

ABSTRACT

XU, XIANGNAN. Optimizing Environmental Parameters for Day-neutral Strawberry Propagation with Precision Indoor System (Under the direction of Dr. Ricardo Hernandez).

The production of day-neutral strawberry has increased in the SE United States; however, strawberry nurseries are challenged with low propagation yield caused by competition of resources allocated to flowering, disease incidence, and variable environmental conditions. A potential alternative to field production is Precision Indoor Propagation (PIP). PIP are closed-type control environment systems that use electrical lighting as the sole-source of light for photosynthesis, and they are often equipped with sensors and controllers to maintain an optimal environment for crop growth. The objective of this project is to characterize the propagation efficiency of 'Albion' day-neutral strawberry in different light intensities, photoperiods, and light spectrums in PIP systems. In the first phase of this project, three light intensities, 250 (low), 350 (medium) and 450 (high) $\mu\text{mol m}^{-2} \text{s}^{-1}$ were tested with 12 h photoperiod. Plants produced 36, 46 and 56 daughter plants in round one (12 weeks) and 40, 44 and 48 daughter plants in round two (9 weeks) for low, medium and high light intensity, respectively. The results indicated that increasing light intensity will significantly increase daughter plant production.

In the second phase, two photoperiods (12 h and 8.5 h) and two spectrums (high blue and low blue) were tested. Plants in 12 h photoperiod had 25% higher daughter plant production than plants in 8.5 h photoperiod, while plants grown under low blue spectrum had an increased rates of stolon elongation and daughter plant production.

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Optimizing Environmental Parameters for Precision Indoor Propagation of Day-neutral
Strawberry

by
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DEDICATION

I would like to say thanks to my parents and grandma, Lu Xu, Jianying Zheng and Shujing Liu, who gave me full support when I decided to devote myself into this field. I would like to give credits to my sincere beloved, Zhaoyuan Zhang, who stand by my side and encouraging me with no reason.

BIOGRAPHY

Xiangnan Xu, born in Beijing, China, 1995. I found my passion in agriculture at the first time when I watched the agriculture education TV show, which is 19 years ago. Soon after that I made the decision of becoming a plant scientist. After first two years of undergrad, I was so determined to become a plant physiologist specialized in control environment, then start my life as a master student in horticultural science.

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CHAPTER 1. REVIEW OF INDUSTRY FACTS AND CURRENT STUDIES IN STRAWBERRY PROPAGATION

1.1. Strawberry Industry and Production Value

Strawberry is an important crop in United States with production area of 53,600 acres (planted in 2016); Among the total planted acreage, California is the largest producer with 72% of the total planting area (\$1.84 billion production value) and more than 90 million kilograms (2 billion lbs) of strawberry fruits are harvested annually, and the total production accounts for about 87% of national production. Florida follows California with 20.15% of the total acreage; North Carolina is third in fresh market production with 1300 acres (National Agricultural Statistics Service, 2017; California Strawberry Commission, 2013). Strawberry is also an important crop in Canada. For example, growing short-day strawberry plants can generate a total income of \$76,671 CAD/ha (two-year production cycle), yielding in average a 15,722 kg and generating \$22,300 CAD net profit per hectare (Galic et al., 2014). North American strawberry varieties are important in terms of market share in the US and around the world. Specialized nurseries in Argentina and Chile distribute North American varieties to nearly all the countries in South America. Brazil has a production area of 3500 ha generating 105,000 metric tons of strawberry every year mainly for the fresh market and ‘Camarosa’, a US cultivar, is still the predominant cultivated strawberry (Antunes and Peres, 2013).

1.2. Strawberry Morphology and Flowering Classification

The strawberry is an herbaceous plant that is classified as a perennial but is often grown as an annual in many production regions. The mature plant consists of leaves, roots, crowns, stolons, flowers and fruit (Figure 1a) (Trejo-Téllez and Gómez-Merino, 2014). Most modern cultivars have flowers that are perfect and the part of the flower that is eaten is the enlarged receptacle. The plant has a shortened mainly underground stem called a crown. The crown enlarges and develops side branches or crowns, and flower primordia, leaf, roots and stolons (A.K.A runners) develop from the crown. The stolons produce daughter plants at regular intervals and the daughter plants are often used as the main propagation material.

In terms of genetics, the *Fragaria* species can be grouped by chromosome numbers, which include diploid, hexaploidy, and octoploid (Longley, 1926). However, strawberries are mainly classified by their complex flowering behavior. In early 20th century, scholars stated that flowering characteristics of strawberry could not be properly classified, concluding that each variety had its own flower developmental pattern and rate (Waldo, 1930). Later, Darrow and Waldo (1933) found that low temperature and photoperiodism also influenced flower induction in strawberry. Their experiments tested more than 50 different varieties demonstrating that the *Fragaria virginiana* and other varieties adapted to northern and eastern states required long-day (more than 12 h) to maintain flower initiation; while *Fragaria chiloensis* and other varieties adapted to northwest and southern states flower mainly under short-days (daylength 9 h).

Strawberries can be classified by their flowering pattern, which includes short-day, long-day, and day-neutral. In general, from initiation of flower tissue to visible flower bud, it takes around five weeks (Taylor et al., 1997). Short-day cultivars only flower when daylength is shorter than the critical photoperiod (<13-14 h) and under long-day conditions (>14 h) they

remain in vegetative growth (production of stolons). Long-day cultivars flower under daylengths of more than 14 h and remain vegetative during short-days. Day-neutral plants typically flower independently of the daylength.

Long-day and short-day plants can be further classified into qualitative or quantitative long-day/short-day. Plants classified as qualitative photoperiod will not flower unless a specific photoperiod is met (critical photoperiod). For example, the ornamental plant *Campanula* ‘Uniform White’ classified as qualitative long-day plant, would only flower after the day length is greater than 9 h (critical photoperiod). Plants classified as quantitative photoperiod can flower under several day lengths; however, the time to flower will decrease or increase based on the photoperiod. For example, the ornamental plant *Petunia* “Dreams Mix” classified as a quantitative long-day plant flowers under short and long photoperiods but the time to flower is reduced with the increase of daylength (Blanchard and Runkle, 2016). However, precise flowering classification of North American strawberry cultivars based on photoperiodic responses is still lacking. Table 1 shows a list of several common North America cultivars and its current flowering classification.

As a high profit fruit, strawberry is popular among growers. The key of business success is to meet market demand at a targeted time. Strawberry can be grown in both open-land (with desired climate) or in controlled environment such as greenhouses and high tunnels. When the market demands strawberry in specific season (or year-round), open-land propagators have to choose the appropriate flowering variety, while control environment propagators have to adjust their environmental settings, to match the seasonality demand. Therefore, accurate flowering classification is imperative to predict and maximize production.

1.3. Strawberry Pest Challenges

Strawberry production is affected by pathogens, parasites, and insects that reduce yield and can result in economic losses. For example, strawberry pallidosis associated virus (SPaV) was responsible of a two-billion-dollar loss for California strawberry growers in 2011 (Blake, 2013). The American Phyto-pathological Society lists fungal pathogen as the main cause for strawberry diseases (Gubler and Converse, 1993). Common strawberry fungal diseases include botrytis gray mold rot (*Botrytis cinerea* Pers. ex Fr.), strawberry leafspot (*Ramularia tulasnei* Sacc.), anthracnose (*Colletotrichum acutatum* and *Colletotrichum fragariae*), powdery mildew (*Sphaerotheca macularis* and etc.), red stele (*Phytophthora fragariae*), crown rot (*Phytophthora cactorum*), leather fruit rot (*Phytophthora cactorum*), verticillium wilt (*Verticillium dahliae*), and black root rot (*Cerbasidium* sp., *Idiella lunata*, *Pythium ultimum*, *P. irregulare*, other *Fusarium* spp. and etc.) (Paulus, 1990) among others. These diseases cause unmarketable fruit, yield reduction, stunted growth, defoliation and plant death among others. In addition to fungal pathogens, virus or virus-like pathogens also represent a significant pest problem for the strawberry industry. Virus or virus-like diseases are spread by insects, parasites and fungal vectors (Gubler and Converse, 1993). Common vectors and associated diseases affecting strawberries are listed in Table 2.

Other diseases and disorders caused by bacteria or nematodes include angular leaf spot, bacterial wilt, cauliflower disease (complex), bulb and stem, dagger, dagger (American), lesion, root-knot, spring dwarf (crimp), sting, and summer dwarf (crimp) (Gubler and Converse, 1993).

1.4. Strawberry Propagation

1.4.1. Current Industry

Strawberry production in the US is mainly vegetative propagated from daughter plants that develop in the stolons of the stock plants. Growers utilize several kinds of strawberry propagation material to produce fruit. The two most common forms are: 1) bare root plant and 2) plug plant produced from a runner daughter plant. A bare root plant is a dormant matured plant with a developed root system and several leaf primordia (Figure 1b). Bare root plants are grown in propagation fields and dug up from the soil after dormancy. As a large plant, bare root propagation material is easier to ship but requires longer time to propagate.

A daughter plant is the common term used to describe a clonal plant (tip) produced by the stock plant. The daughter plant is cut from the stolon connected to the stock plant (Figure 1c) before the root system is developed. Daughter plants are easy to ship in large numbers, but they are sensitive to storage environment. Since daughter plants do not have a developed root system, they need to be rooted by the user (strawberry grower) under specialized conditions (high humidity) for approximately 4-8 weeks before they can be planted in the field. Plug plants are the result of a daughter plant that has been rooted in a tray system (Figure 1d). Plug plant has relatively higher vigor than daughter plant, can be shipped by trays, and typically the cost is higher than daughter and bare root plants.

Another propagation method that ensures plants that are disease free, uniform, season independent, and true-to-type offspring, is micro-propagation (plant tissue culture) (Anis and Ahmad, 2016). Plant tissue culture refers to cultivation of plant cells or tissues on sterile sugar-based medium under aseptic and controlled conditions (Bhojwani and Dantu, 2013). However, tissue culture plants are not used as an input to the strawberry fruit production industry; instead,

tissue culture plants serve as an input to the strawberry nursery industry and are used to replaced and maintain stock plants.

1.4.2. Challenges of Strawberry Nursery Propagation

1.4.2.1. Plant quality and availability

US commercial strawberry fruit industry relies on plants that are asexually propagated by clones. This system depends on a multi-geographical multi-year nursery propagation system with the capacity to grow enough stock plants that can produce clones (daughter plants) to meet the demand by producers in a small window of time. Since strawberry development (reproductive vs vegetative) and propagation yield is highly influenced by environmental conditions (Stewart and Folta, 2010; Smeets, 1980a), it can be difficult to meet the demand if environmental conditions are not favorable for propagation. For example, during summer, long-days inhibits stolon production of long-day cultivars, and short-day cultivars will not have daughter plants in autumn or spring (short-day). In addition, the intrinsic changes in weather conditions also affect propagation yield; for example, excess of water or high humidity can affect yield and disease incidence, fluctuation of temperatures can reduce growth, crop rotation may influence disease development, etc. (Ebihara et al., 2010; Amsalem et al., 2006; Ledesma et al., 2008).

Due to the climate differences, nurseries from different locations produce propagation material under different environmental conditions and environmental conditions highly influence plant physiology. For example, larger (greater than 4g compared to less than 2.5g) transplants perform better in both branch crown development (25% higher) and fruit yield (6% higher) (Takeda, F. et al., 2004). In addition, studies conducted in Florida showed that the geographical location of the nursery influenced the production of strawberries fruits. For example, ‘Sweet Charlie’ strawberry transplants from southern sources (Alabama, Florida) had significant lower

yield than the plants from northern (Canada, Massachusetts, Oregon) and mid-latitude (North Carolina) sources; in addition, plants showed differences in flowering time, initial crown size, foliar disease, pests, mortality, and fruit weight (Stapleton et al., 2001). In contrast, another study showed that differences of geographical propagation (Ontario-Canada, Prince Edward Island-Canada and North Carolina-USA) did not influence plant performance of ‘Chandler’ strawberry (Fernandez, Gina E. et al., 2002).

1.4.2.2. Disease incidence in strawberry nurseries

Another challenge of propagating strawberry in the field is the potential contamination by pests such as insects and diseases. For example, insects including aphid and whitefly can widely spread different viruses such as latent ring spot, mild yellow edge, mottle and necrotic shock, among others (Table 2) (Martin and Tzanetakis, 2013).

Other than virus vectored by insects, fungal diseases can collapse strawberry propagation when weather or environmental conditions are favorable for pathogen development. For instance, anthracnose caused by *C. acutatum* can be spread through rain splash (Madden and Boudreau, 1997). *V. dahlia* (Verticillium Wilt) can also be a severe problem for stolon and daughter plant production. For example, when mother plants are inoculated with *V. dahlia*, susceptible and resistant cultivars had a reduction of 70% and 7-15% in stolon production (Shaw et al., 2005). Experiments have shown that chemical fumigation can be an effective disease control for *V. dahlia* increasing marketable stolon production to 1.5-3 times higher than untreated plots; however, *V. dahlia* colonies were able to recover after several months granting additional fungicide applications (Meszka and Malusà, 2014). Propagating of strawberry in field increases the potential transmission of pathogens from the propagation field to the production field; for example, *C. acutatum* could be present on stolons and old petioles when propagation

material is stored in cold storage (Debode et al., 2015). In other studies, strawberry propagation material was tested before transplanting into the production field, and 43% of the sampled shipments of cold storage transplants showed bacteria disease symptoms (Roberts et al., 1997).

1.5. Environmental Studies

1.5.1. Temperature and Photoperiod

Several studies have characterized the impact of temperature and photoperiod on strawberry growth, flowering, and yield. Low temperature over time triggers strawberry dormancy (chilling), dormancy can influence future vegetative growth, in general, increasing the length of the chilling period, increases vegetative growth after plants are transferred into the greenhouse (Bailey and Rossi, 1965). In contrast, other studies suggested that high temperature (24°C) combined with long-days (16h) stimulate stolon formation (Heide, 1977). Research has shown that the factors promoting vegetative growth are similar among many strawberry varieties (high temperature and long photoperiod) and flower initiation will be inhibited by any factors promoting vegetative growth (Guttridge, 1960).

Strawberry flowering response to temperature is not fully characterized (Heide et al., 2013); for example, in cooler temperatures (23/18 °C compared to 30/25 °C, day/night) strawberry had more flowers and flower earlier but the flowering response to low temperature is known to be cultivar specific (Ledesma et al., 2008). Similarly, higher temperature is associated with an increase on stolon induction and a decrease in flower initiation; however, the responses to high temperature are also species specific. For example, research on stolon production of several strawberry cultivars in different temperatures (17 °C, 20 °C, 23 °C, 26 °C) under natural daylength from April to October found that not all cultivars had an increase stolon formation

with the increased temperature (Smeets, 1956). Studies have also shown the effect of the interaction of temperature and photoperiod on flowering (Sønsteby and Heide, 2008); for example, strawberry 'CHI-24-1' is classified as a long-day plant with a critical daylength of 20 h when the mean temperature is greater than 20 °C. However, when the mean temperature is less than 15 °C, strawberry 'CHI-24-1' acts as a facultative short-day plant (Yanagi et al., 2006). Strawberry day-neutral plants can be further classified in four flowering scenarios: 1) strong-day-neutral: cultivars flower at the same rate in a photoperiod from 12h to 24h; 2) intermediate day-neutral: cultivars have 100% flower under 12h day length; 3) Weak day-neutral: cultivars have significant reduction in flower initiation when photoperiod is shorter than 12h (Hamano et al., 2015) and 4) some of the day-neutral cultivars can show facultative long-day response under lower temperatures (appr. 17°C) (Garcia, 2016).

As previously mentioned, strawberry plants tend to produce more stolons or have a longer stolon producing season under higher temperatures (greater than 26°C) (Serçe and Hancock, 2005; Ledesma et al., 2008; Smeets, 1980a; Taghavi et al., 2016; Sønsteby and Nes, 1998). For short-day strawberry cultivars in particular, high temperature (greater than 26°C) would suppress the effect of short photoperiod and retard flowering or reduce flower initiation ratio (Sønsteby et al., 2016; Sønsteby and Heide, 2006). However, day-neutral strawberries under 12h photoperiod have fewer flowers in high temperatures (26 °C and 30 °C compared to 22 °C and 18 °C) and the decrease in flowering does not increase daughter plant growth (Serçe and Hancock, 2005).

1.5.2. Carbon Dioxide

In addition to temperature and photoperiod, increasing CO₂ concentration is known to increase growth rate in strawberry plants. For example, research investigating the response of elevated CO₂ in vitro culture concluded that higher CO₂ concentration promotes general growth

of strawberry plantlets in vessels and the benefit continued after they were transplanted for acclimatization (Laforge et al., 1991; Navarro et al., 1994; Zhou et al., 2005). In greenhouse production, three, five- and ten-fold of CO₂ enrichment increased “Tioga” strawberry fruit yield by 31%, 43% and 51%, respectively (Enoch et al., 1976). Other studies have characterized the response of strawberry fruit yield to higher concentration of CO₂; however, research on the effect of CO₂ enrichment on strawberry propagation is lacking.

1.5.3. Nutrition

The total amount, proportion of different forms of nutrients, and timing of fertilizer can shift the physiological response of plants. Higher nitrogen can increase everbearing strawberry yield while average fruit weight remains the same (Iatrou and Papadopoulos, 2016) and increasing the ratio of ammonium nitrogen to nitrate nitrogen linearly increases the number of fruits (Cárdenas-Navarro et al., 2006). In addition, strawberry plants will produce more stolons if 50% rather than 100% of the total available nitrogen is from ammonium nitrogen (Cárdenas-Navarro et al., 2006). Timing of nitrogen application can also modify the short-day flower induction of short-day strawberry; for example, research showed that nitrogen given before the beginning of short-day period will delay flowering (Sønsteby et al., 2009).

Higher calcium supply promotes more stolon production and fruit yield for strawberry (Blatt, 1967). Excess phosphorus can cause interveinal chlorosis by reducing uptake of micronutrients (Iron, Copper, Manganese and Zinc) (Choi, J. M. and Lee, 2012). Phosphorus deficiency leads to harder fruits, lower soluble solids, higher phenolic compounds, bioactive compounds and calcium in fruit (Valentinuzzi et al., 2015).

Foliar application of potassium nitrate can help dormant day-neutral strawberry break bud dormancy when combined with chilling, in which potassium nitrate is the inductive factor

(Khayyat et al., 2010). Supplemental potassium also helps strawberry improve yield and quality in high salinity conditions (Kaya et al., 2002).

Tip-burn (leaf tip-burn) is a common strawberry physiological disorder induced by improper nutrition balance and environmental stress and can reduce fruit yield, quality and plant photosynthetic rate. In most cases tip-burn is associated with localized calcium deficiency, especially in shoot daughter plants and flower buds. A study showed that in high humidity environment lower magnesium, phosphorus, and nitrate (compared to standard strength) can reduce tip-burn (Mason and Guttridge, 1975). In addition, research showed that the change of Ca concentration in the nutrient solution did not change leaf calcium content. However, in the leaves with tip-burn, tissue analysis showed high K/Ca ratio or high K/Mg ratio (Palencia et al., 2010); meanwhile, reduction of Mg and K in the fertilizer reduced tip-burn (Bautista et al., 2009).

Nutrition also influences disease severity; for instance, when *Colletotrichum gloeosporioides* is present in hydroponic cultivation system, higher levels of nitrogen and potassium will increase disease severity (Nam et al., 2006).

Micronutrients are effective at very low concentrations and can be used to steer strawberry growth and development. For example, molybdenum can improve strawberry fruit quality and flavor through field spray (Liu et al., 2017); boron influences root daughter plant growth (Bohnsack and Albert, 1977); iron deficiency will significantly reduce the chlorophyll content in strawberry leaves and affect phenolic compounds, zinc, and copper concentration in fruit (Valentinuzzi et al., 2015).

1.5.3. Light Spectrum

Light in the spectral range between 300 nm to 800 nm is important for plant morphology and growth. Under similar light intensity, different light spectrum can regulate the physiological

development of many crops (Kim, H. et al., 2004; Song et al., 2017) including strawberry (Nadalini et al., 2017). With new LED technology it is possible to customize the spectrum to improve plant growth and morphology. For example, a spectral combination of 30% blue (B) and 70 % red (R) photon flux (PF) ratio (30B:70R) promotes strawberry transplant growth (Nhut et al., 2003; Hung et al., 2015). In addition, compared to 0B:100R, 30B:70R also increases strawberry stolon production (Wu et al., 2011). Furthermore, under a more balanced red and blue ratio (spectral ratio red: blue = 0.7,1.1, 1.5 compared to 0.5, 5.5) strawberry leaves have higher photosynthetic efficiency (Piovene et al., 2015).

