SD. $n = 4$ (OX-1), $n = 5$ (RNAi-2), $n = 6$ (RNAi-1 and OX-2), and $n = 7$ (SD *F. vesca*). C and D, Expression of *FvTFL1* (C) and *FvAPI* (D) in the apices of two independent *P35S:FvTFL1-RNAi* (RNAi) lines. Values indicate means \pm SD. $n = 3$ (RNAi-1 and SD *F. vesca*) or $n = 2$ (RNAi-2). Copyright American Society of Plant Biologists, www.plantphysiol.org

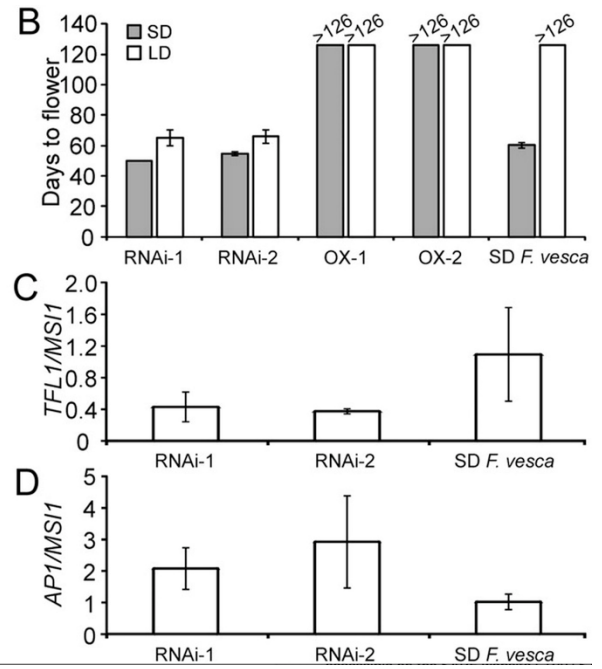
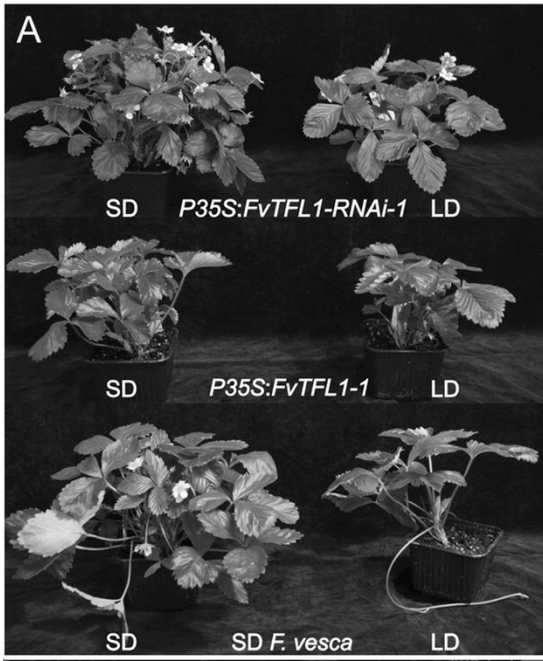


Fig. 14. From Castro et al. (2015): Frequency distribution of number of weeks flowering at Maryland (MD) (a) and California (CA) (b). The phenotypic values of the parents, ‘Tribute’ and ‘Honeoye’ are indicated by arrows.

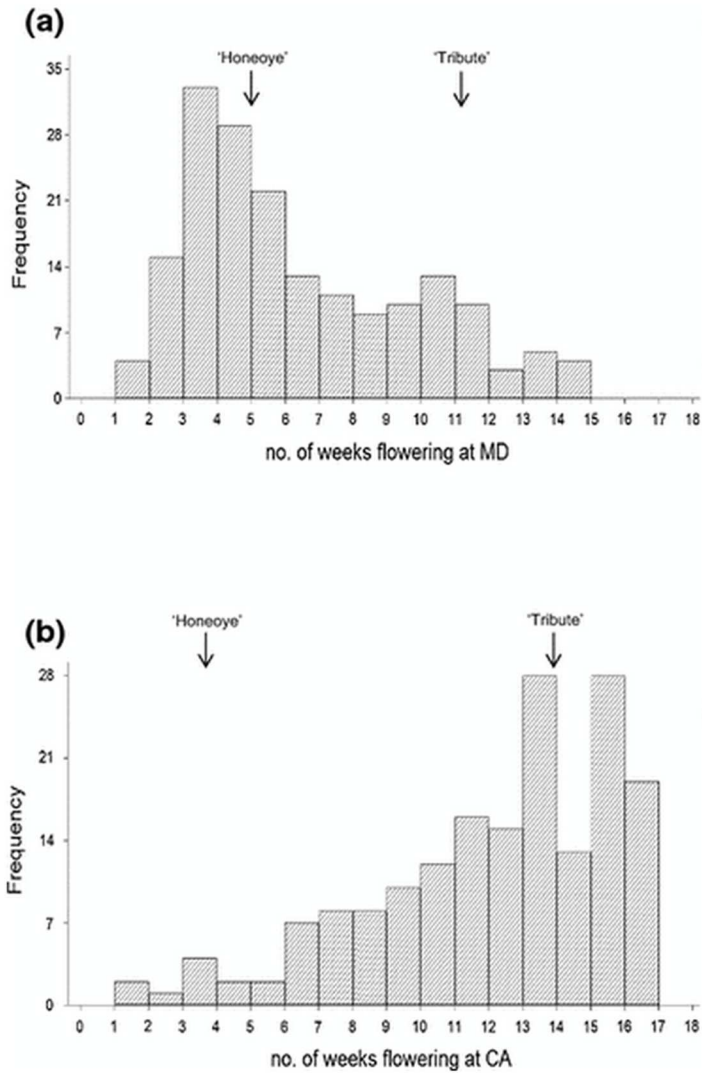


Table 1. From Gaston et al. (2013): Map positions and genetic effect of QTL detected for the PF (perpetual flowering) and RU (runnering) traits for the female (f) parent ‘Capitola’ and male (m) parent ‘CF1116’. QTL identification was based on composite-interval mapping analysis with LOD>LOD threshold (3.1) ($\alpha=0.05$). By permission of Oxford University Press.

| Trait | Linkage group | QTL name ^a | Year ^b | No. QTL ^c | Marker ^d | Position ^e | LOD ^f | r^2 ^g | Effect ^h |
|-------|---------------|-----------------------|---------------------------------|----------------------|---------------------|-----------------------|------------------|--------------------|---------------------|
| PF | Id-m | PF-LGId-m | 2002 | 1 | ccta185 | 6.01 | 3.2 | 10.3 | +5.7 |
| | Id-m | PF-LGId-m | 2002 | 1 | gata148 | 21.11 | 3.4 | 8.7 | -7.6 |
| | Id-m | PF-LGId-m | 2007 | 1 | ccaa267 | 43.71 | 3.7 | 8.5 | +3.4 |
| | IVb-f | PF-LGIVb-f | 2002/2003/2003b/ 2004/2005/2007 | 6 | gatt284 | 2.0/4.0 | 14.3–33.5 | 36.5–59.3 | +6.0–10.7 |
| | IVd-m | PF-LGIVd-m | 2002/2003 | 2 | tgac408/tgta115 | 60.06/55.57 | 3.2/3.7 | 10.5/13.4 | +6.1/4.0 |
| | Va-f | PF-LGVa-f | 2007 | 1 | u009180 | 20.01 | 3.3 | 5.9 | -2.9 |
| | Vic-f | PF-LGVic-f | 2007 | 1 | v013200 | 84.47 | 3.1 | 4.0 | -2.5 |
| RU | Iic-m | RU-LGIic-m | 2005 | 1 | gctg207 | 54.59 | 3.1 | 7.2 | +1.2 |
| | IIIb-m | RU-LGIIIb-m | 2002 | 1 | gtag280 | 54.86 | 3.7 | 7.4 | +1.0 |
| | IIId-m | RU-LGIIId-m | 2002 | 1 | tgac108 | 15.51 | 4.1 | 8.1 | +1.0 |
| | IVb-f | RU-LGIVb-f | 2002/2003/2005 | 3 | gatt284 | 0.0/2.0 | 5.7–26.7 | 12.4–51.1 | -1.5–4.8 |

^a Name of the unique QTL.

^b Year of observation of the QTL.

^c Number of significant QTL.

^d The left marker associated with the QTL is indicated.

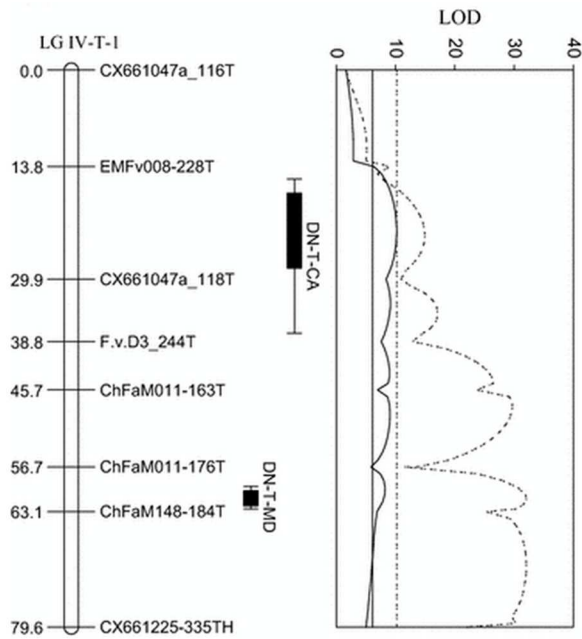
^e Position indicates the distance in cM of the QTL from the top of the chromosome.

^f LOD is the log-likelihood at that position.

^g r^2 is the percentage of phenotypic variation explained by the QTL.

^h Mean effect on a trait mean value of the presence of one allele at a marker by comparison with the presence of the second allele. + and - indicate the direction of the additive effect. A positive effect means a higher value for the Capitola allele on the female map or a higher value for CF1116 on the male map.

Fig. 15. From Castro et al. (2015): Quantitative trait loci (QTL) for number of weeks of flowering detected in the octoploid strawberry (*Fragaria* × *ananassa* Duchesne ex Rozier) population ‘Tribute’ × ‘Honeoye’ evaluated in Maryland (*dotted line*) and California (*solid line*) using interval mapping (IM).



CHAPTER 3

**YIELD AND QUALITY CHARACTERISTICS OF DAY-NEUTRAL
STRAWBERRY IN THE UNITED STATES UPPER MIDWEST USING
ORGANIC PRACTICES**

(submitted to Biological Agriculture & Horticulture)

Andrew Petran

Summary

The Upper Midwest of the United States accounts for an extremely small portion of national strawberry (*Fragaria x ananassa*) production due to the prevalence of ‘June-bearing’ cultivars, which are limited to a short 6-week fruiting window from June to early July. Determination of high yielding and high quality organic management practices for newer day-neutral cultivars in the region was performed. Six day-neutral cultivars were planted at two locations (St Paul, and Morris, MN) 2013 and 2014 and organically managed as annual crops under straw mulch, plastic mulch, and plastic mulch with low tunnel cultural practices. Yield and average berry weight for each cultivar/cultural practice combination was collected and analyzed weekly and cumulatively at each location and year. In addition, total phenolic content (TPC) of fruit was measured as an indicator of nutritional quality, and total soluble solid (TSS) content was measured as an indicator of sensory quality. Total soluble solids and TPC were measured at three different times of harvest. Day-neutral production added an average of fourteen weeks to the June-bearing strawberry season regardless of cultural practice. Plastic mulch and plastic mulch with low tunnel practices yielded more fruit than straw mulch, while all practices yielded more fruit than the 6,160 kg·ha⁻¹ average for June-bearing production in the region. Significant cultivar differences within each practice were observed as well. Average berry weight, TPC and TSS were rarely influenced by cultural practice, while TPC and TSS both displayed significant cultivar and time of harvest effects. When combining yield and quality characteristic data, a trend of high-yielding cultivars having lower sensory and nutritional quality characteristics is observed. Overall our data support

the conclusion that day-neutral strawberries can perform well using organic cultural practices in the Upper Midwest, providing growers the opportunity to extend the season and bolster yield, while providing consumers access to nutritious, locally available fruit for a longer period of time.

Introduction

The common cultivated strawberry (*Fragaria x ananassa*) is a valuable horticultural crop, and an increasingly popular addition to any diet. However only isolated regions in the United States- primarily California and Florida- are capitalizing on this fruit's production. Though the market for fresh strawberries in the United States more than quadrupled from 1990-2012, the Upper Midwest claims only 5.3% of total national acreage- the vast majority of which only produces fruit in a short, 6-week window from June to early July (Perez and Plattner, 2013; Vilsack, 2007). This regional production, accounting for less than 1% of annual national yields, does not fulfill an increased consumer demand for locally produced fruits and vegetables and a concurrent increase in demand for organic produce (Falguera et al., 2012; Frith, 2007; Vilsack, 2009). Approaches that would extend the strawberry season in the Upper Midwest would help meet these demands.

Among the factors influencing the increased demand of fresh, local fruit- and strawberries in particular- is the growing body of literature linking sensory quality with nutritional quality. Strawberries are highly regarded in the consumer market for their sensory attributes, including color and sweetness (Gunness et al., 2009). While the redness induced by the pigment anthocyanin enhances sensory quality, the anthocyanins

themselves have various nutritional and health benefits. Indeed, anthocyanins and other phenolic compounds commonly found in strawberry have been found to contribute to disease prevention due to their anticancer, antioxidant and platelet aggregate suppression properties (Wattenburg et al., 1980; Rice-Evans et al., 1996; Rein et al., 2000; Silva et al., 2007).

Strawberries are notably abundant with nutritionally beneficial compounds even amongst other fruits, and have been found to contain 2 to 11-fold more antioxidant capacity than apples, peaches, pears, grapes, tomatoes, oranges or kiwifruit (Scalzo et al., 2005). In an analysis of 20 fruit and 22 vegetable species, Marinova et al. (2005) found strawberry to be higher in phenolic content than all vegetables and all but six fruits. Research has also shown that strawberry phenolic content is often greater when plants are managed using organic practices; many phenols are antimicrobial in nature, and organically managed plants likely produce more due to increased pest pressure relative to conventional practices (Asami et al., 2003). Capocasa et al. (2008) found that strawberry cultivars with the highest total soluble solid content also had relatively high phenol and antioxidant content. Combining these findings, a consumer could reasonably assume that a sweet strawberry also has considerable nutritional benefits, and if grown using organic practices the potential increases even more.

The desirable sensory and nutritional quality of strawberries substantially decreases, however, as storage time increases. The USDA recommends strawberries be stored at 0° C to extend postharvest shelf life up to seven days (Mitcham, 2014). However Ayala-Zavala et al. (2004) reports significant decreases in overall strawberry

quality including anthocyanin and soluble solid content after seven days of storage, even at 0° C. Thus an extended season of locally produced strawberries that are immediately available for sale after harvest would assist in meeting market demands in addition to maximizing sensory and nutritional quality for consumers.

The short production window for most strawberry cultivars in the Upper Midwest is a function of genetically mediated flowering response to daylength, aka photoperiod. These responses have been grouped into three flowering types: June-bearing, everbearing and day-neutral. June-bearing plants induce flowers under shortening daylengths, optimally from 9.5 to 13 hour days, depending on cultivar (Darrow, 1933). In the Upper Midwest this induction period takes place in early fall prior to dormancy. Savini (2005) notes that most June-bearers will also have flower initials before they enter dormancy. As dormancy breaks the following spring, daylength and temperatures have increased, causing June-bearing plants to stop flower induction and initiation and continue flower development (Salisbury & Ross, 1992). June-bearing cultivars typically stop producing in late June or early July depending on cultivar and location. These plants yield an average of 6,160 kg·ha⁻¹ after their initial ‘establishment year’ in the Upper Midwest (Vilsack, 2009), then enter a vegetative state during the long photoperiods of summer, until shortening days trigger the beginning of the next flowering cycle. Though sub- and supraoptimal temperatures have been shown to interact with photoperiod to influence strawberry flowering habit, this project focuses primarily on photoperiod effects, as daylength is observed to be the major contributing factor to flowering habit in this region of the US (Bradford et al., 2010; Guttridge, 1985; Wagstaffe, 2009).

