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"Evaluation of genotypes for sustainable resource use in strawberry cultivation"

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...Alla mia famiglia

SINTESI IN ITALIANO

Le caratteristiche qualitative della fragola e le performance produttive sono influenzate da molteplici aspetti tra cui genotipo, ambiente e tecniche di coltivazione. Analizzare i comportamenti della fragola in relazione alle dinamiche di tali fattori è di fondamentale importanza nell'ambito produttivo ed economico del settore della fragolicoltura.

La ricerca nasce per acquisire e comprendere la risposta delle piante di fragola alla riduzione di input (idrici e nutrizionali) e al cambiamento climatico; attraverso l'analisi dei parametri vegetativi, produttivi e qualitativi di differenti genotipi, tecniche e aree di coltivazione.

Tre varietà di Single-cropping e tre di Remontant sono state oggetto di studio in un'azienda agricola sperimentale del centro Italia. Mentre nove selezioni provenienti dal breeding del gruppo di ricerca D3A e una varietà spagnola sono state monitorate presso un'azienda agricola privata del Sud della Spagna. Lo studio ha rilevato che una riduzione di percentuale idrica e azotata può essere applicata, senza influenzare negativamente la quantità e qualità del frutto.

La tipologia di pianta, il genotipo, il periodo di coltivazione e l'ambiente incidono sulle reazioni della pianta ai differenti quantitativi d'acqua somministrati. A riguardo le Remontant mostrano una maggiore sensibilità rispetto alla Single cropping. Nel sito della Spagna si è potuto constatare che la risorsa idrica applicata in eccesso o in deficit può causare una diminuzione delle performance sia vegetative che produttive, con conseguente perdita economica. Considerando la prova dell'azoto l'influenza del genotipo si manifesta particolarmente sulle Remontant, non mostrando grandi variazioni dettate dalla riduzione.

Le variazioni di temperatura invernali indotte hanno determinato differenti risposte per i parametri oggetto di studio. Uno dei parametri maggiormente influenzati è la precocità di raccolta sia per le Remontant che per le Single cropping, più evidente in quest'ultime.

I dati ottenuti dalla valutazione di genotipi coltivati secondo un uso sostenibile delle risorse indirizzano gli agricoltori nella scelta di adeguate pratiche agricole che valorizzino non solo la produzione ma anche la sostenibilità ambientale.

ENGLISH SUMMARY

The strawberry quality characteristic and its productive performance is influenced by several factors including genotype, environment and cultivation techniques.

It is extremely important to analyze the plant behavior in relation to the factors previously listed within the productive and economic system of the strawberry cultivation.

The research comes from the understanding of the response of the strawberry plant to the reduction of inputs (nutritional and water-related) and to the climate changing, through the analysis of the qualitative and vegetative parameters of different genotypes, cultivation techniques and growing areas. Three Single-cropping and three Remontant varieties have been studied in an experimental agricultural farm of central Italy.

Another nine selections from the breeding program of the UNIVPM-D3A and a Spanish variety have been monitored in a private agricultural farm in the south of Spain. The study shows how a reduction of the water and nitrogen supplies can be applied without adversely affecting the quality and quantity of the fruit. The type of plant, the genotype, the growing period and the environment affect the reaction of the plant to the different quantity of water supplied.

In this regard the Remontant varieties show an increased sensitivity compared with the Single cropping.

In Spain it was found that a water supply applied in excess or in deficit can cause a vegetative and productive underperformance, resulting in an economic loss. According to the study on the nitrogen supply, the influence of the genotype effect is clear for the Remontant varieties, but great variations among them are not evident.

The induction of the variation in winter temperature causes different responses for the parameters object of the study. One of the mostly influenced is the precocity of the harvest for Remontant and in particular for the Single Cropping varieties.

The data generated from the evaluation of the genotype cultivated following a sustainable use of the resources, could lead the farmers in selecting the appropriate agricultural practices which can valorize not only the production, but also the environmental sustainability.

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1 INTRODUCTION

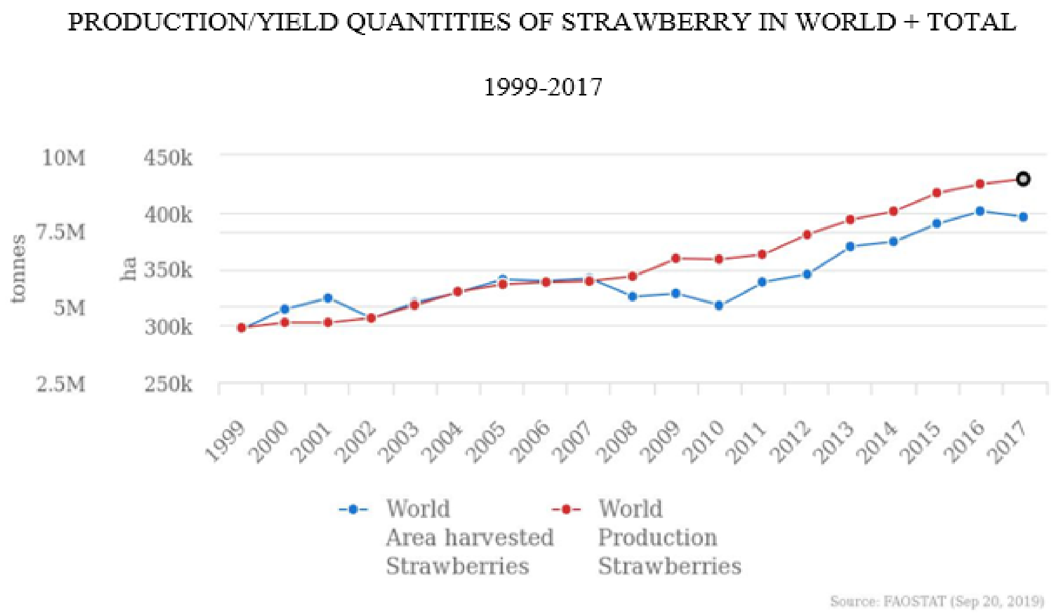
Agronomic and organoleptic qualities and product safety were the main factors that drove the strawberry market choices for several years. In the last few years, the interest for healthy foods and rapid demographic growth is leading to a greater demand of natural product, including fruits and berries. The strawberry fruits are known for their typical succulent aspect, bright red color, juicy texture and aroma. Strawberry fruit plays an important role in the diet, due to its quality and nutritional characteristics (Battino and Mezzetti, 2006). Strawberries are widely consumed either fresh or used in the preparation of transformed foods. Fresh consumption is appropriate to ensure the maximum availability of nutrients, vitamins and fibers. Strawberry fruits have biological properties such as antioxidant, anticoagulant, anticancer, anti-neurodegenerative and anti-inflammatory, cell oxygenation, nervous and immune system improvement (Etminan et al., 2004; Capocasa et al., 2008; Tulipani et al., 2008; Tulipani et al., 2009; Giampieri et al., 2015; Amatori et al., 2016). In addition to this, the fruit can be used in cosmetology and in health sector (perfumes, cosmetics, and hand sanitizers).

The agronomic quality of strawberry plant, as good adaptability, resistance to biotic and abiotic stress and high yield, represents the main important aspects to the farmers. The development and the improvement of new cultivation techniques and the variety innovation have allowed the increase of the plant yield and of the other mentioned characteristics. Overall demographic increase, over the years, has created a big food demand and the agricultural sector is committed to satisfy it. An intensive economic growth has determined an imbalance between the resources' exploitation and their natural regeneration capacity. Consequently, the agricultural sector needs to increase the quantity and the quality of the products, reducing the inputs (water, fertilizers, plants protection) for environmental sustainability. The technological progress is necessary, and it allows to transform an agricultural scientific knowledge into practical innovations. The agricultural knowledge is reached through the collection and analysis of data from research activities. Environmental, climatic and agronomic analysis can accurately define the nutritional, water and temperature needs of crops. Moreover, the genetic improvements have made possible the selection of genotypes with good productive, qualitative and nutritional features and with a good resistance to the extreme environmental conditions.

1.1 Strawberry importance and main productive areas

The strawberry global market amounted to 9.2 M tons (Source: FAOSTAT), increasing by 4% over the last year (Figure 1). Annual world strawberries production has grown steadily through the years.

Figure 1: Worldwide production/yield quantities of strawberry from 1999 to 2017 (Source: FAOSTAT)



The market volume has registered a robust expansion from 2007 to 2017. The strawberry harvested area is about 395,844 ha with a global production of 9,223,815 tons. The trend pattern, however, has indicated some remarkable fluctuations, that have been recorded throughout the analyzed period (FAOSTAT). The figure evidences in the period 2007-2010 (341,440 ha - 317,580 ha) a negative trend regarding the cultivated area, while the global production increased (5,838,608 tons to 6,566,541 tons) at the same time. It is evident an increase in unit yields, due to both varieties innovation and technological development. In general, a positive evolution has been shown from 1999 to 2017, more evident from 2011. The most evident growth rate was recorded in 2014, with an increase of 38% compared to the previous year. Over the period reviewed, global strawberry production has attained maximum level at 9,223,815 tons in 2017. In 2025 the production could reach 11.5 M tons. (FAOSTAT). Strawberries are produced commercially in 76 countries. The highest production is recorded in Asia (49.9%), U.S. (25.2%) and Europe (17.9%) (FAOSTAT). China is the largest producer, followed by USA, Spain, Mexico, Turkey. Nowadays, strawberries production is increasing, particularly in Asia, North and Central America and North Africa. In the last ten years, China showed a strong increase of yield amounted to +1,842,814 tonnes. Instead, in USA, since 1990, the fruit production increased by 17% with the greatest expansion in Florida and California (USDA, 2013), despite a reduction of cultivated surface in California (USDA, 2017, 2018). At the same time, to this expansion correspond also a contemporary reduction of cultivated fields in many other States, due to the inability to adapt to new technologies and to compete on the national marketplace. Additional factors are responsible of this decline such as drought, growth urbanization, labor cost and availability. The 2015/2016 season, in Florida, has been characterized by extreme heat; this factor exerted a negative effect on strawberry production. In 2015, the warm day/night temperatures have conditioned the flower induction and have decreased the plant yield; a moisture excess, also, have promoted the development of diseases. The interaction between climatic change and other environmental (timing and period of distribution of precipitation, warmer season temperature) or social factors interferes on strawberry production system, and make it difficult to take decisions. Those factors are specific for different areas, so they need to be studied in each specific location.

European strawberry market is wide, but the seasonal availability of the fruit requires the importation of strawberries from extra-European countries to cover a supply gap in certain periods of the year. For this reason, the protected cultivation conditions are enhancing the production of fresh strawberry also out of the typical season. The purpose of this cultivation technique was initially to increase the earliness of Single cropping short-day varieties. Currently, the aim is to obtain fruits available all year-round, protecting them from adverse climatic conditions (Neri et al., 2012). In particular, this technique is getting more and more used in Europe, giving also that climatic changes are becoming more common, such as increase of temperatures, spread of drought, and the frequently irregular rainfalls (EEA Report N°1/2017, European Environment Agency). An example is given by the strawberry production in Huelva, one of the most important region for the production of this fruit, that in the last ten years was also affected by climatic changes (Pacheco-Palencia et al., 2009). In 2018, there was a loss of production of 70% compared to the previous harvest due to storms recorded by UPA HUELVA. This meteorological event has delayed production without affecting the quality of the fruit.