Monochromatic B light (100B:0R) increases strawberry leaflet length, petiole length, flower stem elongation, and root growth when compared to 0B:100R (Choi, H. et al., 2015; Wu et al., 2011; Nadalini et al., 2017) or 30B:70R (Choi et al., 2015). In addition, blue light (100B:0R) increases flowering of long-day strawberry ‘HS 138’ after 10 days of anthesis (Yoshida et al., 2016).

Green (G) light can affect plant growth and morphology and plant responses to green light are species specific (Kim, H. et al., 2004; Dougher and Bugbee, 2001; Wang and Folta, 2013). In strawberry research reports, the addition of 10% G PF to 30B:70R (20B:10G:70R) increased the number of daughter plants formed in one strawberry stolon (Wu et al., 2011).

The ratio of red to far-red PF triggers several photomorphogenic responses in plants. In short-day strawberry ‘Paros’ the increase of far-red PF (with end-of-day supplemental far-red light) increased flower induction under long-day (>14h) and high temperature (above 25°C) (Zahedi and Sarikhani, 2017). Another study showed that high red to far red ratio delayed the flowering in short-day cultivar “Festival” (Takeda, Fumiomi et al., 2008). It was found that end-of-day far-red light induced the expression of *FvFT1*, which is a key promoter in the plant

response to light quality for long-day strawberry accession ‘H4’; also, the study showed that compared to the long-day cultivar, short-day *F. vesca* had the inversed response, which means end-of-day red light promotes flowering (Rantanen et al., 2014). In summary, light spectrum can affect flowering and vegetative responses and more research is needed to elucidate the vegetative responses of North American strawberry cultivars to light spectrum.

1.5.4. Light Intensity

In addition to spectral plant responses, light intensity is another environmental component that can influence strawberry growth and development. In *in-vitro* culture, higher light intensity could enhance the growth of strawberry during acclimatization stage (Zhou et al., 2005; Laforge et al., 1991). For example, under same CO₂ concentration, plants acclimatized under light intensity of 125 and 330 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF) had more plant dry mass and leaf area than in 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF (Laforge et al., 1991). Furthermore, research showed that strawberry plants grown in 280 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF had higher stolon production than plants in 140 or 220 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF (Kim, S. K. et al., 2010). However, different cultivars have different responses to light intensity. For example, for strawberry ‘Glasa’ and ‘Sivetta’, a decreased in PPF during flowering reduced stamen development, but for ‘Karina’, the increase or decrease of light did not influence stamen development (Smeets, 1980b; Smeets, 1976).

Daily light integral (DLI: $\text{mol m}^{-2} \text{d}^{-1}$) is another factor commonly used to quantify the amount of light received in one day. Higher DLI can significantly shorten the time of rooting ornamental cuttings and strengthen their growth, and it also accelerates flowering by 20% - 40% (Lopez, Roberto G. and Runkle, 2008). Two or three times higher light intensity can increase strawberry ‘Glasa’ flower production, reduce flower production time, and increase fruiting by 77% to 104% (Smeets, 1980c). Even though several research studies have focused on the

response of strawberry fruit production to light intensity; more research is needed to elucidate the responses of strawberry propagation to light intensity.

1.5.5. Humidity

Adequate humidity is required to maintain optimal plant growth. Humidity and temperature are directly correlated to vapor pressure deficit and plant transpiration rate, optimizing humidity will increase plant growth. Few research studies have focused on the impact of humidity on strawberry. For example, under 30 days of high humidity treatment (90-95% compare to 50-55%) strawberry 'Bigyo' did not show an increase in leaf area and dry mass; however, the N, K, Ca content in leaf increased by 25-28% (Choi, J. H. et al., 1997). Another study showed that continuous high humidity environment in greenhouse strawberry production could enhance fruit size and vegetative growth, but it could also cause flower and leaf tip burn and less firmness and shelf life in fruits (Lieten, 2002).

1.6. Indoor Production and Propagation Systems

Environmental conditions such as temperature, photoperiod, CO₂, nutrient composition, light quality, light intensity, humidity, among others, can greatly influence strawberry growth, development, and morphology. Several studies have focused on characterizing the effect environmental parameters for the production of strawberry fruit; however, research on environmental optimization to maximize propagation is lacking. In addition, with the intrinsic problems of open field strawberry propagation (seasonality, diseases transmission, lack of uniformity) it is necessary to research new propagation strategies.

A potential solution is to optimize the propagation of strawberry by using controlled environment (CE) growing systems. Controlled environment growing systems are defined as the

use of technology to optimize the environment to maximize growth while protecting the crop from outdoor challenges.

One example of CE production systems is medium and high-tech greenhouses. Greenhouses have proven to increase yield and profitability in several horticultural crops; for example, lettuce, tomato, cucumber, and pepper among others (Khan et al., 2006; Cantliffe et al., 2001). For tomato, greenhouse productivity is 10 times higher than open field (Cantliffe et al., 2001). Another example of CE system is precision indoor production (PIP) which is also known as plant factory or vertical farms. The main difference between PIP and greenhouses is the level of environmental control. PIP systems use electrical lighting as the source for photosynthesis instead of solar radiation enabling the control of light intensity and quality, and to better manage temperature, humidity, CO₂ enrichment etc. However, the higher technology in PIP systems makes them more energy and capital intensive.

Many kinds of ornamental and vegetable seedlings can be successfully and affordably produced in PIP systems including impatiens, salvia, petunia, snapdragon, tomato, cabbage, cucumber, stevia, grape, artichoke among others (Rabara et al., 2017; Yao et al., 2017; Simlat et al., 2016; Song et al., 2017; Ohashi-Kaneko et al., 2006; Hernández et al., 2016; Park, Y. and Runkle, 2016; Wollaeger and Runkle, 2014).

Light emitting diodes (LEDs) are commonly used in PIP systems. LED fixtures can be customized to provide unique spectrums and can positively or negatively affect plant growth, development, and photosynthetic rate (Castronuovo et al., 2016; Matsuda et al., 2016; Rabara et al., 2017; Hernández et al., 2016). Strawberry flowering and vegetative growth are highly influenced by the environment and more research is needed to find optimal environmental conditions to maximize strawberry propagation in PIP systems.

PIP system has several advantages to the field and greenhouse systems:

1) The user has full control of all environmental factors, reducing the uncertainty of outside climate, eliminating season restrictions on production yielding high uniformity and quality of the propagated material.

2) PIP system can be installed in any location and provide desirable propagation conditions without spatial or temporal restrictions. Propagation material can be available for the planting season for those areas using daughter plants and plugs and even supplement bare-root plants since several benefits of plugs over bare-roots have been reported (Kamperidou and Vasilakakis, 2006).

3) PIP systems can reduce the incidence of pathogens and insects since the facility is an enclosed structure.

4) The environment could be optimized to maximize stolon production from the stock plant and produce affordable plantlets of higher quality and vigor.

1.7. Current Study

Most studies focusing on the environmental optimization (light intensity, light quality, photoperiod and CO₂ enrichment) are for *in-vitro* propagation and *ex-vitro* acclimatization for strawberry micro-propagated material (Zhou et al., 2005; Laforge et al., 1991; Nhut et al., 2003; Hidaka et al., 2013); or for the production of strawberry fruit (Enoch et al., 1976; Hidaka et al., 2017; Hidaka et al., 2013). However, there is a gap of knowledge on the optimal environmental conditions to maximize stolon and daughter plant production in PIP systems. While the impact of temperature and photoperiod on promoting or inhibiting strawberry flowering is better understood; research in the interactions between light quality, light intensity, photoperiod, and temperature to maximize propagation output is lacking. The objective of the present proposal is

to optimize light intensity, light quality, and photoperiod to maximize stolon and daughter plant production under PIP growing conditions.

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CHAPTER 2. OPTIMUM LIGHT INTENSITY FOR ALBION STRAWBERRY PROPAGATION IN PRECISION INDOOR SYSTEM

Introduction

The strawberry production industry in the US requires a vast availability of propagative material to meet the geographical and seasonal demand. In order to have enough planting material, strawberry stock plants are reproduced in multiple years and in multiple geographical locations in the US and Canada. The current field propagation system is vulnerable to several problems such as decrease in plant quality after long storage, low availability of plant material at certain times of the year, and most importantly, pathogen transmission between nursery fields and the production fields. For example, anthracnose has been identified as a typical nursery transmitted disease that remains symptomless during propagation; however, devastating symptoms appear late in the fruit production season (Debode et al., 2015).

In North Carolina, anthracnose (*Collectotricum acutatum* and *C. gleosporioides*) Phytophthora (*P. cactorum*) and angular leaf spot (*Xanthomonas fragariae*) are amongst the most common diseases that growers in this state encounter as a result of nursery infected plants (Louws, 2018). In many cases, plants arrive from the nursery to the growers' location with no symptoms, yet when exposed to the hot humid conditions we often have in the last summer and early fall, quiescent infections can erupt after daughter plants begin to root in plugging nurseries or after they are planted into the production fields.

Propagating strawberry plants in a complete enclosed controlled environment using precise environmental strategies (precision indoor propagation: PIP) could be an alternative and complement to open-field propagation system. PIP systems have several advantages over greenhouse and open field propagation. For example, all key environmental conditions can be adjusted to control desirable outcomes such as initiate or prevent plant flowering (Mobini et al., 2016); change plant morphology (compactness, extension) (Mishra et al., 2012); and maintain higher consistency of plant quality (Yokoi et al., 2008). In addition, PIP systems have higher protection from the inoculum of outside pest or pathogens. Researches reports have shown the ability of increasing the efficiency and profitability of several nursery crops. For example, snapdragon (Park, Y. and Runkle, 2016), petunia, impatiens and salvia (Wollaeger and Runkle, 2014), tomato (Wollaeger and Runkle, 2014; Hernández et al., 2016), cabbage (Sato and Okada, 2014), grape (Yao et al., 2017), artichoke (Rabara et al., 2017), cucumber (Hernández and Kubota, 2016; Song et al., 2017), stevia (Simlat et al., 2016) among others.

One of the of the main challenges for the adoption of indoor growing systems such as PIP is the high energy demand and operational cost mainly driven by the use of electrical lighting as the sole-source-light for photosynthesis. Thus, research studies in other crops have focused on strategies to increase light-use-efficiency in indoor production systems; for example, adjusting photoperiod in leafy green production to reduce electricity cost (Urairi et al., 2017) and increasing CO₂ concentration (to 700 µl L⁻¹) to reduce the light quantity (Kjaer et al., 2011).

In order to make PIP systems more affordable for the propagation of strawberry, it is imperative to find the optimal light intensity to increase vegetative output (daughter plants) and maximize light-use-efficiency. Few studies have focused on light intensity for strawberry propagation. For example, Wu et al. (2011) compared the influence of different light sources and

spectrums on stolon production of 'Toyonoka' (short-day cultivar) strawberry and found that high intensity ($110\text{-}122 \mu\text{mol m}^{-2} \text{s}^{-1}$) white color light (fluorescent: 5000 and 6500K) increases stock plant dry mass when compared to plants in lower light intensity ($50\text{-}55 \mu\text{mol m}^{-2} \text{s}^{-1}$) and warmer color (fluorescent: 4000 and 3000K). In addition, plants in 30% blue:70% red photon flux (30B:70R) treatment produced the highest number of stolons while in 20%blue:10% green:70% red (20B:10G:70R) treatment plants had the highest daughter plants per stolon. Researchers found that 'Maehyang' (short-day cultivar) strawberry produced significantly greater number of daughter plants under $280 \mu\text{mol m}^{-2} \text{s}^{-1}$ compared to $140 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $220 \mu\text{mol m}^{-2} \text{s}^{-1}$ fluorescent light (photoperiod 16h) with a daily productivity of 0.27 daughter plant per mother plant (Kim, S. K. et al., 2010). Tsuruyama and Shibuya (2018) use an indoor propagation system to grow two strawberry cultivars ('Elan' and 'Yotsuboshi') (day-neutral cultivar) from seed under different photoperiods while maintaining the same daily light integral ($10 \text{ mol m}^{-1} \text{ d}^{-1}$) and found that plugs in 16 h and 24 h photoperiods had greater growth rate and shorter time until flower-bud-initiation than in 8 h and 12 h photoperiods. These studies have illustrated a baseline of responses to light conditions for short day strawberry cultivars, however, limited studies have focused on optimizing the light intensity to maximize the asexual propagation of day-neutral strawberry cultivars.

'Albion' similar to other day-neutral cultivars is used in south east United States for extended season production; in addition, 'Albion' has been used as a strawberry model crop on research focused on fruit production. 'Albion' is classified as day-neutral since it flowers under both long-day and short-day light conditions; however, recent research has shown that 'Albion' shows a facultative long-day response that flower initiate faster under long-day condition (Garcia, 2016). 'Albion' often produce low number of daughter plants in open-field

propagation system (Pers. comm. with expert) (Kubota, 2017). The primary objective of the present study is to investigate the influence of different light intensities on ‘Albion’ strawberry daughter plant production (propagation) and overall morphology and growth rate of both the stock plant and daughter plants.

Materials and Methods

1. Material preparation

On 16 Oct. 2017 one thousand ‘Albion’ strawberry daughter plants obtained from Cottle Farm Inc. (Faison, NC) were planted into 50-cell trays (96ml) filled with C/P growing mix (Jolly Gardener: Pro-Line) and placed in a greenhouse under mist to root (temperature set to 21°C). Planted daughter plants received controlled mist for 42 days to promote rooting and become plug plants. The misting frequency was 1 min every 5 min for the first 7 days, 3 min per hour for following 14 days, and 10 min per day for last 21 ddays. Plug plants received over-top irrigation every other day with customized nutrient recipe (see section 2.2) and tap water.

Prior to moving the plant material into NC State Phytotron (2 Feb. 2018), foliage and substrate was removed from 90 plug plants with uniform crown diameter, and plants were dipped in an insecticide/miticide (Mavrik Aquaflow, Central Life Sciences, Schaumburg, IL, USA) and transplanted into individual pots (240 ml). Foliage regeneration process took two weeks, under 75% humidity, 22/18 °C (day/night) and $\sim 250 \mu\text{mol m}^{-2} \text{s}^{-1}$ (12 h photoperiod). Plants received deionized water for two weeks then a customized nutrient recipe (see section 2.2) every other day.

On 17 Feb. 2018, 45 of the most uniform plug plants with two expanded leaves and similar crown diameter at the soil level (7.29 ± 0.75 mm) were selected for the experiment.

2. Environmental control and growing conditions

Plants were grown in a growth chamber (8.6 m²) with environmental control (chamber A18, 2731 Pilsbury Cir, NCSU Phytotron, Raleigh, NC 27607). A network of sensors (14 per chamber) was deployed in the chamber to measure and maintain the environment. Temperature, relative humidity (Sensirion SHT75, Sensirion, Laubisruetistrasse 50, 8712 Staefa ZH, Switzerland) and carbon dioxide (Vaisala CARBOCAP, Vaisala, P.O. Box 26, FI-00421 Helsinki, Finland) sensors were installed in an aspirated sensor box. Nine fine-wire thermocouples (type T, gauge 24, Omega Inc., Stamford, CT, USA) were deployed (one per treatment and repetition) to measure canopy temperature. All environmental data was recorded every three minutes (aspirated box) and every minute (thermocouples) using a datalogger (CR1000, Campbell Scientific, Logan, UT, USA). Environmental parameters are reported in Table 3.

Plants were subject to three different light intensity treatments, PPF250 (241 ± 13), PPF350 (337 ± 13), and PPF450 (443 ± 17) photosynthetic photon flux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) at 12 h photoperiod (Table 4) provided by cool white fluorescent lamps (4100K). Spectral curve and distribution are shown in Figure 2 and Table 4. Pieces of horticultural shade cloth (Harmony 4647FR, Harmony 3315oFR and Luxous 1347, Svensson Textile, Sweden) were used to precisely adjust the light intensity under each treatment (LI 1500 Sensor Logger and LI 190R Quantum Sensor, LI-COR, Lincoln, NE, USA). In addition, the shade cloth was adjusted every three weeks to ensure that photon flux at the canopy level was stable ($\pm 10\%$ from initial intensity).

A customized nutrient recipe with (mg L^{-1}) 80 NO₃-N, 20 NH₄-N, 21.4 P, 145.2 K, 60 Ca, 12.2 Mg and 39 S, as well as micro-nutrients was delivered using a custom drip-irrigation system

controlled by three soil moisture sensors (ECH₂O EC-5, METER Environment, Pullman, WA, USA). Electrical conductivity, pH, and percent drainage were measured daily (Table 3). Each plug was put into 1-gallon pot with customized substrate composed of a mix of 50% perlite, 25% coconut coir and 25% of peat moss.

3. Experimental design and data collection

All 45 potted plants were randomly divided into nine groups of five plants each. Each experimental treatment was applied to three groups (5 plants per group) for a total of three repetitions per treatment. Every week, crown diameter, leaf number, stolon number and number of daughter plants were recorded on each stock plant.

In week 11, net photosynthetic rate (P_n) was measured for all stock plants using a gas exchange system (LI-COR 6800, LI-COR, Lincoln, NE, USA). The first fully expanded leaf at the center of canopy of each plant was chosen, measuring chamber was clipped at the center of middle leaf-let. Measurement was taken following the order of PPF250-PPF350-PPF450 for repetition 1, then for repetition 2 and 3 with the same order. All plants within experiment groups were measured.

After 12 weeks of cultivation (nine weeks of daughter plants production period), all stolons were cut from the base of the stock plant. Daughter plants were cut off the stolons and measured for fresh mass, crown diameter, leaf count, leaf area (LI-COR 3100 Area Meter, LI-COR, Lincoln, NE, USA) and dry mass (at least 48 h in oven with air circulation set to 70 °C). All daughter plants were classified in 6 categories, and counted, including primordia (see below and Figure 3). Any leaf wider than 1cm was counted.

In addition, dry mass and internode length between daughter plants were measured on each stolon. This first 12-week cycle is defined as ‘round one’ in this experiment.

After removing all the stolons, stock plants were returned to their positions. After an additional nine weeks of growing, same parameters than round one were measured ('round two'). In addition, stock plants were cut at the substrate level to quantify fresh mass, leaf area, crown diameter, leaf count (>1 cm) and dry mass.

For both rounds all harvested daughter plants were classified by crown diameter. The classification is only for the data analysis of presented study. No industrial meaning is implied. Daughter plants were grouped into six categories (Figure 3): extra-large (XL) crown diameter greater than 11 mm, large (L) crown diameter 8.5 mm to 11 mm, medium (M) crown diameter 6 mm to 8.5 mm, small (S) crown diameter 3.5 mm to 6 mm, extra-small (XS) crown diameter smaller than 3.5 mm and non-developed (ND) daughter plant primordia without leaf.

All data collected were analyzed using JMP software (SAS Institute, Cary, North Carolina, USA). Linear regression was used to model the plant response to light intensity and growing time. For identifying the difference among treatments, significance level $\alpha = 0.05$ was used. Tukey-Kramer HSD ($\alpha = 0.05$) was used when analyzed mean separation.

Results and discussion

1. Daughter plants and stolons

1.1. Daughter plant number, morphology, and growth

Total number of daughter plants produced per plant increased with the increase of light intensity (Figure 4). For round one (12-weeks), the total number of daughter plants in low light ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$), medium light ($350 \mu\text{mol m}^{-2} \text{s}^{-1}$) and high light ($450 \mu\text{mol m}^{-2} \text{s}^{-1}$) was 38.7, 45.7, 56.7, respectively. For round two (21-weeks), the total number of daughter plants for low light, medium light, and high light was 41.9, 47.2, 50.6, respectively.

The effect of light intensity on total daughter plant dry mass and fresh mass (sum of all the daughter plants per stock plant) is shown in Figure. 5a and 5b. In round one, the total dry mass and fresh mass of all daughter plants per stock plant increased with the increase of light intensity. In round two, the increase of total daughter plants dry mass and fresh mass with the increased of light intensity was less evident but the trend was still present ($P=0.070$ and $P=0.068$). In addition, the total area of all daughter plants per stock plant increased with the increase of light intensity in round one only (Figure 5c).

Generally, higher DLI (day light integral) is associated with higher yield, including fruits, bulbs, cut flowers, roots and potted plants, in general 1% increase of light increases yield by 0.25% to 1.5% (Marcelis et al., 2006). In the current experiment, plants under the PPF450 treatment produced 34.1% and 19.7% greater daughter plant dry mass and 44.7% and 20.8% greater number of daughter plants in round one and round two, respectively, compared to plants under the PPF250 treatment. However, plants in PPF450 (actual DLI: 19.14) were exposed to 84% more light than plants under the PPF250 treatment (actual DLI: 10.41) suggesting that a 1% increase in light only yield 0.23-0.4% increase in total daughter plant dry mass and 0.25-0.53 % increase in number of daughter plants. Similarly to studies in cucumber (Hovi-Pekkanen and Tahvonon, 2008), tomato (Demers et al., 1998), and ryegrass (Warringa and Marinissen, 1996), in the present study the percent increase in the amount of light was not directly converted to the same percent increase of target organ yield.