The day-neutral flowering phenotype offers potential for extending the strawberry fruiting season in the Upper Midwest. Unlike June-bearing cultivars, the crowns, branch crowns and rooted runners of day-neutral strawberry plants fruit prolifically in their first year of commercial production if planted as bare-rooted transplants (Ahmadi & Bringhurst, 1991). Under desirable temperature conditions, day-neutral cultivars also show insensitivity to photoperiod, flowering and fruiting at the same rate throughout a growing season of dynamic changes in daylength (Durner et al., 1984). The interaction of these factors- first year flowering and photoperiod insensitivity- would allow growers in the Upper Midwest to change their cultural practices regarding strawberries, from cultivating short-season perennial June-bearers to extended season annual day-neutrals. This shift in practice would allow growers to enjoy an extended fruiting season, incorporate strawberry plants into an annual rotation, and avoid the risks of overwintering crops in the bitter cold climates typical of the region (USDA hardiness zones 3 & 4, USDA, 2014). Organically managed strawberry fields in particular experience yield benefits when treated as an annual versus a perennial crop (Conti et al., 2014).

Despite the flowering advantages, older day-neutral cultivars such as ‘Tribute’ and ‘Tristar’ have not performed well in the Upper Midwest, evidenced by poor yields in Midwestern climates, soft fruit and propagation difficulties (Durner et al., 1984; Luby et al., 1987, Luby 1989). Historically, day-neutral cultivars only performed well in California and portions of Florida, but newer day-neutral cultivars have shown promising yields in regions other than California (Lewers, 2012). However no studies using these cultivars have been performed in the Upper Midwest. Thus our objectives were to revisit

the yield and quality performance of day-neutral strawberry production in the Upper Midwest. Six newer day-neutral strawberry cultivars were managed under three organically certified cultural practice treatments during the 2013 and 2014 growing seasons at two sites, St Paul, MN and Morris, MN.

The objectives of this paper are to present and analyze the performance of each cultivar/practice combination, and discuss the benefits and challenges observed with each practice. Emphasis was placed on the interaction of cultivar and cultural practices on yield, berry weight and quality characteristics. Total soluble solid (TSS) content was chosen as an indicator of sensory quality and total phenolic content (TPC) was chosen as an indicator of nutritional quality. Multi-factorial analysis by these categories allows us to determine if a certain cultural practice outperforms others, if a certain cultivar outperforms others, and if the performance of a cultivar depends on the cultural practice in which it was produced. We show in this project that newer day-neutral strawberry cultivars can be managed as annual crops under an array of organically approved cultural practices in the Upper Midwest, extending the season and producing yields considerably higher than the documented averages of perennially managed June-bearing strawberries typical to the region.

Materials and Methods

Plant Materials, Cultural Practice and Experimental Design.

Six newer day-neutral strawberry cultivars ‘Monterey’, ‘Evie-II’, ‘Albion’, ‘Portola’, ‘San Andreas’ and ‘Seascape’ were chosen for this study, which was carried

out in St Paul, MN (44.9442° N, 93.0936° W, silty clay soil) and Morris, MN (45.5861° N, 95.9139° W) during the 2013 and 2014 growing seasons. The plants at both sites were grown and managed on USDA certified organic land. Plants were acquired as chilled, bare rooted transplants from Nourse Farms (South Deerfield, MA). Planting occurred at both sites in the spring, when soils had thawed and drained sufficiently for tractor activity. For the St Paul site this was 10 May 2013 and 23 May 2014. Morris site planting was the week of 3 May 2013 and 14 May 2014. Three organically certified cultural practice treatments were selected for analysis: plastic mulch with low tunnel coverings, plastic mulch without low tunnel coverings, and in St Paul an added treatment of straw mulch. Plastic mulch was 4' wide, 1 mm thick white on black plastic (Berry Plastics, Evansville, IN), and low tunnel plastic was 12' wide, 4mm thick clear greenhouse film (Ag Resource Inc., Detroit Lakes, MN). Cultural practices were prepared by initially rotovating the soil, followed by raised bed preparation with a model 2121-D bed shaper and plastic mulch/drip tape application with a model 2133 mulch layer, both from Buckeye Tractor Company (Columbus Grove, OH).

Treatments were fertigated using drip tape applicators underneath each mulch type, at the rate of $6.1 \text{ kg} \cdot \text{ha}^{-1}$ of fertility solution per week. Fertility source was OMRI-approved AgGrand Organic Series fertilizer, 4N-1.3P-2.5K. Fungicides were never used, but PyGanic® 5.0 was sprayed via backpack sprayer to control spider mites near the beginning of each season, 2 July in 2013 and 5 July in 2014. PyGanic was applied once a week for 2 weeks at the rate of 14.8 mL per 5 gallons of water. Plastic mulches and tunnels were removed from the field at the end of each season to maintain organic

compliance (NOP regulation §205.206). The plots at the St Paul site were preceded by dry beans (*Phaseolus vulgaris*) each year. The plots at the Morris site were planted on previously fallow land each year.

We used a modified split-plot experimental design, assigning each cultural practice treatment to the main plots and each cultivar to the split plots. Each main plot consisted of six 30.5m long strips. Strips contained a 1.2m wide raised bed with plants assembled in a double row with 30.5cm within-row and 35.6cm between-row plant spacing, and a 0.6m walkway. Each strip was split into 4 equal lengthwise sections, with each section containing one of 4 replicates of each cultivar. Thus, each main plot contained 24 sections: 4 replicates of each cultivar, for a grand total of 72 cultural practice/cultivar units in St Paul and 48 cultural practice/cultivar units in Morris, which did not have a straw mulch main plot. Yield and berry weight data was only collected on the interior 28 plants within each section replicate, the ‘selection zone’, to reduce possible interaction effects between cultivars. Thus selection zones within each section served as the experimental units in the project.

Yield Parameters

Inflorescences were removed until July 1 at each site, each year, to ensure vegetative growth and root establishment immediately after planting. At the St Paul site, harvest began 10 July and concluded 9 October in 2013, and began 23 July and concluded 30 October in 2014. At the Morris site, harvest began 23 July and concluded 11 October in 2013, and began 31 July and concluded 1 October in 2014. Plots were harvested twice each week all season, but only once each week for the first and last

harvest weeks of each season, when yields were low. During each harvest, all berries picked within a selection zone were weighed for experimental unit yield. Unit yield from each of the two harvests a week were combined to calculate weekly yield per unit. Twenty berries in each selection zone were randomly selected, weighed, and divided by 20 to gauge average berry weight for that unit.

Quality Parameters

Total soluble solid content was measured in % Brix (Atago PAL series refractometer, Bellevue, WA). Samples were taken at the St Paul site during three dates in 2014: 18 August, 7 September and 6 October, hereon referred to as ‘early’, ‘mid’ and ‘late’ season, respectively. Five berries from the selection zone of each cultivar/cultural practice unit were selected randomly and measured for % Brix.

Total phenolic content was taken at the St Paul site during three dates in 2013: 25 July, 22 August, and 24 September, also referred to respectively as ‘early’, ‘mid’ and ‘late’ season. At each harvest, five berries from the selection zone of each cultivar/cultural practice unit were randomly selected, wrapped in aluminum foil and placed directly from the field into a cooler containing dry ice. The wrapped berries were then transported and stored in a -62 C freezer for subsequent testing. Once removed from storage, frozen strawberries were soaked in 80% ethanol proportional to the weight of the berries for 48h in order to solubilize the phenolic compounds. Thawed and soaked strawberries were homogenized in a blender, and centrifuged. Total phenolic content was analyzed using the Prussian Blue spectrophotometric method (Hagerman, 2002).

Absorbance values were quantified at 720 nm based on a Gallic acid standard curve referred to as Gallic acid equivalents (GAE (mg/g fresh weight)).

Statistical Analysis

All statistical analyses were conducted using R statistical software version 3.0.2. Analysis of Variance was conducted with each measured factor to determine the presence of significant treatment differences $p < 0.05$. Pairwise comparisons were conducted using Tukey's Honest Significant Difference test, significance at $p < 0.05$. Linear regression analyses comparing yield with % brix over time was performed using the R linear model function.

Results

An ANOVA of all main effect factors (year, site, cultivar and cultural practice) and their interactions influencing total yield was performed (Table 1). The number of significant main effects and interactions indicated the need for further investigation of within-factor and between-factor relationships. Thus pairwise comparisons of total yield, berry weight, TSS and TPC were conducted among cultural practices for each cultivar, and among cultivars within each cultural practice at each site and year. While between-cultural practice pairwise comparisons of each cultivar was performed at each site and year (i.e. comparison of 'Monterey' under straw mulch, plastic mulch and plastic mulch with low tunnels at the St Paul site in 2014), between-cultural practice pairwise comparisons as a whole could not be performed (i.e. comparison of straw mulch vs. plastic mulch vs. plastic mulch with low tunnels at the St Paul site in 2014) because the

modified split plot design at each site and year had only one replication of the main plots, cultural practice.

Effect of Cultivar/Cultural Practice Combinations on Total and Weekly Yields

Data from both sites and years shows no difference in total yield per plant between plastic mulch with or without low tunnel coverings for almost every cultivar (Table 2). There were two exceptions to this trend: at the Morris site in 2013, when an August hailstorm destroyed the fruit of all cultivars not protected by low tunnels, and at the St Paul site in 2014, when ‘Monterey’ had higher yields per plant when plastic mulch was covered with low tunnels. However when comparing plastic mulch practices to straw mulch, total yield differences are more pronounced. No cultivar averaged a higher average total yield per plant when managed under straw mulch practices compared to plastic mulch, with or without low tunnels (Table 2), and in many instances the cultivar averages under straw mulch were significantly lower than plastic.

[Insert Table 2 here]

Differences were also observed comparing total cultivar yields within each cultural practice at either site. At the St Paul site, ‘Evie-II’ was the highest yielding cultivar for every cultural practice both years, with the exception of the straw mulch practice in 2013, where it was the second highest yielding cultivar behind ‘Portola’. At Morris, ‘Portola’ was the highest yielding cultivar for both cultural practices each year. Significant pairwise comparisons can be seen in Table 3.

[Insert Table 3 here]

Harvests occurred continuously from July through October at both sites, 2013 and 2014, with weekly harvest data over the course of a field season can be plotted in addition to end-of-season cumulative data (Figures 1 & 2). At each site and year, yield fluctuations over time were apparent, but there were no observable differences based on cultivar or cultural practice (Figures 1 & 2). At the St Paul site, yields increased continuously from the first harvest until mid-August (day 240±5), regardless of year, cultivar or cultural practice (Figure 1). This was the highest yielding portion of the field season, with weekly harvests averaging 1000-1500 g per unit, depending on cultivar or cultural practice. After this point, yields dropped and fluctuated around 500 g per cultural practice/cultivar unit per week, until the end of the season. At the Morris site in 2013, the same general trend was observed, with the highest yielding week centering on late August (day 250±5), approx. 10 days later than the highest yielding week in St Paul (Figure 3). Again, yields dropped substantially after this point, and yield fluctuation differences did not appear to be influenced by cultivar or cultural practice.

[Insert Figures 1, 2 & 3(a&b) here]

Low tunnels only extended the season once, at the St Paul site in 2014, when harvests extended to 30 October, whereas the plastic mulch without tunnel and straw mulch cultural practices stopped producing 9 October (Figure 1).

Effect of Cultivar/Cultural Practice Combinations on Berry Weight

Cultural practice occasionally had an effect on berry weight. At the St Paul site in 2013, berry weight of ‘Evie-II’ was higher in plastic mulch with low tunnel coverings than straw mulch, and ‘Albion’ averaged significantly larger fruit when managed under

straw mulch practices, compared to plastic mulch with or without low tunnels (Table 4). Cultural practice had no significant effect on berry weight of any cultivar at the St Paul site in 2014. At Morris, both ‘Evie-II’ and ‘Portola’ averaged significantly larger berries when plastic mulch was covered with low tunnels in 2013, and ‘Seascape’ averaged significantly larger berries under low tunnels in 2014 (Table 4).

[Insert Table 4 here]

There were several differences in berry weight among cultivars within each cultural practice. While no differences occurred at the St Paul site in 2013, there were several in 2014, with ‘San Andreas’ producing the largest berries in both plastic mulch practices, and ‘Portola’ producing the largest berries under straw mulch (Table 5). At the Morris site, ‘Portola’ had the largest berry weight regardless of cultural practice or year, and ‘Seascape’ the smallest berry weight regardless of cultural practice or year (Table 5).

[Insert Table 5 here]

Relationship of Weekly Yield with Weekly Berry Weight

An analysis of the relationship of weekly yield and weekly berry weight over time was conducted (Figures 3a & 3b). Yield and berry weight data from 2013 and 2014 at both sites was combined, and linear regression analysis comparing these factors indicated a loose relationship ($R^2 = 0.10$, Figure 3b). Berry weight increased with yield at the beginning of the field season, but starting in early August (day 220±5 from Jan. 1) began to fluctuate independently of weekly yield (Figure 3a).

Total Soluble Solid (TSS) Analysis

Fruit TSS content was influenced by both cultivar and time of harvest (Table 6). Average % Brix of early, mid and late season harvested fruit was 8.8, 7.9 and 9, respectively. Pairwise comparisons showed that both early and late harvested fruit had higher Brix levels than fruit harvested mid season ($p=0.005$ and $p=0.0005$, respectively). In addition the % Brix of each strawberry measured was compared against the weekly yield of the cultivar/cultural practice unit it belonged to. Though a significant relationship was found ($p=6e^{-4}$), correlation r and adjusted R^2 values were modest, -0.28 and 0.05 , respectively.

[Insert Table 6 here]

Among cultivars, ‘Albion’ had higher TSS content than ‘Evie-II’ and ‘Portola’. The average TSS of ‘Albion’ was 2.11% Brix more than ‘Portola’, the cultivar with the lowest TSS tested. All pairwise comparisons are in Table 6. Cultural practice treatments did not influence TSS content (ANOVA, $p = 0.88$).

Total Phenolic Content (TPC) Analysis

Fruit TPC was measured during the 2013 field season, and the effect of cultivar, cultural practice and season (early, mid or late) on TPC was determined. Pairwise comparisons reveal that while cultural practice did not influence average fruit TPC, there were seasonal and cultivar effects. Average TPC of early and mid season fruit were similar, before a significant drop during late season harvests (Table 7).

[Insert Table 7 here]

An ANOVA test revealed a strong cultivar effect on TPC, $p= 4.77e^{-10}$. Pairwise comparisons were conducted to determine differences of cultivar treatment means. The

TPC of ‘Portola’ was lower than all other cultivars with a mean value of 0.129 mg GAE/mL, followed by ‘Albion’, which had lower TPC than ‘Monterey’ (Table 7). ‘Monterey’ had the highest TPC of all cultivars tested with a mean value of 0.193 mg GAE /g. Cultural practice treatments did not influence total soluble solid content ($p = 0.3244$).

Discussion

The results of this project demonstrate the viability of extended season strawberry production in the Upper Midwest using organic practices and the selected day-neutral cultivars. This discussion will cover the potential of our methodology to extend strawberry harvest in the Upper Midwest, compare our total yield and berry weight figures with the averages of June-bearing cultivars in the region and analyze the relationship of yield and berry weight over time. For fruit quality, we will compare TSS and TPC of our fruit with previous research, along with the effect of cultivar, cultural practice and time of harvest on TSS and TPC. Finally we will make cultivar and cultural practice recommendations based on our two years of multi-site research.

The cultural practices observed have the potential of increasing social and economic facets of sustainability. The typical June-bearing strawberry season ends near the 4th of July in the Upper Midwest (Poppe, personal communication). Both sites in this project harvested fruit from mid-July through October in 2013 and 2014 (Figures 1 & 2). Coupled with an already-present June-bearing season, day-neutral production using these practices would more than triple the length of the strawberry season in the region, with

the indirect outcome of increased job availability and satiating an increased social demand for local produce, and organically managed produce specifically (Falguera et al., 2012; Frith, 2007).

The extended production observed could yield economic benefits for growers as well. USDA production census data (Vilsack, 2009) indicate that perennial June-bearing strawberry systems yield an average of 6,160 kg·ha⁻¹ annually after an initial establishment year in the Upper Midwest. These averages can be coupled with over 20 years of June-bearing production data recorded at the University of Minnesota-Morris, where cultivars average approximately 7,840 kg·ha⁻¹ per year after the establishment year (Luby et al., 2013). The day-neutral cultivars- managed organically as an annual rotation crop in this project- outperformed these averages regardless of year, site, cultivar or cultural practice. Average total yields, when combining sites, cultivars and years, were on average 0.5 kg fruit/plant/year when managed under plastic mulch with low tunnels, 0.4 kg fruit/plant/year when managed with plastic mulch without low tunnels, and 0.3 kg fruit/plant/year when managed with straw mulch. Plots managed similar to the treatments in this project, with raised bed strips and double row planting, will average near 45,052 plants/hectare. Applying our yield data, this translates to average yields of 22,472 kg·ha⁻¹ per year, 18,386 kg·ha⁻¹ per year and 12,257 kg·ha⁻¹ per year when managing under plastic mulch with low tunnel, plastic mulch without low tunnel and straw mulch practices, respectively. Day-neutrals managed in this fashion also do not require an initial establishment year. Thus, while June-bearing cultivars have historically averaged 2.2 kg fruit/m of row over 4 years (Luby et al., 2013), yield data from the day-

neutral cultivars tested indicate they would average 19.68 kg fruit/m of row, 16.4 kg fruit/m of row and 10.8 kg fruit/m of row when managed as plastic mulch with low tunnel, plastic mulch without low tunnel and straw mulch practices, respectively, over the same time.