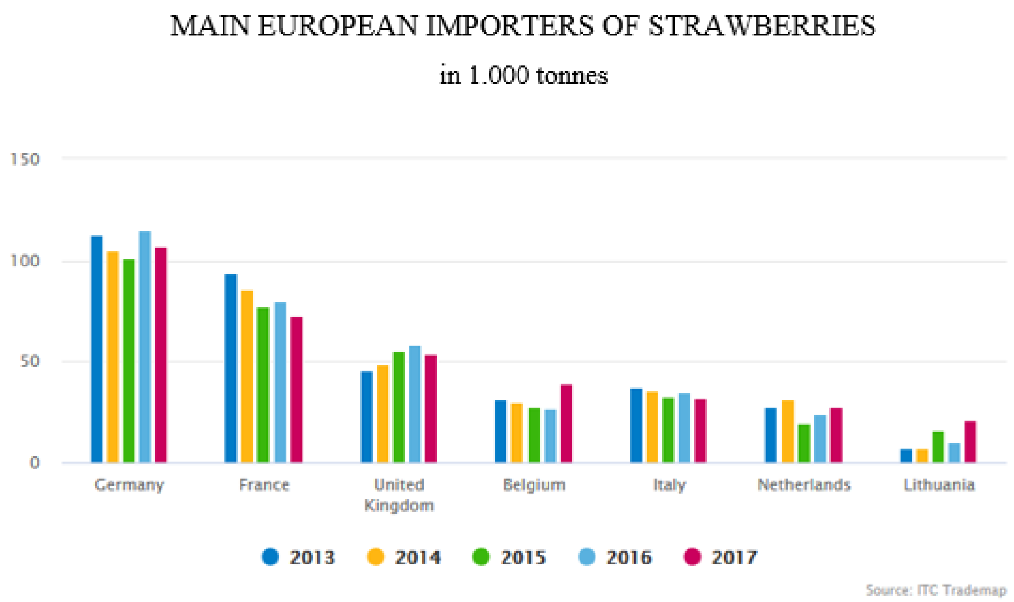
The consumption of strawberries in Europe is estimated to be around 1.3 million tonnes, and differs according to different countries. Italy, Germany and the United Kingdom have the highest consumption per capita with 3 kilos per year. The Netherlands is far below the average, with 0.7 kilos. Belgium is at 2.15 kilos per person per year.

The total European import and internal trade amounted to 503,000 tonnes of strawberries in 2017 (Center for the Promotion of Imports of Developing Countries - CBI, in 2017), but well over 90% can be attributed to re-export and internal trade of local products.

In 2017, developing countries exported almost 35,700 tonnes of strawberries across Europe to compensate the supply shortages. The largest importers are Germany (107,000 tonnes) and France (73,000 tonnes), United Kingdom (54,000 tonnes), Belgium (39,000 tonnes), Italy

(32,000 tonnes). Europe also imports strawberries that come from outside the EU (Figure 2), mainly Morocco, Egypt and the US.

Figure 2: Main European importers of strawberries from 2013 to 2017 (Source: ITC Trademap)



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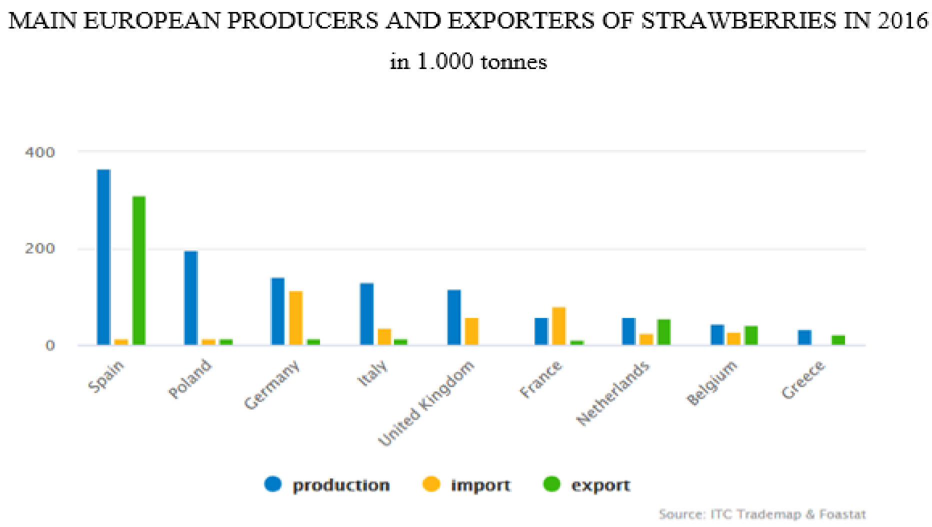
Three biggest suppliers can be identified in Europe: Spain (311,000 tonnes), Poland (197,000 tonnes) and Germany (143,000 tonnes) (Figure 3). Spain provides 80% of the production in three months (March, April and May). The European export market has 5 main destinations: Belarus, Switzerland, Norway, Serbia and the UAE. The European growers are increasing the use of more efficient and controlled production systems, and many new varieties. The collection season is facing an extension with early or late - season growing varieties. In this way, the annual internal production of European countries is almost reaching the consumer demand with less help from external suppliers. It is clear that the growers should increase the capacity to understand the consumer tastes, in particular in terms of fruit quality. This includes the tendency to eat fruits that are produced by sustainable farming methods, taking into account the environmental and social issues that are becoming more relevant.

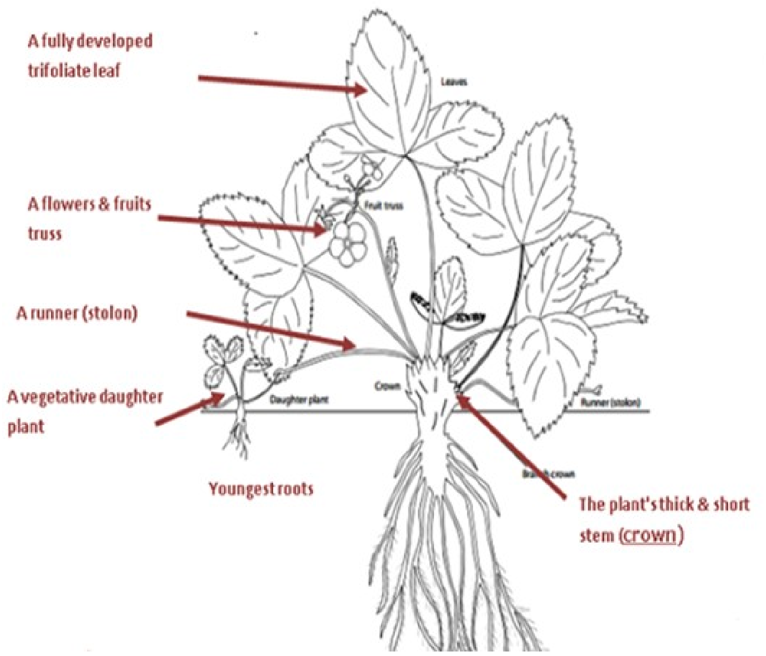
Figure 3: Main European producers, importers and exporters of strawberries in 2016 (Source: ITC Trademap and FAOSTAT)



1.2 Plant structure relations

Strawberry belongs to the *Rosaceae* family, genus *Fragaria*, in which many species are included, both cultivated and spontaneous.

Figure 4 Strawberry Plant Structure Source: Ellis et al., Ohio, 2006



The strawberry is a perennial herbaceous plant, and the propagation takes place both by seeds (sexually) and runners (vegetative).

The plant consists of a root system, a primary stem (rhizome or crown), runners and foliage (Figure 4). The crown is the primary stem: the leaves grow in rosette at the top, while roots and stolons develop at the base of the crown. Some of the axillary buds can become branch crowns or long internodes called runners. The runners generate a new rosette, with a node having small roots. Generally, the runner is formed by two nodes and internodes, the first has a dormant or sterile bud, while the second can generate a new runner. The plants obtained by runners have an identical genetic characteristic compared to the mother plant. The vegetative development (e.g.: the formation of runners and floral initiation) is influenced by the environmental factors (temperature, nutrition, water) and their interactions.

The root system originates from the rhizome. The roots are responsible for the absorption and transport of nutrients and water to the crown and for the storage of reserve substances.

The roots are subdivided in primary and secondary, of variable number based on the species and the cultivars considered. The primary, unlike the secondary ones, are periennial and thicker, therefore the main absorption of water and nutrients is carried out by these roots. The primary roots may achieve 35 cm of depth, but most of them develop in the first 15-20 cm.

The secondary roots have fews week of life and are continuously replaced by new ones.

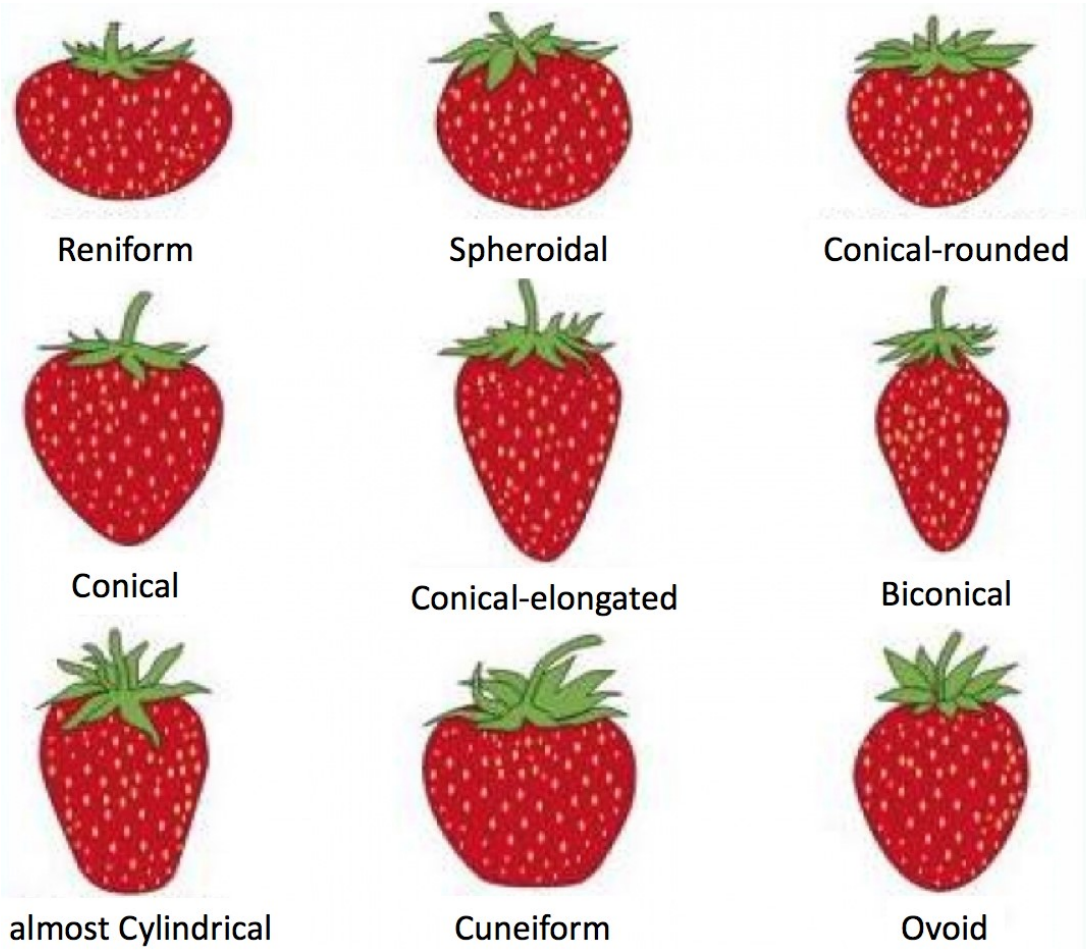
The development of the root length depends on the physical characteristics of the soil, and it is greater in clay soils than in sandy soils.