Another method to evaluate the propagation response to light is by estimating the crop specific efficacy to PPF. Crop specific efficacy to cumulative PPF (grams of yield per mol of PPF: g mol^{-1}) is frequently described with a linear equation of the relationship between cumulative PPF (mol m^{-2}) and cumulative yield (g m^{-2}) for the length of the production time. The

slope of the linear equation can be used as a good estimation for crop specific efficacy to PPF (g mol^{-1}) (Kubota et al., 2016; Acock et al., 1971; Cockshull et al., 1992). For example, Kubota et al. (2016) listed crop productivity values of $7.6\text{-}14 \text{ g mol}^{-1}$, $4.6\text{-}6.5 \text{ g mol}^{-1}$, and $3.7\text{-}6.9 \text{ g mol}^{-1}$ for cluster tomato, cherry tomato, and lettuce, respectively. In addition, specifically for strawberry, Kubota et al. (2016) reported a $1.5\text{-}2.1 \text{ g mol}^{-1}$ for strawberry fruit. If we translate this methodology to the propagation of daughter plants, the crop specific efficacy to PPF in our experiment was $0.081 \text{ daughter plants mol}^{-1}$ and $0.097 \text{ daughter plants mol}^{-1}$ for round one and round two, respectively.

The number of daughter plants and total daughter plant dry mass under different PPF in round two was 1.1% and 27.1% lower than in round one. The potential cause for the lower response could be explained by plant aging. Previous study concluded that the stock plant with smaller crown diameter (10-15mm compared to greater than 15mm) had higher potential to produce more daughter plant biomass (Lisiecka et al., 2014); which agree with the presented results since the plant crown diameter in round two was larger than round one (around 30 mm compared 7.5 mm at the beginning of each round).

1.2. Weekly daughter plant production

The relationship between number of weeks and total number of daughter plants on each stock plant is shown in Figure 6. The cumulative number of daughter plants increased with time for both round one and round two for all light intensities. In addition, the light intensity treatment had a significant influence in the rate of daughter plant production (Figure 6). In round one, the greater the light intensity the greater the rate of daughter plant production ($\text{PPF450} > \text{PPF350} > \text{PPF250}$). In round two, high and medium light intensity had a greater rate of daughter plant production than low light intensity (Figure 6).

The number of daughter plants per week per light treatment is shown in Figure 7. In round one, the average number of daughter plants developed per week significantly increased over time for all light treatments. In addition, the greater the light intensity, the more daughter plants per week (PPF450>PPF350>PPF250). In round two, the number of daughter plants per week also increased over time for all light treatments. However, the number of daughter plants produced per week was not different between the light intensity treatments.

In the present study we also measured the light intensity around the cascade of hanging daughter plants at different heights below the stock plant (data not shown) and the average light intensity was 146.2, 146.2 and 142.6 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for PPF250, PPF350 and PPF450, respectively. We observed that plants with higher number of daughter plants generally produced new daughter plants faster, independently of the light intensity treatment provided to the stock plant. We hypothesize that daughter plants contributed to the development of new daughter plants most likely serving as a source organ in terms of photosynthetic activity. Although, previous research in strawberry indoor propagation concluded that stock plant photosynthesis was the major source of daughter plants production and that daughter plants have little contribution to the development of new daughter plants while connected to the stolon (Park, Seon Woo et al., 2017), the difference in results could be explained by the difference of the strawberry cultivar (short day vs day-neutral) or propagation strategy since Park et al. (2017) was rooting new daughter plants while still attached to the stock plant.

The difference in daughter plants per stolon production between round one and round two could be explained by plant photo-assimilate partitioning between daughter plants and stolon sections. Stock plant produced more stolons in round two than in round one (Figure 8), but the total number of daughter plants were not very different (Figure 9). The photosynthates were

transferred to maintain the growth of existing stolons, which compromised the new daughter plants formation per stolon in round two. Therefore, new daughter plant formation was more active in round one than in round two. Another possibility may be a potential root space restriction on round two; however, we do not have quantifiable measurements to compare root development between round one and round two.

1.3. Daughter plants classification and stolon morphology

The uniformity of daughter plant size was not influenced by light intensity in either round one or round two (Figure 9). In round one, small size (S) daughter plant was the largest group among all six different categories ($35.5\pm 8.5\%$, $35.0\pm 7.5\%$ and $35.8\pm 5.3\%$ for low, medium and high light, respectively). While in round two, medium size (M) daughter plant was the largest group among all six different categories in all three treatments ($34.6\pm 5.9\%$, $34.7\pm 6.0\%$ and $32.4\pm 7.3\%$ for low, medium and high light, respectively).

Non-developed daughter plant rate (number of daughter plants with no leaf/total number of daughter plants) was also not altered by different light intensities. All three treatments gave around 20-22% of non-developed daughter plants in round one ($20.4\pm 8.7\%$, $21.4\pm 7.1\%$ and 21.8 ± 6.7 for low, medium and high light, respectively) and around 24-26% in round two ($26.3\pm 5.3\%$, $24.4\pm 4.0\%$ and $25.0\pm 8.0\%$ for low, medium and high light, respectively).

When a new stolon emerges from strawberry crown, the first daughter plant (oldest daughter plant on that stolon) is the one closest to the base of the stock plant (first daughter plant of stolon). New daughter plants grow along the elongated stolon, when new daughter plant becomes functional, it develops a continuation of the same stolon (a branch). The stolon will continue to develop new daughter plants until is removed from the stock plant. At the end of the main stolon is the daughter plant primordium (youngest daughter plant on that stolon). When the

stolon (with multiple daughter plants) is removed from the stock plant, usually the youngest daughter plant is not functional (not useful for transplanting). In theory, a stock plant with more stolons and less daughter plants per stolon will overall have more uniform daughter plants than a stock plant with fewer stolons and more daughter plants per stolon. Plants developed more stolons at a faster rate in round two than in round one (Figure 11). In round two, the younger stolons was visible after first stolon was developed while in round one an additional stolon was initiated only after the existing stolon had several daughter plants. Therefore, the stolons in round two are more similar in age and speed of daughter plants formation. The total daughter plant productivity of stock plant in same light level was not different between two rounds of experiment, but non-developed rate was higher in round two. The increase in non-developed rate potentially associated with the increase of stolon number, we hypothesize that if round two was harvested at a later time, the number of functional daughter plants and the uniformity of the daughter plants would be greater than in the round one.

1.4. Relation between flower development and stolon generation

The number of flowers removed decreased as the number of weeks increase in both round one and later weeks in round two (Figure 12). Generally, strawberry flower takes around 35 days from initiation to exposed outside the crown (Taylor et al., 1997). All the flowers removed during that week initiated at least 5 weeks ago. In round one, the flowers removed from week 1 to week 5 initiated before the start of treatment (under much lower temperature), potentially some of the flowers removed from week 6 to week 7 also initiated before plants were moved into the chamber. A distinct drop of flower number was noticed in week 7. Then, after stolons from round one were harvested, there was a clear increase in number of flowers removed from week 13 to week 16. Our hypothesis is that the drop of flower number in week 7 was caused by both

the treatment environmental conditions and the stolons serving as a strong sink organ; the visible flowers noticed from week 13 to week 16 were formed from week 8 to week 11 (round one) but not visible since the stolons during round one were acting as a stronger sink for available photo-assimilates. After the stolons were removed (end of round one), the dormant flowers became the main sink organs until the new formed stolons started competing for photo-assimilates again, which was after week 17 in round two.

1.5. Internode length

The internode length between daughter plants were measured to study the influence of light intensity on stolon morphology. Linear regression models are shown in Figure 10. No linear relation between light intensity and internode length daughter plants was found in round one. In round two, the internode length between daughter plants slightly decreased with the increase of light intensity.

In round one, stock plants with 39-57 daughter plants had stolons longer than 2 meters which hang down from elevated benches (1.5m) and touched ground very soon, while in round two, with 42-51 number of daughter plants, stolons were shorter and touched the ground later. In case of PIP system for strawberry, space efficiency is an important factor. Shorter stolons reduce the required space for each stock plant, increasing yield per unit of area. Under the spacing in current experiment (five stock plants per linear meter) every 0.1 m reduction in stolon length can save 0.003 m³ (0.1m x 0.15m x 0.2m) of chamber space per stock plant. For every 1000 stock plants with 2 m long stolons, the space saved by reducing 0.1 m stolon length can allow to fit 50 more stock plants and increase production per space by 5%. Future research will investigate the influence of light quality on the distance between daughter plants and overall length of the stolon in order to maximize daughter plant production per meter square.

2. Stock plant growth, morphology, and net photosynthetic rate

The plant crown diameter (Figure 13a) and leaf count (Figure 13b) did not increase with the increased of light intensity in round one, but the it was increased by the end of experiment (round two). Plant leaf area, canopy fresh mass, and canopy dry mass all increased with the increase of light intensity (Figure 14).

Stock plant net photosynthetic rate was measured before harvesting the daughter plants from round one under 250, 350 and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 15). Net photosynthetic rate (Pn) increased with the increase of light intensity ($P < 0.0001$) for all plants grown under the three experimental light treatments. Under the same light intensity measurement, Pn of plants from all three treatments were not significantly different ($P = 0.4511$, $P = 0.6062$ and $P = 0.6007$ for 250, 350 and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$, respectively). The Pn was not measured at the end of round two due to unexpected equipment failure.

Higher light intensity increased stock plant growth, which matches other studies about light intensity and strawberry plant growth (Zhou et al., 2005; Laforge et al., 1991). In the present study, long term exposure to a specific light intensity did not increase the plant's efficiency under that specific light intensity, nor decrease the plant photosynthetic efficiency under other (either higher or lower) light intensities. For example, a plant grown in PPF250 treatment had the same Pn than a plant grown in PPF450 when Pn was measured at PPF of 250, 350 and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Previous study on *Chenopodium album* showed that mature leaf will increase its light saturation point when transferred from low light environment (70 $\mu\text{mol m}^{-2} \text{s}^{-1}$) to high light environment (700 $\mu\text{mol m}^{-2} \text{s}^{-1}$); however, the transferred plant had a lower Pn capacity than plants grown under 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ due to differences in leaf anatomy (Oguchi et al., 2003).

We hypothesize that the difference of light intensity between the treatments in the current experiment is not big enough to generate the change in leaf anatomy.

Conclusion

Precision indoor propagation has the potential to increase the production yield of strawberry daughter plants. It is reasonable to use this PIP system to supplement open-field propagation. Efficiency of propagation can be furtherly improved by increasing light intensity, and high light condition can increase daughter plant production by 50%, reduce production space and improve stock plant vigor. Potentially, high light treatment can also reduce the stolon length increasing the space using efficiency in PIP system. Future studies will focus on optimizing other environmental conditions such as photoperiod, light quality, temperature, CO₂ and nutrient dynamics.

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CHAPTER 3. THE EFFECT OF TWO LIGHT SPECTRUMS AND TWO SHORT PHOTOPERIODS ON ALBION STRAWBERRY PROPAGATION

Introduction

Strawberry is mainly asexually propagated, and nurseries rely on the capability of stock plants to produce clones (daughter plants). The amount of daughter plants per stock plant will determine the availability of propagation material for fruit production. In addition, the quality/physiological stage of the daughter plant can influence the production of strawberry fruit. For example, daughter plants with 60% or higher fresh mass can increase branch crown development by 25% of 'Chandler' strawberry (Takeda, F. et al., 2004). Propagation of strawberry in the U.S. is located in several states to different environmental conditions to enhance vegetative production (daughter plants). Commercial strawberry propagation is mainly located in California, Florida, Oregon, and North Carolina and parts of Canada (United State Department of Agriculture 2007); however, strawberry propagation is highly restricted by climate and season, and variability on climate may affect nursery yield and plant quality. In addition, the flowering physiology of most strawberry cultivars is not fully characterized since vegetative growth and flower initiation are both sensitive to several environment conditions including temperature, day length (photoperiod), and their interaction (Heide et al., 2013).

Optimal environmental conditions including temperature, photoperiod, light intensity, spectrum, and their interaction are critical factors to maximize nursery production. One alternative is to optimize these environmental factors using highly controlled propagation environments which we define as precision indoor propagation (PIP) systems.

Indoor production requires solely electrical light, which can be provided Light emitting diodes (LED). LEDs are energy efficient and fixtures can be built with different spectrum combinations. Recent studies in a range of plants have shown that under similar light intensity, different light quality can regulate the physiological development of many crops like cucumber (Song et al., 2017), lettuce (Kim, H. et al., 2004) and strawberry (Nadalini et al., 2017).

Previous studies showed that in vitro culture, the total dry mass of strawberry ‘Camarosa’ was 21% to 36% greater when grown under a light spectrum of 30% blue (B) photon flux (PF) and 70% red (R) PF (30B:70R) than in 10B:90R, 50B:50R, and 70B:30R (Hung et al., 2015). Under CO₂ enrichment conditions, strawberry ‘Akihime’ had 16% to 35% higher dry mass when grown under 30B:70R than in 10B:90R and 20B:80R, respectively (Nhut et al., 2003). Furthermore, under a more balanced red and blue ratio (spectral ratio red: blue = 0.7,1.1, 1.5 compared to 0.5, 5.5) strawberry leaves had higher fresh mass and fruit yield per energy input (g kW⁻¹) (Piovene et al., 2015).

Monochromatic B spectrum (100B:0R) increased strawberry leaflet length (‘Daewang’), petiole length (‘Elsanta’) and flower stem elongation (‘Elsanta’) compared to monochromatic red light (0B:100R) (Nadalini et al., 2017; Choi, H. et al., 2015; Wu et al., 2011). In addition, long day strawberry ‘HS138’ grown in 100B:0R had up to 100% greater flowering rate after 10-day of anthesis than in 0B:100R spectrum (Yoshida et al., 2016). Research also showed that the early flowering induced by end-of-day blue light requires gene FVSOIC mediation in addition to gene

FvFT1 when long-day accession strawberry 'Hawaii-4' was growing in short-day condition (Rantanen et al., 2014). In greenhouse supplemental lighting, 100B increases stolon number by 25% to 163% of strawberry 'Festival' compared to plants in 100R (Jamal Uddin, A. F. M. et al., 2018).

Even though there are several studies focused on the effect of light quality, photoperiod, and intensity in fruit strawberry production and light intensity in strawberry propagation; the influence of light quality and varied short photoperiods (under same DLI) on day-neutral strawberry asexual propagation is lacking. Short photo periods were chosen because 'Albion' strawberry was defined as quantitative long-day cultivar (Garcia, 2016). The objective of current experiment is to explore the impact of two light qualities and two short photoperiods on the propagation efficiency of day-neutral strawberry 'Albion' when grown in Precision Indoor Propagation (PIP) systems.

Material and Methods

1. Material preparation

On 15 June 2018, 150 'Albion' strawberry daughter plants harvested from an indoor propagation chamber (A18, 2731 Pilsbury Cir, NCSU Phytotron, Raleigh, NC 27607) were rooted in 48-cell germination trays with customized substrate (see below). The rooting process was completed in an incubator with light intensity of $\sim 250 \mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux provided by fluorescent lamps. The chamber temperature was set to 20°C and photoperiod was 12/12 hours (day/night). Rooting daughter plants were irrigated with deionized water every other day. After three weeks, plants received customized nutrition solution (see below) every

other day. On 21 August, 2018, 80 rooted daughter plants with crown diameter of 7.87 ± 1.52 mm were selected for the experiment.

2. Experiment conditions and cultivation environment

Two 8.6 m² growth chambers were used for the experiment (A19 and A18, 2731 Pilsbury Cir, NCSU Phytotron, Raleigh, NC 27607). Sensors were independently installed in the two chambers to record temperature and humidity (Sensirion SHT75, Sensirion, Laubisruetistrasse 50, 8712 Staefa ZH, Switzerland) and CO₂ (Vaisala CARBOCAP, Vaisala, P.O. Box 26, FI-00421 Helsinki, Finland). Also, eight thermocouples (type T, gauge 24, Omega Inc., Stamford, CT, USA) evenly spread in each chamber tracked temperature for the different treatments and reps. All readings were recorded and logged using a data logger (CR1000, Campbell Scientific, Logan, UT, USA). A summary of the environmental condition is showed in Table 5.

Rooted plants were transplanted into 1-gallon plastic pots, filled with substrate with 50% perlite and 50% coconut coir. A moisture sensor (ECH₂O EC-5, METER Environment, Pullman, WA, US) per chamber was used to monitor substrate moisture and trigger irrigation in both chambers. Fertilizer was injected using an injections system (Model D14MZ2, Dosatron International, Inc. 2090 Sunnydale Blvd. Clearwater, FL, US). Customized nutrient recipe provided (mg L⁻¹) 80 NO₃-N, 20 NH₄-N, 21.4 P, 145.2 K, 60 Ca, 12.2 Mg and 39 S, as well as micro-nutrients. pH and EC of both irrigation and percent drainage were tracked daily (Table 5).

Plants received two different light quality treatments, high blue (HB) and low blue (LB) under two different short photoperiods (12 hours and 8.5 hours) with same day light integral (DLI). Light was provided by LED fixtures (Model FL 100, Osram Gmbh, Marcel-Breuer-Straße 6, 80807 Munich, Germany). Using a spectroradiometer (Black Comet PF200, Stellar, Inc, Tampa, Florida, USA) light quality was measured at the center of each pot and averaged by

treatment and chamber (Figure 16) with spectroradiometer (Black Comet PF200, Stellar, Inc, Tampa, Florida, USA). Light intensity was measured as the average of five measurement points on the pot surface with quantum sensor (MQ-500, Apogee Instruments, Logan, UT, USA). Two light intensities were used under two photoperiods to achieve similar DLI (Table 5). High blue treatment under 12 hours is defined as 12HB, low blue treatment under 8.5 hours is defined as 8.5 HB and similarly for the low blue treatments 12LB and 8.5LB.

3. Experimental design and data analysis

Eighty uniform plants with crown diameter of 7.87 ± 1.52 mm were randomly assigned to each treatment. Four repetitions of each light quality were assigned to each photoperiod and the photoperiod treatments were repeated in time.

Plant height, crown diameter, leaf number, stolon number, and daughter plant number were measured weekly on each stock plant. Average plant height was measured weekly and the height of lights was adjusted accordingly to maintain light intensity between spectrum and photoperiod treatments. At the beginning of week 12, all stolons were cut from the base of the stock plant. Each daughter plant was removed from stolon and crown diameter, fresh mass, leaf area, leaf number and dry mass were measured from each daughter plant. The distance between daughter plants on each stolon was also measured. Stolons were dried in a 70 °C oven and at the end of 2 days, stolon dry mass was measured.

Harvested daughter plants were classified based on crown diameter: extra-small (XS) crown diameter smaller than 3.5mm, small (S) crown diameter 3.5mm to 6mm, medium (M) crown diameter 6mm to 8.5mm, large (L) crown diameter 8.5mm to 11mm, extra-large (XL) crown diameter greater than 11mm and non-developed (ND) daughter plants with no leaf (Figure 17). These categories are used to delineate sizes, and in most commercial operations, medium and

small sizes produce the highest quality plants (Fernandez, G., 2019). At the end of the experiment all stock plants were harvested and canopy fresh mass, leaf number (>1cm), crown diameter, and dry mass were measured. In addition, chlorophyll concentration was quantified based on Moran and Porath (Moran and Porath, 1980) by cutting one 27 mm² leaf circle from one leaf of each mother plant.

All data collected were analyzed using JMP software (SAS Institute, Cary, North Carolina, USA). Linear regression was used to model the quantitative relation between results and time. Analysis of variance (P=0.05) was conducted to determine the difference between two light qualities within each photoperiod. Tukey-Kramer HSD (p=0.05) was used to analyze mean separation.

Result and Discussion

1. Stolons and daughter plants

1.1. Total number of stolons and daughter plants

In the present experiment, the light quality treatments did not have a significant influence on total number of daughter plants, functional number of daughter plants, and number stolons in either photoperiod (Table 7).

However, plants grown in 12 h photoperiod had 23-28% greater total number of daughter plants, 21-23% greater functional daughter plants, and 17% greater number of stolons (LB treatment) than in 8.5 h photoperiod.

In our original hypothesis, we suggested that plants in shorter photoperiods will generate more stolons and/or daughter plants earlier and faster since ‘Albion’ strawberry is classified as a quantitative long-day plant (Garcia, 2016); however, the shorter photoperiod treatment (8.5 h)

had lower propagation output than the 12 h photoperiod. This result disagrees with the study on 'Elan' strawberry, which produced significantly higher number of daughter plants in 10 hour photoperiod than in 14 hour photoperiod (Sønsteby, A. and Heide, 2007).