While formal economic analyses were not performed for this production paper, we believe the yield advantages of these annual, day-neutral management practices over perennial, June-bearing practices are more than enough to make up for the extra labor costs a grower would accumulate for harvesting over a longer period of time. This is especially true considering the price premium a grower could likely fetch for supplying locally produced strawberries at an unconventional time for the US Upper Midwest. It is interesting to note that our improved yields were observed using organic practices, while the cited June-bearing yields were all managed using conventional practices. If our practices were modified to conventional rather than organic management, we believe it would still provide annual yield improvement over the cited June-bearing figures due to the season extension inherent in our practices.

There were yield differences within the management practices depending on cultivar. The highest yielding cultivar/practice was ‘Portola’ at the Morris site in 2013 using low tunnels, while the lowest yielding cultivar/practice was ‘Seascape’ at the St Paul site in 2014, managed under straw mulch (Tables 1 & 2). It is likely the lower yield of ‘Seascape’ in 2014 was influenced by a root rot present at at planting; indeed, ‘Seascape’ was the lowest yielding cultivar at the St Paul site under every management practice (Table 3). As the plants were managed under organic practices, fungicide dips

were not available to treat ‘Seascape’ plants. Interestingly, ‘Seascape’’s low yield at the St Paul site in 2014 translates to $7,150 \text{ kg}\cdot\text{ha}^{-1}$, still higher than the $6,160 \text{ kg}\cdot\text{ha}^{-1}$ Upper Midwest average for June-bearing strawberries cited by the USDA (Vilsack, 2009).

Observing total yield, fluctuations over time were consistent among cultivars and cultural practices (Figures 1 & 2). Regardless of site, cultural practice or cultivar, weekly yields increased from the first harvest until mid- to late-August, followed by a yield drop over the next 1-2 weeks and subsequent, marginal recovery. Since no cultivar or cultural practice treatment influenced yield fluctuations differently than others, we cannot recommend a ‘mix’ of cultivars or practices that ensure at least one combination is producing at its highest rate throughout the growing season.

In addition to favorable yields, berry weight of the day-neutral cultivars measured in this project compare well to June-bearing fruit grown in the region. While cultural practice rarely had an effect on berry weight, there were several significant cultivar effects (Tables 3 & 4). Combining sites and years, mean berry weight of cultivar/cultural practice treatments ranged from 9.8 to 18.1g, with a grand mean of 13.8g. Comparatively, mean berry weight among 18 June-bearing cultivars grown in Morris, MN in 2013 ranged from 2.4 to 16.4 grams, with a grand mean of 9.3 grams (Luby et al., 2013). Combined with total yield data, these figures show that the day-neutral cultivars selected, when managed under the organic practices observed in this project, consistently produce higher yields of equal-to-larger size fruit than conventionally managed June-bearing cultivars grown in the Upper Midwest.

When plotted on the same graph, it becomes apparent that weekly yields and berry weight do not have a strict linear relationship; while weekly yields visit the same unit weight several times over the course the season, their corresponding weekly berry weights are typically quite different (Figure 3a). This data implies that growers cannot always expect smaller fruit when yields are high, and vice versa.

The TSS content of fruit grown in this project compares favorably with related strawberry research. Cordenunsi et al. (2003) and Sturm et al. (2003) conducted projects in Brazil (23.1172° S) and Slovenia (46.0500° N), respectively, measuring average TSS on a combined 18 strawberry cultivars. The cultivar in our research with the lowest average TSS content ('Portola', 7.29 % Brix) was higher than all but 6 of the cultivars in those projects. 'Albion' and 'San Andreas', the two cultivars in our project with the highest average TSS content (9.4 & 8.98 % Brix, respectively) measured higher than all 18 cultivars measured by the other studies. Thus day-neutral strawberries grown in the Upper Midwest have the potential to be of higher sensory quality than fruit grown in similar and more southern latitudes.

Other important observations are discerned when analyzing TSS. Our results summarized in Table 6 imply both genotype and environmental effects can influence TSS. When combining TSS data with the yield data, the cultivar with the lowest TSS content ('Portola') also consistently had the highest yields (Table 3). A linear regression showed us that although there is a negative relationship between weekly yield and % brix ($p = .0007$), the relationship is likely more complex than a single-factor regression can explain. Indeed, research implies that a multifactorial model may explain a greater deal of

this variance. Shaw (1990) found variance in strawberry TSS to be influenced by pedigree in addition to yield, and increased TSS could be obtained through selective breeding measures. Our data supports these findings, as significant differences in TSS were observed among cultivars (Table 6).

An ANOVA tested on the relationship of TSS with cultural practice treatments revealed no significance ($p = 0.88$). Previous research on the influence of plastic coverings on TSS content is mixed. Voća et al. (2007) found that strawberry cv. 'Elsanta' grown in high tunnels had no effect on TSS or TSS:total acidity ratios, compared to fruit grown in the open field. However Salamé-Donoso et al. (2010) found that strawberries often had higher TSS when managed under high tunnels. It is possible that low tunnels do not provide enough of a modified microclimate for a difference in TSS to occur compared to high tunnels, or that TSS in this project was not measured at a time that would have captured tunnel effects. Salamé-Donoso et al. (2010) specifies that late season TSS differences were at their greatest after freezing events had occurred. TSS for this project was measured on 6 October 2014, while the first freezing event at the St Paul site occurred 9 October 2014 (Boulay, personal communication), perhaps explaining why a significant low tunnel effect was not observed even in the late season measurement (ANOVA, $p=0.14$).

The most likely explanation for the discrepancy in the literature is based on location of research. The research by Salamé-Donoso et al. (2010) was based in Balm, FL (27.76° N), where the climate is more moderate, compared to St Paul (44.94° N) and Morris, MN (45.59° N). Thus passive heat retention practices, such as high tunnels,

would likely have a greater effect on microclimate. Research by Voća et al. (2007), whose findings coincide with the results of this project, was conducted in Zagreb, Croatia (45.82° N). It seems climatic similarities may play a role in the effect of tunnel research.

The results of our TPC analysis support previous research that there is notable variability in the presence of bioactive compounds even within individual crops (Table 7, Capocasa et al., 2008). This implies that while a strawberry bought from a farmers market today may have more phenolic content than a grape, a strawberry bought next week may not. Both genetic and environmental factors have been proposed to contribute to this intraspecific variability, and the results obtained in this study support the influence of genotype/cultivar on the quality characteristics of strawberry. Cultivar had a significant effect on sensory (TSS) and nutritional (TPC) aspects of fruit, while cultural practice did not (Tables 5 & 6). Such findings provide useful insight to growers; choice of cultural practice can influence the yield of a strawberry crop, but likely won't impact nutritional or sensory quality.

Our TSS and TPC findings suggest that strawberry cultivar/genotype influence sensory and nutritional quality characteristics and that strawberry TSS content can be used as a loose predictor for TPC, and vice versa (Tables 5 & 6). Capocasa et al. (2008) found that both TSS and TPC are significantly affected by cultivar in strawberry; a significantly positive TSS~TPC correlation was found as well ($r=0.38$). In addition, TPC is highly correlated with total antioxidant capacity (Proteggente et al., 2002), suggesting that the strawberry cultivars in this study with high TPC may also be high in other nutritional characteristics as well. Combining yield data with TSS and TPC, we observed

that the consistently highest yielding cultivar had the consistently lowest measured quality characteristics (Tables 2, 5 & 6). This implies that high producing cultivars in this project may have less sugars and bioactive compounds to invest into each strawberry, and further supports the reported impact of plant genetics on nutrition (Black et al., 2002). A method to further clarify this relationship would be to thin the inflorescences of half of the plants in each cultivar, and examine if there are TSS or TPC differences in the fruit produced by thinned vs. non-thinned plants.

While our TSS and TPC observations among cultivar/cultural practice combinations can provide useful insight to growers in the Upper Midwest, the lack of a conventionally managed treatment keeps us from discerning if organically managed strawberries using these practices are more or less nutritious than conventionally managed counterparts. Thus we cannot support or contrast the claims of Asami et al. (2003), that organically managed strawberries have higher phenolic content than conventional due to an increase in fruit phenols with antimicrobial properties, potentially fending off the increased amount of pest pressure typical in organic operations. However since our work helps solidify the commercial viability of organic strawberry production in the region, a future project examining that question becomes justified.

There are many considerations to take into account before choosing a cultivar, cultural practice or cultivar/cultural practice combination for growers in the Upper Midwest. ‘Portola’ consistently boasted the highest or second highest yield regardless of site, cultural practice and year, and had average to above-average berry weight (Tables 3 & 4). However research implies that ‘Portola’ may have slightly lower fruit quality than

other day-neutral cultivars. Similar to our findings, Ruan et al. (2013) found ‘Portola’ to have the lowest TSS and organic acid content in a comparative analysis that included ‘Albion’, ‘Monterey’ and ‘San Andreas’. In that analysis ‘Albion’ also had the highest TSS content and soluble solid/organic acid ratio. Thus ‘Portola’ may be a good fit for Upper Midwest growers concerned with producing the most fruit, while ‘Albion’ may be considered an optimal ‘all-around’ cultivar that performs well across all parameters (Tables 1-4). Combining the findings of Ruan et al. (2013) with our own, ‘Albion’ appears to be the most well adapted cultivar tested, and while all cultivars were productive, any day-neutral grower in the Upper Midwest that incorporates ‘Portola’ and ‘Albion’ into a mix of cultivars can likely expect high yields, large fruit size and a sweet fruit with relatively abundant phenols.

Although ‘Portola’ and ‘Albion’ stood out as exceptional cultivars in this project, we believe that recommending a single best cultural practice among those tested is not possible due to the variance in our results, along with the variability of environmental conditions present in the Upper Midwest. The only instance where it appeared low tunnels resulted in a consistent yield advantage over plastic mulch without low tunnels was at the Morris site in 2013 (Table 2). This advantage was very likely due to an August hailstorm that destroyed two harvests for plants that were not protected by low tunnels. Therefore low tunnels offer a type of quality and yield insurance, especially in areas where hailstorms are likely. The straw mulch cultural practice consistently yielded less fruit than either plastic mulch practice (Table 2). This reduction may be attributed to poor weed suppression and/or the tendency of straw mulch to immobilize soil nitrate-N

(Döring et al., 2005). As such, we cannot recommend straw as a mulch source for organic growers primarily concerned with optimal yields. Observations during the 2013 & 2014 harvests lead us to believe that low tunnels may also contribute benefits not measured in this paper, such reductions in pest pressure and increased proportions of undamaged fruit. Indeed, reduced incidence of *Botrytis cinerea* and spotted wing drosophila (*Drosophila suzukii*) have been reported on fruits grown under tunnel systems (Burrack et al., 2013; Xiao et al., 2001). Additionally Kadir et al. (2006) found tunnel systems to significantly improve fruit quality of strawberry cv. ‘Sweet Charlie’ and ‘Chandler’.

While there were clear differences in yield and quality performance among the cultural practice/cultivar combinations tested, the potential for economic advantages exists for any combination. ‘Seascape’, when managed under straw mulch at the St Paul site in 2014 was the lowest average yielding combination in the project. Yet when scaled up to production by acreage, that combination would have averaged 7,150 kg·ha⁻¹ per year. This is still more than the 6,160 kg·ha⁻¹ per year Upper Midwest average for June-bearing cultivars (Vilsack, 2009). Combining year and cultivar data and scaling up for acreage, straw mulch management averaged 12,257 kg·ha⁻¹ per year, compared to 22,472 kg·ha⁻¹ per year and 18,386 kg·ha⁻¹ per year when managing under plastic mulch with low tunnels and plastic mulch without low tunnels, respectively. Thus, while straw mulch management did not deliver optimal yields in this project, it still may prove economically viable for organic growers who wish to minimize plastic used on their land.

Conclusions

Compiling all data from each year and location, we can conclude any organic or conventional grower in the Upper Midwest who wishes to incorporate day-neutral strawberries into their farm system would benefit from an annual rotation utilizing plastic mulch with low tunnels as described in this project, and planting a mix of cultivars that includes ‘Portola’ and ‘Albion’. Averaging our data across sites and years suggest an annual low tunnel practice that houses ‘Portola’ and ‘Albion’ at a 1:1 ratio would consistently extend harvests from July into October and yield approximately 0.53 kg/plant/year, scaling up to an impressive 23,647 kg·ha⁻¹ per year. This is a substantial yield advantage over the 6,160 kg·ha⁻¹ average for traditional June-bearing practices in the region. Such a mix would also likely have fruit with high TSS and TPC content, and tunnel practices helping ensure the highest proportion of quality produce (Burrack et al., 2013; Kadir et al., 2006; Ruan et al., 2013; Xiao et al., 2001). Utilizing plastic mulch without low tunnels still result in high yield while sacrificing potential protection from environmental pressures. Straw mulch practices, while consistently yielding less fruit than plastic mulch to a significant degree (Figure 1) may nonetheless provide a viable opportunity to organic growers looking to extend the strawberry season while minimizing plastic inputs. Taken as a whole, our study has indicated that high yielding, high quality day-neutral strawberry cultivars can be grown organically in the Upper Midwest long past the traditional 6-week fruiting season, and a careful selection of cultivar and cultural practice can maximize growing success.

Table 1. ANOVA of all significant main effects and interactions influencing total yields.

* = $P \leq 0.05$, *** = $P \leq 0.001$.

| | <u>Df</u> | <u>F value</u> | <u>p value</u> | |
|-----------------------------|-----------|----------------|----------------|-----|
| Year | 1 | 70.6 | 2.20E-16 | *** |
| Site | 1 | 467.4 | 2.20E-16 | *** |
| Cultivar | 8 | 315.1 | 2.20E-16 | *** |
| Cultural Practice | 2 | 55.6 | 2.20E-16 | *** |
| Year:Site | 1 | 6.4 | 1.10E-02 | * |
| Year:Cultivar | 4 | 12.4 | 5.03E-10 | *** |
| Site:Cultivar | 5 | 6.7 | 3.29E-06 | *** |
| Year:Cultural Practice | 2 | 11.8 | 7.74E-06 | *** |
| Site:Cultural Practice | 1 | 15.4 | 8.59E-05 | *** |
| Cultivar:Cultural Practice | 15 | 3.8 | 1.05E-06 | *** |
| Year:Site:Cultural Practice | 1 | 18.9 | 1.34E-05 | *** |

Table 2. Average total yields of cultivars among each cultural practice in St Paul, MN and Morris, MN, 2013 & 2014.

| Cultivar | Treatment | St Paul | | Morris | |
|-------------|-----------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| | | 2013 (kg/plant) ^z | 2014 (kg/plant) ^z | 2013 (kg/plant) ^z | 2014 (kg/plant) ^z |
| Albion | Plasticulture-low tunnel | 0.39 | 0.48 a | 0.56 a | 0.39 |
| | Plasticulture-no tunnel | 0.29 | 0.39 ab | 0.42 b | 0.38 |
| | Straw mulch | 0.28 | 0.28 b | | |
| Evie-II | Plasticulture-low tunnel | 0.49 ab | 0.57 | 0.60 | 0.45 |
| | Plasticulture-no tunnel | 0.56 a | 0.44 | 0.55 | 0.42 |
| | Straw mulch | 0.32 b | 0.43 | | |
| Monterey | Plasticulture-low tunnel | 0.42 a | 0.55 a | 0.59 a | 0.46 |
| | Plasticulture-no tunnel | 0.42 a | 0.38 b | 0.34 b | 0.42 |
| | Straw mulch | 0.20 b | 0.34 b | | |
| Portola | Plasticulture-low tunnel | 0.42 | 0.49 a | 0.95 a | 0.52 |
| | Plasticulture-no tunnel | 0.49 | 0.42 ab | 0.61 b | 0.50 |
| | Straw mulch | 0.36 | 0.34 b | | |
| San Andreas | Plasticulture-low tunnel | 0.35 a | 0.43 a | 0.55 a | 0.35 |
| | Plasticulture-no low tunnel | 0.32 a | 0.36 ab | 0.42 b | 0.29 |
| | Straw mulch | 0.17 b | 0.22 b | | |
| Seascape | Plasticulture-low tunnel | 0.41 a | 0.28 | 0.72 a | 0.36 |
| | Plasticulture-no tunnel | 0.38 ab | 0.24 | 0.47 b | 0.33 |
| | Straw mulch | 0.23 b | 0.16 | | |

^z Pairwise comparisons (in columns, separated by lines) by Tukey's Honest Significant Difference test at $P \leq 0.05$.