The foliar structure of the strawberry plant consists of petioles (in the presence or not of stipules) supporting leaves in groups of three seperate leaflets, called trifoliate leaves. The size of the petioles is usually utilized as a plant vigor parameter, as an indicator of plant response to environmental conditions. The length of the petiole can vary from 3 cm to 40 cm. The shape of the leaves can vary and be trilobate, of oval shape, more or less elongated, toothed and joined to rosette. The colour and shape may vary depending on the cultivar.

The inflorescences are ramified and represent the terminal structures of the development of the crown axis. The inflorescences end with the flowers, and are usually formed by a primary, two secondary, four tertiary and eight quaternary axes (Anderson and Guttridge, 1975). The flowers consist of 5 sepals with small green leaflike structures, at the base of 5 petals. The petals are white but the color may vary according to the genotype. The total percentage of anthesis of primary and secondary flowers decreases by 20% with the tertiary and 50% with the quaternary (Anderson and Guttridge, 1975). The stamens correspond to the male part of the flower, containing the pollen to fertilize the pistils, the female part placed on the receptacle. Generally, strawberries have perfect or hermaphrodite flowers, self-compatible or compatible with varieties of similar genetic characteristics. In some cases, cross pollination is required for imperfect or unisexual flowers. After fecundation, the achenes, commonly called seeds, are generated, and represents the true fruit. The achenes are brought by the floral receptacle, which develops after the fecundation and originates the so called "strawberry fruit", that is a false fruit. The primary fruits are brought on primary axes and have bigger size, with high amount of seeds and ripen earlier than the other fruits. Primary fruits are followed by secondary and tertiary berries maturation, which present smaller size than primary fruits.

The ripening takes place 20 or 30 days after the flowering, but depends on the environmental conditions. The characteristics of the fruit, such as size and colour are influenced from environmental parameters, the varieties and the techniques of cultivation adopted. The fruit shape depends on the genotype, and could be: reniform, spheroidal, conical, oblate, cordiform, cuneiform, biconic and ovoid (Figure 5). The uniformity of the fruit depends mainly on the pollination process (Ariza et al., 2011): misshapen fruits result from an insufficient pollination due to negative climatic or enviromental condition.

Figure 5 - Different form of strawberry fruits



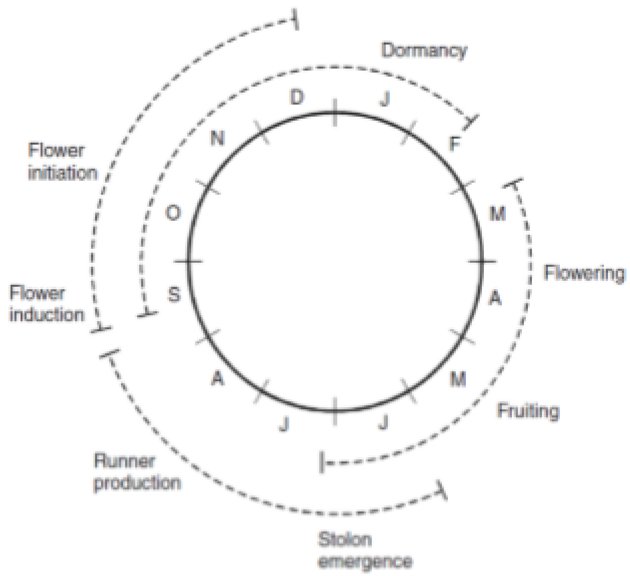
1.3 Growth cycle, flowering and fruiting habits

The expansion of the strawberry growing market has led researchers to study and select cultivars that produce several times a year or that adapt to grow and produce in different environmental conditions. There are many factors that influence the flowering induction, such as genetic aspects, agricultural practices (irrigation, nutrition) and climatic conditions.

The cultivars belonging to the species *Fragraria x ananassa* Duch. may be classified according to the sensitivity to the thermophotoperiod, as demonstrated by their flowering and fruiting habits. On the light of this, strawberry genotypes could be divided into **Single cropping** (non-remontant, seasonal flowering, monoflowering, Junebearing, short day) and **Remontant** (multiple-cropping, perpetual-flowering, everbearing, long day and day neutral).

Single cropping cultivars produce once a year in spring-summer, and the floral induction occurs in the previous summer-autumn season. The plants generate vegetative shoots during the summer, and the latter produce flowers or flower-bearing shoots in the next spring-early summer (Kurokura et al., 2013). Strawberries enter in semi-dormancy from autumn and this phase is characterized by stunted vegetative growth. In spring, after winter chilling, inflorescences grow out and bear fruits. The Cycle of annual growth and development (Darrow, 1966; Guttridge, 1985; Carew and Battey, 2005; Kurokura et al., 2013) is represented in Figure 6.

Figure 6: Annual cycle of seasonal flowering *Fragaria* (Carew and Battey, 2005)



The vegetative phase is characterized by asexual reproductive organs, namely stolons (runners). The runners originate in late spring and continue to develop in summer until autumn, if growing conditions are favorable (Darrow, 1966). In autumn, the runner production is interrupted, and the axillary buds differentiate into axillary leaf rosettes (branch crowns), another form of asexual reproduction (Guttridge, 1965; Darrow, 1966). Initiation and differentiation occur in apical crown meristem and axillary leaf rosettes on upper nodes; with the winter season, the growth is slowed (Kurokura et al., 2013). Entrance into semi-dormancy can also occur during the flower initiation period for single cropping cultivars. The reduction of photoperiod with low temperatures accelerate the start of dormancy (Jonkers, 1965). The overcoming of the semi-dormancy with low winter temperatures allows a vigorous vegetative development in spring (Sønsteby and Heide, 2006). Numerous studies identify the dormancy of the strawberry as semi-dormancy because the plant growth did not stop, but is limited, with a restart under favorable conditions (Guttridge, 1985; Konsin et al., 2001; Sønsteby and Heide, 2006; Rohde e Bhalareo, 2007, Kurokura et al., 2013). Chilling is a physiological process with strong connection with dormancy and affects subsequent development. The satisfaction of chilling requirement and the warm temperatures during spring allows the exit from the dormancy, promoting the growth of buds, elongation of petioles, new stolons and leaves formation (Guttridge, 1958). The cooling requirement to overcome dormancy changes depending on the genotypes.

In spring, it is possible to register a development of the leaves originating from the main crowns and the inflorescences that generate fruits. In addition, the uppermost axillary shoots initiate the next growth cycle as compressed vegetative branches (crowns) (Kurokura *et al*., 2005; 2013; Perrotte et al., 2016). The satisfaction of the thermophotoperiod requirements for the stimulation of flower induction is due to short-day condition and low temperatures (Heide et al. 2013). Cultivar and environmental conditions play a key role on the timing of flowering start, which in the northern hemisphere temperate zone occurs in September-October (Jahn and Dana, 1970). Numerous studies confirm an optimum flowering induction at 18°C (Manakasem and Goodwin, 2001; Verheul et al. 2006; 2007; Opstad et al., 2011) while the performance decrease with temperature ≤12 °C or >22 °C (Sønsteby and Heide, 2006; 2008; Verheul et al., 2006; 2007; Opstad et al. 2011). A demonstration on how the response of cultivars depends on the temperature-related photoperiod is present in Heide (1977). At temperature of 12 °C, the scandinavian *F.* x *ananassa* plants were indifferent to the photoperiod (12-24h) but with the increasing of the temperatures, the initiation of flowers occurred mainly under short days for the studied cultivars. The cultivars selected at higher latitudine need low temperatures (15-18 °C) regardless of the photoperiod for the flower induction. Other varieties prefer the satisfaction

of photoperiod in respect to thermoperiod, because the induction of flowering does not happen at 9 °C (Sønsteby and Nes, 1998; Sønsteby and Heide, 2006) or 12 °C (Verheul et al., 2006) in long days. As an example, Konsin et al. (2001) showed that the flower induction in the cultivar "Korona" strongly depends on the photoperiod; in fact, it did not took place in the optimal temperature of 18 °C at 18 hours of day-lenght, but only between 12 and 15 hours, with a reduction of efficiency with increasing hours.
On the contrary, other cultivars showed the inhibition of flowering at high temperatures (27-30 °C) and optimal photoperiod (Hideo and Saito, 1962; Verheul et al., 2006, 2007). In mild subtropical climates area (Subramanium and Iyer, 1974), too hot temperatures reduce bud formation (Strik, 1985); in fact, exceeding 26 °C (Durner et al., 1984) and 30 °C (Hideo and Saito, 1962) causes the interruption of floral induction. Temperatures, genetic characteristics and photoperiod may influence the number of cycles required for flowering induction (Sønsteby and Heide, 2006; 2008, Verhuel et al. 2007). The optimal range of induction cycles varies from 7 to 14 (Hideo and Saito, 1962, Jonkers, 1965). The photoinduction period is extended with higher temperatures. Ten cycles (16-h) at 9 °C are necessary for floral induction, whereas 16 cycles at 17 °C (Hideo and Saito, 1962). Gutridge (1985) said that short photoperiods and low temperatures show similar numbers of induction cycles. The precocity of some cultivars compared to others is manifested with flowering induction with less cycles and at lower temperatures. The night temperatures affect the flowering induction, as shown by Sønsteby and Heide (2008), and the cultivars from different places of origins subjected to an optimal day temperature show an increase in flowering at different night temperatures. In general, the low night temperatures can compensate the high daytime temperatures, for the flowering induction.

Remontant genotypes bloom and produce constantly during the strawberry crop cycle, many times per year (Heide et al., 2013). This condition is visible under moderate temperature conditions and when the length of the day is more than 12 hours (Hancock, 1999). Flower induction depends on the different sensitivity of the perpetual-flowering cultivar to environmental conditions: day length and temperature (Nishiyama and Kanahama, 2000, Sønsteby and Heide, 2007). According to Sønsteby and Heide (2007), the flower bud initiation is favored in long-days at temperature of 15-21 °C, while with increasing temperatures at around 27°C, the flowering is stimulated by long-day conditions only. Therefore, at high temperature conditions flowering can be activated by passing from short day to long day. On the contrary, at low temperatures, such as 9 °C, the photoperiod does not influence the flowering which appear to be delayed. The remontant are qualitative (obligatory) Long days (LD) plants at high temperatures, and quantitative LD plants at intermediate temperatures (Sønsteby and Heide, 2007). These cultivars are defined as day neutral only at temperatures below 10°C, since they have initiated flower buds independently from the photoperiod (Nishiyama and Kanahama, 2000; Sønsteby and Heide, 2007). The term dayneutral was created and used by American horticultural literature (Galletta et al., 1990; Durner et al., 1984; Nicoll and Galletta, 1987; Durner and Poling,1988; Galletta and Bringhurst, 1990; Sakin et al., 1997; Dale et al.,2002; Stewart and Folta, 2010). In studies such as from Nicoll and Galletta (1987), day neutral genotypes are classified in strong, medium and weak, based on the flowering capacity. The strong day neutrals are not very vigorous with small leaves, few runners and bloom poorly during the summer (Pritts and Dale, 1989). While the intermediate and weak day-neutrals (Pritts and Dale, 1989) produce high amounts of runners, bloom more and the plants are more vigouros. Conditions of high temperature and short photoperiod determine a decrease of flowering for day neutral plants (Heide, 1977; Durner et al., 1984; Serçe and Hancock, 2002). In a study of Manakasem and Goodwin (2001), a scarcity of production is shown for the day-neutral cultivars that have been subjected to short-day conditions at 25-20 °C, during flower initiation. The optimum temperature is between the range 18-13 °C and 21-16 °C, and allows good amount of inflorescence, fruit set and fruit weight.