1.2. Cumulative number of stolons and daughter plants and weekly daughter plant new growth

The relation between number of weeks to total number of stolons and daughter plants is shown in Figure 18 and Figure 19, respectively. The number of stolons and daughter plants increased over time in all treatments. Plants in 12 h photoperiod had significantly higher rate of stolon and daughter plant production than in 8.5 h photoperiods, independent of the light spectrum. In both photoperiods, plants under different spectrums produced stolons at comparable rates, but plants under low blue spectrum had a higher daughter plant production rate than plants in high blue spectrum. When comparing four treatments together, the stolon and daughter plant production rate ranking was 12 LB=12 HB>8.5 LB=8.5 HB (stolon: $P<0.05$; daughter plant: $P<0.01$).

The number of daughter plants per week per plant is shown in Figure 20. Weekly daughter plant production increased over time in all treatments. Similar to the total number of stolons, plants in 12 h photoperiod had more daughter plants, developed more daughter plants, and stolon production generation (per week) than plants in 8.5 h photoperiod independently of light quality treatment. Also, in both photoperiods, plants under low blue spectrum generate new daughter plants faster than plants under high blue spectrums. When comparing the four treatments together, plants in 12 HB generated new daughter plants with faster speed than the plants in 8.5 h photoperiod, but not faster than the plants in 12 LB. Furthermore, plants in 12 LB also generated daughter plants faster than plants in 8.5 LB, but not faster than the plants in 8.5 HB.

The transition between strawberry flowering and vegetative growth is affected by the interaction of several environmental factors, including temperature and photoperiod (Heide et al., 2013). For example, 'CHI-24-1' flowers under long days when temperature is greater than 20°C, but it will also flower under short days (facultative short-day) when temperature is lower than 15°C (Yanagi, Yachi, & Okuda, 2006). The average temperature of this study was 25.9°C, higher than temperature normally in favor of flower production (Sønsteby and Heide, 2006; Sønsteby et al., 2016; Ledesma et al., 2008); therefore, in the present study the warmer temperature could have influenced the flowering rate at a greater extent than the difference in photoperiod. Another possibility is that 'Albion' may still produce higher number of daughter plants under shorter photoperiods (<12h) but 8.5 hours may be too short of a photoperiod for 'Albion' strawberry normal development and growth.

In the present study, plants in low blue spectrum (11B:4G:85R) showed a better performance in promoting vegetative growth compared to plants in high blue spectrum (38B:12G:50R). In addition to the differences in the B PF between treatments, the treatments also had different amounts of G and R photon flux (PF); the spectrum of HB treatment was composed of 38B:12G:50R percent PF ratio and the spectrum of the LB treatment was composed of 11B:4G:85R percent PF ratio. McCree (1971) quantified the relative quantum efficiency (RQE) of different plants grown in the field and growth chambers and concluded that B (400-500nm) and G (500-600nm) wavelengths had lower RQE than R (600-700nm) wavelengths. Based on McCree (1971), it is expected for plants grown in the LB treatment (higher R PF in spectrum) to have greater RQE, photosynthetic rate, and growth than plants under the HB treatment.

Wu et al., (2011) grew strawberry ‘Daewang’ under different white spectrums and under B, G, and R LED spectrums. In contrast to the present study, Wu et al., (2011) showed that plants in white spectrums with higher B PF (6500K) produced more stolons than plants in white spectrums with lower B PF (3000K and 4000K) (42 days of treatment). In addition, strawberry plants in 30B:70R had higher number of stolons than plants in 20B:10G:70R and 100R (28 days after treatment) (Wu et al. 2011). Another possible explanation for the reduction in growth is the difference in the amount of green light between the two treatments (12% G in HB and 4% G in LB). Research has shown that green light suppresses the growth as well as chlorophyll formation of several plant species (Klein, 1964; Klein et al., 1965; Folta and Maruhnich, 2007; Dougher and Bugbee, 2001) and the greater amount of green PF in HB treatment could have contributed to the reduction in plant growth.

1.3. Daughter plants classification

The amount of blue light and different photoperiods had no significant influence on functional daughter plant rate (Figure 21). Averagely, each stock plant produced 85.8%, 88.6%, 83.1% and 83.5% functional daughter plants for 8.5HB, 8.5LB, 12HB and 12 LB, respectively. The amount of blue light had no influence on daughter plants crown diameter under either of the photoperiod. Generally, medium size daughter plants are the main group in all treatments, which are around 27.3%, 27.6%, 31.6% and 33.7% for 8.5HB, 8.5LB, 12HB and 12 LB, respectively.

2. Stock plant growth and morphology

2.1. Leaf chlorophyll content

Leaf chlorophyll concentration of the stock plant is shown in Table 8. Plants in 12 h photoperiod had 16%-23% higher chlorophyll concentration than the plants in 8.5 h photoperiod; however, light quality did not have an effect on chlorophyll concentration.

In present study, longer photoperiod increased chlorophyll concentration, which means under same DLI, lower light intensity provided at a longer time promoted chlorophyll accumulation more than higher intensity with provided at a shorter time. Our results match a similar study done on *Arabidopsis* were plants not only showed growth reduction but also showed that chlorophyll was lower under 6 h photoperiod compared to 12 h photoperiod with same DLI (Mengin et al., 2017).

The increase of B PF is known to increase chlorophyll concentration in several plant species (Hernández and Kubota, 2016; Kopsell et al., 2014). For example, broccoli sprouts in 20B:80R had significantly higher chlorophyll content than in 5B:95R and 5B:10G:85R (Kopsell et al., 2014). However, in the present study, plants grown in higher B PF (HB) did not have greater chlorophyll concentration. Green light is known to mitigate blue-light mediated responses (Folta and Maruhnich, 2007; Wang and Folta, 2013), one possible explanation is that the greater G PF in the HB treatment reduced the plant's ability to increase chlorophyll concentration.

2.2. Internode length

No differences in internode length were detected in plants grown under the two light spectrums and photoperiods (Table 8). In PIP systems, any reduction in the growing space increases the economic viability of the system. In the present experiment, the internode length between each daughter plant was relatively long (~41.4 cm); for an indoor system, a significant reduction of the internode length will allow for PIP systems to be built with a lower height and consequently increase economic viability; therefore, additional research is granted. Several studies have shown the impact of light quality on internode and stem length (Snowden et al., 2016; Runkle and Heins, 2001; Xiong et al., 2002; Kurepin et al., 2011; Jeong et al., 2014). For example, it was reported a reduction in stem length of tomato, pepper, soybean, and cucumber,

when the percent B PF increased from 11% to 28%; however, other plant species such as wheat, lettuce, and radish did not have the same response (Snowden et al., 2016).

2.3. Stock plant growth and biomass

Stock plant fresh mass, dry mass, crown diameter, leaf area, and leaf number were similar under the two photoperiods (12h and 8.5h) (Table 8). However, plants in LB spectrum had 20.5%, 16.3%, 15.8% and 14.8% higher canopy fresh mass, canopy dry mass, leaf area and leaf number, respectively than plants in HB spectrum only when grown in 12 h photoperiod (Table 8).

Plant growth is directly correlated to DLI which is the cumulative number of photons received in one day ($\text{mol m}^{-2} \text{d}^{-1}$) (Lopez, R. and Runkle, 2017). In the present study, plants under different photoperiod received the same DLI ($10.9 \text{ mol m}^{-2} \text{d}^{-1}$) by adjusting the PPF ($\mu\text{mol m}^{-2} \text{s}^{-1}$) and since stock plants were grown under the same DLI, no differences in total plant growth were expected.

Due to the relatively lower sensitivity of ‘Albion’ strawberry to blue light change, the limitation of experiment facilities may contribute to the no differences found in 8.5 h photoperiod. In 12 h photoperiod, high blue spectrum had 34% higher blue light content than low blue spectrum, while in 8.5 h photoperiod the gradient is only 21.5%. Therefore, the critical point of triggering the plant reaction to blue light change could be around 30% gradient, which means at least 30% difference in blue light amount can induce biomass increase and or decrease for ‘Albion’ strawberry.

2.4. Weekly stock plant growth

2.4.1. Weekly flower removed

The relation between number of weeks and flower removed per stock plant per week is shown in Figure 22. The number of flowers removed decreased over time. No differences were found between all four treatments. Plants produced 2.7 ± 1.1 , 2.7 ± 1.4 , 2.9 ± 1.3 and 3.1 ± 1.8 total number of flowers for 8.5 HB, 8.5 LB, 12 HB and 12 LB, respectively.

2.4.2. Cumulative functional leaf number and crown diameter of stock plant

Stock plant crown diameter (Figure 23) and functional leaf number (Figure 24) increased with the increase of the number of weeks for all treatments. Under low blue spectrum, plants in 12 h photoperiod had higher crown diameter growth rate and higher leaf growth rate than plants in 8.5 h photoperiod. Also, in 12 h photoperiod, plants under low blue spectrum had higher crown diameter and leaf growth rate than plants under high blue spectrum. When compared four treatments together, plants in 12 LB had significantly higher crown diameter growth rate and leaf number increase than the plants in 8.5 LB.

In presented study, the longer photoperiod (12h) promotes stock plant growth and daughter plant production; thus, lower light intensity with longer daylength (up to 12 h) is more favorable than high light intensity with shorter daylength in 'Albion' strawberry plant propagation.

Conclusion

In this study, the 12 h photoperiod and low blue spectrum resulted in the most 'Albion' strawberry daughter plants. Plants under these conditions had a higher total propagation yield and rate of production. Plants in 12 h and high blue spectrum had better performance in daughter plants production than plants in 8.5 h photoperiod under both spectrums. The 8.5 h photoperiod and high blue spectrum had lowest daughter plant production rate and new generation speed.

Stock plants also have higher growth rate, leaf chlorophyll content and higher vigor in 12 h photoperiod than in 8.5 h photoperiod. Different light spectrums didn't have influence on stock plant growth in general.

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TABLES

Table 1. Flowering classification of common strawberry cultivars in North America.

Cultivar	Species	Season	Patent	Publisher
Radiance	<i>Fragaria x ananassa</i>	Short-day	USPP20363P2	Univ. of Florida, 2009
Diamante	<i>Fragaria x ananassa</i>	Day-neutral	USPP10435P	Univ. of California, 1996
San Andreas	<i>Fragaria x ananassa</i>	Day-neutral	USPP19975P2	Univ. of California, 1997
Chandler	<i>Fragaria x ananassa</i>	Short-day	USPP5262P	Univ. of California, 1984
Camarosa	<i>Fragaria x ananassa</i>	Short-day	USPP8708P	Univ. of California, 1994
Albion	<i>Fragaria x ananassa</i>	Day-neutral	USPP16228P3	Univ. of California, 2006
Sweet Charlie	<i>Fragaria x ananassa</i>	Short-day	USPP8729P	Univ. of Florida, 1994

Table 2. Common disease transmitting vectors affecting strawberry

Aphid transmitted	Leafhopper transmitted	Nematode transmitted	Fungus transmitted	Pollen transmitted	Vector unknown
Strawberry chlorotic fleck	Aster yellows MLO	Arabis mosaic virus*	Tobacco necrosis virus in <i>Fragaria vesca</i>	Strawberry pallidosis	Necrotic shock
Strawberry crinkle	Strawberry green petal*	Raspberry ringspot virus*			Strawberry leafroll
Strawberry vein banding	Strawberry lethal decline	Strawberry latent ringspot virus*			Strawberry feather-leaf
Strawberry pseudo mild yellow-edge	Strawberry mycoplasma yellows disease*	Tomato black ring virus*			
Strawberry mottle	Strawberry rickettsia yellows disease*	Tomato ringspot virus			
Strawberry mild yellow-edge	Strawberry witches'-broom				
Strawberry latent C virus in <i>Fragaria</i>					

Table is regenerated from the list showed on The American Phytopathological Society website, last edited in 1993 by Gubler and Converse.

<https://www.apsnet.org/publications/commonnames/Pages/Strawberry.aspx>

*Indicates the disease is not known in North America.

Table 3. Light intensity and environmental parameters measured inside the growth chamber

Average and standard deviations of the three repetitions (mean \pm standard deviation) for two growing cycles (round one 1-12 weeks and round two 13-21 weeks).

treatment	PPF*	Photoperiod	Ave. Temp.	VPD	RH	CO ₂	Irrigation		Drainage		Drainage
	$\mu\text{mol m}^{-2} \text{s}^{-1}$		h				$^{\circ}\text{C}$	kPa	%	$\mu\text{mol mol}^{-1}$	
First round (1-12 weeks)											
PPF 250	241 \pm 13	12	25.7 \pm 0.2	0.98 \pm 0.24	70.6 \pm 8.0	438 \pm 37	6.8 \pm 0.7	1.2 \pm 0.2	5.0 \pm 0.9	1.0 \pm 0.3	29 \pm 14
PPF 350	337 \pm 13		25.8 \pm 0.2								
PPF 450	443 \pm 17		25.7 \pm 0.2								
Second round (13-21 weeks)											
PPF 250	241 \pm 8	12	25.8 \pm 0.1	0.92 \pm 0.05	76.9 \pm 2.3	450 \pm 41	5.5 \pm 0.3	1.1 \pm 0.2	5.7 \pm 0.4	1.3 \pm 0.3	15 \pm 12
PPF 350	336 \pm 12		25.7 \pm 0.2								
PPF 450	440 \pm 15		25.7 \pm 0.2								

*Photosynthetic photon flux measured at the plant canopy every 3-weeks

Table 4. Light quality composition for the three light intensity treatments

Light intensity under fluorescent fixtures, the percent photon flux is presented for ultraviolet (UV), blue (B), green (G), red (R), and far-red (FR) on 100 nm increments.

Wavelength (nm)	300-400	400-500	500-600	600-700	700-800
Treatment	UV (%)	B (%)	G (%)	R (%)	FR (%)
PPF 250	0.68±0.07	17.05±0.16	40.78±0.24	36.10±0.23	5.37±0.23
PPF 350	0.54±0.04	17.98±0.38	41.02±0.23	35.65±0.35	4.80±0.19
PPF 450	0.33±0.10	18.77±0.36	41.59±0.20	35.21±0.45	4.09±0.25

Table 5. Environmental parameters and light intensity measured inside growth chambers.

Average and standard deviation of four repetitions (mean \pm standard deviation) for four treatments (two light qualities under two photoperiod).

treatment	PPF $\mu\text{mol m}^{-2} \text{s}^{-1}$	Photoperiod h	Day light integral $\text{mol m}^{-2} \text{d}^{-1}$	Ave. Temp. $^{\circ}\text{C}$	VPD kPa	RH %	CO ₂ $\mu\text{mol mol}^{-1}$	Irrigation pH	EC	Drainage pH	EC	Drainage %
8.5 HB	355 \pm 23	8.5	10.9 \pm 0.7	25.9 \pm 0.3	0.86	74 \pm 2	427 \pm 66	5.8	1.2	5.8	1.27	47.0 \pm 10%
8.5 LB	354 \pm 26		10.8 \pm 0.9	25.9 \pm 0.3								
12 HB	252 \pm 14	12	10.9 \pm 0.6	25.9 \pm 0.2	0.88	74 \pm 4	431 \pm 66	5.7	1.2	5.7	1.32	43.2 \pm 9%
12 LB	253 \pm 20		10.9 \pm 0.9	25.9 \pm 0.3								

Table 6. Light quality composition for 4 treatments

Light quality of high blue (HB) and low blue (LB) treatments under 12 hours (12) and 8.5 hours (8.5) photoperiods. The percent is presented for blue (B), green (G), red (R) on 100 nm increments.

Wavelength (nm) Treatment	PSS (P_{fr}/P_{total})	400-500 B (%)	500-600 G (%)	600-700 R (%)
8.5 HB	0.87	32.74±0.69	11.34±1.95	55.92±2.11
8.5 LB	0.88	11.39±1.51	3.86±1.97	84.70±3.37
12 HB	0.85	44.32±1.31	12.78±3.24	42.89±2.29
12 LB	0.88	10.30±1.35	3.81±1.35	85.89±2.26

Table 7. Effects of light quality and photoperiod on stolon and daughter plant production

Within-columns means followed by different letters (A, B, a, b) are significantly different according to mean separations (Tukey-Kramer HSD, $\alpha = 0.05$). Letters represent the impact of light quality. Within-rows means followed by (*) are significantly different according to Anova ($\alpha = 0.05$). (*) represents the impact of photoperiod.

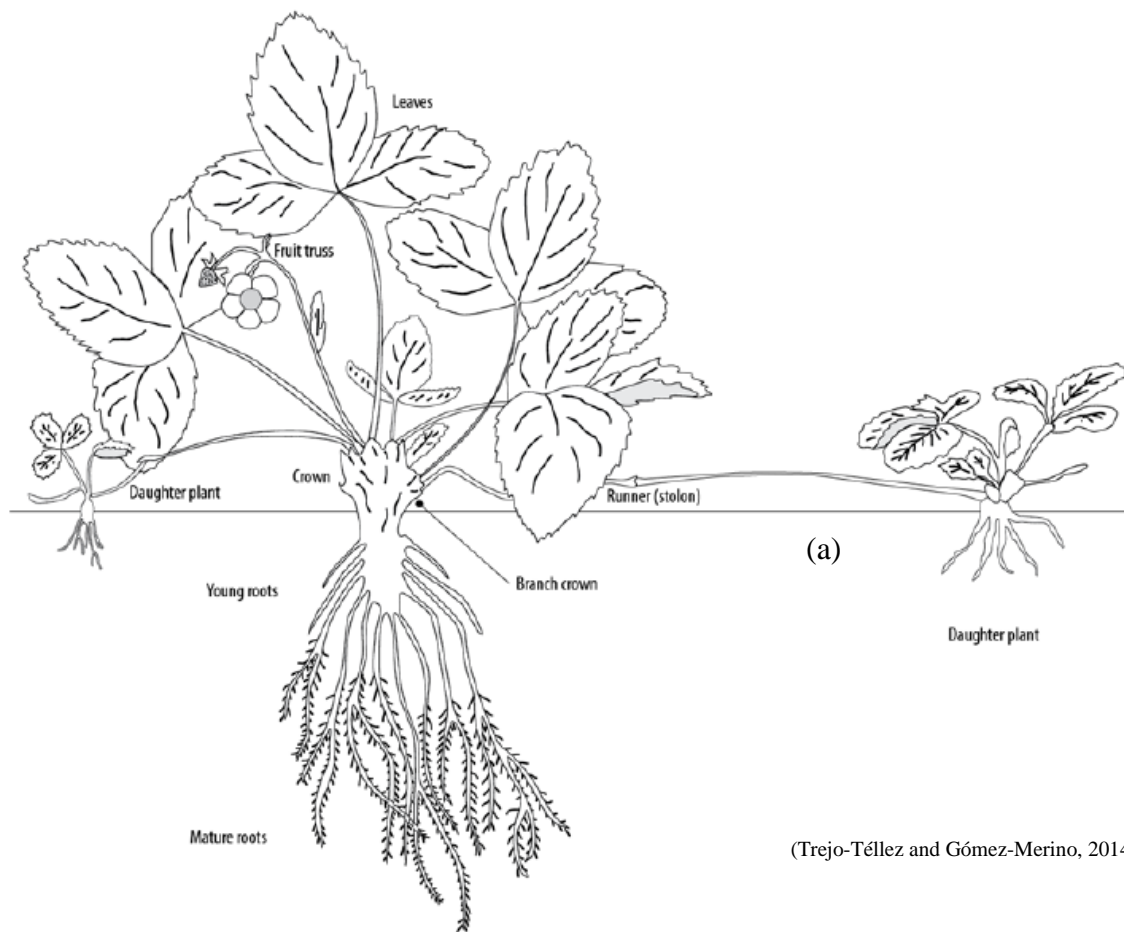
Light quality (LQ)	Photoperiod (PP)	
	8.5 hours (8.5)	12 hours (12)
Total number of daughter plants		
High blue (HB)	16.7±5.0 A	20.6±5.4 a *
Low blue (LB)	17.5±6.5 A	22.4±6.3 a *
<i>Significance</i>	<i>PP: P_(HB)=0.019, P_(LB)=0.006 LQ: P_(8.5)=0.484, P₍₁₂₎=0.301 PPxLQ: P=0.675</i>	
Number of functional daughter plants		
High blue (HB)	13.5±3.9 A	16.3±4.7 a *
Low blue (LB)	14.8±5.6 A	18.2±5.0 a *
<i>Significance</i>	<i>PP: P_(HB)=0.037, P_(LB)=0.010 LQ: P_(8.5)=0.270, P₍₁₂₎=0.134 PPxLQ: P=0.709</i>	
Number of stolons		
High blue (HB)	2.9±0.9 A	3.4±1.0 a
Low blue (LB)	3.0±0.8 A	3.5±0.9 a *
<i>Significance</i>	<i>PP: P_(HB)=0.078, P_(LB)=0.034 LQ: P_(8.5)=0.685, P₍₁₂₎=0.862 PPxLQ: P=1.000</i>	

Table 8. Effects of light quality and photoperiod on stock plant and stolon morphology and biomass.