Table 3. Average total yields of each cultivar within cultural practices in St Paul, MN and Morris, MN, 2013 & 2014.

| Cultivar | St Paul | | Morris | |
|----------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| | Plasticulture- Low tunnels | | Plasticulture- Low tunnels | |
| | 2013 (kg/plant) ^z | 2014 (kg/plant) ^z | 2013 (kg/plant) ^z | 2014 (kg/plant) ^z |
| Albion | 0.39 | 0.48 a | 0.58 c | 0.39 ab |
| Evie-II | 0.49 | 0.57 a | 0.60 bc | 0.45 ab |
| Monterey | 0.42 | 0.55 a | 0.59 bc | 0.46 a |
| Portola | 0.42 | 0.49 a | 0.95 a | 0.52 a |
| San | | | | |
| Andreas | 0.35 | 0.43 ab | 0.55 c | 0.35 b |
| Seascape | 0.41 | 0.28 b | 0.72 b | 0.36 ab |
| Cultivar | Plasticulture- No tunnels | | Plasticulture- No tunnels | |
| | 2013 (kg/plant) ^z | 2014 (kg/plant) ^z | 2013 (kg/plant) ^z | 2014 (kg/plant) ^z |
| | | | | |
| Albion | 0.29 c | 0.39 a | 0.42 bc | 0.38 abc |
| Evie-II | 0.56 a | 0.44 a | 0.55 ab | 0.42 ab |
| Monterey | 0.42 abc | 0.38 ab | 0.34 c | 0.42 abc |
| Portola | 0.49 ab | 0.42 a | 0.61 a | 0.50 a |
| San | | | | |
| Andreas | 0.32 c | 0.36 ab | 0.42 bc | 0.29 c |
| Seascape | 0.38 bc | 0.24 b | 0.47 bc | 0.33 bc |
| Cultivar | Straw mulch | | | |
| | 2013 (kg/plant) ^z | 2014 (kg/plant) ^z | | |
| | | | | |
| Albion | 0.28 | 0.28 abc | | |
| Evie-II | 0.32 | 0.43 a | | |
| Monterey | 0.20 | 0.34 abc | | |
| Portola | 0.36 | 0.34 ab | | |
| San | | | | |
| Andreas | 0.17 | 0.22 bc | | |
| Seascape | 0.23 | 0.16 c | | |

^z Pairwise comparisons (in columns) by Tukey's Honest Significant Difference test at $P \leq 0.05$.

Table 4. Average berry weight of cultivars among each cultural practice in St Paul, MN and Morris, MN, 2013 & 2014.

| Cultivar | Treatment | St Paul | | Morris | |
|-------------|--------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | | 2013 (g/fruit) ^z | 2014 (g/fruit) ^z | 2013 (g/fruit) ^z | 2014 (g/fruit) ^z |
| Albion | Plasticulture-low tunnel | 12.33 b | 14.05 | 15.16 | 18.25 |
| | Plasticulture-no tunnel | 11.82 b | 12.62 | 14.32 | 17.55 |
| | Straw mulch | 13.59 a | 13.56 | | |
| Evie-II | Plasticulture-low tunnel | 12.63 a | 12.90 | 14.86 a | 17.09 |
| | Plasticulture-no tunnel | 11.61 ab | 12.25 | 12.96 b | 16.28 |
| | Straw mulch | 10.81 b | 12.44 | | |
| Monterey | Plasticulture-low tunnel | 13.11 | 13.44 | 14.93 | 16.75 |
| | Plasticulture-no tunnel | 11.53 | 13.91 | 13.87 | 16.55 |
| | Straw mulch | 10.39 | 12.91 | | |
| Portola | Plasticulture-low tunnel | 13.00 | 13.78 | 18.61 a | 19.91 |
| | Plasticulture-no tunnel | 12.87 | 13.89 | 14.78 b | 17.83 |
| | Straw mulch | 13.41 | 14.17 | | |
| San Andreas | Plasticulture-low tunnel | 13.35 | 14.30 | 15.48 | 18.11 |
| | Plasticulture-no tunnel | 13.22 | 14.42 | 16.52 | 16.61 |
| | Straw mulch | 10.40 | 12.55 | | |
| Seascape | Plasticulture-low tunnel | 11.31 | 11.50 | 14.40 | 13.81 a |
| | Plasticulture-no tunnel | 10.07 | 10.77 | 13.20 | 12.12 b |
| | Straw mulch | 10.69 | 9.79 | | |

^z Pairwise comparisons (in columns) by Tukey's Honest Significant Difference test at $P \leq 0.05$.

Table 5. Average berry weight of each cultivar within cultural practices in St Paul, MN and Morris, MN, 2013 & 2014.

| Cultivar | St Paul | | Morris | |
|----------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | Plasticulture- Low tunnels | | Plasticulture- Low tunnels | |
| | 2013 (g/fruit) ^z | 2014 (g/fruit) ^z | 2013 (g/fruit) ^z | 2014 (g/fruit) ^z |
| Albion | 12.33 | 14.05 a | 15.16 b | 18.25 ab |
| Evie-II | 12.63 | 12.90 ab | 14.86 b | 17.09 b |
| Monterey | 13.11 | 13.44 ab | 14.93 b | 16.75 b |
| Portola | 13.00 | 13.78 ab | 18.61 a | 19.91 a |
| San Andreas | 13.35 | 14.30 a | 15.48 b | 18.11 ab |
| Seascape | 11.31 | 11.50 b | 14.40 b | 13.81 c |
| Cultivar | Plasticulture- No low tunnels | | Plasticulture- No low tunnels | |
| | 2013 (g/fruit) ^z | 2014 (g/fruit) ^z | 2013 (g/fruit) ^z | 2014 (g/fruit) ^z |
| | Albion | 11.82 | 12.62 abc | 14.32 b |
| Evie-II | 11.61 | 12.25 bc | 12.96 b | 16.28 a |
| Monterey | 11.53 | 13.91 ab | 13.87 b | 16.55 a |
| Portola | 12.87 | 13.89 ab | 14.78 ab | 17.83 a |
| San Andreas | 13.22 | 14.42 a | 16.52 a | 16.61 a |
| Seascape | 10.07 | 10.77 c | 13.20 b | 12.12 b |
| Cultivar | Straw mulch | | | |
| | 2013 (g/fruit) ^z | 2014 (g/fruit) ^z | | |
| | Albion | 13.59 | 13.56 a | |
| Evie-II | 10.81 | 12.44 ab | | |
| Monterey | 10.39 | 12.91 ab | | |
| Portola | 13.41 | 14.17 a | | |
| San Andreas | 10.40 | 12.55 ab | | |
| Seascape | 10.69 | 9.79 b | | |

^z Pairwise comparisons (in columns) by Tukey's Honest Significant Difference test at $P \leq 0.05$.

Table 6. Average TSS (measured in % Brix) of fruit within each cultivar, time of harvest and cultural practice in 2014.

| Cultivar | TSS ^z | Time of Harvest | TSS ^z | Cultural Practice | TSS ^z |
|----------------|------------------|-----------------|------------------|------------------------------|------------------|
| Albion | 9.4 a | Early | 8.8 a | Plastic Mulch- Low Tunnel | 8.66 |
| Evie-II | 8.14 bc | Mid | 7.9 b | Plastic Mulch | 8.43 |
| Monterey | 8.67 ab | Late | 8.99 a | Straw Mulch | 8.59 |
| Portola | 7.29 c | | | | |
| San Andreas | 8.98 ab | | | | |
| Seascape | 8.88 ab | | | | |

^z Pairwise comparisons (in columns) by Tukey's Honest Significant Difference test at $P \leq 0.05$.

Table 7. Average TPC (measured as gallic acid equivalents, mg/g fresh weight) of fruit within each cultivar, time of harvest and cultural practice in 2013.

| Cultivar | GAE ^z | Time of Harvest | GAE ^z | Cultural Practice | GAE ^z |
|----------|------------------|-----------------|------------------|------------------------------|------------------|
| Albion | 0.124 b | Early | 0.146 a | Plastic Mulch- Low Tunnel | 0.127 |
| Evie-II | 0.14 ab | Mid | 0.138 a | Plastic Mulch | 0.13 |
| Monterey | 0.15 a | Late | 0.109 b | Straw Mulch | 0.136 |
| Portola | 0.101 c | | | | |
| San | | | | | |
| Andreas | 0.133 ab | | | | |
| Seascape | 0.138 ab | | | | |

^z Pairwise comparisons (in columns) by Tukey's Honest Significant Difference test at $P \leq 0.05$.

Figure 1. Weekly average yields of each cultivar/cultural practice combination, St Paul site, 2013 & 2014. LT, PL, and SM denote plastic mulch with low tunnels, plastic mulch without low tunnels, and straw mulch cultural practices, respectively.

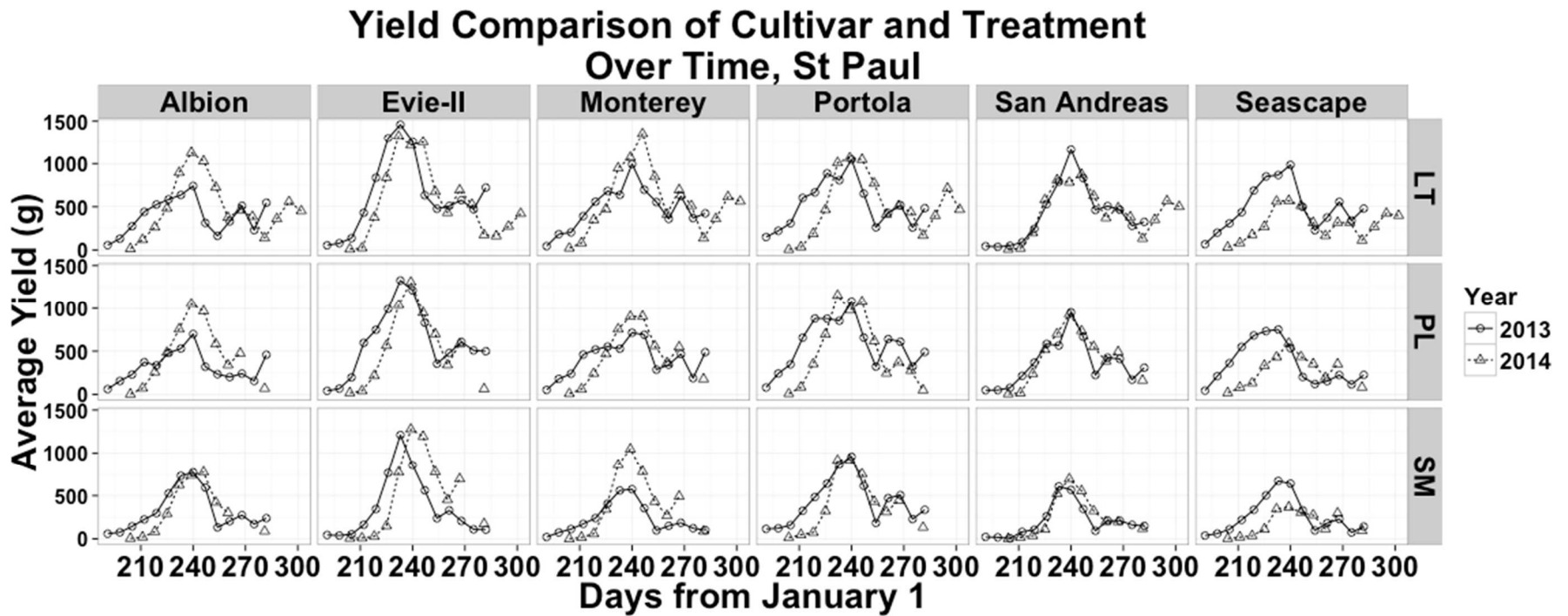


Figure 2. Weekly average yields of each cultivar/cultural practice combination, Morris site, 2013 & 2014. LT and PL denote plastic mulch with low tunnels and plastic mulch without low tunnels, respectively.

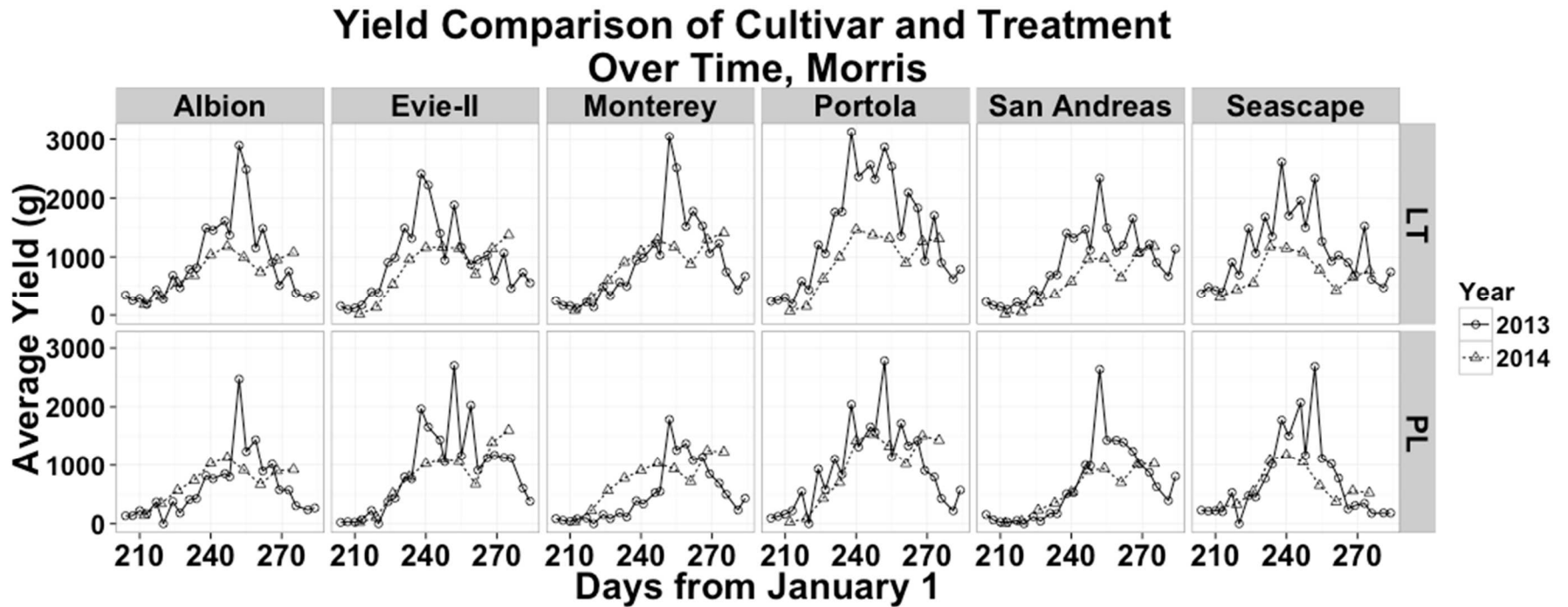
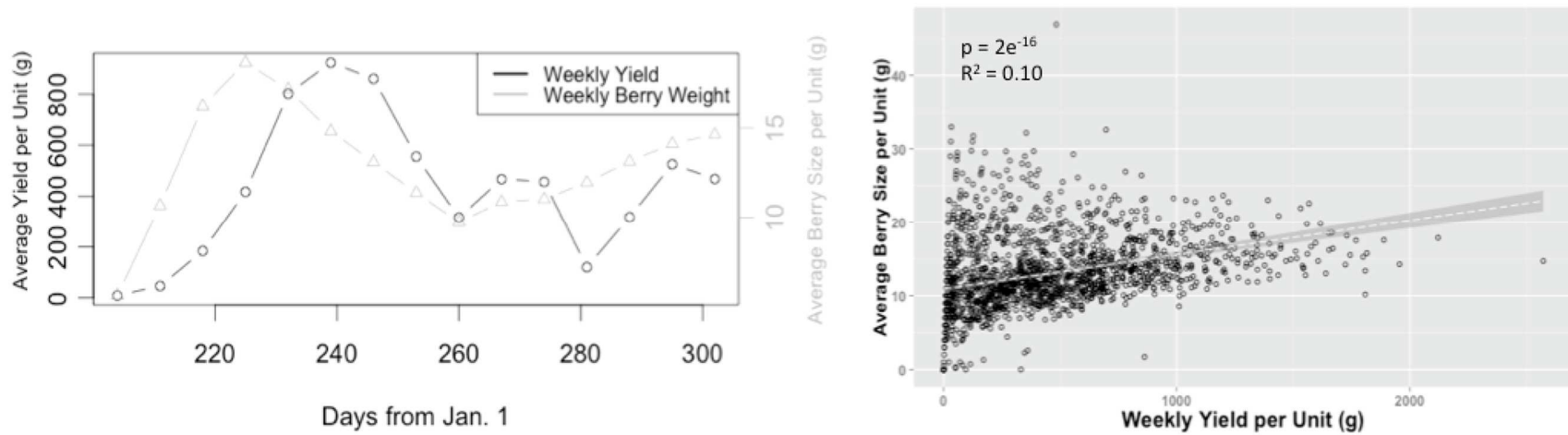


Figure 3. (a) Relationship of average weekly yields and average berry weight over time, St Paul site 2014. (b) Linear regression comparing weekly yield of each experimental unit with it's corresponding berry weight taken at the St Paul site in 2014.