1.4 Type of plants and production cycles

The strawberry is a plant characterized by a high plasticity in the process of flower differentiation and in the management of the architecture of the plant. These characteristics have allowed the nursery production to obtain differences in production potential for the same genotypes.

The nursery techniques have evolved with the programmed cycles and with different types of plants to face the plant demand of the areas of strawberry cultivation from different latitudes, altitudes and several production systems.

The diversification of the vegetative material allows to adapt the plants to specific growing cycles. In the past years, the bare-rooted freshly dug stolon plants were used in polyannual crops area. In recent years, the spread of annual production system has increased the interest in cold-stored plant (Neri et al., 2012). The cold-stored plants are produced in soil with good drainage and optimal nutritional supply. When the plants are in dormant phase, they are transplanted and stored. The storage of the plants is preceded by the elimination of leaves. Once the plants are divided in classes, according to the diameter of the crown, they are preserved in an appropriate refrigeration system. The cold-stored plants have a terminal bud with a single differentiated inflorescence. The root systems are quickly re-created after the next planting, allowing to overcome the relative shock. The classification of the plants is made on the basis of plant crown diameter: class B (6-9 mm), class A (10-14 mm), class A+ (15-20 mm) and class A++ (above 20 mm). The plants are characterized by excellent vegetative capacities but a low quality of flowers due to the storage in cold conditions. So, an appropriate refrigeration is important to avoid plant quality loss (Musacchi et al, 2012). Cold stored plants of strawberry are cultivated in many countries during the summer. In the last years, the cold stored plants were mainly used for spring cropping. The good plant yield has allowed the possibility to extend the strawberry production season (Dolgun, 2007). The vegetative material can be planted from January until late August and the harvest can be made from mid-March to end-December (Lieten et al., 2005). Some authors affirm that the transplanting realized with high temperatures could determine a loss of yield compared with earlier and later plantings (Kinet et al., 1992). Stressful climatic conditions can determine a physiological deterioration of the plant and consequently worse productive performances (Lieten et al., 1995). Once planted, the growing strategy consists of removal of the first flowers and lateral buds to stimulate the growth of new vegetation (Savini et al., 2005). For example, the production of ever-bearing cultivars starts at July and continues until October; in the month of March, the flowers are detached from inflorescences.

In the planning of the strawberries crop, the waiting bed and A+ cold-stored plants are more used for the out-of-season production in central Europe. Waiting-Bed (WB) is a plant with a large caliber, located in waiting-beds. The plant with bare roots is placed in a cold storage room. After this period, the vegetative material is planted in the field or in the greenhouse.

Generally, the fruits begin to ripe between 5 to 8 weeks after the plantation, based on the genotype and the conditions of plantation. This type of plant is mainly used in programmed crop; an optimal production (500-800 g of fruit per plant) and quality and uniform size of fruits are obtained in short period. The high capacity of production is guaranteed from planting in advance, that stimulates the development (Kirschbaum et al., 2000).

In Tray plants (TP), the runners are taken from mother plants. The apexes of runners are cut and stuck into modular peat tray; then in August, this vegetative material is placed to root in protected conditions. The plants, in full vegetative rest, are packed and stored in refrigerators. The tray plants are then ready to be planted in different seasons with chilling requirement already met. The strawberry growers of the Mediterranean region (South Italy and Spain) tend to cultivate fresh bare-rooted plants with a production that begin in the month of December or January. In the same geographical region, the cold-stored plants are used as planting material for the summer planting system (Kamperidou and Vasilakakis., 2006). In September/October, the cold-stored plants are no longer planted, because they suffer for excessive period in cold storage, and are replaced by fresh plants. Fresh plants are cultivated in runner beds and in mid October are uprooted and located in warm areas. The winter planting allows to include strawberries in crop rotation systems (Palha et al., 2000). The plug plant and bare root are often compared in term of yield, but for the bare root the collection is not concentrated in some spring season as plug plants. (Poling et al., 2005) The plug plants produced from runner tips are an increasing viable alternative to traditional bare root plants (Lieten, 1998; Durner et al., 2002). Plug plants may be available earlier than fresh-dug (bare-root) plantlets (Durner et al., 2002), because they develop more quickly after transplanting (late August or early September). The plug plants enable the greater control over transplant dates with mechanical transplanting opportunity. In general, the growers locate the plants into a plastic mulch, consequently the water management or overhead watering is essential to allow the proper development of strawberry plant. Another advantage of plug plant is to not require the constant overhead irrigation during the first week after transplanting, because root systems are not demanged.

The vegetative growth of fresh plants continues concomitantly with fruiting (Kamperidou and Vasilakakis, 2006). The plantation management is cheaper than cold-stored plants because they do not need the removal of runners and inflorescences, and allow a greater saving of water use. Productive and qualitative performance of fresh and cold-stored plant were compared in Voth and Bringhurst (1990). Results showed greater yield from cold-stored plants (summer planting system) but better quality and earlier harvest from fresh plants. In contrast, in Palha et al. (2000) any significant difference on fruit quality and yield was detected. The vegetative development was higher in cold-stored plants than in fresh plants. The possible explanation is that in fresh plants there is the contemporary vegetative development and fruit production (Kamperidou and Vasilakakis, 2006).

1.5 Main factors influencing strawberry performance

The vegetative, qualitative and nutritional characteristics of strawberry are influenced by the genotype; in fact, the breeding activity has allowed improvements on fruits characteristics and vegetative performance. The increase of fruit size, firmness, sweetness, aroma and bioactive compounds concentration, together with plant yield and rusticity, are the mainly aimed parameters for the new selected material. However, these features result from many factors (agronomic, environmental, climatic), that interact with each other. In the last years, the climate changes have pushed more and more the researcher activity to find solutions for the new genotype-environmental interactions. The interactions between plant-soil and environmental factors (temperature, light and water resource…), combined with climate changes knowledge, are currently object of many studies.

1.5.1 Environmental factors

Effect on plant Development

Temperature and light are environmental factors influencing flowering induction and vegetative development (leaf area, inflorescence, petiole length and number/size of runners).

Some strawberry classifications relie on flowering response to the termophotoperiod: in SD (short day) genotypes, the flower induction occurs at less than 11-16 h or at low temperature (9-21°C, optimal 15-18°C) (Heide, 1977; Opstad et al., 2011; Konsin et al., 2001; Verheul et al., 2007); this condition is present in the summer-autumn period. Instead, the runners' production and the maintainment of vegetative stage of the apex of the main crown in the single cropping is stimulated by day length >14-16 hours and high temepartures (17-20 °C). Flower initiation of LD (long day) genotypes takes place to day length >12 h, DN (day neutral) are relatively indifferent (Nishiyama and Kanahama, 2000; Sønsteby and Heide, 2007).

In SD is present a positive correlation between the percentage of leaf area exposed to short day photoperiod and the number of inflorescences per plant.

The high temperature (above 26-30°C) may inhibit the effect of photoperiod on the behavior of the plant, for both SD and DN genotypes (Kadir et al., 2006; Verheul et al., 2006). In tropical or equatorial areas, the strawberries' profitable cultivation occurs in places where it is reached low temperatures or with cold season. Another factor that affects the plant's physiology is chilling (0-10°C), preceded by the start of semi-dormancy.

In this phase, due to the extension of the short day, new runners are not generated, while short leaves and petioles are formed (Guttridge, 1985).

The compact habit of the plants is overcome by the refrigeration that allows the development of long petioles, the formation of new runners and leaves. In chilling temperatures conditions, the flower induction is inhibited (Guttridge, 1985) and the floral differentiation is enhanced (Durner and Poling, 1987). The extension of the period at low temperatures reduces the number of inflorescences and flowers, due to a lower concentration of starch and sugar (Sønsteby and Hytonen, 2005).

The light quality and intensity influence the flowering process, especially in SD plants (Guttridge, 1985). Therefore, it is possible to intervene trough the use of coverings and artificial light. Reduction in light intesity and temperature through shading stimulates the floral induction by 75-95% (Kumakura and Shishido, 1995) and decreases the size of the crown, the number of the inflorescences and the leaves (Awang et. al 1993). Contrarily, an increase in light intensity can induce flower differentation (Chabot, 1978). Application of photoselective (red and blue) nets over the plants create long day conditions and can inhibit or delay the flower bud initiation. This is made possible by the combined use of far red or light red light with low red light (Guttridge, 1985).

Effect on fruit quality

Important environmental factors to take into account for the berry fruit quality determination are the light, the temperature and their interaction. Low temperatures (Sistrunk and Moore, 1971; Nelson et al., 1972; Kader, 1988; Morris et. al., 1991;) during pre-harvest, reduce solid soluble content and increase the acidity of the fruits (Kidmose et al., 1996). Day/night temperatures below 30/22°C between the end of flowering and the beginning of harvest, led to production of fruits with lower sugar and acidity content. In Wang and Camp (2000), the same parameters increased with temperature values ≤25 °C, and a thermal excursion between day and night of 7–8 °C. The fruit maturation is accelerated by high temperature and protracted light. The reduction of the crop cycle duration is connected to high temperature. High temperatures determine greater early production and drop in total yield (Pacheco-Palencia et al., 2009). Furthermore, high temperatures in spring stimulate earlier flowering, but the occurrence of spring frost reduce the quality and quantity of the successive production (Kampuss et al., 2009).

The firmness value decreases with rainfall at the end of harvest period, while increases with low temperatures and light (Kader, 1988). The aroma development of ripe strawberry fruits is stimulated by cool nights and sunny days (Morris et al., 1991). Also the nutritional profile is affected by climatic conditions during the harvest period: the content of ascorbic acid is highest in fruit collected in sunny days and 18/25 °C of temperature. The rise in night temperature (minimum and maximum) has an impact on improvement of total polyphenols (Wang and Camp, 2000; Wang and Zheng, 2001; Davik et al., 2006). The antioxidant activity is higher in fruits of plant cultivated at high temperatures (Wang, 2006).

As reported by Anagnostou and Vasilakakis (1995), light intensity influenced firmness, acidity, soluble solids content and anthocyanins concentration. Low light intensity could lead to small size and color inhomogeneities of fruits, while an excess could increase the temperature and consequently fruit damage (Sams, 1999). A high light intensity corresponds to enhancement of fruits antioxidants (Atkinson et al., 2005). Different cultivars, studied by Cervantes et al. (2019), respond in different manner to the environmental light changes. For example, "Sabrina" has a conservative behavior, so the standard quality traits of fruits are less affected by changes in the light value. This conclusion is very important for the farmers, because this type of cultivar does not change its good characteristics, even if one of the environment parameters change.

1.5.2 Genotype effect

The aim of strawberry breeding consists of originating new cultivars, which join great plant resilience with good plant production, fruit quality and nutritional characteristics (Mazzoni et al., 2020). Obviously, it is important to maintain these characteristics in other areas that are different from the origin countries. The success of strawberry crop is deeply influenced by cultivar choice; it depends by each region environmental characteristics, mainly temperature and photoperiod (Duerte Filho et al., 2007). Farmers promote and use the varieties that stands out for optimal characteristic in each region (Fagherazzi et al., 2016). Some cultivars show a high productivity and good fruit quality in their origin country, but they do not exhibit the same potential in other areas. Before promoting the advanced selections of a specific breeding program to commercial variety, it is essential to test their potential in private and/or farmer associations' fields. The aim is to achieve new cultivars that are the most possible adapted to different growing areas, by testing them in different climatic and environmental locations. The cultivars with best results (vegetative, productive and qualitative parameters) could be chosen for subsequent tests in larger growing areas.