Within-columns means followed by different letters (A, B, a, b) are significantly different according to mean separations (Tukey-Kramer HSD, $\alpha = 0.05$). Letters represent the impact of light quality. Within-rows means followed by (*) are significantly different according to mean separations (Tukey-Kramer HSD, $\alpha = 0.05$). (*) represents the impact of photoperiod.

Light quality (LQ)	Photoperiod (PP)	
	8.5 hours (8.5)	12 hours (12)
Total chlorophyll content (g m^{-2})		
High blue (HB)	0.395±0.04A	0.484±0.03a *
Low blue (LB)	0.381±0.06A	0.442±0.06a *
<i>Significance</i>	<i>PP: P_(HB)<0.001, P_(LB)=0.041 LQ: P_(8.5)=0.882, P₍₁₂₎=0.162 PPxLQ: P=0.393</i>	
Internode length (cm)		
High blue (HB)	40.5±1.7 A	41.6±2.4 a
Low blue (LB)	41.2±2.2 A	42.2±2.7 a
<i>Significance</i>	<i>PP: P_(HB)=0.090, P_(LB)=0.145 LQ: P_(8.5)=0.357, P₍₁₂₎=0.307 PPxLQ: P=0.947</i>	
Canopy fresh mass (g)		
High blue (HB)	19.3±6.7 A	17.7±4.5 b
Low blue (LB)	21.1±5.8 A	21.3±5.9 a
<i>Significance</i>	<i>PP: P_(HB)=0.383, P_(LB)=0.885 LQ: P_(8.5)=0.450, P₍₁₂₎=0.013 PPxLQ: P=0.423</i>	
Canopy dry mass (g)		
High blue (HB)	4.1±1.3 A	4.3±1.1 b
Low blue (LB)	4.5±1.3 A	5.0±1.5 a
<i>Significance</i>	<i>PP: P_(HB)=0.641, P_(LB)=0.251 LQ: P_(8.5)=0.367, P₍₁₂₎=0.025 PPxLQ: P=0.554</i>	
Crown diameter (mm)		
High blue (HB)	16.7±4.3 A	16.1±3.9 a
Low blue (LB)	17.1±4.6 A	17.5±5.1 a
<i>Significance</i>	<i>PP: P_(HB)=0.572, P_(LB)=0.692 LQ: P_(8.5)=0.720, P₍₁₂₎=0.266 PPxLQ: P=0.502</i>	
Leaf area (m^2)		
High blue (HB)	0.041±0.01A	0.040±0.01b
Low blue (LB)	0.044±0.01A	0.046±0.00a
<i>Significance</i>	<i>PP: P_(HB)=0.799, P_(LB)=0.391 LQ: P_(8.5)=0.419, P₍₁₂₎=0.004 PPxLQ: P=0.451</i>	
Leaf number		
High blue (HB)	8.0±2.5 A	8.1±1.5 b
Low blue (LB)	9.0±2.8 A	9.3±2.8 a
<i>Significance</i>	<i>PP: P_(HB)=0.745, P_(LB)=0.653 LQ: P_(8.5)=0.252, P₍₁₂₎=0.027 PPxLQ: P=0.800</i>	

FIGURES



(pictures from internet)

Figure 1. Strawberry morphology and strawberry propagation material examples

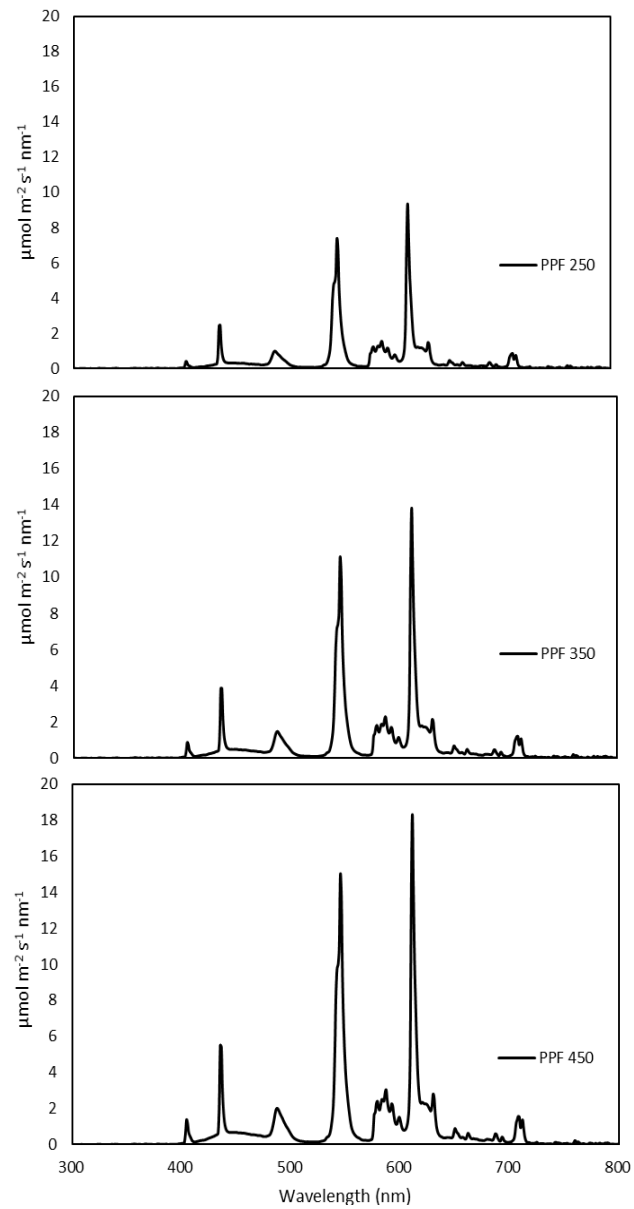


Figure 2. Spectral distribution for the three fluorescence light treatments of 250 (PPF 250), 350 (PPF 350) and 450 (PPF 450) ($\mu\text{mol m}^{-2} \text{s}^{-1} \text{nm}^{-1}$).



Figure 3. Daughter plant classifications based on crown diameter.

Extra-large (XL) crown diameter greater than 11mm; Large (L) crown diameter 8.5mm to 11mm Medium (M) crown diameter 6mm to 8.5mm; Small (S) crown diameter 3.5mm to 6mm; Extra small (XS) crown diameter smaller than 3.5mm; and Non-developed (ND) daughter plant primordia with no leaf.

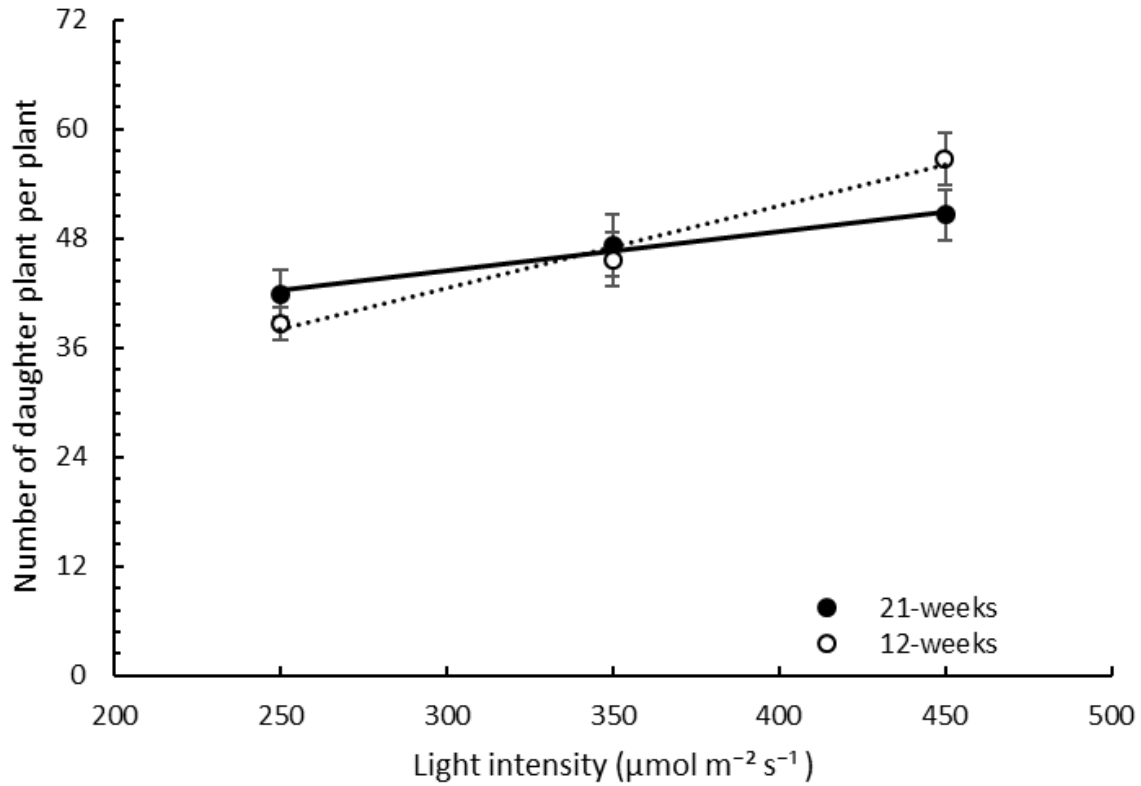


Figure 4. Effect of light intensity on the total number of daughter plants per stock plant of day-neutral strawberry 'Albion'.

Open circles (○) represent data collected when stock plant was 12-weeks old (round one) and filled circles (●) represent data collected when stock plant was 21-weeks old (round two). Dotted or solid lines represent significant linear regression ($P \leq 0.05$).

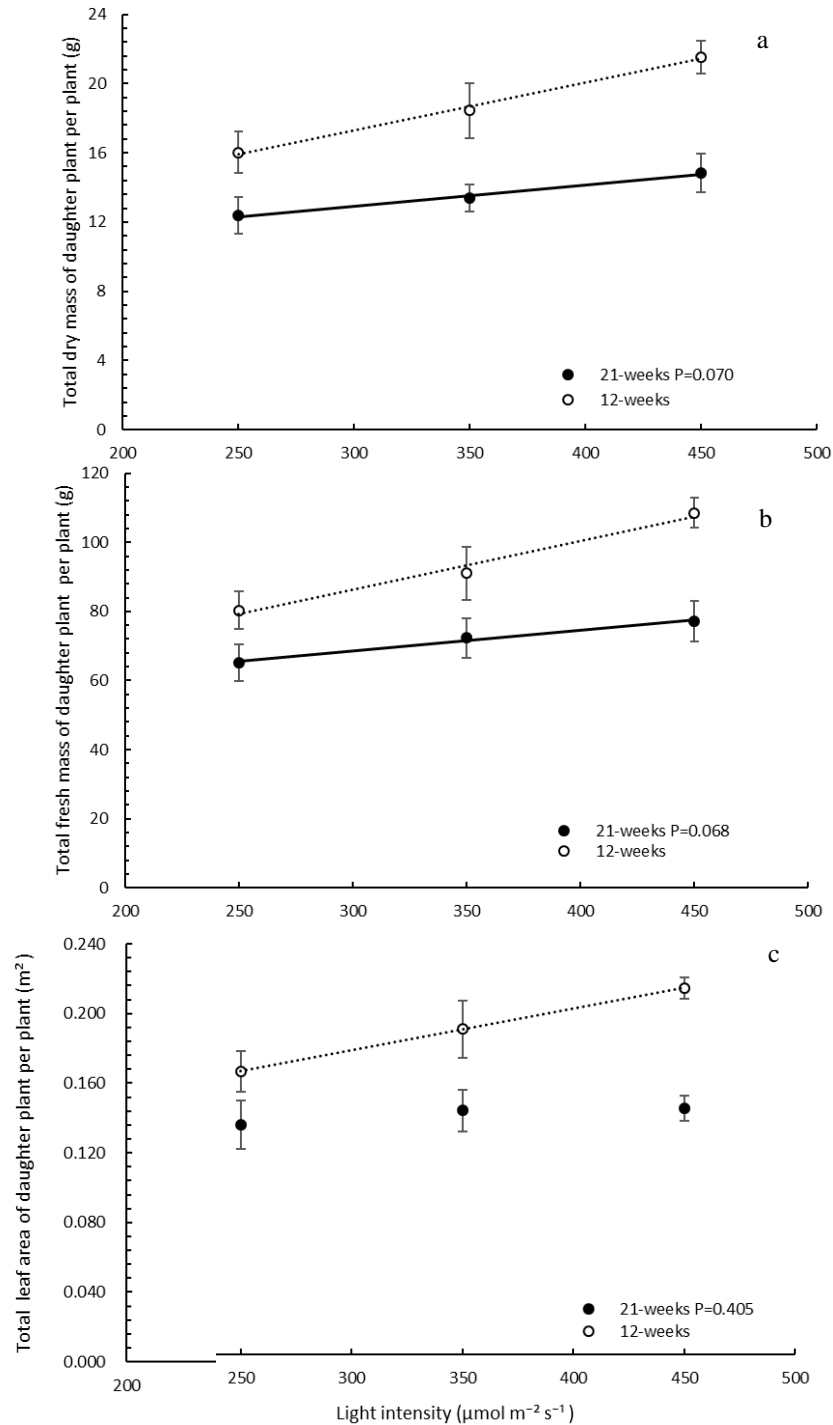


Figure 5. Effect of light intensity on the total dry mass (a), fresh mass (b) and leaf area (c) of all daughter plants per stock plant of day-neutral strawberry 'Albion'.

Open circles (\circ) represent data collected when stock plant was 12-weeks old (round one) and filled circles (\bullet) represent data collected when stock plant was 21-weeks old (round two). Dotted or solid line represents significant linear regression ($P \leq 0.05$) and respective P-value is shown next to the legend.

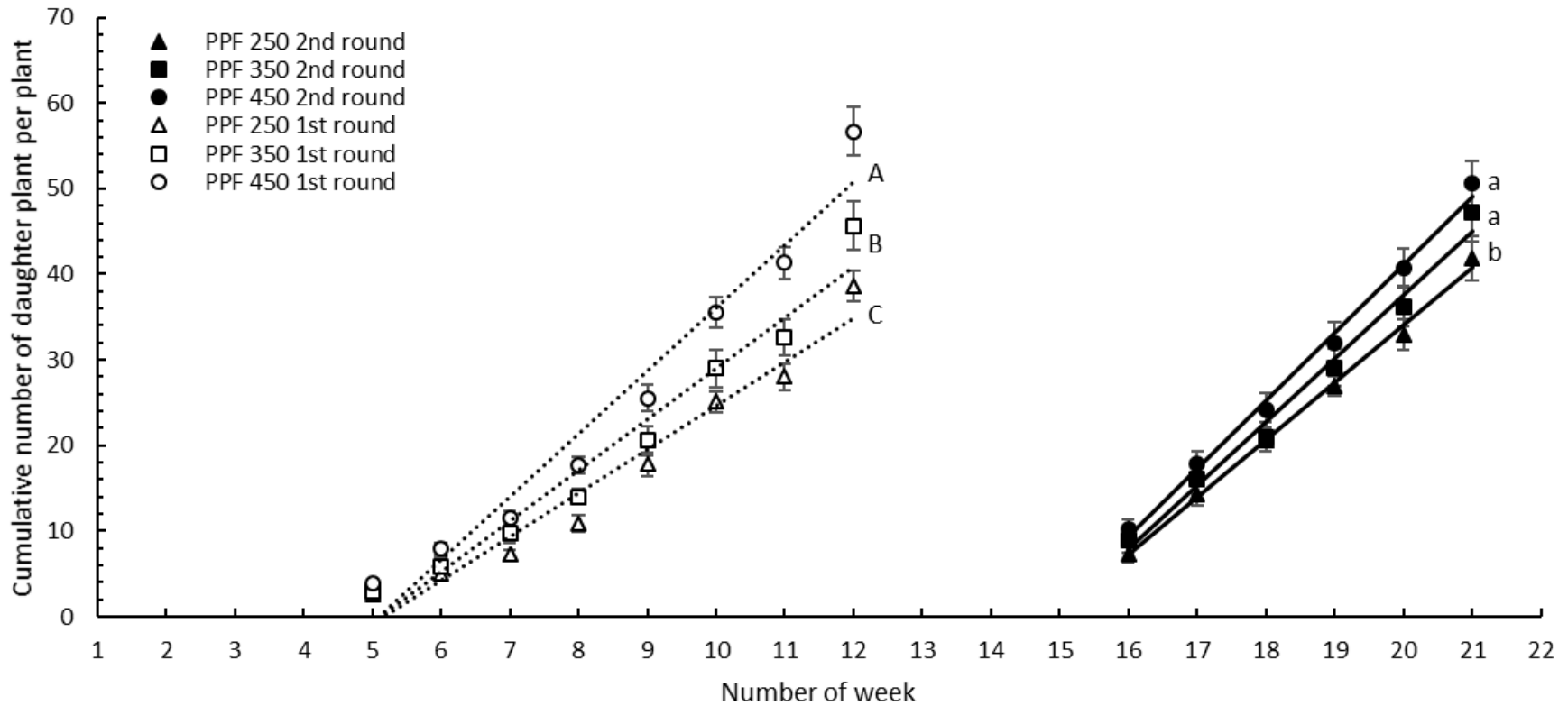


Figure 6. Cumulative number of daughter plants per stock plant of day-neutral strawberry 'Albion'

Open markers (Δ , \square , \circ) represent data collected from start of experiment to week 12 (round one), and filled markers (\blacktriangle , \blacksquare , \bullet) represent data collected from week 13 to week 21 (round two). Triangles (\blacktriangle , Δ), squares (\blacksquare , \square ,) and circles (\bullet , \circ) represent plants grown under 250, 350, and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, respectively. Dotted or solid line represents significant linear regression $P \leq 0.05$. Letters (A, B, C and a, b) represent mean separation using multiple comparisons $P \leq 0.05$.

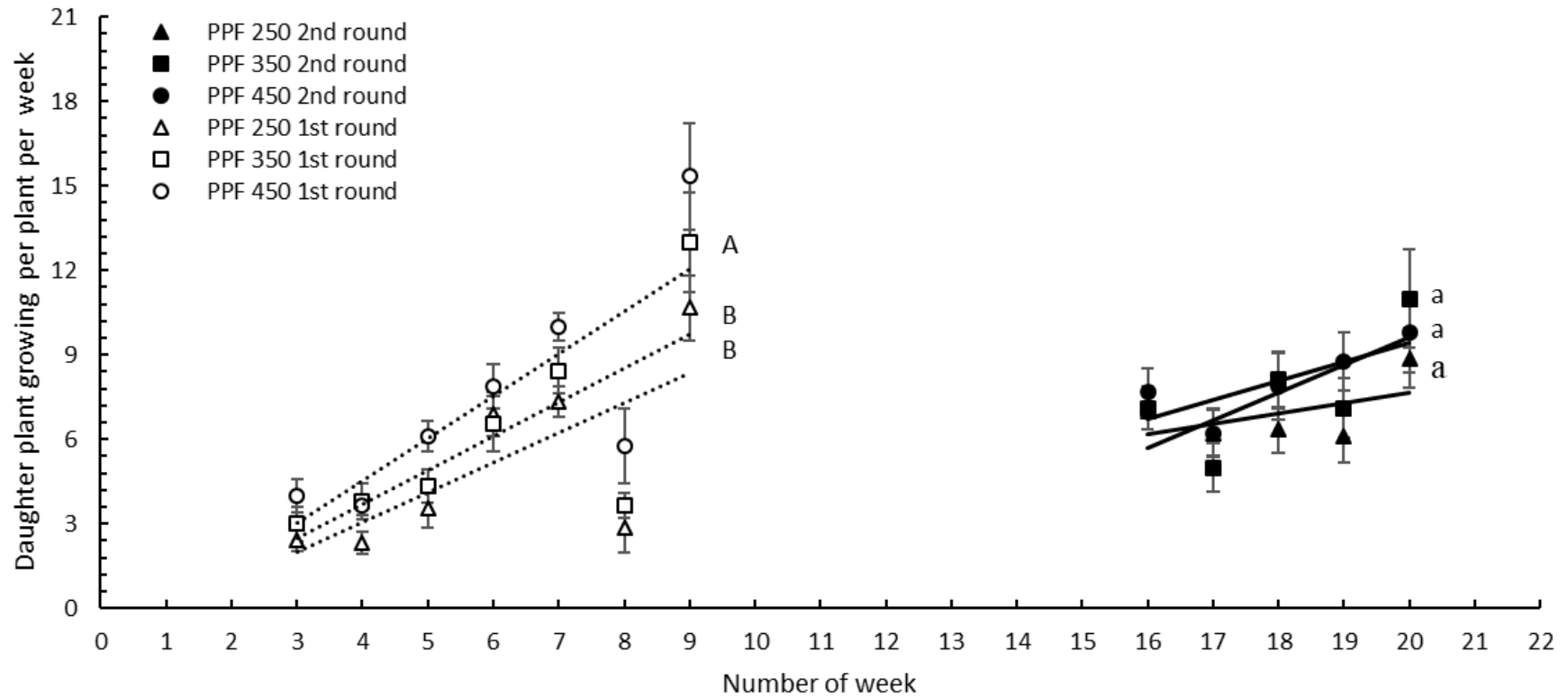


Figure 7. Number of daughter plants per week per stock plant of day-neutral strawberry 'Albion'.