CHAPTER 4

**THE INFLUENCE OF POST-TILLAGE CULTURAL PRACTICES ON SOIL
HEALTH INDICATORS AND YIELD IN ORGANIC DAY-NEUTRAL
STRAWBERRY**

(to be submitted to *Biological Agriculture & Horticulture*)

Andrew Petran

Abstract

Organic growers employ cultural practices that support soil health for certification, to increase crop quality and yields, and to improve environmental sustainability. The soil microbial ecosystem is an aspect of soil health that growers can alter, but adjusting a practice may or may not result in diversifying the soil microecosystem, or affecting crop yield. This project compared three post-tillage cultural practices for annual, day-neutral strawberry production (plastic mulch with low tunnels, plastic mulch without low tunnels and straw mulch) to determine 1) possible differences in soil microbial biomass (SMB-Nitrogen and SMB-Carbon) and permanganate oxidizable carbon (POX-C), known indicators of soil health, and 2) possible correlations of soil health indicators with yield. Samples were taken twice (early season and late season) from each practice over 2 years (2014 and 2015); harvests occurred weekly. ANOVA and pairwise comparisons revealed that post-tillage cultural practice rarely influenced SMB or POX-C levels. Year of planting was the only significant main effect for both soil health indicators. SMB-Nitrogen was the only soil health factor significantly correlated with yield, but was likely confounded by year. We conclude that while mulch type or low tunnels may increase total yield and crop quality for day-neutral strawberry, those benefits are likely not the result of the practices influencing a change in SMB or POX-C.

Introduction

Improving soil health has an impact on crop production; healthier soils often lead to higher yields and more nutritious crops (Borowicz, 2009; Moore-Kucera et al., 2008). The United States National Organic Program (NOP) has recognized this relationship, and requires farmers to actively build soil health in order to retain organic certification (NOP regulation § 205.203). However much like human health, soil health is a broad topic that is difficult to succinctly define. Moore-Kucera et al. (2008) lists empirically measurable soil functions including “...the capacity to supply nutrients, retain optimal water content, support soil food webs, recycle nutrients, maintain microbial diversity, remediate pollutants, and suppress plant pathogens”. Since farmers are concerned with how soil health impacts the yield and sustainability of crop production, they typically focus on soil functions that directly impact those ends (Abawi & Widmer, 2000).

The quantity of microbial life in soils is typically referred to as soil microbial biomass (SMB), and is one of the most important soil functions for crop production (Doran, 19080). An abundant SMB leads to increased availability of soil nutrients to plants; more than 90% of all nutrients in the soil food web are cycled through microbial biomass to higher trophic levels (Kennedy, 1999). Because of this microbial benefit to crops, organic farmers usually desire high levels of SMB in their soils. Organic farming practices in particular are sensitive to SMB levels since NOP and international guidelines limit the selection of supplemental fertility, and organically approved fertility sources often require microbially mediated reactions to become plant available (Stockdale et al., 2002).

Fortunately, farmers can adjust cultural practices to optimize the SMB microenvironment (Abawi and Widmer, 2000; Peters et al., 2003). SMB levels can be affected by farm cultural practices such as tillage (Doran, 1980), cropping system (Acosta-Martinez et al., 2003) and fertilizer regimen (Kramer et al., 2006). Organisms functioning within higher trophic levels of the soil microecosystem, such as nematodes, exhibit similar responses to cultural practices (Forge et al., 2005; Neher and Olson, 1999). Cultural practices, thus, affect the makeup of the soil microbial and faunal ecosystem, additionally affecting the availability of nutrients in organically managed soils. A wise selection of soil health building cultural practices can prime intrinsic fertility for plants.

The common cultivated strawberry (*Fragaria x ananassa*) is a valuable horticultural crop; international production has exceeded 4 million tonnes per year since 2007 and the market for fresh strawberries in the United States more than quadrupled from 1990-2012 (Wu et al., 2012; Perez & Plattner, 2013a). Organically produced strawberries have become especially valuable in the United States. While total organic strawberry acreage increased 4% from 2008-2011, total gross value of sales increased 52% over the same time (Perez & Plattner, 2013b). The most common and high yielding cultural practice for strawberries in the United States is annual raised-bed production, however this practice requires intensive tillage techniques known to reduce SMB in other crops (Shi et al., 2013; Murugan et al., 2014). While there is a general lack of research examining the effect of organic strawberry cultural practices on soil microbial communities, Pešaković et al. (2013) observed increased yields in strawberries fertilized

with supplements that increase microbial activity relative to fertilizers that did not. Such work implies that organic strawberry growers who employ annual raised-bed practices may experience further yield benefits from post-tillage management that works to replenish SMB lost during bed preparation.

Soil organic material (SOM) is heterogeneous, consisting of older, more recalcitrant humus, and younger pools of labile, actively decomposing plant material (Janssen, 1984). The younger pool disproportionately contributes to soil biological activity as it has high rates of decomposable material that serves to fuel microbial processes. The permanganate oxidizable carbon (POX-C) assay measures this pool, and like SMB has high sensitivity to changes in management or environmental variation (Culman et al., 2012). POX-C has displayed strong correlations with several commonly used indicators of soil health such as SMB, particulate organic matter and soluble carbohydrate C (Weil et al., 2003; Melero et al., 2009a; Melero et al. 2009b). However despite the history of research on soil health indicators it is difficult to establish SMB and POX-C recommendations for growers because the ranges vary based on soil type, and cultural practice driven separation takes longer to observe in soils with higher natural SMB and POX-C levels (Weyers et al., 2013). Thus accurate recommendations would need to be customized by region and soil type.

The purpose of this project was to determine if organic strawberry cultural practices employed post-tillage affected soil health indicators in alfisol soils common to the Upper Midwest United States. Two soil health indicators were chosen, SMB and permanganate oxidizable carbon (POX-C). Petran et al. (2016) recently demonstrated the

potential yield differences of the chosen post-tillage cultural practices. If differences in soil health indicators are observed among post-tillage cultural practices as well, it may contribute a more empirical perspective to our understanding of the relationship between soil health and strawberry crop performance or yield.

Materials & Methods

Plant Materials

Day-neutral strawberry cultivar ‘Albion’ was planted, in St Paul, MN (45° 0’ 31.09” N, 93° 18’ 56.313” W, alfisol soil) during the 2014 and 2015 growing seasons. The plots at the site were preceded by dry beans (*Phaseolus vulgaris*) each year. The plants were managed as annuals, working into a yearly rotation on USDA certified organic land. Plants were acquired as chilled, bare rooted transplants from Nourse Farms (South Deerfield, MA). Planting occurred in the spring when soils had thawed and drained sufficiently for tractor activity, 23 May in 2014 and 21 May in 2015. Plants were uprooted and composted in the first week of November each year.

Cultural Practices

Three organically certified post-tillage cultural practice treatments were selected for analysis: plastic mulch with low tunnel coverings, plastic mulch without low tunnel coverings, and straw mulch. Plastic mulch was 1.22 m wide, 1 mm thick white on black plastic (Berry Plastics, Evansville, IN), and low tunnel plastic was 3.66 m wide, 4mm thick clear greenhouse film (Ag Resource Inc., Detroit Lakes, MN). Raised beds were prepared by initially rotovating the soil, followed by bed preparation with a model 2121-

D bed shaper and plastic mulch/drip tape application with a model 2133 mulch layer, both from Buckeye Tractor Company (Columbus Grove, OH).

Treatments were fertigated using drip tape applicators underneath each mulch type, at the rate of $6.1 \text{ kg} \cdot \text{ha}^{-1}$ of fertilizer per week. Fertility source was OMRI-approved AgGrand Organic Series fertilizer, 4N-1.3P-2.5K, incorporated via 7.57 L injection tank (EZ-Flo Fertilizing Systems, Rocklin, CA). Fungicides were never used, but PyGanic® 5.0 was sprayed via backpack sprayer to control spider mites near the beginning of each season, 2 July in 2013 and 5 July in 2014. PyGanic was applied once a week for 2 weeks at the rate of 14.8 mL per 5 gallons of water. Plastic mulches and tunnels were removed from the field at the end of each season to maintain organic compliance (NOP regulation §205.206).

Plot Design and Harvesting

Each cultural practice, arranged in a randomized complete block design, consisted of 30.5m long strips. Strips contained a 1.2m wide raised bed with plants assembled in a double row with 30.5cm within-row and 35.6cm between-row plant spacing, and a 0.6m walkway covered with landscape fabric (DeWitt Company, Sikeston, MO).

Inflorescences were removed until July 1 at each site, each year, to ensure vegetative growth and root establishment immediately after planting. Harvest began 23 July and concluded 11 October in 2013, and began 21 July and concluded 31 October in 2014. Plots were harvested twice each week all season, but only once each week for the first and last harvest weeks of each season, when yields were low. During each harvest, all

berries picked within a selection zone were weighed for experimental unit yield. Unit yield from each of the two harvests a week were combined to calculate weekly yield per unit.

C and N Dynamics

Four random 30.48cm soil cores were taken within the raised beds of each cultural practice at the beginning (post-tillage) and end of each growing season- 24 May and 1 November in 2014, and 22 May and 1 November in 2015. These samples were tested for POX-C, SMB-Carbon (SMB-C) and SMB-Nitrogen (SMB-N). The POX-C assay followed the methodology of Culman et al. (2012), a slightly modified version of the original method by Weil et al. (2003). Briefly, an oxidized permanganate solution, which is purple in color, was combined with the reduced labile carbon present in the SOM of a soil sample; the ensuing redox reaction cause the permanganate to lose its purple color, which was measured on a spectrophotometer. SMB was extracted following the chloroform direct extraction method documented by Setia et al. (2012) and quantified using a TOC-TN analyzer (Shimadzu, Kyoto, Japan) to determine SMB-C and SMB-N.

Statistical Analysis

All statistical analyses were conducted using R statistical software version 3.0.2. Analysis of Variance was conducted with each measured factor to determine to the presence of significant treatment differences $p < 0.05$. Pairwise comparisons were

conducted among cultural practices within each sampling date using Tukey's Honest Significant Difference test, significance at $p < 0.05$.

Results & Discussion

A 2-way ANOVA of all factors and measurements shows that environmental, rather than cultural factors appear to influence SMB and POX-C in our study of annual organic management of day-neutral strawberries (Table 1). Statistical significance by cultural practice only occurred once, during the initial sampling in 2014 for SMB-C and POX-C (Tables 2 and 4). As this sampling occurred immediately after tillage, it is likely the separation was due to microbial variance already present within the soil rather than post-tillage cultural practices. Year was the only significant main effect shared among all measurements, while cultural practice and sampling time were less consistent and appeared to bear more significance when interacting with year (Table 1). These results are clarified in Tables 2-4. If our post-tillage cultural treatments were to effect soil health indicators we would observe statistical separation of cultural practice, but there are no differences in SMB-C, SMB-N or POX-C among cultural practices at the end of the growing season in either year (Tables 2 & 4). SMB and POX-C consistently decreased between sampling times in 2014, but similar results were not observed in 2015, where POX-C increased for all cultural practices over the course of the growing season, as did SMB-N and SMB-C for the straw mulch treatment (Tables 3 & 4). These observations further emphasize the interaction effect of sampling time with year (Table 1).

The general decrease in SMB and POX-C in 2014 framed with certain increases in 2015 is difficult to evaluate because previous research appears to only compare levels

between years and not within them (Kandeler et al., 1999; Chan et al., 2001; Chu et al., 2007; Culman et al., 2012; Bruun et al. 2013; Grandy et al. 2013). This is due to the focus on tillage practices themselves and not post-tillage management. However previous work allows an opportunity to process our observed results in context. Kandeler et al. (1999), performing an 8-year, factorial analysis found that both tillage and year had significant effects on microbial biomass, with less intensive tillage techniques increasing SMB in the top soil fraction over time. Our lack of SMB or POX-C separation among post-tillage cultural practices in the late season imply that mulch type or tunnel presence do not influence the recovery of soil health indicators after their likely disturbance from intensive tillage practices (Tables 1-4). Culman et al. (2012) reviewed 13 POX-C studies analyzing an array of experimental factors and found that environmental factors such as year, rotation and field constantly had a significant effect on POX-C, while cropping system never did. Our findings coincide with this meta-analysis, with POX-C being affected by year as a main effect but not cultural practice (Table 1). While early season differences in SMB and POX-C were observed among cultural practices in 2014 (Tables 2 & 4), the overall lack of significance of post-tillage cultural practice (Table 1) implies that these differences may have been the result of confounding environmental variance. This seems especially true given that early season samples were taken immediately after cultural practices were implemented, before any practice-driven influence could have conceivably taken place.

Tillage technique, fertilizer rate and time (over years) have greater observed management effects on SMB and POX-C than cropping system (Kandeler et al., 1999;

Culman et al., 2012). Our results agree; strawberry growers with annual day-neutral systems looking to maximize SMB or labile C in their soil organic matter will possibly find more success adjusting their fertility regimens (Pešaković et al., 2013) or adopting less invasive tillage techniques than choosing a specific mulch or tunnel type for their raised beds. The cultural practices employed in this project required intensive topsoil tillage; plots were initially rotovated each spring before bed shapers further disturbed the topsoil to create raised beds. Previous work investigating the effect of tillage technique on soil health imply that a single season is not long enough for SMB to recover after an intensive tillage event, regardless of mulch practice employed (Havlin et al., 1990; Karlen et al., 1994; Hussain et al., 1999). This may explain why late season measurements were not different among cultural practices in either year of the study (Figures 2 & 4).

Petran et al. (2016) observed total yield differences among post-tillage cultural practices in Minnesota alfisols and mollisols. This is contrasted by the lack of SMB or POX-C separation among cultural practices at the end of the growing season observed in this project. Despite this lack of separation we can still investigate possible correlations between yield and soil health factors. An ANOVA revealed SMB-N to be the only soil health indicator related to yield in this project (Table 5, $p = 0.043$), so that factor was chosen for regression analysis. SMB-N and total yields displayed a negative relationship (Figure 1). While this may imply increasing SMB-N could result in lower yields, we do not agree, as correlation r and R^2 levels were modest, -0.46 and $.21$ respectively. It is likely SMB-N can only account for 21% of the variation in yield because of the

confounding effect of year, as year affected both SMB-N (Table 1) and yields ($p = 7.6 \times 10^{-8}$). Thus we conclude that it is unlikely low tunnel or mulch driven effects on SMB or labile carbon in the SOM explain the yield and quality differences among cultural practices of an annual, rotational strawberry production system with raised beds described by Petran et al. (2016).

Other post-tillage management factors have yet to be investigated in this system. Although previous research has investigated the effect of N rate on POX-C (Grandy et al. 2013), no work has been conducted with organically approved fertility sources, or how different SMB or POX-C levels in the soil could potentially affect yields. In addition, creating an integrated fertility regimen that combines inorganic fertilizers with organic amendments and green manures has been shown to increase SMB even in the short term (Goyal et al., 1999). Such a regimen may be accomplished in this system by replacing the landscape fabric walkways with short, living mulches like clover or mowed down green manures. Improving soil health often results in increased crop quality and yields (Borowicz, 2009; Moore-Kucera et al., 2008), but more research is needed to determine cultural practice combinations that optimize both yield and soil health indicators for different crops.