1.5.3 Cultivation system

Another important factor that influence the strawberry performance is the cultivation method. Many researches study the cultivation methods effect on strawberry plant growth, yield and fruits quality. However, the genotype influences at greater extent the biochemical compounds composition of strawberry fruits than the cultivation strategies, as stated in Capocasa et al. (2008).

The two most important production systems are named hill and matted rows. It is possible to modify these systems through specific requirements of individual producing regions. The cultivation system (hill plasticulture/matted row) and genotype interactions affect the strawberry fruit quality (Wang et al., 2002); for example, plants cultivated with the matted row system originates fruits with fewer nutritional compounds concentration. In the other hand, in the hill cultivation systems, the plants increase their height of about 20 cm, compared to a low bed system (Karhu et al., 2017).

In Laugale et al. (2010), the high-density plantation determines a plants number reduction in the second year of trial. During this second year of growth, the marketable yield of high-density plantation is still higher than the low-density plantation, but this difference is smaller than the first year. In order to increase the fruit size and plant yield, and to reduce the *Botrytis* incidence, in both density plantation type, it can be used the mulching practice.
Actually, the most used and common agricultural practice is the annual system, so the farmers, after one year of strawberries cultivation, eliminate the plants. An alternative strategy is to maintain the plants for a second harvesting period, but according to the results achieved in Braet et al. (2016), this solution is very work-expensive during the winter.
Open field cultivation is the conventional method of farming, but in order to expand the productive season, the use of plastic tunnels and greenhouse is increasing. The effects of greenhouse concerns: the early ripening, the crop protection against the weather damages (Neri et al., 2012), the potential yield increment through reduction of losses caused by precipitation (Grijalba et al., 2015) and a better control of diseases (Andriolo et al., 2002). The presence of waste fruit, mainly due to malformation, could be solved with pollinator insects (Lopez-Galarza et al., 1996). Comparative studies between open-field and greenhouse cultivation show how the adopted system influences the fruit quality (D'Antuono et al., 2000; Recamales et al., 2007; Voća et al., 2006). In the province of Cesena (Italy), the early fruit ripening obtained in protected cultivation was characterized by bigger and brighter fruits but with poorer quality (less sweetness, acidity, texture) than fruits obtained in open field cultivation. Other studies, carried out in Greece, demonstrated that fruits obtained in protected cultivation possessed similar quality, and plants had significant greater yield than those at open field conditions (Paraskevopoulou-Paroussi et al., 1993).
The continuous technological improvement creates new protected crop system. For example, the soilless cultivation is used in protected agriculture. Its advantages are the early ripening, a more adequate mineral nutrients supply, better conditions for plant development and better control of diseases and pests (Schuch and Peil, 2011). The soilless cultivation can help to optimize the hydric and nutritional requirements, and it has a very small influence from the environmental variables. The productivity, quality and vegetative features are in correlation with different cultivation strategies (soil and soilless culture) (Paraskevopoulou-Paroussi et al., 1993). The results of soil cultivation show higher yield (467 g/plant) than for soilless culture (315 g/plant), for cultivar "Selva". Fruit color is better in soilless conditions, due to higher temperatures (Voća et al., 2006).

1.5.4 Nutrition

The strawberry plants, subjected to different fertilizing techniques and products, are able to produce better productive and qualitative results, due to a balanced ratio between supply (fertilizers) and plant demand (nutritional requirements) (Tagliavini et al., 2004). Knowledge of the plant's nutritional requirements is a fundamental aspect for environmental and economic sustainability. However, fertilizers should be applied efficiently, taking seasonal conditions into account. This means to apply the proper amount of nutrients for good crop growth without providing excess nutrients, that may be lost into groundwater or surface waterways.

Even if the nutritional elements are contained in the different plant organs (roots, collar, leaves, petioles, flowers and fruits), as expressed in Lieten and Misotten (1992), it is possible to find the main quantity in leaves, fruits and roots. Strawberry is a perennial plant, but it is generally cultivated in an annual cycle, so it is subjected to different cultivation methods, and the plants type, and variety change according to the production areas. Nutrients removal depends on genotype and agronomic practices. In Tagliavini et al. (2002) the yield and biomass of three cultivars ("Idea", "Marmolada" and "Elsanta") are reported. The first two varieties are cultivated in Italy and "Elsanta" in France. Nitrogen (N), Phosphorus (P), and Potassium (K) removal is similar for the studied cultivars, but "Idea" has a higher production and biomass than "Marmolada" and "Elsanta". A balanced ratio between N, P, and K macroelements is essential for a satisfactory plant growth (Boyce and Matlock, 1966; John et al., 1975).

Nitrogen is an essential nutrient for plant development and for a good qualitative and quantitative production. The runners and shoot formation are stimulated by nitrogen availability; an excess of this element, due to fertilization, accelerates their development at the expense of flower induction (Fujimoto, 1972; Furuya et al., 1988). Therefore, the reduction of nutrients can determine a retarded plant growth and a stimulation of the floral induction (Guttridge, 1985). The excessive supply of nitrogen can lead to low fruit firmness and plant yield, to fruits rot and malformation, to a retarded maturation and to pests and diseases increase (Voth et al., 1967; May and Pritts, 1993). Nitrogen deficiency can manifest symptoms such as undersized fruits and yellowish-green foliage. There is also a reduction in vegetative growth and runner production (Johanson, 1980; Pritt et al., 1998). The increase of N level in the nutrient solution (40, 80, 120 and 160 mg L^{-1}) has a significant impact on increment of runner number and on decrease of fruits soluble solid content (Trejo-Tellez and Gomez-Merino, 2014). Faedi et al. (2006) show that the effects of nitrogen fertilization depend on the doses and administration periods. In autumn season, the Single cropping cultivation does not show differences in percentage of nitrogen concentration in biomass (kg/ha). In spring season, the plant with N 100 and N 55 (kg/ha) shows the highest value in comparison with N 0 (kg/ha); the same trend concerns fruit firmness and soluble solids content. Rodas et al. (2013) has tested four nitrogen doses (100-200-300-400 kg ha^{-1}) and the higher °Brix value was obtained with 200-400 kg ha^{-1} of N. As D'Anna et al. (2012) showed, the different response to the nitrogen quantity depends from genotype. The nutrient satisfaction is genotype-dependent and might be used to adjust fertilization programs and to characterize new cultivars in breeding programs. Generally, however, the firmest and the highest °Brix values are found in fruits with lowest doses of nitrogen (120-170 kg/ha).

Potassium plays an important role in the regulation of the cell osmotic potential and of photosynthetic activity, and of the mechanisms of stress tolerance. Symptoms of potassium deficiency are evident in leaves, where the margins become necrotic but the basal portions remain green. All plants that have a good level of potassium, synthetize more sugar and produce sweeter fruits. Moreover, potassium content has an influence on fruit weight, number and size, on plant yield, and on root weight and length, as demonstrated by Ebrahimi et al. (2012). In this work, the tested cultivars have different responses to potassium fertilization and "Parus" has the best performance.

The phosphorus has a higher impact on fruit quality and on defensive mechanisms initiation than on the plant yield. There is a good relation between soluble solids content and phosphorus content in mature fruits (Cao, 2015). In Medeiros et al. (2015), the fruit number increases directly with the N and P nutrient supply, even with or without K presence. The K presence allows a higher nutritional supply than the fertilization without this element. Deficit in phosphorous supply can determine a limited growth (Choi et al., 2013) while phosphorous excess can reduce branch crowns size and can determine dark-green foliage with a bluish-purple coloration (Choi and Lee, 2012). In case of elevate deficiency, the bluish color covers the whole leaf and it is possible to see a fruit size reduction during the harvest (Domoto, 2011).

The plant performance is more affected by the presence of some elements than others (Pritt et al., 1998). The growth cycle of the plant requests a constant supply of nitrogen and potassium. The dose of phosphorous is usually applicated before the planting. A low calcium content slows down maturation and senescence phases in many fruits, influencing the firmness and the shelf-life (Singh et al., 2009). The fruits that ripe in plants subject to calcium deficiency conditions, are small and acidic and have high firmness (Singh et al., 2007). The calcium deficiency is determined by the difficulty of plant absorption due to soil conditions, environment, moisture. Another microelement that promote a correct root development and a good production of pollen is boron (B). If boron element is present in excessive amount could be toxic for the plants, while its deficiency decreases flowers size and generates small leaves with a yellowish margin. There is a positive interaction between B and P; in fact, the average fruit weight and the plant yield are bigger when P level is high and B level increases (May and Pritts, 1993). Phosphorus, Iron, Magnesium, and Manganese presence is less limiting than Nitrogen, Potassium, and Boron on plant performances. The genotype and the environmental variations could influence deficiency symptoms of micronutrients previously described.

1.5.5 Water

The strawberry plant has an extremely shallow root system; the roots have an extension of about 20-25 cm deep in soils. The depth of soil with available water changes according to the soil type: for example, it is 10 mm in sandy soils and 24 mm in medium-textured soils. The susceptibility to water stress is mainly due to the limited depth and poor efficiency of the root system. The critical stage of growth is the transplant because the plant does not have yet a good fibrous root, with thin root's hairs for water absorption. The absorbent roots are sparse and not long enough to penetrate the surrounding sub-soil, so this situation can lead to the transplant stress.

Some researches have shown the importance of avoiding water stress in the first stage of plant growing after rooting. Water deficiency can be defined an insufficient availability of water that lead to the reduction of numerous physiological and biochemical process in all plant organs. This condition leads to stunted growth, difficulty in the formation of reproductive organs and in the accumulation of reverse substances (Singer et al., 2002). The insufficient amount of water causes a limited starch accumulation in the roots and the reduction in the buds' number, that turns in a yield loss greater than 25%. On the other hand, a water excess can cause root asphyxia or plant collar rot, that brings significant losses in production. The marked deterioration in the fruit sensory qualities (a reduction in sugar content, an increase in acidity) is determined by excessive water application. The determination of correct irrigation requirements and appropriate irrigation system choice are the prerequisite to obtain good productive results in strawberries, especially in protected system, where the only water input is the irrigation. Therefore, the irrigation