Open markers (Δ , \square , \circ) represent data collected from start of experiment to week 12 (round one), and filled markers (\blacktriangle , \blacksquare , \bullet) represent data collected from week 13 to week 21 (round two). Triangles (\blacktriangle , Δ), squares (\blacksquare , \square) and circles (\bullet , \circ) represent plants grown under 250, 350, and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, respectively. Dotted or solid line represents significant linear regression $P \leq 0.05$. Letters (A, B, C and a, b, c) represent mean separation using multiple comparisons $P \leq 0.05$.

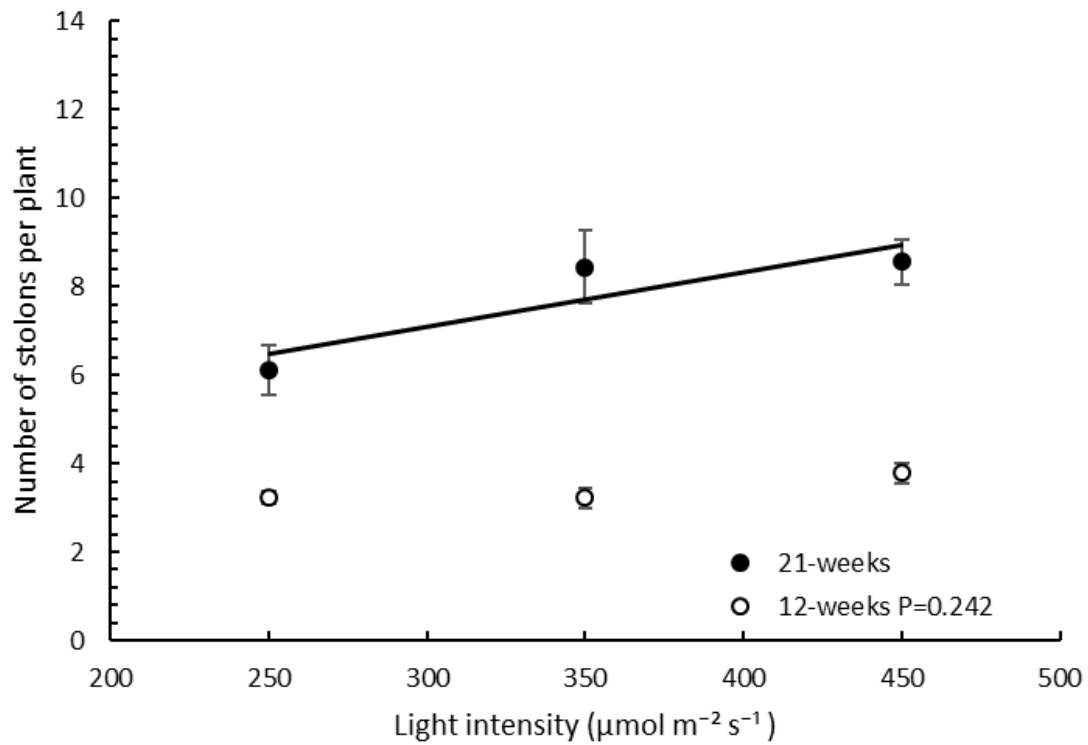


Figure 8. Effect of light intensity on stock plant stolon number of day-neutral strawberry 'Albion'

Open circles (○) represent data collected when stock plant was 12-weeks old (round one) and filled circles (●) represent data collected when stock plant was 21-weeks old (round two). Dotted or solid lines represent significant linear regression ($P \leq 0.05$) and respective P-value is shown next to the legend.

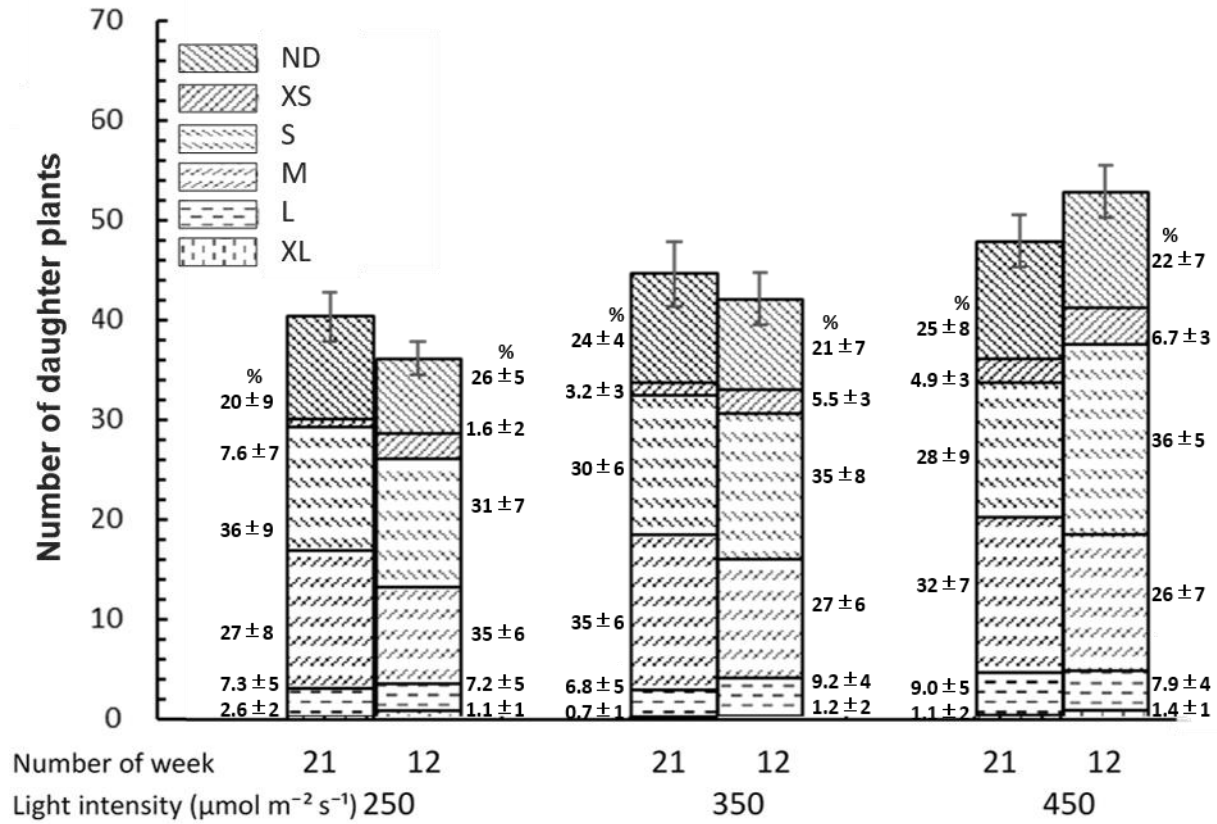


Figure 9. Total number of daughter plants per light intensity in each round of treatments.

Round one: 12 weeks, round two: 21 weeks. Each column is divided to represent the different daughter plant sizes. The number next to each column is the percentage of each category out of total number of daughter plants.

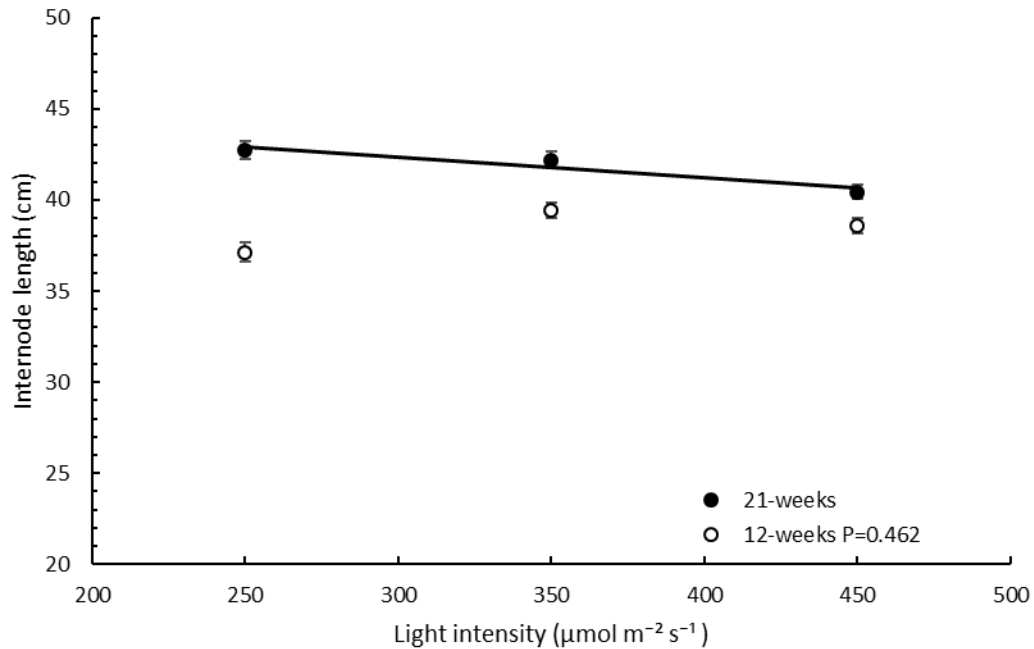


Figure 10. Effect of light intensity on the internode length of day-neutral strawberry 'Albion'.

Y axis is distance by centimeter. X axis is light intensity by micro moles per meter square per second, representing three different treatments. The distance between two functional daughter plants were measured. Open circles (\circ) represent data collected when stock plant was 12-weeks old (round one) and filled circles (\bullet) represent data collected when stock plant was 21-weeks old (round two). Dotted or solid line represents significant linear regression ($P \leq 0.05$) and respective P-value is shown next to the legend.

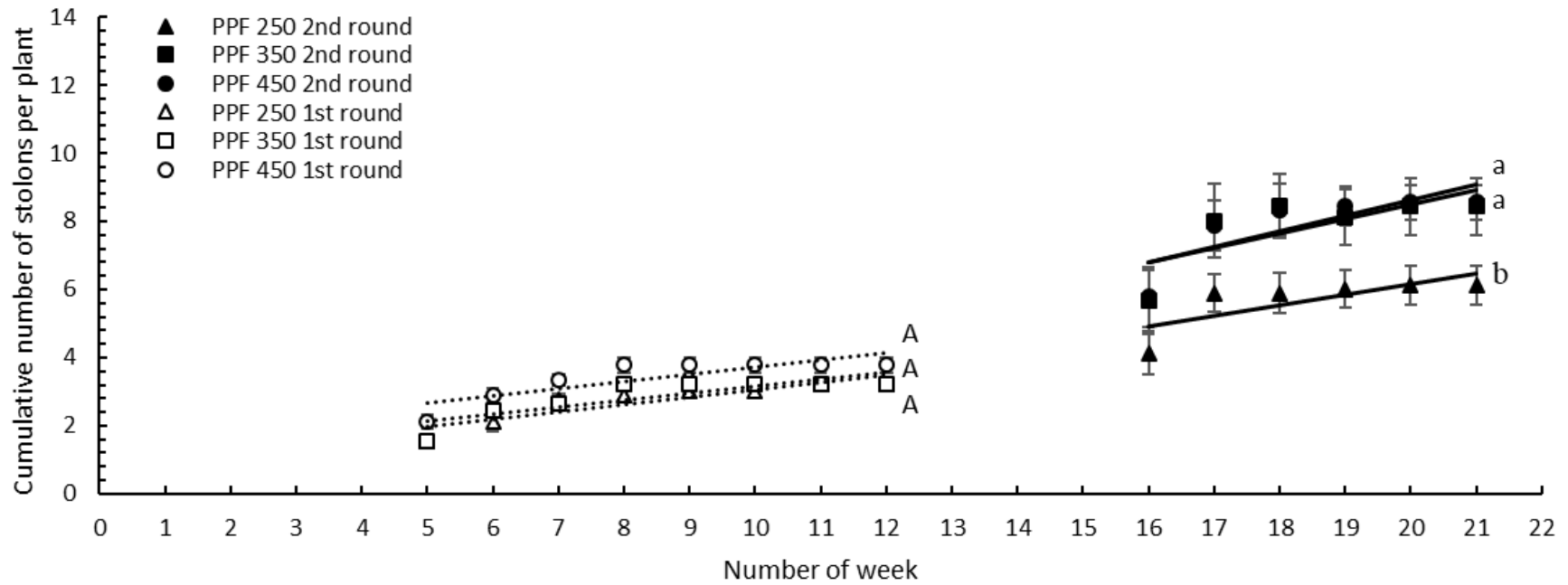


Figure 11. Cumulative number of runners per stock plant of day-neutral strawberry 'Albion'.

Open markers (Δ , \square , \circ) represent data collected from start of experiment to week 12 (round one), and filled markers (\blacktriangle , \blacksquare , \bullet) represent data collected from week 13 to week 21 (round two). Triangles (\blacktriangle , Δ), squares (\blacksquare , \square) and circles (\bullet , \circ) represent plants grown under 250, 350, and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, respectively. Dotted or solid line represents significant linear regression $P \leq 0.05$. Letters (A, B, C and a, b, c) represent mean separation using multiple comparisons $P \leq 0.05$.

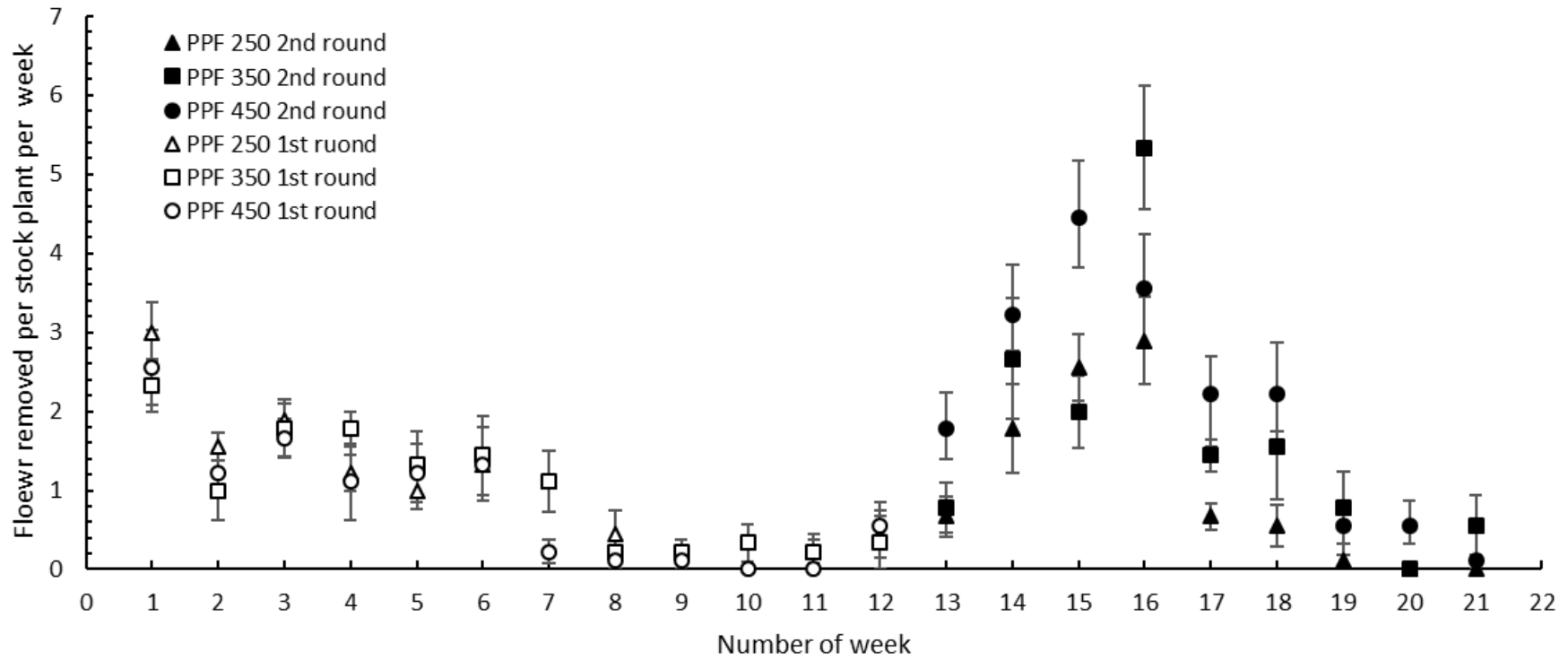


Figure 12. Flower removed per stock plant per week of day-neutral strawberry 'Albion'.

Open markers (Δ , \square , \circ) represent data collected from start of experiment to week 12 (round one), and filled markers (\blacktriangle , \blacksquare , \bullet) represent data collected from week 13 to week 21 (round two). Triangles (\blacktriangle , Δ), squares (\blacksquare , \square) and circles (\bullet , \circ) represent plants grown under 250, 350, and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, respectively. Dotted or solid line represents significant linear regression $P \leq 0.05$. Letters (A, B, C and a, b, c) represent mean separation using multiple comparisons $P \leq 0.05$.

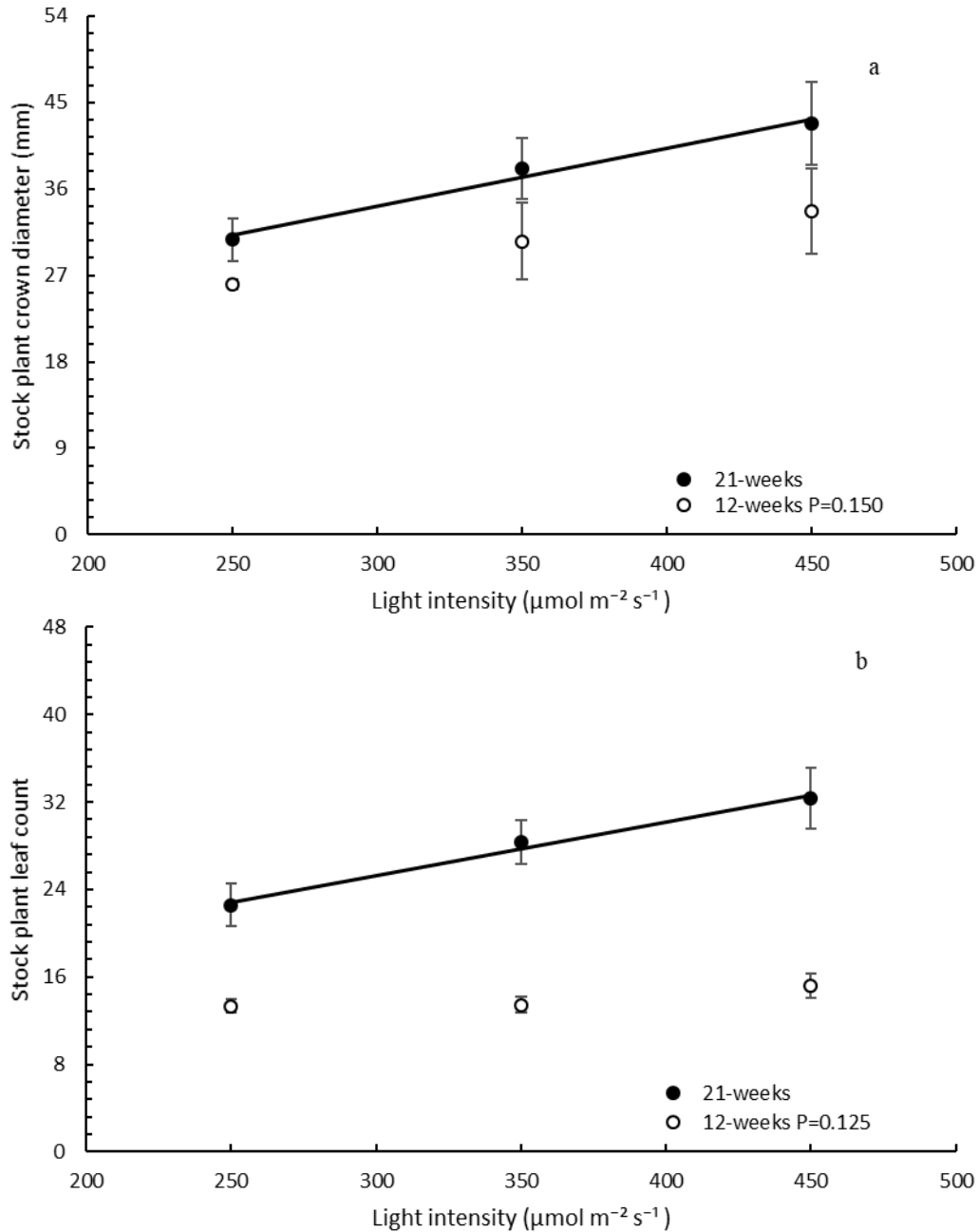


Figure 13. Effect of light intensity on stock plant crown diameter (a) and leaf count (b) of day-neutral strawberry 'Albion'.