Interestingly, even if late season separation had occurred among cultural practices, it would be difficult to use those differences to make categorical recommendations to growers. This is because of the variance of soil type even within the Upper Midwest United States, and the effect that variance has on the levels of soil health indicators. For example, previous research has observed 443-784 mg/kg⁻¹, 78 mg/kg⁻¹ and

400 mg/kg⁻¹ as levels associated with increased yields for SMB-C, SMB-N and POX-C, respectively, in other crops (Franco-Otero et al., 2012; Liang et al., 2012; Jannoura et al., 2014). Based on our data and the history of research documenting cultural practice effects on SMB and POX-C, it is conceivable that a grower in Minnesota alfisol soils similar to those on our site could use those levels as recommendations and modify their practices to reach them, potentially achieving greater yields. However if a Minnesota grower was located on a prairie mollisol soil they would experience natural SMB-C, SMB-N and labile C levels that are on average up to 10 times higher than what we observed in this experiment; such high levels already exceed the above recommendations and can take up to 6 years to observe cultural practice driven separation (Weyers et al., 2013). Thus any annual, post-tillage practice recommendations based on projects conducted on alfisol soils may not be useful for a grower with mollisol soils. Growers would be better served to explore customized practice recommendations based on research conducted on their native soil type.

Conclusions

Organic strawberry growers looking to gauge the general microbial health of their soil can do so by measuring SMB or POX-C, among other indicators. The POX-C assay in particular can be performed relatively quickly, is correlated with an array of other soil health measurement techniques and often shows greater sensitivity to change in environment and management (Culman et al., 2012). However our findings indicate that the post-tillage cultural practices of plastic mulch, straw mulch or the presence of low

tunnels do not alter the soil microenvironment enough to result in pairwise SMB or POX-C differences among treatments over the course of a growing season on our Minnesota alfisol soil. These practices often have observed yield differences (Petran et al., 2016), so we can reasonably assume they are not caused by mulch type or tunnels modifying SMB or labile C in the soil organic matter. Based on our observations we raise 2 propositions: to investigate the effect of other post-tillage management techniques such as fertility rate, organic amendments and green manures on SMB and POX-C in this annual, organic day-neutral system, and to customize grower recommendations based on native soil type. The implementation of these propositions may assist in determining best practices for both economic and environmental sustainability.

Table 1. ANOVA models of SMB-C, SMB-N and POX-C among all measured main effects, including significant interaction effects.

| SMB-C | | | | |
|--------------------|----|---------|----------------------|-----|
| | df | F value | p value ^z | |
| Cultural Practice | 2 | 3.98 | 0.028 | * |
| Year | 1 | 9.05 | 0.005 | ** |
| Sampling Time | 1 | 13.35 | 8.60E-04 | *** |
| Year:Sampling Time | 1 | 5.86 | 0.021 | * |

| SMB-N | | | | |
|--------------------|----|---------|----------------------|----|
| | df | F value | p value ^z | |
| Cultural Practice | 2 | 1.19 | 0.316 | |
| Year | 1 | 2.43 | 0.128 | |
| Sampling Time | 1 | 1.21 | 0.279 | |
| Year:Sampling Time | 1 | 8.35 | 0.007 | ** |

| POX-C | | | | |
|------------------------|----|---------|----------------------|-----|
| | df | F value | p value ^z | |
| Cultural Practice | 2 | 0.81 | 0.45279 | |
| Year | 1 | 46.5817 | 5.57E-08 | *** |
| Sampling Time | 1 | 0.2005 | 0.657 | |
| Year:Cultural Practice | 2 | 4.4819 | 0.01828 | * |
| Year:Sampling Time | 1 | 36.1823 | 6.63E-07 | *** |

^z Stars indicate significance at * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$.

Table 2. Average SMB-C and SMB-N levels for each factor combination, comparing cultural practices within each sampling time.

| 2014 | | SMB-C (mg/kg soil) ^z | SMB-N (mg/kg soil) ^z |
|--------------|------------------------------|---------------------------------|---------------------------------|
| Early Season | Plasticulture-low tunnels | 129.5 b | 14 |
| | Plasticulture-no low tunnels | 148 ab | 17.95 |
| | Straw mulch | 159 a | 12.7 |
| Late Season | Plasticulture-low tunnels | 99.5 | 6.6 |
| | Plasticulture-no low tunnels | 88.5 | 4.4 |
| | Straw mulch | 126.5 | 7.05 |
| 2015 | | SMB-C (mg/kg soil) ^z | SMB-N (mg/kg soil) ^z |
| Early Season | Plasticulture-low tunnels | 115 | 11.2 |
| | Plasticulture-no low tunnels | 101 | 7.3 |
| | Straw mulch | 108 | 7.25 |
| Late Season | Plasticulture-low tunnels | 98.5 | 45.3 |
| | Plasticulture-no low tunnels | 88.5 | 24.2 |
| | Straw mulch | 116 | 14.55 |

^z Presence of letters indicate pairwise differences among cultural practices within sampling times at $p \leq 0.05$.

Table 3. Average SMB-C and SMB-N levels for each factor combination, comparing sampling times within each cultural practice.

| 2014 | | SMB-C (mg/kg soil) ^z | SMB-N (mg/kg soil) ^z |
|----------------------------------|--------------|---------------------------------|---------------------------------|
| Plasticulture- low tunnels | Early Season | 129.5 a | 14 a |
| | Late Season | 99.5 b | 6.6 b |
| Plasticulture- no low tunnels | Early Season | 148 a | 17.95 a |
| | Late Season | 88.5 b | 4.4 b |
| Straw mulch | Early Season | 159 | 12.7 a |
| | Late Season | 126.5 | 7.05 b |
| 2015 | | SMB-C (mg/kg soil) ^z | SMB-N (mg/kg soil) ^z |
| Plasticulture- low tunnels | Early Season | 115 | 11.2 |
| | Late Season | 98.5 | 45.3 |
| Plasticulture- no low tunnels | Early Season | 101 | 7.3 |
| | Late Season | 88.5 | 24.2 |
| Straw mulch | Early Season | 108 | 7.25 b |
| | Late Season | 116 | 14.55 a |

^z Presence of letters indicate pairwise differences between sampling times within each cultural practice at $p \leq 0.05$.

Table 4. Average POX-C of cultural practices during early and late season samplings, 2014 and 2015.

| 2014 | |
|--|---------|
| Early Season POX-C (mg C/kg soil) ^z | |
| Plastic Mulch- Low Tunnel | 481.9 b |
| Plastic Mulch- No Low Tunnel | 683.9 a |
| Straw Mulch | 607.4 a |
| Late Season POX-C (mg C/kg soil) ^z | |
| Plastic Mulch- Low Tunnel | 354.1 |
| Plastic Mulch- No Low Tunnel | 463.5 |
| Straw Mulch | 349.7 |
| 2015 | |
| Early Season POX-C (mg C/kg soil) ^z | |
| Plastic Mulch- Low Tunnel | 190.8 |
| Plastic Mulch- No Low Tunnel | 238.5 |
| Straw Mulch | 192.2 |
| Late Season POX-C (mg C/kg soil) ^z | |
| Plastic Mulch- Low Tunnel | 479.7 |
| Plastic Mulch- No Low Tunnel | 316.2 |
| Straw Mulch | 351.7 |

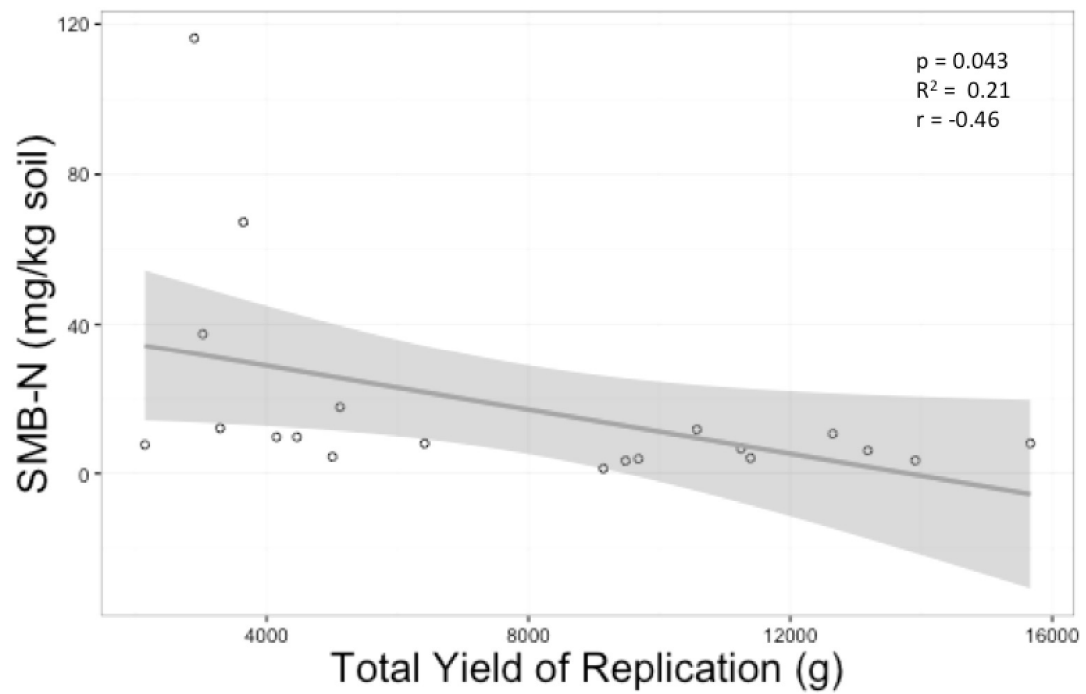
^z Presence of letters indicate pairwise differences among cultural practices within sampling times each year at $p \leq 0.05$.

Table 5. ANOVA table showing main effects of late season soil health indicator on total strawberry yields during 2014-2015 field seasons.

| | df | F value | p value ^z |
|-------|----|---------|----------------------|
| SMB-C | 1 | 0.09 | 0.76 |
| SMB-N | 1 | 5.1 | 0.04 * |
| POX-C | 1 | 0.35 | 0.57 |

^z Star indicates significance at * = $p \leq 0.05$

Figure 1. Relationship of SMB-N with total strawberry yields of each selection zone replication, 2014-2015.



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APPENDIX

Summary Statistics & R Code

Chapter 3

St Paul Summary Statistics- 2013

Projected **lb/acre** of each cultivar and treatment based on 4 replications, with standard deviation and standard error

| | <u>Cultivar</u> | <u>Treatment</u> | <u>N</u> | <u>mean</u> | <u>sd</u> | <u>se</u> |
|----|-----------------|------------------|----------|-------------|-----------|-----------|
| 1 | Albion | LT | 4 | 14562.800 | 3697.8636 | 1848.9318 |
| 2 | Albion | PL | 4 | 11085.081 | 2163.7686 | 1081.8843 |
| 3 | Albion | SM | 4 | 10464.552 | 932.6965 | 466.3483 |
| 4 | EV2 | LT | 4 | 18244.540 | 5319.0767 | 2659.5384 |
| 5 | EV2 | PL | 4 | 20961.086 | 2807.6627 | 1403.8314 |
| 6 | EV2 | SM | 4 | 12095.375 | 3978.3379 | 1989.1690 |
| 7 | Monterey | LT | 4 | 15810.622 | 3375.9177 | 1687.9588 |
| 8 | Monterey | PL | 4 | 15795.028 | 2477.9305 | 1238.9653 |
| 9 | Monterey | SM | 4 | 7595.421 | 4945.2546 | 2472.6273 |
| 10 | Portola | LT | 4 | 15766.299 | 6299.4649 | 3149.7324 |
| 11 | Portola | PL | 4 | 18189.557 | 2820.6034 | 1410.3017 |
| 12 | Portola | SM | 4 | 13636.959 | 4672.6576 | 2336.3288 |
| 13 | San Andreas | LT | 4 | 13065.058 | 1096.1901 | 548.0951 |
| 14 | San Andreas | PL | 4 | 11872.450 | 2146.4202 | 1073.2101 |
| 15 | San Andreas | SM | 4 | 6476.660 | 3216.2381 | 1608.1191 |
| 16 | Seascape | LT | 4 | 15447.487 | 3208.8764 | 1604.4382 |
| 17 | Seascape | PL | 4 | 14128.128 | 2437.0623 | 1218.5311 |
| 18 | Seascape | SM | 4 | 8706.368 | 3127.1944 | 1563.5972 |

Projected lb/plant of each cultivar and treatment based on 4 replications, with standard deviation and standard error

| | <u>Cultivar</u> | <u>Treatment</u> | <u>N</u> | <u>mean</u> | <u>sd</u> | <u>se</u> |
|----|-----------------|------------------|----------|-------------|------------|------------|
| 1 | Albion | LT | 4 | 0.8543436 | 0.21693948 | 0.10846974 |
| 2 | Albion | PL | 4 | 0.6503192 | 0.12694001 | 0.06347001 |
| 3 | Albion | SM | 4 | 0.6139152 | 0.05471773 | 0.02735886 |
| 4 | EV2 | LT | 4 | 1.0703372 | 0.31204984 | 0.15602492 |
| 5 | EV2 | PL | 4 | 1.2297065 | 0.16471481 | 0.08235740 |
| 6 | EV2 | SM | 4 | 0.7095893 | 0.23339383 | 0.11669692 |
| 7 | Monterey | LT | 4 | 0.9275486 | 0.19805215 | 0.09902607 |
| 8 | Monterey | PL | 4 | 0.9266338 | 0.14537068 | 0.07268534 |
| 9 | Monterey | SM | 4 | 0.4455943 | 0.29011913 | 0.14505957 |
| 10 | Portola | LT | 4 | 0.9249483 | 0.36956545 | 0.18478273 |
| 11 | Portola | PL | 4 | 1.0671115 | 0.16547399 | 0.08273699 |
| 12 | Portola | SM | 4 | 0.8000281 | 0.27412691 | 0.13706345 |
| 13 | San Andreas | LT | 4 | 0.7664769 | 0.06430927 | 0.03215464 |
| 14 | San Andreas | PL | 4 | 0.6965111 | 0.12592224 | 0.06296112 |
| 15 | San Andreas | SM | 4 | 0.3799608 | 0.18868436 | 0.09434218 |
| 16 | Seascape | LT | 4 | 0.9062448 | 0.18825248 | 0.09412624 |
| 17 | Seascape | PL | 4 | 0.8288431 | 0.14297310 | 0.07148655 |
| 18 | Seascape | SM | 4 | 0.5107693 | 0.18346051 | 0.09173025 |

Average **berry weight (g)** of each cultivar and treatment based on 4 replications, with standard deviation and standard error

| | <u>Cultivar</u> | <u>Treatment</u> | <u>N</u> | <u>mean</u> | <u>sd</u> | <u>se</u> |
|----|-----------------|------------------|----------|-------------|-----------|------------|
| 1 | Albion | LT | 4 | 12.33238 | 0.2030970 | 0.10154848 |
| 2 | Albion | PL | 4 | 11.82217 | 0.7878947 | 0.39394736 |
| 3 | Albion | SM | 4 | 13.58793 | 0.1799770 | 0.08998848 |
| 4 | EV2 | LT | 4 | 12.62646 | 0.8059077 | 0.40295384 |
| 5 | EV2 | PL | 4 | 11.61244 | 0.7718892 | 0.38594460 |
| 6 | EV2 | SM | 4 | 10.80586 | 1.1249237 | 0.56246186 |
| 7 | Monterey | LT | 4 | 13.11452 | 0.9069769 | 0.45348847 |
| 8 | Monterey | PL | 4 | 11.53073 | 1.5572568 | 0.77862842 |
| 9 | Monterey | SM | 4 | 10.39081 | 2.8865628 | 1.44328142 |
| 10 | Portola | LT | 4 | 12.99658 | 1.3187922 | 0.65939609 |
| 11 | Portola | PL | 4 | 12.87134 | 0.9250679 | 0.46253393 |
| 12 | Portola | SM | 4 | 13.40901 | 1.2085917 | 0.60429586 |
| 13 | San Andreas | LT | 4 | 13.34504 | 1.8663904 | 0.93319521 |
| 14 | San Andreas | PL | 4 | 13.21969 | 1.0430436 | 0.52152179 |
| 15 | San Andreas | SM | 4 | 10.39808 | 2.7367952 | 1.36839759 |
| 16 | Seascape | LT | 4 | 11.30788 | 0.6433915 | 0.32169576 |
| 17 | Seascape | PL | 4 | 10.06641 | 1.2137257 | 0.60686287 |
| 18 | Seascape | SM | 4 | 10.69123 | 1.9400072 | 0.97000360 |

Projected lb/acre based on treatment alone...