management must be carried out in the most effective way, in order to save water and maximize its utilization (Fereres and Sorano, 2007; Xiloyannis et al., 2012).
Even if a correct water supply is necessary for optimize the plant production, the water stress after the beginning of blossoming may allow flower induction (Naumann, 1961). According to Naumann (1964), the strawberry yield is particularly influenced by an efficient water management during the flower bud initiation and differentiation, which occurs in autumn or in spring in some crop system. Many studies demonstrate that irrigation influences plant yield and fruit quality.
The results reported in Kr*ü*ger et al. (2000) show that maximum plant yield is obtained in slight water deficit (-20 kpa). Fruit firmness decreases in case of lower water supply. Furthermore, Kirnak et al. (2003) demonstrated that there is a loss of fruits weight and a minor production in severe drought conditions, while a little reduction of water does not generate any effect on yield. High water reduction causes a decrease of fruit number and weight and of total production (Davies and Albrigo, 1983; Gehrmann, 1984; Yuan et al., 2004; Johnson et al., 2008). In addition to this, the water stress duration also has an impact on leaf, crown, runner and root growth (Gehrman, 1984; Serrano et al., 1992). Genotype, growth phases, stress period and cultivation system influence strawberry behavior under water deficiency conditions (Bota et al., 2001; Adak et al., 2017). The nutritional parameters (Total Antioxidant Capacity, Total Phenolic Content) are positively influenced by water stress (Adak et al., 2017). Bordonaba and Terry (2008) explains that an irrigation system focused on reducing water consumption between flowering and harvest phases can be a right compromise for increasing fruit quality in "Elsanta", "Sonata", "Simphony" and not have a negative influence on berry size in "Christine" and "Florence". The irrigation method could influence the water amount optimization: the surface drip irrigation positively stands out, unlike other methods. In geographical regions characterized by water deficiency, the use of irrigation allows to counteract this scarcity and to achieve a rational strawberry production. In Tunc et al. (2019), different watering amounts (full irrigation until 75% deficit irrigation) were applied with different irrigation systems. Surface drip irrigation with black polyethylene mulch (MD) resulted the best system for plant productivity and fruit quality; mild stress (-25% irrigation amount than control) with MD showed high plant yield and fruit quality similar to full irrigation. Fruit firmness, soluble solids content, and acidity had higher values in the lowest irrigation trial. Therefore, the mild stress has been tested as a method to reduce the water use, but the qualitative and productive performances depend on many variables (genotype, irrigation

system, crop cycle phase, stress duration). The realization of new types of sustainable crop system, such as the soilless cultivation, aims to improve the plant yield, through an efficient use of nutrients and water. Consequently, this method reduces the environmental impact of greenhouses and nurseries (Putra and Yuliando, 2015). Even today, many cultivation methods applied have a low efficiency of use of water (40%-50%), while the diffusion of better cultivation practice, with localized irrigation methods (drip-irrigation), allows to reach a watering efficiency of 90-95% (Xiloyannis et al., 2005, 2006, 2012).

2 AIM OF STUDY

One of the most important challenges of the agricultural production system is to reduce the use of precious and limiting resource (e.g water, nutritional elements) and the effects of climate change (e.g rising temperature). In the last thirty years, the acceleration of changing climatic conditions, with more intense forecast for the next future (IPCC 2014), requires the transformation of the agricultural systems towards less vulnerability and greater resilience. The objective of the study was to test innovative solutions to manage the multiple and variable exogenous factors (climate-environmental changes, reduction of non-renewable resources), using different cultivation strategies and strawberry genotypes that maintain the optimal production values and fruit quality. In order to achieve this objective, the strawberry plants responses to nutritional, water and environmental changes was investigated. These changes involved a decrease in terms of water and nutritional supply in two different cultivation enviroment (Centre of Italy and South of Spain). An additional study was set to evaluate the effect of chilling hours variation, during the winter season, on plant performance. The work considered different cultivars and has allowed to identify, among them, the more suitable genotypes to these resilient conditions. The study can be summarized in three research lines:

- **Research line 1:**

- Testing of vegetative, productive and qualitative parameters of Single-cropping ("Cristina", "Romina", "Sibilla") and Remontant ("Albion", "S. Andreas", "Monterey") cultivars under different water regimes;

- Testing the phenotypic, productive and qualitative responses of strawberry selections (from the genetic improvement of the D3A group) in the South-Spanish environment in water-reduced conditions. The performance of these selections, under same water reduction, were compared with the results of the Spanish commercial variety "Rociera".

- **Research line 2:**

- Evaluating the vegetative, productive and qualitative parameters of Single-cropping ("Cristina", "Romina", "Sibilla") and Remontant ("Albion", "S. Andreas", "Monterey") cultivars under different nitrogen fertilization regimes.

- **Research line 3:**

- Monitoring the temperature effects on vegetative and productive parameters of Single-cropping ("Cristina", "Romina", "Sibilla") and Remontant ("Albion", "S. Andreas", "Monterey") cultivars. The comparison is made between plants subjected to different amounts of chilling hours during the winter resting.

3 RESEARCH LINE I: IDENTIFYING STRAWBERRY GENOTYPES WITH REDUCED WATER DEMAND

Abstract

Strawberries cultivation systems generally require high amounts of water supply (about 7000 m^3/ha in hot-summer conditions, or "South" conditions, http://congresoreganteshuelva.org/contenidos/comunicaciones-libres), especially in some phases of the cultivation cycle. New strawberry cultivars with lower water demand are now requested to reduce its use in the cultivation process, costs and to save this precious resource and to increase adaptability to changing climatic conditions.

With the aim of studying the interaction between the genotype and the water amount, the performance of different strawberry cultivars grown under two levels of water restrictions were compared with the usual water amount. To monitor the response to these treatments, vegetative, productive, and qualitative parameters were observed.

The research activity is focused in two areas of production: central part of Italy and south of Spain. In the central part of Italy, three Single-cropping ("Romina", "Sibilla", "Cristina") and three remontant ("S. Andreas", "Albion", "Monterey") cultivars were grown, while in the south of Spain, nine selections originating by the breeding program of Università Politecnica delle Marche (D3A) have been compared with commercial variety "Rociera". All the tests were performed in field cultivation.

The vegetative and productive parameters showed negative responses to the highest water restriction. The fruit sugar content increased at decreasing amounts of water supply. In particular, the remontant cultivars showed also an increase of fruit firmness and brightness. The results confirmed the importance of genotype rusticity for the adaptation to reduced water use in strawberry cultivation systems. In fact, "Sibilla" maintained similar plant yield in the different water supply regimes, while "S. Andreas" already presented limited production in the light water limitation. The main parameters analyzed for Ancona selections and "Rociera" cultivar showed interesting results originated by the treatment with water reduction instead of full irrigation rates.

3.1 Introduction – Italian trial

The strawberry is a Rosacea plant belonging to the *Fragaria* genus, which includes many species, both spontaneous and cultivated. *F.* x *ananassa* is the cultivated strawberry. In 2012 the allocated areas for the worldwide production of the strawberry reached 241.000 ha (FAOSTAT; Diel et al., 2016). Strawberry is taking on an increasingly important role in the market for the sensorial value and importance for the diet due to high content of minerals, phenolic compounds and vitamins and the related nutraceutical properties, which allow to prevent the uprising of many chronic-degenerative diseases (Giampieri et al., 2012). As the strawberry is one of the most produced and consumed fresh fruits in the world, the environmental factors that influence the good performance and quality of strawberries are extensively studied.

One of the abiotic factors that significantly limit the crop production is drought (Cattivelli et al., 2008). Irrigation for the strawberry growth is necessary in all the Italian cultivation areas, as the strawberry is a plant that needs a significant amount of water, especially in some phases of the crop cycle. Its characteristic root system (Goulart and Funt, 1986) makes it sensitive to water stress (Krüger et al., 1999). Therefore, it is necessary to intervene with the correct irrigation management in order to guarantee a sufficient return of water to the plants in line with their real needs, and to achieve an optimal balance between production, quality and environmental aspects. The climatic changes and the increasing demand for water resources are the main responsible for the reduction of irrigation water availability in the last decades. So nowadays and in the future, the agricultural sector must work to reduce the waste of water and to optimize the water use. Regulated Deficit Irrigation (RDI) is an interesting technique of irrigation used to control water stress in specific periods of crop cycle, particulary when the plant stage is less sensitive to water deficit (Liu et al., 2007, Grant et al., 2010, Arash et al., 2015). If correctly used, it does not show any negative effects on production and quality of the fruit. However, in some cases, a loss in yield corresponds to an increased quality of the product (Kang and Zhang, 2004; Costa et al., 2007; Stefanelli et al., 2010, Kumar and Dey, 2011). In many studies, the water stress on the strawberry plants was monitored with several probes, measuring the soil water potential at different depths. The soil water potential at the root zone is significantly related to plant hydric status indicators such as stem water potential (Intrigliolo and Castel, 2004) and predawn leaf water potential (Lebon et al., 2003; Pellegrino et al., 2005; Ghrab et al., 2013;). However, there are contrasting results on the maximum efficiency of plant production based on water soil potential, because it derives from many variables like environmental conditions, nature of the soil, type of the plant, cultivation techniques. The results of these studies showed an optimum in strawberry quality and production at -10 kPa, but in the same time it presented a significant decrease at -30/-50/-70 kPa (Serrano et al., 1992). In other works, the maximum yield was reach at -15 kPa and the maximum efficiency in the use of water was shown through threshold for irrigation of -30/-50 kPa (Serrano et al., 1992; Guimerà et al., 1995; Hoppula and Salo, 2007). In contrast with previous studies, Pires et al. (2006) highlighted similar values of soil potential for vegetative and productive optimum, between -10 kPa and -35 kPa (depth 10 cm). As shown in Ançay et al. (2016), manual and automatic irrigation efficiency was evaluated, based on optimal water potential at -20 kPa (depth 25 cm). These probes were used to avoid a water decrease that exceeds the real needs of the plants, resulting in negative responses.

An insufficient supply of water determines numerous physiological and morphological responses of the plant. The strawberry activates some defense mechanisms to water stress for optimize its use, including the stomal closure, the reduction of water potential, transpiration and photosynthesis (Chaves et al., 2002). Several studies analyzed the phenotypic symptoms to evaluate the water stress; for example, the reduction of the number of leaves and dimensions, the decrease of the runners, the number and the weight of the fruits (Renquist et al., 1982; Gehrmann, 1984; Serrano et al., 1992). The reproductive development, such as the production of flowers and fruits, is intensively limited by water stress. Drought during blossoming, ripening and harvesting, causes a reduction of fruit yield and quality (Hartz, 1997). However, the plants resistance to drought is strongly influenced by the genotypes, and the responses could be different according to the considered species and cultivar. By knowing the different responses of the varieties to water stress allows us to understand the mechanisms of adaptation and to select the less sensitive cultivars to the extreme climatic conditions for plant production and fruit quality. Therefore, the goal of this study is to verify the behavior of different cultivar of strawberries in response to water input reduction by analyzing the vegetative, productive and qualitative parameters.

3.2 Materials and methods

3.2.1 Field trials

The experimental trials took place at the ASSAM (Agenzia Servizi al Settore Agroalimentare delle Marche) experimental farm in Petritoli, Marche region, (Italy) (43°04’01.56’’N; 13°41’19.22’’E). Tests were carried on single cropping and remontant varieties. Single cropping varieties’ trial was realized in two cycle of cultivation: 2016/2017 and 2017/2018. In

the first year, the planting took place on 28/07/2016 in open field, covered with a plastic tunnel (Figure 7-8) (oriented in east-west direction) on 24/02/2017 and the fruits were harvested in spring 2017. The same experiment was run for another cycle (2017/2018): planting (26/07/2017) and coverage of the green house (21/02/2018). The Single cropping varieties "Romina", "Sibilla", and "Cristina" have been used as cold-stored plants category A+. Remontant varieties' trial was realized in one cycle of cultivation, the plants were planted on 24/04/2019 directly under green house and fruit collected in summer 2019. The type of the plant was cold-stored plants: "Albion" (A+), "S. Andreas" (A++) and "Monterey" (A+).
The vegetative material was obtained from Coviro Soc. Cons. a r.l. (Cervia, Italy). It is an Italian nursery company, that produces and markets certified nursery material. Cold-stored plants (preserved at -2°C for 7 months from nursery before planting) were planted in double row with a distance of 30 cm between the rows and 35 cm between the plants along the row. The distance between the hills measured 1.5 m. The planting density was 5.5 plants/m^2. The non-fumigated soil was composed of 30% clay, 30% sand and 40% silt at a pH value of 8.14 (Table 1). The plants were fertilized with total amount of N (120 unit/ha), P (100 unit/ha) e K (150 unit/ha), during the cultivation cycle. The fertigation has been controlled with a Dosatron® D8R (Dosatron SAS, Tresses, FR). The irrigation system for each line was composed of two dripline hoses Toro® Acqua-Traxx at the distance of 20 cm with 1.1 L/hour, covered by plastic film. The cultivation was based on the Standard Integrated Pest Management (IPM) (Directive128/2009). The soil characteristics were the plants took place are reported in table 1. The soil analysis was carried out in the laboratory of the "Assam".