Open circles (\circ) represent data collected when stock plant was 12-weeks old (round one) and filled circles (\bullet) represent data collected when stock plant was 21-weeks old (round two). Dotted or solid lines represent significant linear regression ($P \leq 0.05$) and respective P-value is shown next to the legend.

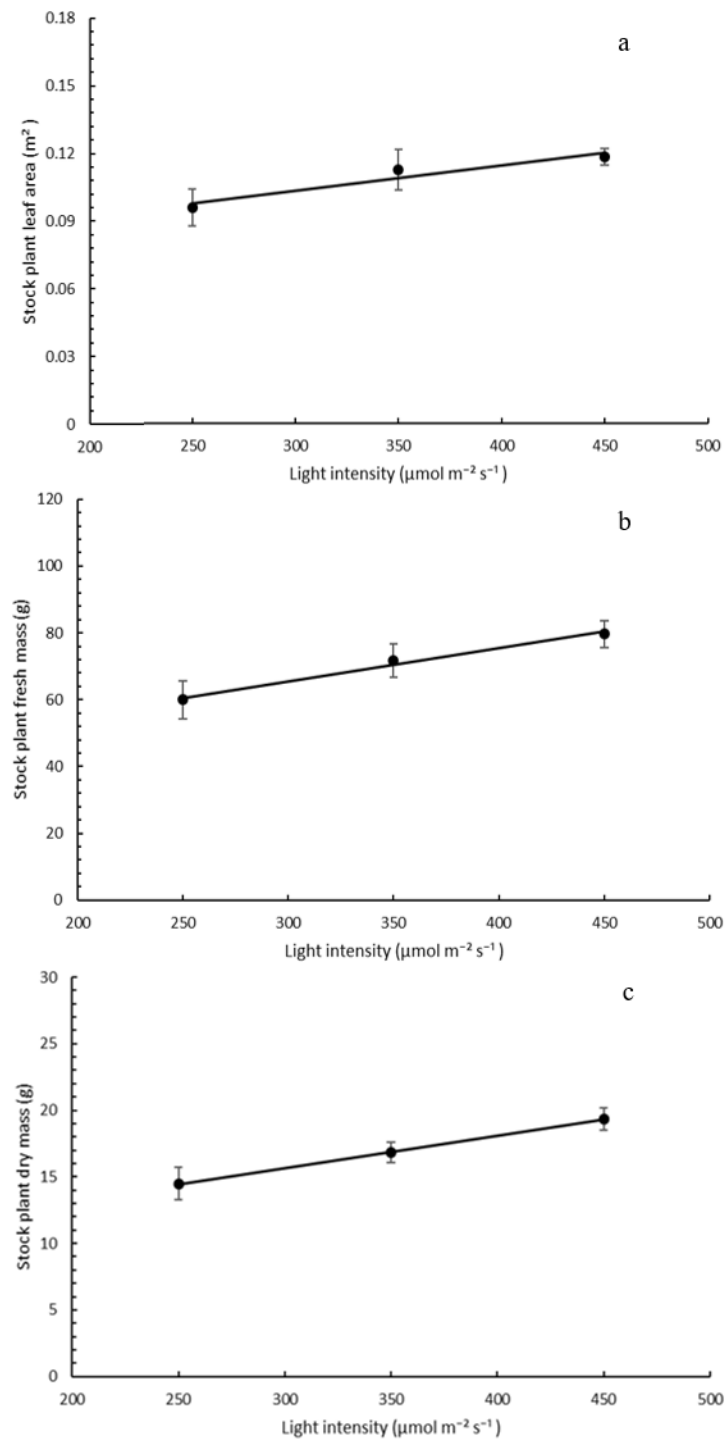


Figure 14. Effect of light intensity on the total dry mass (a), fresh mass (b) and leaf area (c) of stock plants of day-neutral strawberry ‘Albion’.

Data collected on week 21 (round 2). Solid line represents significant linear regression ($P \leq 0.05$) and respective P-value is shown in each graph.

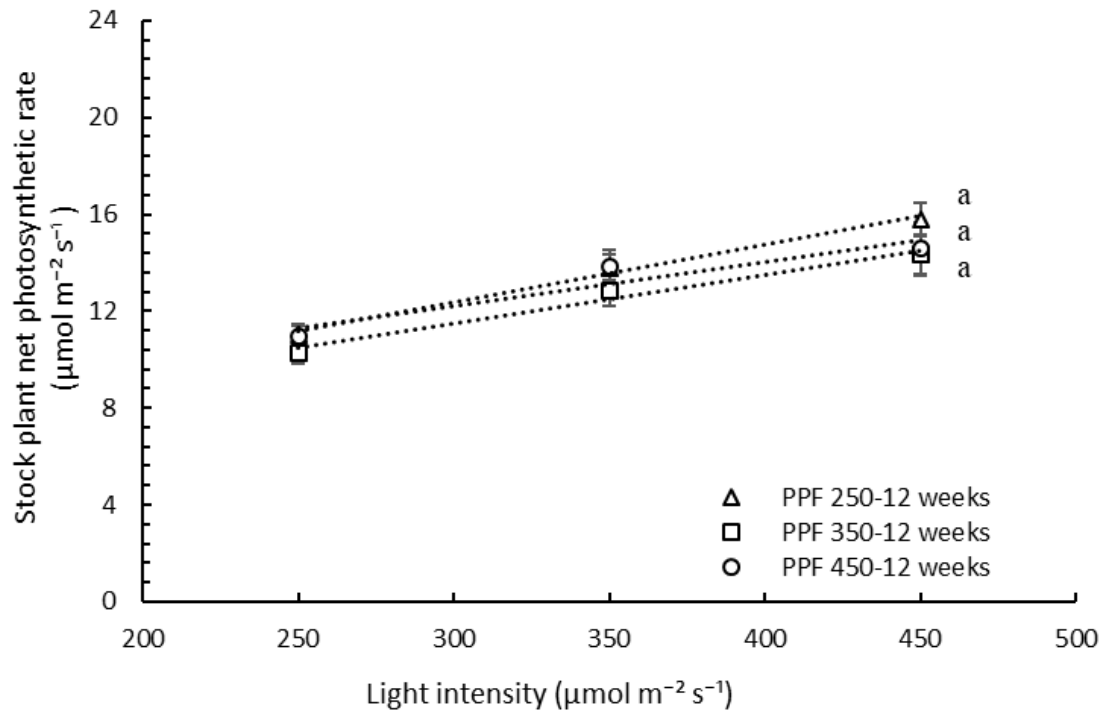


Figure 15. Effect of light intensity on net photosynthetic rate of stock plants of day-neutral strawberry 'Albion'.

Data collected on week 12 (round 1). Triangles (Δ), squares (\square) and circles (\circ) represent plants grown under 250, 350, and 450 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity, respectively. Dotted line represents significant linear regression ($P \leq 0.05$). Letters (A, B, C) represent mean separation using multiple comparisons $P \leq 0.05$

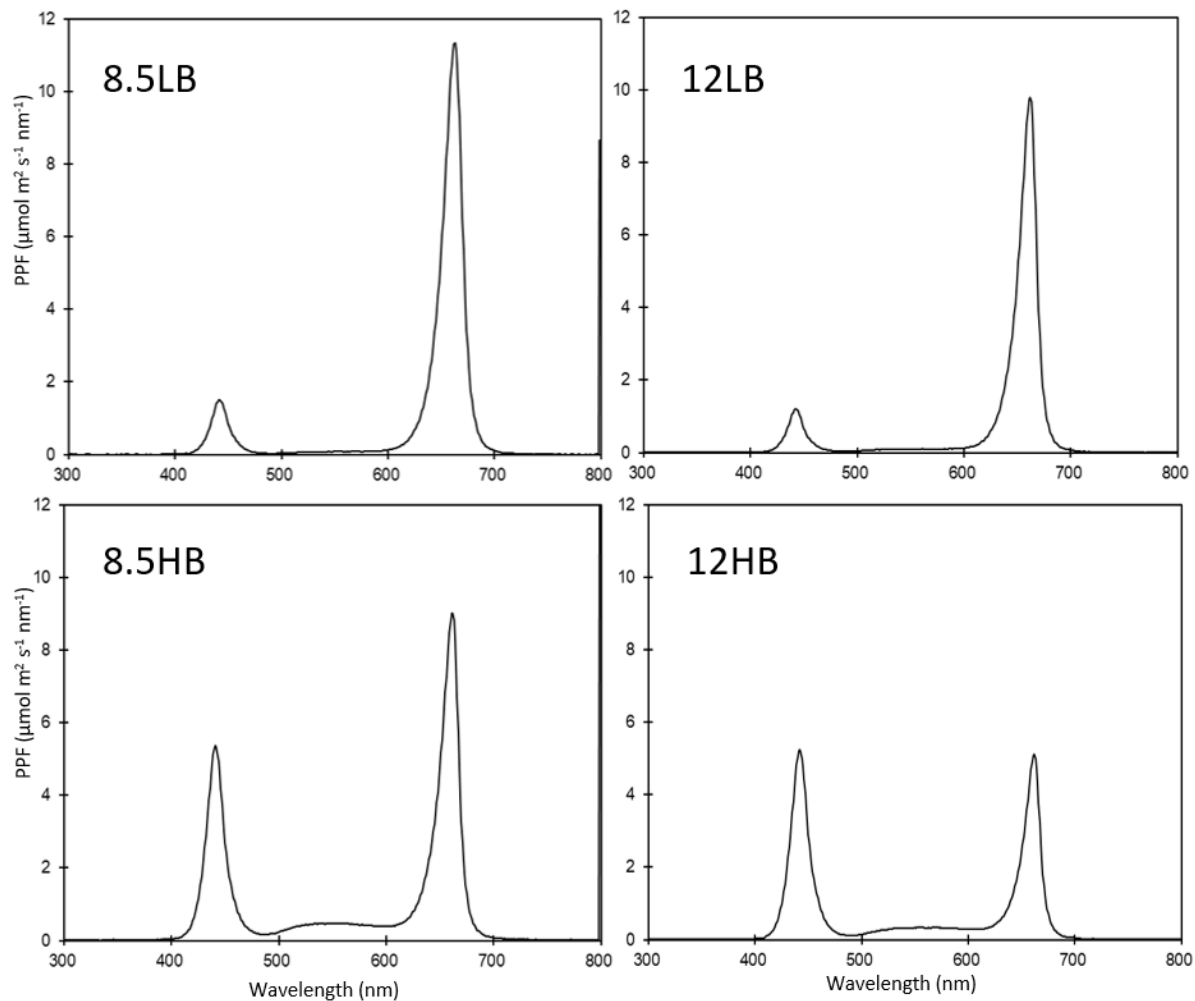


Figure 16. Spectral distribution for the four light treatments and two photoperiods.

12 and 8.5 represent the photoperiod in hours. HB represents high blue light treatment and LB represents the low blue light treatment. Spectra were measured using a spectroradiometer at the beginning and end of each repetition averaged at center of the pot at plant canopy height.



Figure 17. Daughter plant classifications based on crown diameter

Extra-large (XL) crown diameter greater than 11mm; Large (L) crown diameter 8.5mm to 11mm; Medium (M) crown diameter 6mm to 8.5mm; Small (S) crown diameter 3.5mm to 6mm; Extra small (XS) crown diameter smaller than 3.5mm; and Non-developed (ND) daughter plant primordia with no leaf.

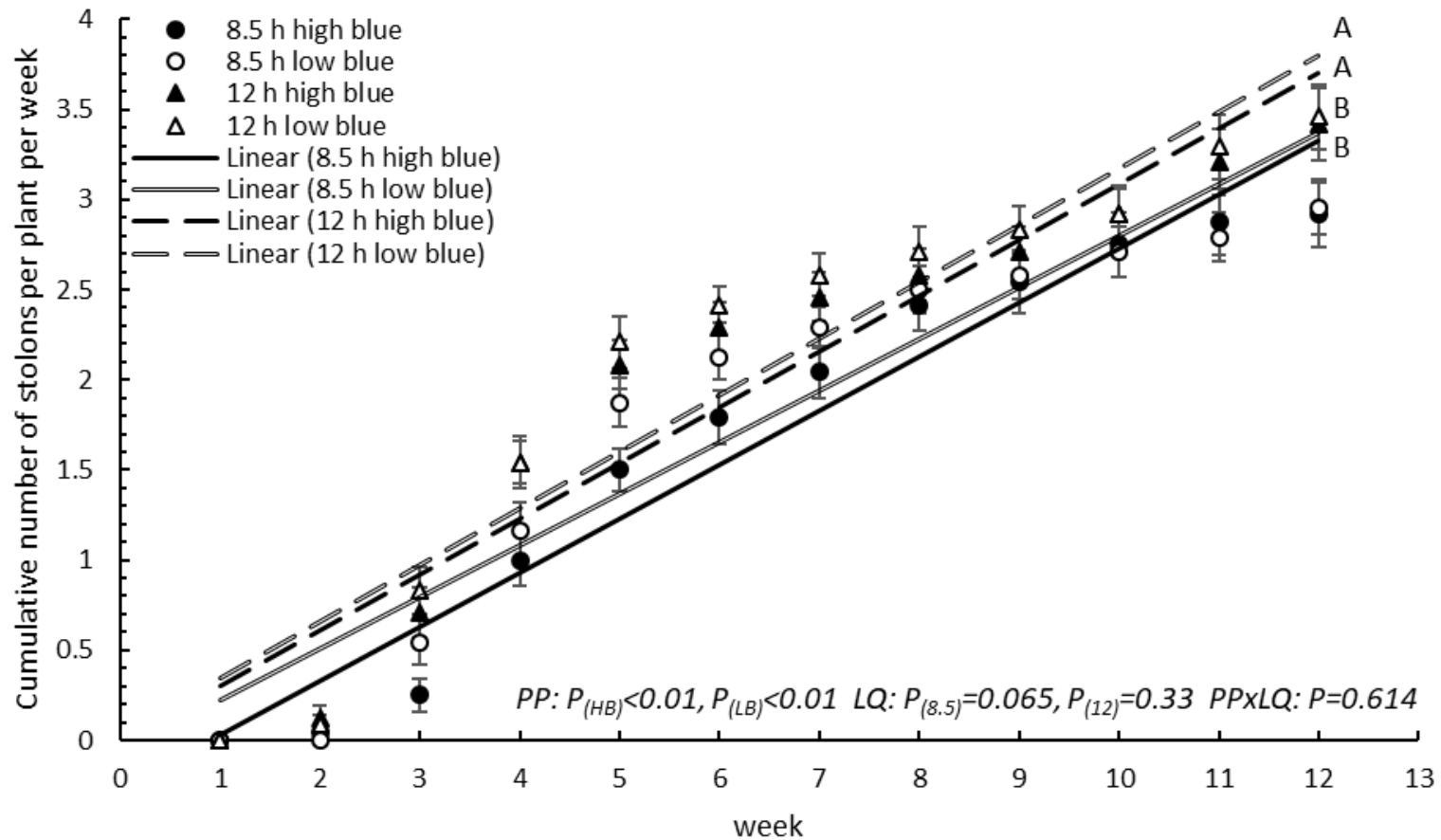


Figure 18. Cumulative number of stolons per stock plant of day-neutral strawberry 'Albion'

Solid markers (▲, ●) represent data collected from plants under high blue treatment, open markers (△, ○) represent data collected from plants under low blue treatment. Dotted and solid lines represent significant linear regression ($P \leq 0.05$) of the data collected from the plants treated under 12 hours photoperiod and 8.5 hours photoperiod, respectively. Letters (A, B) represent mean separation using multiple comparisons $P \leq 0.05$. PP and LQ mean treatment influence of photoperiod and light quality, respectively. The p-values of mean separation using ANCOVA are showing above.

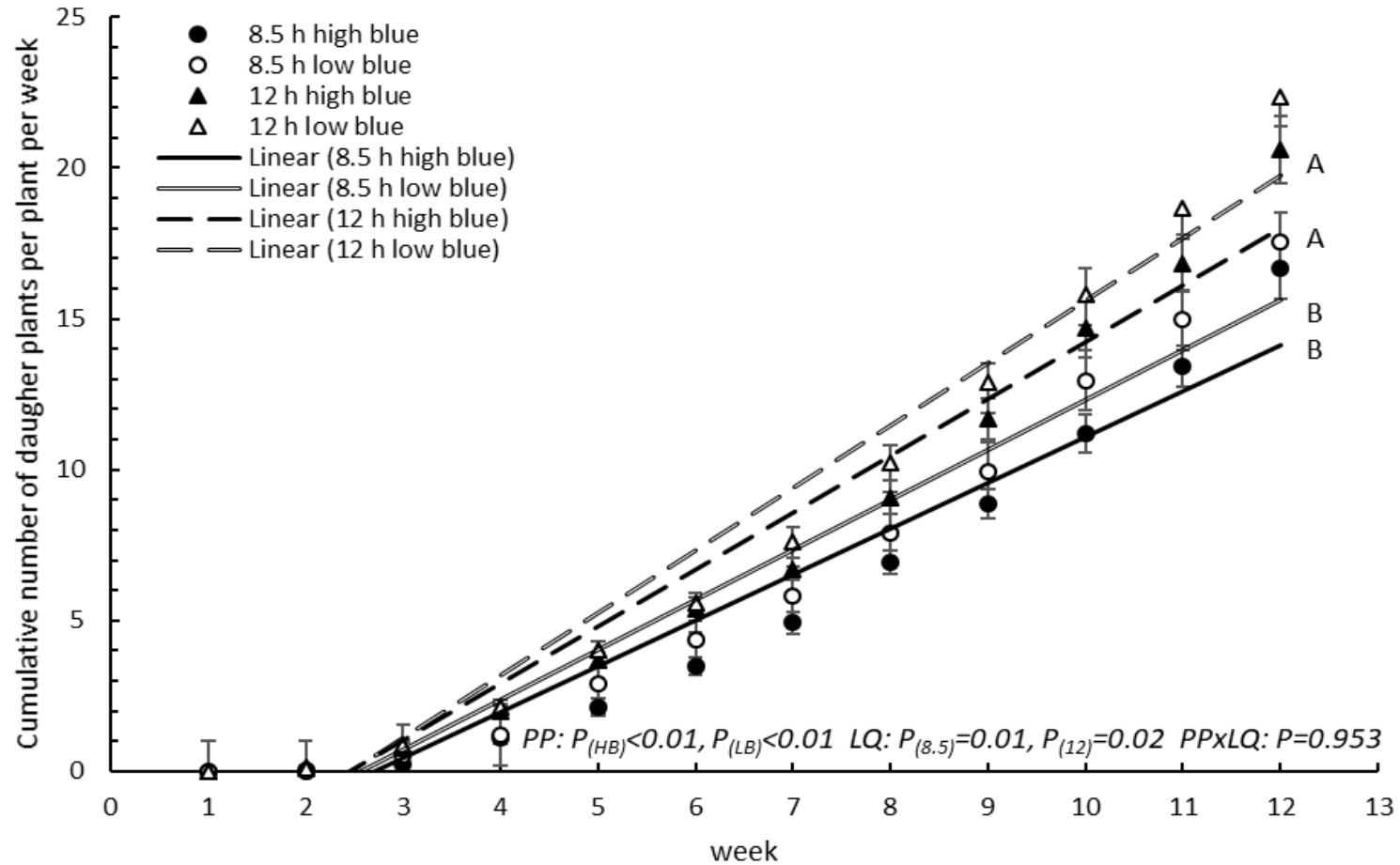


Figure 19. Cumulative number of daughter plants per stock plant of day-neutral strawberry 'Albion'.

Solid markers (▲, ●) represent data collected from plants under high blue treatment, open markers (△, ○) represent data collected from plants under low blue treatment. Dotted and solid lines represent significant linear regression ($P \leq 0.05$) of the data collected from the plants treated under 12 hours photoperiod and 8.5 hours photoperiod, respectively. Letters (A, B) represent mean separation using multiple comparison comparisons $P \leq 0.05$. PP and LQ mean treatment influence of photoperiod and light quality, respectively. The p-values of mean separation using ANCOVA are showing above.