| | <u>Treatment</u> | <u>N</u> | <u>mean</u> | <u>sd</u> | <u>se</u> |
|---|------------------|----------|-------------|-----------|-----------|
| 1 | LT | 24 | 15482.801 | 4019.890 | 820.5565 |
| 2 | PL | 24 | 15338.555 | 4158.300 | 848.8094 |
| 3 | SM | 24 | 9829.223 | 4167.383 | 850.6635 |

Projected lb/plant based on treatment alone...

| | <u>Treatment</u> | <u>N</u> | <u>mean</u> | <u>sd</u> | <u>se</u> |
|---|------------------|----------|-------------|-----------|------------|
| 1 | LT | 24 | 0.9083166 | 0.2358315 | 0.04813891 |
| 2 | PL | 24 | 0.8998542 | 0.2439515 | 0.04979639 |
| 3 | SM | 24 | 0.5766428 | 0.2444844 | 0.04990517 |

Morris Summary Statistics- 2013

Projected **lb/acre** of each cultivar and treatment based on 4 replications, with standard deviation and standard error

| | Cultivar | Treatment | N | mean | sd | se |
|----|-------------|-----------|---|----------|-----------|-----------|
| 1 | Albion | LT | 4 | 22365.00 | 2039.3343 | 1019.6671 |
| 2 | Albion | PL | 4 | 16970.55 | 1247.1712 | 623.5856 |
| 3 | Evie 2 | LT | 4 | 24001.02 | 1871.1507 | 935.5753 |
| 4 | Evie 2 | PL | 4 | 22068.04 | 4081.4902 | 2040.7451 |
| 5 | Monterey | LT | 4 | 23730.15 | 1282.0717 | 641.0358 |
| 6 | Monterey | PL | 4 | 13675.99 | 2989.8487 | 1494.9243 |
| 7 | Portola | LT | 4 | 38292.93 | 3553.3305 | 1776.6653 |
| 8 | Portola | PL | 4 | 24649.42 | 276.9987 | 138.4994 |
| 9 | San Andreas | LT | 4 | 22195.30 | 1785.8934 | 892.9467 |
| 10 | San Andreas | PL | 4 | 16774.89 | 2056.0328 | 1028.0164 |
| 11 | Seascape | LT | 4 | 28870.29 | 2789.2976 | 1394.6488 |
| 12 | Seascape | PL | 4 | 18874.66 | 2347.2215 | 1173.6108 |

Projected **lb/plant** of each cultivar and treatment based on 4 replications, with standard deviation and standard error

| | Cultivar | Treatment | N | mean | sd | se |
|----|-------------|-----------|---|-----------|------------|-------------|
| 1 | Albion | LT | 4 | 1.2261512 | 0.11180561 | 0.055902803 |
| 2 | Albion | PL | 4 | 0.9304031 | 0.06837562 | 0.034187808 |
| 3 | Evie 2 | LT | 4 | 1.3158455 | 0.10258501 | 0.051292507 |
| 4 | Evie 2 | PL | 4 | 1.2098704 | 0.22376591 | 0.111882955 |
| 5 | Monterey | LT | 4 | 1.3009949 | 0.07028902 | 0.035144509 |
| 6 | Monterey | PL | 4 | 0.7497799 | 0.16391714 | 0.081958572 |
| 7 | Portola | LT | 4 | 2.0993930 | 0.19480979 | 0.097404894 |
| 8 | Portola | PL | 4 | 1.3513937 | 0.01518633 | 0.007593167 |
| 9 | San Andreas | LT | 4 | 1.2168474 | 0.09791082 | 0.048955411 |
| 10 | San Andreas | PL | 4 | 0.9196758 | 0.11272110 | 0.056360549 |

| | | | | | |
|----|----------|------|-----------|------------|-------------|
| 11 | Seascape | LT 4 | 1.5828009 | 0.15292202 | 0.076461009 |
| 12 | Seascape | PL 4 | 1.0347948 | 0.12868539 | 0.064342696 |

Average **berry weight (g)** of each cultivar and treatment based on 4 replications, with standard deviation and standard error

| <u>Cultivar</u> | <u>Treatment</u> | <u>N</u> | <u>Berry Weight</u> | <u>sd</u> | <u>se</u> |
|-----------------|------------------|----------|---------------------|-----------|-----------|
| Albion | LT 4 | 15.15531 | 0.8359639 | 0.4179819 | |
| Albion | PL 4 | 14.31632 | 0.6367413 | 0.3183707 | |
| Evie 2 | LT 4 | 14.85566 | 0.3429895 | 0.1714948 | |
| Evie 2 | PL 4 | 12.95905 | 0.7098855 | 0.3549428 | |
| Monterey | LT 4 | 14.92924 | 0.8383550 | 0.4191775 | |
| Monterey | PL 4 | 13.86776 | 1.4254143 | 0.7127071 | |
| Portola | LT 4 | 18.61097 | 0.3490045 | 0.1745022 | |
| Portola | PL 4 | 14.78197 | 0.2385950 | 0.1192975 | |
| San Andreas | LT 4 | 15.47705 | 0.5625202 | 0.2812601 | |
| San Andreas | PL 4 | 16.52106 | 0.9120133 | 0.4560066 | |
| Seascape | LT 4 | 14.40064 | 0.7786018 | 0.3893009 | |
| Seascape | PL 4 | 13.20249 | 1.3643627 | 0.6821813 | |

Projected lb/plant based on treatment alone...

| | <u>Treatment</u> | <u>N</u> | <u>mean</u> | <u>sd</u> | <u>se</u> |
|---|------------------|----------|-------------|------------|-----------|
| 1 | LT 24 | 1.457005 | 0.3382530 | 0.06904560 | |
| 2 | PL 24 | 1.032653 | 0.2357509 | 0.04812244 | |

Projected lb/acre based on treatment alone...

| | <u>Treatment</u> | <u>N</u> | <u>mean</u> | <u>sd</u> | <u>se</u> |
|---|------------------|----------|-------------|-----------|-----------|
| 1 | LT 24 | 26575.78 | 6169.734 | 1259.3917 | |
| 2 | PL 24 | 18835.59 | 4300.096 | 877.7534 | |

Morris Summary Statistics & R Code- 2014

```
> anl<- lm(Cumulative.Weight..g.~Cultivar*Treatment)
> anova(anl)
Analysis of Variance Table
```

```
Response: Cumulative.Weight..g.
          Df      Sum Sq  Mean Sq F value  Pr(>F)
Cultivar    5 254414622 50882924  9.6031 6.9e-06 ***
Treatment    1  8953633  8953633  1.6898  0.2019
Cultivar:Treatment  5 10588302  2117660  0.3997  0.8458
Residuals   36 190748457  5298568
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

This shows that in Morris in 2014, Cultivar has a significant effect on yield, but Treatment (low tunnel or no tunnel) does not.

Low Tunnel Average total yield per rep in grams:

```
> tapply(Cumulative.Weight..g.,Cultivar,mean)
      Albion      EV2      Monterey      Portola San Andreas      Seascape
15254.00    16628.00    17975.50    18875.50    12083.50    14335.25

          diff          lwr          upr          p adj
EV2-Albion      1374.00   -4223.81   6971.8103  0.9674766
Monterey-Albion  2721.50   -2876.31   8319.3103  0.6417617
Portola-Albion   3621.50   -1976.31   9219.3103  0.3515342
San Andreas-Albion -3170.50   -8768.31   2427.3103  0.4898497
Seascape-Albion   -918.75   -6516.56   4679.0603  0.9945653
Monterey-EV2     1347.50   -4250.31   6945.3103  0.9700466
Portola-EV2      2247.50   -3350.31   7845.3103  0.7937940
San Andreas-EV2  -4544.50  -10142.31  1053.3103  0.1531632
Seascape-EV2    -2292.75   -7890.56   3305.0603  0.7804411
Portola-Monterey    900.00   -4697.81   6497.8103  0.9950609
San Andreas-Monterey -5892.00 -11489.81 -294.1897 0.0358161
Seascape-Monterey -3640.25   -9238.06   1957.5603  0.3463075
San Andreas-Portola -6792.00 -12389.81 -1194.1897 0.0124877
Seascape-Portola  -4540.25  -10138.06   1057.5603  0.1538088
```

```
Seascape-San Andreas 2251.75 -3346.06 7849.5603 0.7925540
```

```
> lines(pairwise(an2,Cultivar))
```

```
5 -3775 |
6 -1523 | |
1 -605 | | |
2 769 | | |
3 2117 | | |
4 3017 | | |
```

This shows that in the low tunnels, Portola and Monterey (the first and second highest yielders on average) have a significantly higher yield than San Andreas, the lowest yielder.

Plasticulture Average total yield per rep in grams:

| | Albion | EV2 | Monterey | Portola | San Andreas | Seascape |
|----------------------------|-------------|------------|-----------------|--------------------|-------------------|------------------|
| | 14615.75abc | 16606.75ab | 15332.75abc | 18982.50a | 11532.75c | 12898.50bc |
| | | | diff | lwr | upr | p adj |
| EV2-Albion | | | 1991.00 | -2718.5181 | 6700.5181 | 0.7579994 |
| Monterey-Albion | | | 717.00 | -3992.5181 | 5426.5181 | 0.9961696 |
| Portola-Albion | | | 4366.75 | -342.7681 | 9076.2681 | 0.0783393 |
| San Andreas-Albion | | | -3083.00 | -7792.5181 | 1626.5181 | 0.3396169 |
| Seascape-Albion | | | -1717.25 | -6426.7681 | 2992.2681 | 0.8500577 |
| Monterey-EV2 | | | -1274.00 | -5983.5181 | 3435.5181 | 0.9513616 |
| Portola-EV2 | | | 2375.75 | -2333.7681 | 7085.2681 | 0.6068783 |
| San Andreas-EV2 | | | -5074.00 | -9783.5181 | -364.4819 | 0.0305231 |
| Seascape-EV2 | | | -3708.25 | -8417.7681 | 1001.2681 | 0.1751002 |
| Portola-Monterey | | | 3649.75 | -1059.7681 | 8359.2681 | 0.1871894 |
| San Andreas-Monterey | | | -3800.00 | -8509.5181 | 909.5181 | 0.1574205 |
| Seascape-Monterey | | | -2434.25 | -7143.7681 | 2275.2681 | 0.5831262 |
| San Andreas-Portola | | | -7449.75 | -12159.2681 | -2740.2319 | 0.0010513 |
| Seascape-Portola | | | -6084.00 | -10793.5181 | -1374.4819 | 0.0073839 |
| Seascape-San Andreas | | | 1365.75 | -3343.7681 | 6075.2681 | 0.9357550 |

```
> lines(pairwise(an2,Cultivar))
```

```
5 -3462 |
6 -2096 | |
1 -379 | | |
3 338 | | |
2 1612 | | |
4 3988 | | |
```

Brix Data- Summary Stats & R Code

Difference by cultivar:

| | | | | | |
|----------|----------|----------|----------|-------------|----------|
| Albion | Evie-II | Monterey | Portola | San Andreas | Seascape |
| 9.400000 | 8.141667 | 8.672222 | 7.288889 | 8.988889 | 8.877778 |

Response: Brix

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-----------|-----|--------|---------|---------|----------------------|
| Cultivar | 5 | 100.58 | 20.1156 | 7.3878 | 2.108e-06 *** |
| Residuals | 210 | 571.79 | 2.7228 | | |

\$Cultivar

| | diff | lwr | upr | p adj |
|----------------------|-----------|------------|----------|-----------|
| Evie-II-Portola | 0.8527778 | -0.2658762 | 1.971432 | 0.2455857 |
| Monterey-Portola | 1.3833333 | 0.2646794 | 2.501987 | 0.0061068 |
| Seascape-Portola | 1.5888889 | 0.4702349 | 2.707543 | 0.0008778 |
| San Andreas-Portola | 1.7000000 | 0.5813461 | 2.818654 | 0.0002784 |
| Albion-Portola | 2.1111111 | 0.9924572 | 3.229765 | 0.0000023 |
| Monterey-Evie-II | 0.5305556 | -0.5880984 | 1.649209 | 0.7483654 |
| Seascape-Evie-II | 0.7361111 | -0.3825428 | 1.854765 | 0.4095528 |
| San Andreas-Evie-II | 0.8472222 | -0.2714317 | 1.965876 | 0.2523226 |
| Albion-Evie-II | 1.2583333 | 0.1396794 | 2.376987 | 0.0174882 |
| Seascape-Monterey | 0.2055556 | -0.9130984 | 1.324209 | 0.9949774 |
| San Andreas-Monterey | 0.3166667 | -0.8019873 | 1.435321 | 0.9646858 |
| Albion-Monterey | 0.7277778 | -0.3908762 | 1.846432 | 0.4228226 |
| San Andreas-Seascape | 0.1111111 | -1.0075428 | 1.229765 | 0.9997410 |
| Albion-Seascape | 0.5222222 | -0.5964317 | 1.640876 | 0.7607994 |
| Albion-San Andreas | 0.4111111 | -0.7075428 | 1.529765 | 0.8976543 |

| | | | |
|------|--------|--|--|
| Port | -1.273 | | |
| EV-2 | -0.420 | | |
| Mont | 0.111 | | |
| Seas | 0.316 | | |
| S.A. | 0.427 | | |
| Albn | 0.838 | | |

Difference by Time of Harvest:

| | | |
|----------|----------|----------|
| Early | Late | Mid |
| 8.798611 | 8.987500 | 7.898611 |

Response: Brix

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-----------|-----|--------|---------|---------|--------------------|
| Season | 2 | 48.75 | 24.3763 | 8.3258 | 0.00033 *** |
| Residuals | 213 | 623.62 | 2.9278 | | |

\$Season

| | diff | lwr | upr | p adj |
|------------|------------|------------|------------|-----------|
| Late-Early | 0.1888889 | -0.4841963 | 0.8619741 | 0.7855475 |
| Mid-Early | -0.9000000 | -1.5730852 | -0.2269148 | 0.0051864 |
| Mid-Late | -1.0888889 | -1.7619741 | -0.4158037 | 0.0005153 |

Midl -0.663

Erly 0.237 |

Late 0.426 |

Difference by Cultural Practice:

| LT | PL | SM |
|----------|----------|----------|
| 8.662500 | 8.431944 | 8.590278 |

Response: Brix

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|-----------|-----|--------|---------|---------|---------------|
| Treatment | 2 | 2.00 | 1.0013 | 0.3181 | 0.7278 |
| Residuals | 213 | 670.37 | 3.1473 | | |

Chapter 4

POX-C R Code

```
#Find the data
> setwd("~/Dropbox/strawberry papers/PhD Thesis/Soil data
2015")
> dat <- read.csv("POXC_Both_Years.csv",header=T)

#Load the appropriate packages
> library("Stat5303",
lib.loc="/Library/Frameworks/R.framework/Versions/3.0/Resou
rces/library")
> library("Stat5303libs",
lib.loc="/Library/Frameworks/R.framework/Versions/3.0/Resou
rces/library")

#Lets focus on 2014 first
> dat_2014 <- subset(dat, Year=="2014")

#Zoom in further to just the initial readings
> dat_2014_Initial <- subset(dat_2014, Reading=="Initial")
> attach(dat_2014_Initial)

#Treatment means
> tapply(POX..mg.C.kg.soil.,Treatment,mean)
      LT      PL      SM
481.9150 683.9352 668.8088

#ANOVA
> an1<- lm(POX..mg.C.kg.soil.~Treatment)
> anova(an1)
Analysis of Variance Table

Response: POX..mg.C.kg.soil.
```

```

          Df Sum Sq Mean Sq F value Pr(>F)
Treatment  2 101294   50647   6.2244 0.02008 *
Residuals  9  73232    8137

```

#It's significant, so lets look at some pairwise comparisons

```

> an2<- aov(POX..mg.C.kg.soil.~Treatment)
> TukeyHSD(an2)
  Tukey multiple comparisons of means
    95% family-wise confidence level

```

```
Fit: aov.default(formula = POX..mg.C.kg.soil. ~ Treatment)
```

```

$Treatment
      diff          lwr          upr      p adj
PL-LT 202.02022  23.933964 380.1065 0.0278675
SM-LT 186.89379   8.807538 364.9800 0.0403107
SM-PL -15.12643 -193.212682 162.9598 0.9695765

```

```
> lines(pairwise(an2,Treatment))
```

```

LT -129.6
SM  57.3 |
PL  72.4 |

```

#So for the initial readings, PL and SM are sig. higher than LT. Lets go to the late season readings

```

> dat_2014_Final <- subset(dat_2014, Reading=="Final")
> attach(dat_2014_Final)

```

#Treatment means

```

> tapply(POX..mg.C.kg.soil.,Treatment,mean)
      LT      PL      SM
354.0833 463.4733 406.4631

```

#ANOVA

```

> an3<- lm(POX..mg.C.kg.soil.~Treatment)
> anova(an3)

```

Analysis of Variance Table

Response: POX..mg.C.kg.soil.