Figures 7-8: Strawberries cultivation before and after the plastic tunnel application

Table 1: Soil analysis of the experimental field (Assam laboratory)

Legend: U.M. unit of measurement

Trial field	U.M.	Results	Judgment	Method
pH	Unit pH	8.14	Slightly alkaline	D.M. 13/09/99 GU SO n.248 del 21/10/1999 III.1
Sand	g/Kg	304		D.M. 13/09/99 GU SO n.248 del 21/10/1999 II.5
Silt	g/Kg	399		D.M. 13/09/99 GU SO n.248 del 21/10/1999 II.5
Clay	g/Kg	297		D.M. 13/09/99 GU SO n.248 del 21/10/1999 II.5
Active Limestone	g/Kg	61	high	D.M. 13/09/99 GU SO n.248 del 21/10/1999-V.2
Total Limestone	g/Kg	174	Moderate limesteone content	D.M. 13/09/99 GU SO n.248 del 21/10/1999-V.1
Phosphorus assimilable	mg/Kg	3.7	Very low	D.M. 13/09/99 GU SO n.248 del 21/10/1999_XV.3
Exchangeable sodium	mg/Kg	15		D.M. 13/09/99 GU SO n.248 del 21/10/1999 III.2, XIII.2.6
Exchangeable calcium	mg/Kg	4597		D.M. 13/09/99 GU SO n.248 del 21/10/1999 III.2, XIII.2.6
Cation exchange capacity	meq/100g	21.9	high	D.M. 13/09/99 GU SO n.248 del 21/10/1999 III.2
Assimilable iron	mg/kg	9.7	low	D.M. 11/05/92 GU n.121 del 25/05/1992 Method n.37
Assimilable manganese	mg/kg	4.1	Very low	D.M. 11/05/92 GU n.121 del 25/05/1992 Method n.37
Assimilable zinc	mg/kg	0.52	low	D.M. 13/09/99 GU n.248 del 21/10/1999 XII.1
Assimilable copper	mg/kg	2.7	medium	D.M. 13/09/99 GU n.248 del 21/10/1999 XII.1
Boron soluble		0.1	low	D.M. 13/09/99 GU n.248 del 21/10/1999 XII.1
C/N		7.7	low	
Organic Matter	g/Kg	11.9	low	D.M. 13/09/99 GU SO n.248 del 21/10/1999-VII.3. VII.3.6
Total N	g/kg	0.90	Poorly equipped	D.M. 13/09/99 GU SO n.248 del 21/10/1999-XIV.2+XIV.3 mod D.M. 25/03/2002 GU n.84 del 10704/2002
Mg/K		2.7		
Exchangeable Manganese	mg/Kg	155	medium	D.M. 13/09/99 GU SO n.248 del 21/10/1999-XIII.2, XIII.2.6
Exchangeable Potassium	mg/Kg	410	high	D.M. 13/09/99 GU SO n.248 del 21/10/1999 XIII.2, XIII.2.6

3.2.2 Strawberry cultivar evaluation

The following Single-cropping cultivars were analyzed in this study: “Cristina” and “Romina”, developed by the breeding program of UNIVPM-D3A and belonging to late and early ripening group respectively, and “Sibilla”, a medium-ripening cultivar developed by the breeding program of “Consorzio Italiano Vivaisti” (CIV) (Figure 9).

“Romina" AN99,78,51 (95.617.1 x Darselect): Single-cropping variety, with high adaptability to non-fumigated soil. Very early ripening. Fruit of conic or bi-conic shape. Good sweet taste (high sugar and low acidity) (Capocasa et al., 2016). High firmness and shelf life. High fruit nutritional quality determined by high polyphenol, anthocyanin, vitamin C and folate contents (Tulipani et al., 2008).

"Sibilla": Single-cropping variety, mid-late and high chilling variety, suitable for European continental climates. Rustic plant, resistant to diseases and stresses. Very high productive potentiality. Attractive, conical long fruit. Bright red color with high firmness. (http://civ.it/strawberries/)

"Cristina" (CN 95,602,8 x CN 95,419,4): Single-cropping variety, with high adaptability to non-fumigated soil. Very late ripening. Very high productivity, large fruit of conical shape, with good taste (Capocasa et al., 2016).

Figure 9: Cristina, Romina, Sibilla fruits.

For the Remontant study, "Albion", "Monterey" and "S. Andreas", originating from strawberry breeding program carried out by the University of California, were chosen (Figure 10).

"San Andreas" is an early Remontant cultivar and produces high quality fruits. The production period is very similar to "Albion". At the beginning of the season, the plant vigor is higher than "Albion" but berry size throughout the fruiting season is similar to "Albion". This variety has a good productivity, while the fruit color is slightly lighter than "Albion". The plant is rustic, and resistant to diseases and stresses. The fruit taste is good. This variety produces few runners (https://research.ucdavis.edu/industry/ia/industry/strawberry/cultivars/).

"Albion" is a Remontant cultivar. The fruit is long, conical and symmetrical. "Albion" produces consistently throughout the season. This variety produces a lot of runners and is necessary to cut them in order to increase plant yield (https://research.ucdavis.edu/industry/ia/industry/strawberry/cultivars/).

"Monterey" is a Remontant cultivar, with slightly stronger flowering than "Albion". The production is similar to "Albion". Its plant is vigorous. The fruit is slightly larger but less firm than "Albion", and quite sweet. "Monterey" is considered to be have good resistance to diseases (https://research.ucdavis.edu/industry/ia/industry/strawberry/cultivars/).

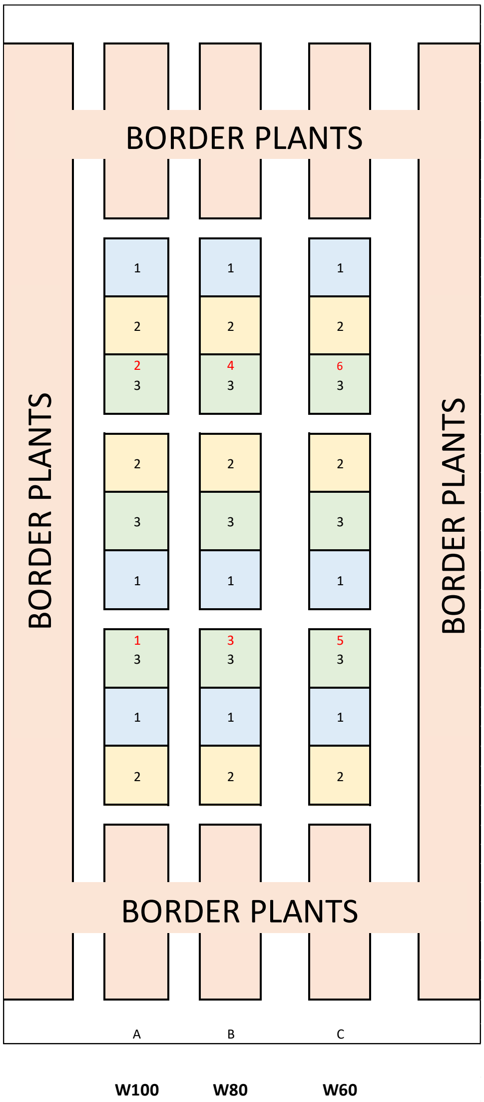
Figure 10: S. Andreas, Albion, Monterey fruits.

The cultivars previously described, were chosen for their rusticity and their ability to resist in exhausted soils and subjected to water shortages.

3.2.3 Experimental design

The experimental design is a split-plot design, with 3 different levels of water supply (main plots) and 3 varieties (sub plots) for each year of study. Each sub plot of single variety containing 8 plants x 3 replicates. Each water treatment comprised a total of 72 plants (Figure 11).

Figure 11: Experimental design



N° Main plot	N° SubPlot	N° plants/plot	Total plants
A (100%)	3(replica)x3(variety)	8	72
B (80%)	3(replica)x3(variety)	8	72
C (60%)	3(replica)x3(variety)	8	72

Legend: 1,2,3 each number represents the variety
1,2,3,4,5,6 each number represents the tensiometer
A, B, C, each letter represents treatment. A=100%, B=80%, C=60%

<u>Water volumes of Single-cropping and Remontant cultivars</u>

Water savings represent a priority against sustainability issue. However, it is necessary to evaluate how the water reduction impacts the fruit production and quality, usually an excessive stress may cause significant losses (Marsal et al., 2016). The water reductions trial was applied during the season of greatest vegetation until the harvest of the fruit. The total amount of water administered through irrigation and precipitation before starting the reduction water trials (Single Cropping and Remontant) was calculated (Table 2).

Table 2: Quantitates of water administered before starting the test (Single cropping, Remontant)

Water administered (m^3/ha) before starting the test		
Year 2017 (m^3/ha) Single Cropping	**Year 2018(m^3/ha) Single Cropping**	**Year 2019 (m^3/ha) Remontant**
1121	1081	1378

The water reduction trial for "Romina", "Sibilla", "Cristina" started on the 1st March and lasted until the 14th of June 2017 and was repeated the following year. Instead, for "Albion", "Monterey", and "S. Andreas" the trial started on the 5th July 2019 and finished on the 20th September 2019.

The table 3 shows the total quantities of water supplied during the three different water regimes (100W, 80W, and 60W). W100 corresponds to the maximum quantity of water given in this test, according to the guidelines from the Delibera 786-10/07/2017 of Marche Region, 20% less corresponds to W80 and 40% less to W60.

Table 3: Quantitates of water administered during the experimental of Single cropping and Remontant

Treatment	**Total Water administered (m^3/ha) During the differentiation water amount**		
	Year 2017 (m^3/ha) Single Cropping	**Year 2018(m^3/ha) Single Cropping**	**Year 2019 (m^3/ha) Remontant**
100%	1104	1135	1182
80%	899	883	957
60%	700	631	665

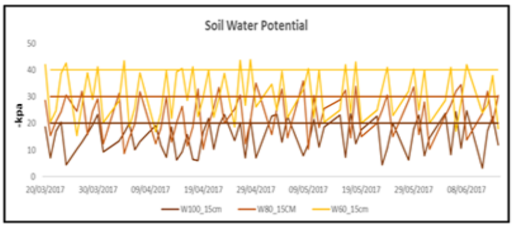
During the trial, the water stress was monitored by measurement of soil water potential. The soil water potential was monitored from the second week of March to the second week of June, for both the years 2017 and 2018, and from July to September for the year 2019. This parameter was recorded by six tensiometers, two for each treatment. These instruments were positioned between two plants in a row, at a soil depth of 15 cm. This depth corresponds to roots exploration area. The probe used were Watermark® and connected to datalogger that recorded the measurements daily (Figures 12-13).