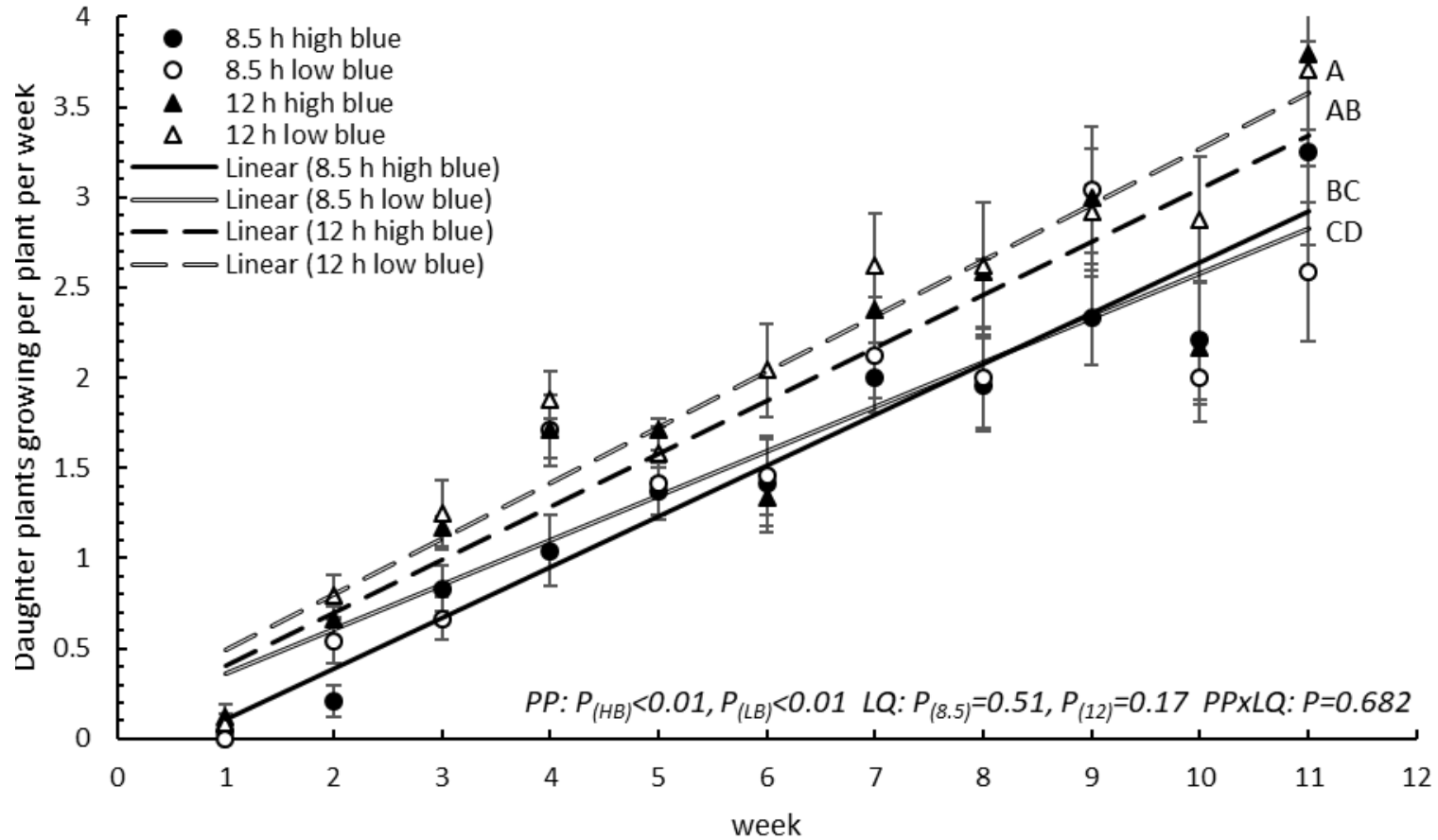


Figure 20. Number of daughter plants per stock plant per week of day-neutral strawberry 'Albion'.

Solid markers (▲, ●) represent data collected from plants under high blue treatment, open markers (△, ○) represent data collected from plants under low blue treatment. Dotted and solid lines represent significant linear regression ($P \leq 0.05$) of the data collected from the plants treated under 12 hours photoperiod and 8.5 hours photoperiod, respectively. Letters (A, B, C and D) represent mean separation using multiple comparisons $P \leq 0.05$. PP and LQ mean treatment influence of photoperiod and light quality, respectively. The p-values of mean separation using ANCOVA are showing above.

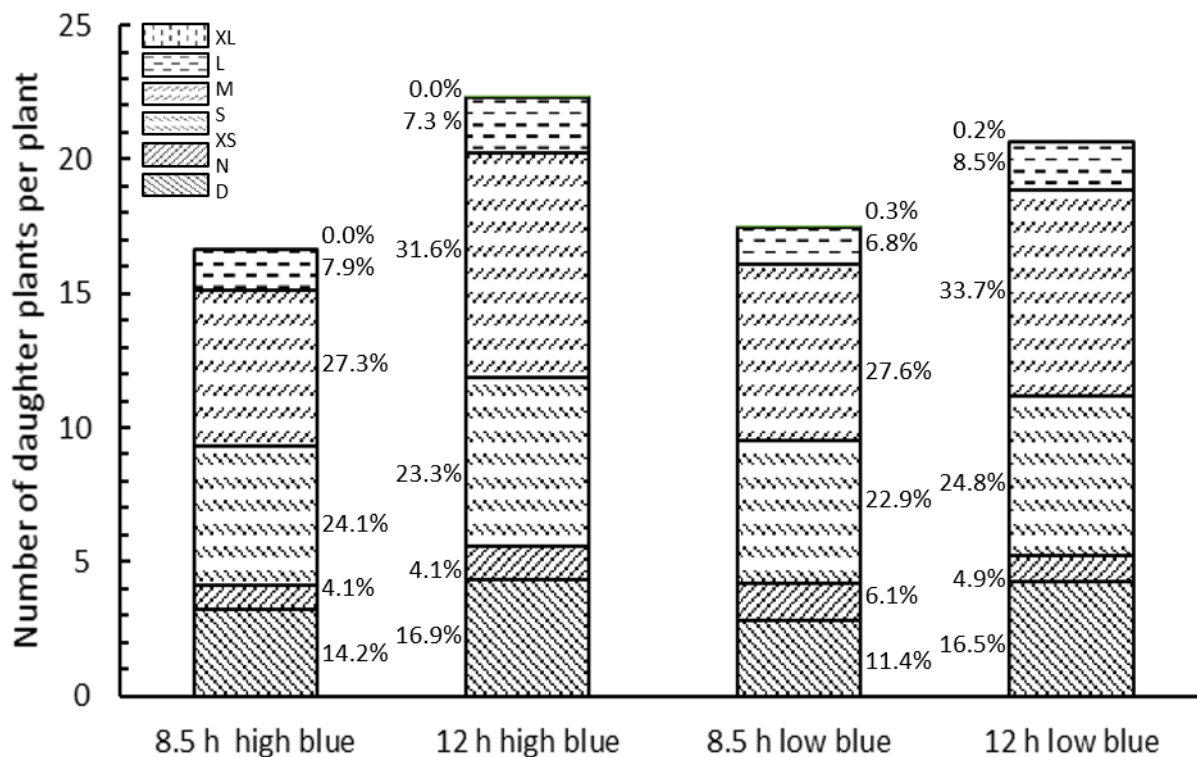


Figure 21. Total number of daughter plants per light quality and photoperiod.

Each column is divided to represent the different daughter plant sizes (see Figure 17). The number next to each column is the percentage of each category out of total number of daughter plants.

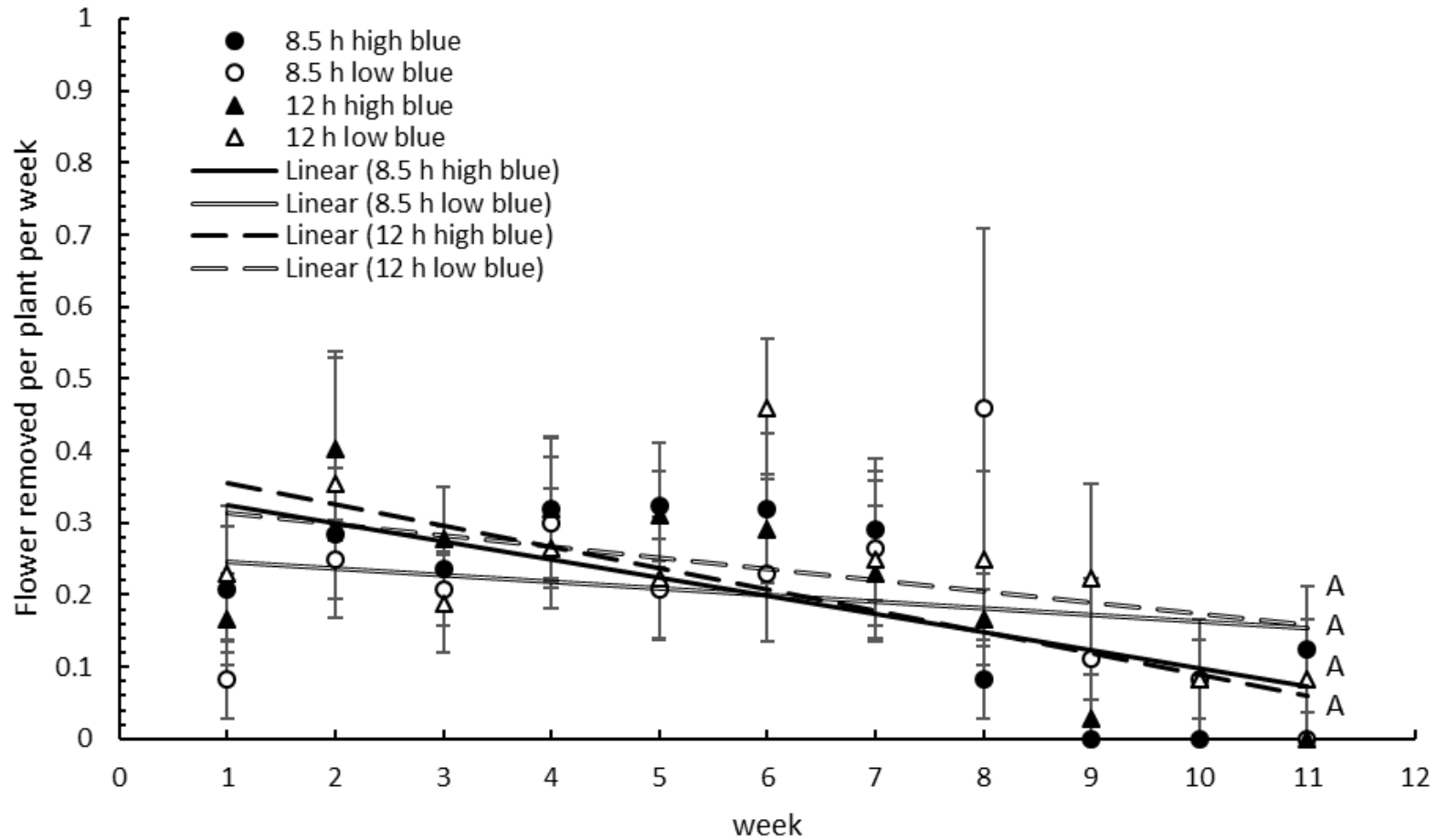


Figure 22. Number of flowers removed per stock plant per week of day-neutral strawberry 'Albion'

Solid markers (▲, ●) represent data collected from plants under high blue treatment, open markers (△, ○) represent data collected from plants under low blue treatment. Dotted and solid lines represent significant linear regression ($P \leq 0.05$) of the data collected from the plants treated under 12 hours photoperiod and 8.5 hours photoperiod, respectively. Letters (A) represent mean separation using multiple comparison comparisons $P \leq 0.05$.

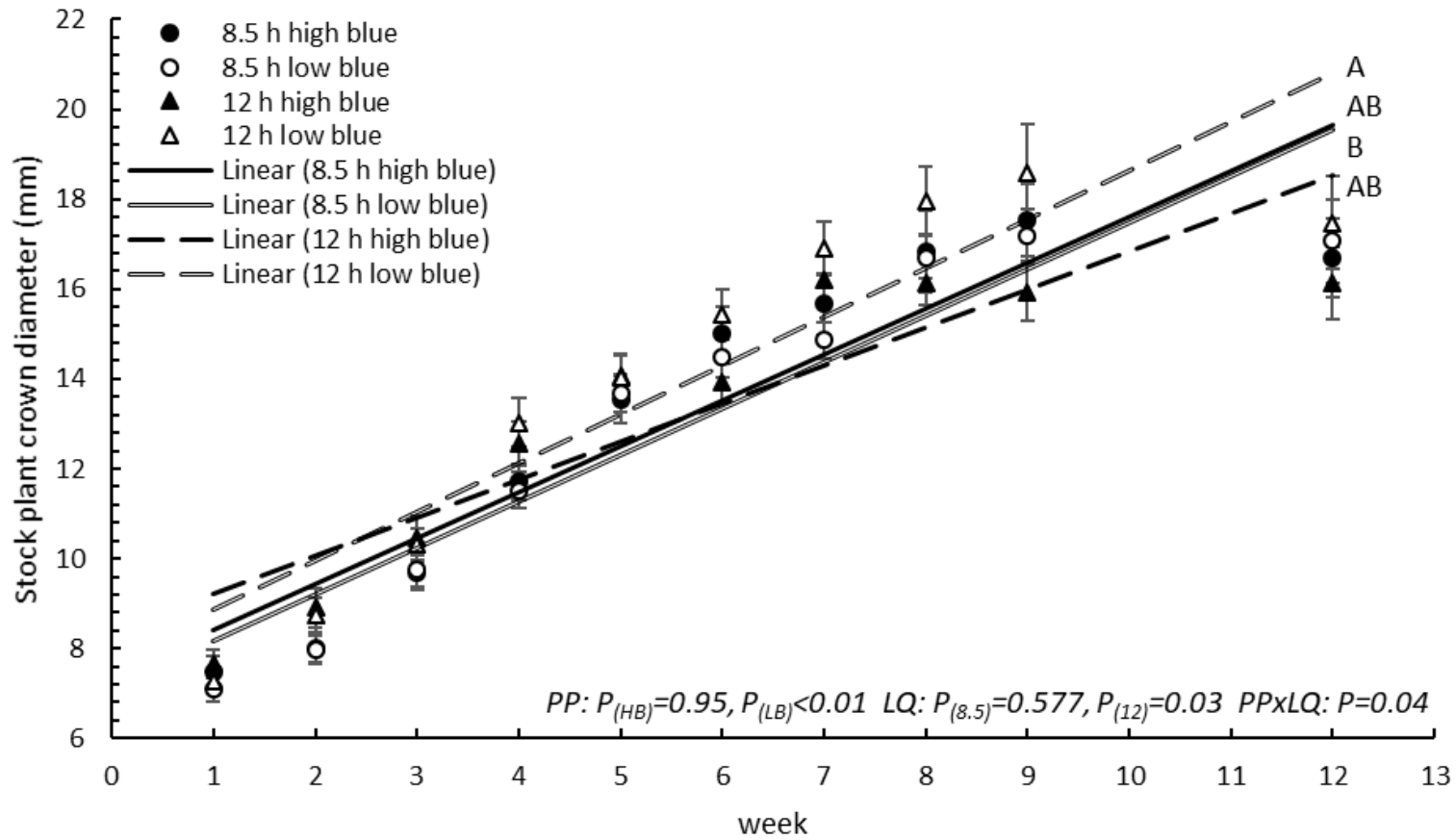


Figure 23. Stock plant crown diameter per week of day-neutral strawberry 'Albion'

Solid markers (\blacktriangle , \bullet) represent data collected from plants under high blue treatment, open markers (\triangle , \circ) represent data collected from plants under low blue treatment. Dotted and solid lines represent significant linear regression ($P \leq 0.05$) of the data collected from the plants treated under 12 hours photoperiod and 8.5 hours photoperiod, respectively. Letters (A, B) represent mean separation using multiple comparisons $P \leq 0.05$. PP and LQ mean treatment influence of photoperiod and light quality, respectively. The p-values of mean separation using ANCOVA are showing above.

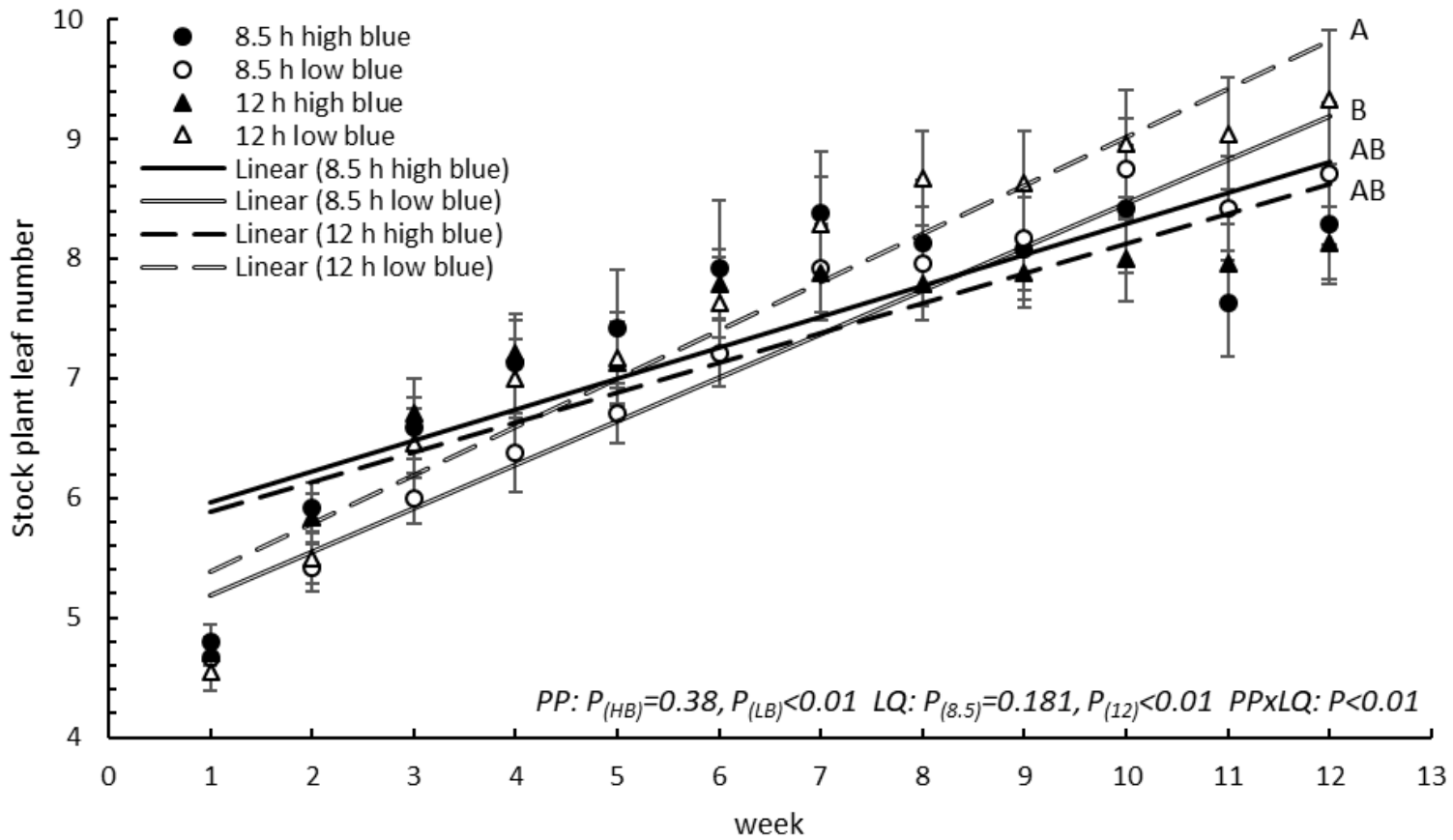


Figure 24. Stock plant leaf number per week of day-neutral strawberry ‘Albion’

Solid markers (▲, ●) represent data collected from plants under high blue treatment, open markers (△, ○) represent data collected from plants under low blue treatment. Dotted and solid lines represent significant linear regression ($P \leq 0.05$) of the data collected from the plants treated under 12 hours photoperiod and 8.5 hours photoperiod, respectively. Letters (A, B) represent mean separation using multiple comparisons $P \leq 0.05$. PP and LQ mean treatment influence of photoperiod and light quality, respectively. The p-values of mean separation using ANCOVA are showing above.