```

          Df Sum Sq Mean Sq F value Pr(>F)
Treatment  2  23947 11973.3   2.4295 0.1433
Residuals  9  44355  4928.4

```

```

#No difference. Lets switch to 2015
> dat_2015 <- subset(dat, Year=="2015")

#Focus in on the initial readings
> dat_2015_Initial <- subset(dat_2015, Reading=="Initial")
> attach(dat_2015_Initial)

#Treatment means
> tapply(POX..mg.C.kg.soil.,Treatment,mean)
      LT      PL      SM
190.7744 238.4820 192.2162

#ANOVA
> an4<- lm(POX..mg.C.kg.soil.~Treatment)
> anova(an4)
Analysis of Variance Table

Response: POX..mg.C.kg.soil.
      Df Sum Sq Mean Sq F value Pr(>F)
Treatment  2  5891.5  2945.74   3.3026  0.08402 .
Residuals  9  8027.6   891.96

#ANOVA shows no difference. Lets switch to the Final
readings
> dat_2014_Final <- subset(dat_2014, Reading=="Final")
> attach(dat_2014_Final)

#Treatment means
> tapply(POX..mg.C.kg.soil.,Treatment,mean)
      LT      PL      SM
479.6551 316.1606 351.6816

#ANOVA
> an5<- lm(POX..mg.C.kg.soil.~Treatment)
> anova(an5)
Analysis of Variance Table

Response: POX..mg.C.kg.soil.
      Df Sum Sq Mean Sq F value Pr(>F)
Treatment  2  59159   29580   0.8789  0.448
Residuals  9 302883   33654

#NO difference again!

```

```
#Lets look at all the data and run an ANOVA of all
treatments to analyze for main effects and interactions
> attach(dat)
> an8 <- lm(POX..mg.C.kg.soil.~Year*Treatment*Reading)
> anova(an8)
Analysis of Variance Table
```

Response: POX..mg.C.kg.soil.

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|------------------------|----|--------|---------|---------|------------------|-----|
| Year | 1 | 554449 | 554449 | 46.5817 | 5.574e-08 | *** |
| Treatment | 2 | 19283 | 9641 | 0.8100 | 0.45279 | |
| Reading | 1 | 2387 | 2387 | 0.2005 | 0.65700 | |
| Year:Treatment | 2 | 106693 | 53347 | 4.4819 | 0.01828 | * |
| Year:Reading | 1 | 430668 | 430668 | 36.1823 | 6.633e-07 | *** |
| Treatment:Reading | 2 | 54522 | 27261 | 2.2903 | 0.11580 | |
| Year:Treatment:Reading | 2 | 9793 | 4897 | 0.4114 | 0.66581 | |
| Residuals | 36 | 428498 | 11903 | | | |

#This ANOVA tells us that Year is the only main effect that influences POXC, and from an interaction POV, the influence of Treatment and Reading on POXC is dependent on Year.

Chapter 4
R Code
Yield/Soil Health Comparisons

```
# import data
> setwd("~/Dropbox/strawberry papers/PhD Thesis/Soil data
2015")
> dat <- read.csv("Soil Yield Comparison Both Years.csv",
header=T)
```

```
# 2-way ANOVA
> anova(an1)
Analysis of Variance Table
```

```
Response: Total.Weight..g.
Df      Sum Sq  Mean Sq F value
Pr(>F)
SMB.C..mg.kg.soil.      1  1692416  1692416  0.0943
0.76402
SMB.N..mg.kg.soil.      1  90813148  90813148  5.0612
0.04402 *
POX.C..mg.kg.soil.      1   6205108   6205108  0.3458
0.56739
SMB.C..mg.kg.soil.:SMB.N..mg.kg.soil.      1  23733842  23733842  1.3227
0.27251
SMB.C..mg.kg.soil.:POX.C..mg.kg.soil.      1   6569195   6569195  0.3661
0.55640
SMB.N..mg.kg.soil.:POX.C..mg.kg.soil.      1   1615292   1615292  0.0900
0.76928
SMB.C..mg.kg.soil.:SMB.N..mg.kg.soil.:POX.C..mg.kg.soil.      1   4200970   4200970  0.2341
0.63719
Residuals                12 215314865 17942905
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# 1-way ANOVA with just SMB-N
> anova(an2)
Analysis of Variance Table
```

```
Response: Total.Weight..g.
Df      Sum Sq  Mean Sq F value  Pr(>F)
SMB.N..mg.kg.soil.      1  72867593  72867593  4.7303 0.04321 *
Residuals                18 277277243 15404291
```

```
# Summary data with R-squared, along with correlation r
```

```
> summary(an2)
```

```
Call:
lm.default(formula = Total.Weight..g. ~ SMB.N..mg.kg.soil.)
```

```
Residuals:
    Min       1Q   Median       3Q      Max
-6407.2 -3503.4   390.6  2599.8  7144.6
```

```
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)    9092.73   1046.16   8.692 7.38e-08 ***
SMB.N..mg.kg.soil.   -70.72    32.52  -2.175  0.0432 *
```

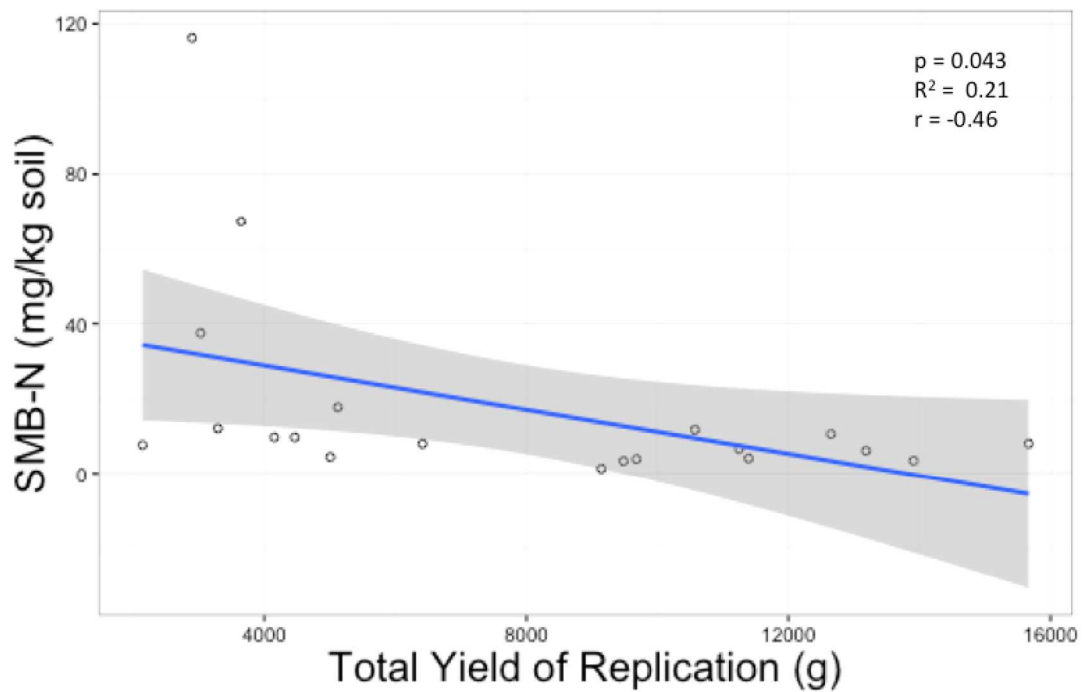
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

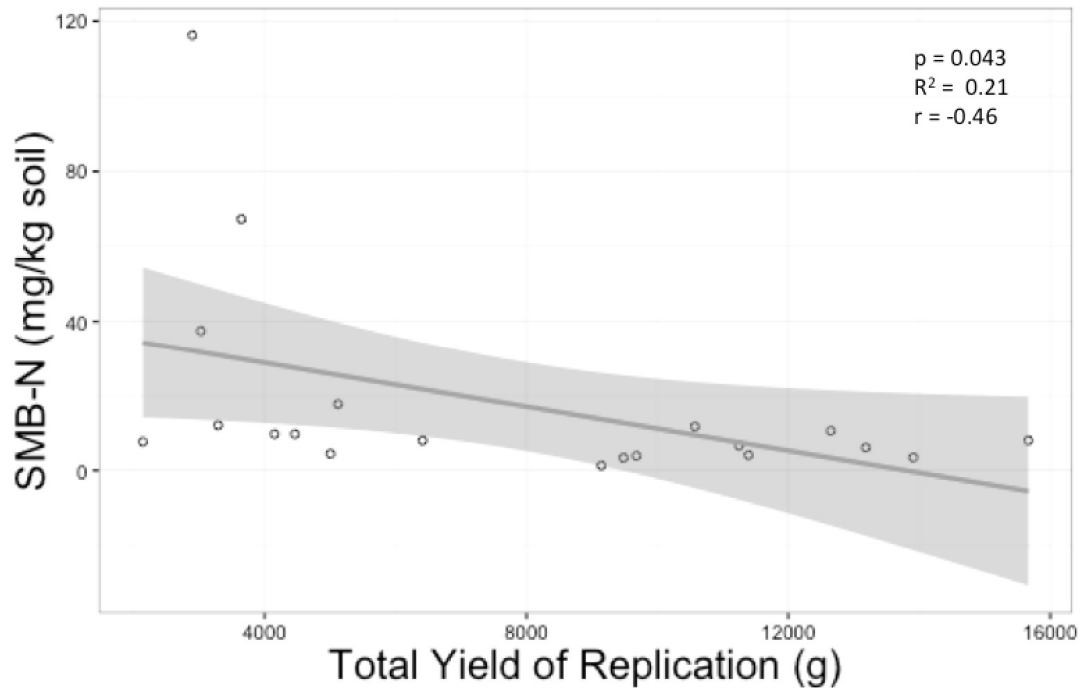
Residual standard error: 3925 on 18 degrees of freedom
Multiple R-squared: 0.2081, Adjusted R-squared: 0.1641
F-statistic: 4.73 on 1 and 18 DF, p-value: 0.04321

```
> cor(Total.Weight..g.,SMB.N..mg.kg.soil.)  
[1] -0.4561875
```

Plot

```
> ggplot(dat, aes(x=Total.Weight..g.,  
y=SMB.N..mg.kg.soil.))+geom_point(shape=1)+geom_smooth(method=lm)+theme_  
bw()+ylab("SMB-N (mg/kg soil)")+xlab("Total Yield of Replication  
(g)")+theme(axis.title.x = element_text(size=20))+theme(axis.title.y =  
element_text(size=20))
```





```
# remove 2 outliers, retest
```

```
> an3<- lm(Total.Weight..g.~SMB.C..mg.kg.soil.*SMB.N..mg.kg.soil.*POX.C..mg.kg.soil.)
> anova(an3)
```

```
Analysis of Variance Table
```

```
Response: Total.Weight..g.
```

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|--|----|-----------|----------|---------|--------|
| SMB.C..mg.kg.soil. | 1 | 16749727 | 16749727 | 0.7845 | 0.3966 |
| SMB.N..mg.kg.soil. | 1 | 55676174 | 55676174 | 2.6077 | 0.1374 |
| POX.C..mg.kg.soil. | 1 | 2877191 | 2877191 | 0.1348 | 0.7212 |
| SMB.C..mg.kg.soil.:SMB.N..mg.kg.soil. | 1 | 537930 | 537930 | 0.0252 | 0.8770 |
| SMB.C..mg.kg.soil.:POX.C..mg.kg.soil. | 1 | 6363854 | 6363854 | 0.2981 | 0.5971 |
| SMB.N..mg.kg.soil.:POX.C..mg.kg.soil. | 1 | 5037171 | 5037171 | 0.2359 | 0.6376 |
| SMB.C..mg.kg.soil.:SMB.N..mg.kg.soil.:POX.C..mg.kg.soil. | 1 | 2422884 | 2422884 | 0.1135 | 0.7432 |
| Residuals | 10 | 213507799 | 21350780 | | |

```
> an4<- lm(Total.Weight..g.~SMB.N..mg.kg.soil.)
```

```
#Retest without outliers just looking at SMB-N
```

```
> anova(an4)
Analysis of Variance Table
```

```
Response: Total.Weight..g.
```

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) |
|--------------------|----|-----------|----------|---------|---------|
| SMB.N..mg.kg.soil. | 1 | 58566804 | 58566804 | 3.8309 | 0.06799 |
| Residuals | 16 | 244605926 | 15287870 | | |

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(an4)
```

```
Call:
```

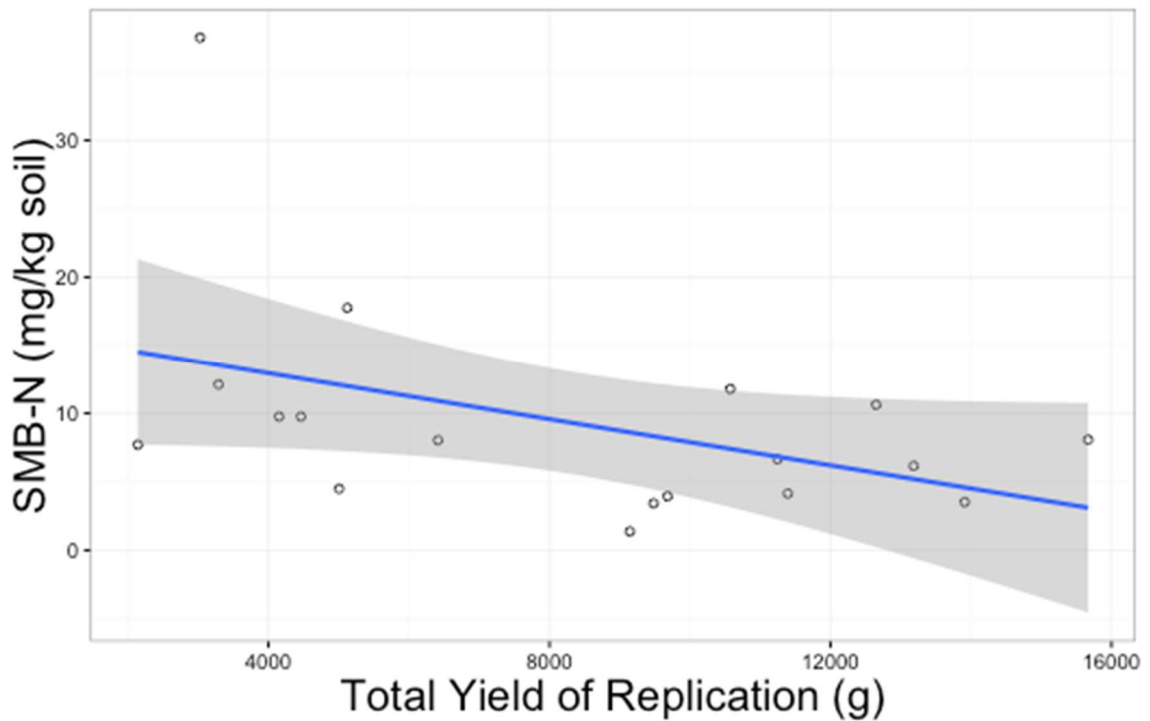


```
lm.default(formula = Total.Weight..g. ~ SMB.N..mg.kg.soil.)
Residuals:
    Min       1Q   Median       3Q      Max
-6580  -3399   -61    2659   7030

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)    10485.9    1423.0     7.369 1.58e-06 ***
SMB.N..mg.kg.soil.  -229.2     117.1    -1.957  0.068 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 3910 on 16 degrees of freedom
Multiple R-squared:  0.1932, Adjusted R-squared:  0.1428
F-statistic: 3.831 on 1 and 16 DF, p-value: 0.06799

> cor(Total.Weight..g.,SMB.N..mg.kg.soil.)
[1] -0.4395221
```



```
> an1 <- lm(Total.Weight..g.~Year*Treatment)
> anova(an1)
Analysis of Variance Table

Response: Total.Weight..g.
```

| | Df | Sum Sq | Mean Sq | F value | Pr(>F) | |
|----------------|----|-----------|-----------|----------|-----------|-----|
| Year | 1 | 288524196 | 288524196 | 132.5934 | 7.648e-08 | *** |
| Treatment | 1 | 10304100 | 10304100 | 4.7353 | 0.05025 | . |
| Year:Treatment | 1 | 4284900 | 4284900 | 1.9692 | 0.18588 | |
| Residuals | 12 | 26112079 | 2176007 | | | |

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1