Figures 12-13: Record-keeping system of tensiometers and the measurement site with tensiometer probe

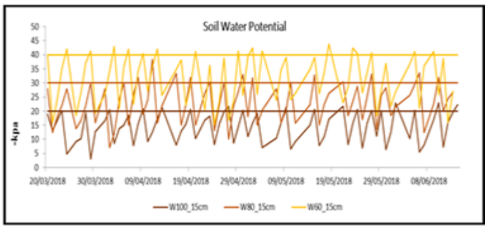
The scheduling irrigation was based on soil water potential. The irrigation for W100 started at -20 kPa (Serrano et al., 1992; Pires, 2006; Ançay et al., 2016), for W80 at -30 kPa, and for W60 at -40 kPa. This is illustrated by sharp peaks in Figure (Figure 14). Monitoring and irrigation operations were operated by Assam field manager.

Figure 14: Soil water potential: a) first cycle and b) second cycle cultivation of Single cropping c) first cycle cultivation Remontant.

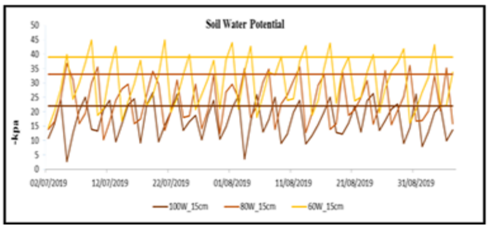
a)



b)



c)



The total water administered (Table 4) for each cycle of strawberry cultivation derives from the summation of irrigation before starting (Table 2) and during the test (Table 3).

Table 4: Total amount water during the complete cycle of the crop (Single cropping, Remontant)

Total water administered (m^3/ha)			
Treatment	**First cycle cultivation (2016/2017)**	**Second cycle cultivation (2017/2018)**	**Remontan (2018/2019)**
100%	2225	2216	2560
80%	2020	1964	2335
60%	1821	1712	2043

3.2.4 Analyzed parameters

Vegetative parameters

The N° of branch crowns/plants, N° inflorescences /plants, N° of leaves/plants were counted. The size of leaves and plant height were measured manually with the scale (ruler), expressed in cm. Size of leaves was detected from lenght and width of the median leaf. Plant height was measured from the base of the branch crown to the apex of the primary leaves. Measurements were made for 8 plant in each subplot included in three main plots (treatments). One measurement date per year (27/04/2017-30/04/2018) was made for the single-cropping. The values obtained in two year (2017-2018) were averaged.

Three measurement dates of number of leaves and plant height were made for the remontant, in order to verify the trend of the vegetative aspects subjected to different water amounts. The measurements were taken on 07/07/2019, 07/08/2019, 07/09/2019. One measurement date (07/08/2019) was applied for the remaining parameters.

Productive parameters

- Ripening period: measured as Precocity Index (IP); IP represents the average of the weighted days number needed to collect the whole production of a cultivar, from January 1, according to the following equation:

  $IP = \Sigma (Zqx)/Q$

  Where Z=number of elapsed days since January 1, q=total harvests production at the date Z, Q=total Production of all harvests.
- Average Fruit Weight (AFW): for each plot at each harvest date, twenty commercial fruits were selected randomly (or all marketable fruits in the case they did not reach the number of twenty) to calculate the average weight (AFW) of the fruit. This parameter was obtained according to the following equation:

  $PMP = \Sigma(pxq)/Q$

Where p=average fruit weight of one harvest, q=commercial production of the same harvest; Q=Total production of all harvests.

- Total production: The total production harvested from all plants of each plot has been evaluated by weighing all ripe fruits detached from plants at each harvest date. Fruits were harvested twice a week. The total production was subdivided into commercial production (healthy whole fruit with diameter of >22mm) and waste production, that included the undersized (diameter < 22 mm), deformed (not regular shape or cracked) and rotten fruits (affected by rot):

  Total production per plant: Σ *commercial production plant*$^{-1}$ + Σ *waste plant*$^{-1}$
- Commercial production: measured as the amount of homogeneous, healthy, and big (diameter >22mm) fruits produced during the harvest season;
- Unmarketable production: measured as the amount of small (diameter <22mm), misshapen fruits and fruits with mechanical and pathological damages.

The dates of harvest time are presented in Table 5 (for Single-cropping cultivars) and in Table 6 (for Remontant cultivars).

Table 5: Description of the date of harvest fruits of single-cropping cultivar studied

Year	Month (Harvest Time)	Date
Season 2016/2017	April	18-21-26-28
	May	2-4-8-10-11-12-15-17-19-22-25-26-29
	June	1-6
Season 2017/2018	April	30
	May	3-7-9-11-14-16-18-21-23-25-28-30
	June	1-4-6-8-12

Table 6: Description of the date of fruit harvest of remontant cultivars studied

Month (Harvest Time)	Date
July 2019	2-9-15-22-29
August 2019	05-12-19-26
September 2019	2-9

Qualitative parameters

Fruits were harvested fully red, at the first, second, and third main pickings and stored at -20 °C until analyses.

- Sugar content (Brix°), or Solide Soluble (SS): determined using a hand-held refractometer (ATAGO, Japan), with temperature compensation. The results are expressed as °Brix. The strawberry juice must be spread on the refractometer's lens, with a Pasteur pipette (3 ml), without the formation of air bubbles. The juice was obtained by previous squeezing of fruits. After each reading of the value, the instrument was cleaned with distilled water, to eliminate residues that could be responsible for changing the values of following samples. The values recorded for the different harvests were averaged for each treatment, for each variety, for each year.
- Titratable acidity (TA): determined from 10 mL of juice diluted with distilled water (1/2:v/v) and titrated with 0.1N NaOH solution. Changing in color is determined by a solution of Bromothymol blue at pH 8.2, when the color changes from green to blue. The value is expressed as mEQ of NaOH per 100g Fresh Weight (FW). The values recorded by the different harvest were averaged for each treatment, for each variety, for each year.
- Fruit color: determined as Chroma by a Minolta Chromameter CR 400, on two side of 10 ripe, undamaged, and uniform fruits. The instrument measured three parameters: L* (luminescence), a* (red tone), b* (yellow tone). L* parameter: elevated value indicates clear and light color (L*=0 represent white color, while L*= 100 represent black color). The value a* and b* are chromaticity coordinates that identify the color: a* indicates the red (from 0 to +60) or green (from -60 to 0) tone, while b* represents the yellow (from 0 to +60) or blue (from -60 to 0) tone. The Chroma was evaluated from a and b values [(a*2+b*2)1⁄2]. The Chroma index measures the saturation of the color: higher Chroma value represents pale fruits and low Chroma represents dark fruits. The different values of Chroma depend on variety and stage of ripeness.
- Firmness: measured by a penetrometer 327 (Effegì, Ravenna, IT). The penetrometer is equipped with a star-shape tip (6 mm diameter). The star tip is necessary to contrast the elasticity of the epidermis. The results are expressed as grams/cm^2.

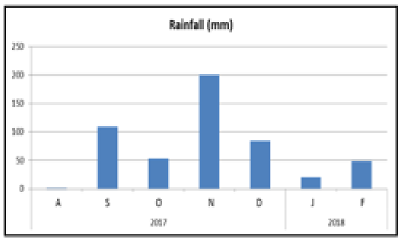
3.2.5 Metereological data

Rainfall (only for Single-cropping cultivars, from August to February, before the protection of the culture) (Figure 15), air and soil temperatures (from March to June for Single-cropping cultivars; from July to September for Remontant cultivars) (Tables 7-8), solar radiation (from July to June for Single cropping; from April to September for Remontant cultivars) (Figure 16) and monthly hours of daylength (Table 9) were registered.

Rainfall data

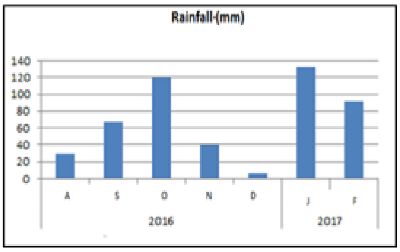
Figure 15: a) Rainfall data of 1°year test –b) Rainfal data of 2°year test

a)



a)1°year test: 264.2 mm of rain (August 2016 to December 2016) and 213.8 mm (January 2017 to Februa 2017)

b)



b) 2°year test: 448 mm of rain (August 2017 to December 2017) and 69 mm (January 2018 to February 2018)

Tables 7-8: Air and soil temperature data 1°year-2° year-3°year test

Air temperature data:

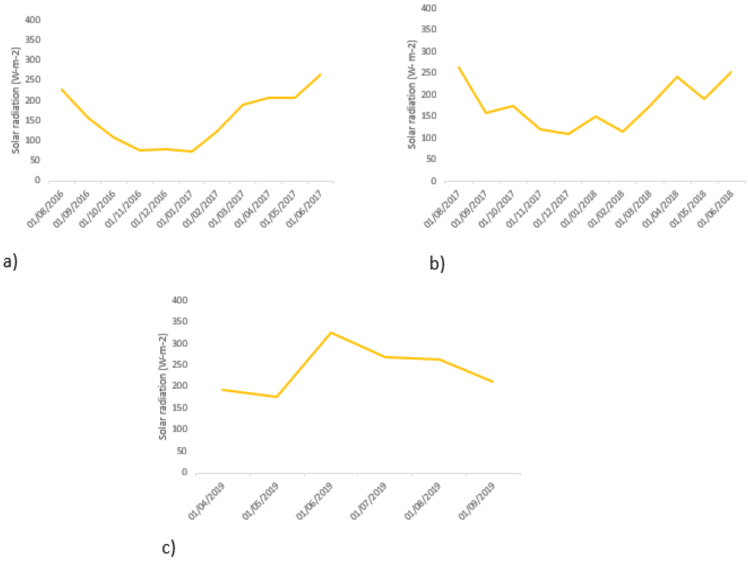
Year	Month	Medium Temperature (°C)	Maximum Temperature (°C)	Minimum Temperature (°C)
2017	March	12.2	27.6	0.2
	April	14.6	31.4	0.1
	May	19.8	36.1	4.1
	June	25.6	42	10.9
2018	March	8.5	20.1	0
	April	15.9	26.6	7
	May	18.6	29.6	9.9
	June	22.0	31	14
2019	July	28.2	45.7	13.8
	August	28.0	47	15.2
	September	24.4	40.5	14.7

Soil temperature data:

Year	Month	Medium Temperature (°C)	Maximum Temperature (°C)	Minimum Temperature (°C)
2017	March	18.0	26.9	9.5
	April	16.1	19.6	11.3
	May	19.5	24.3	14.3
	June	23.8	27.2	19.0
2019	July	28.19	33.1	22.4
	August	28.12	33.3	25.1
	September	25.9	30.8	23.4

Solar radiation data

Figure 16 a) b) c) Solar radiation data 1°-2°-3° year test



Hours of daylight data

Table 9: Hours of daylight monthly

Month	Hours of Daylight Junebearing - Remontant
January	9:32
February	10:38
March	12:2
April	13:31
May	14:47
June	15:27
July	15:08
August	14:02
September	12:37
October	11:10
November	9:52
December	9:11

3.2.6 Statistical analysis

Results for strawberry fruit vegetative, productive, and qualitative parameters are presented as average ± standard deviation (SD) for each cultivar/irrigation treatment. Two-way analysis of variance is used for Single-cropping cultivars to test the differences among cultivation years, cultivar, irrigation treatment, and corresponding interactions. Two-way analysis of variance is used for Remontant cultivars to test the effect of cultivar and treatment, and corresponding interactions. Statistically significant differences in means are determined with Fisher (Least Significant Difference, LSD) ($p \leq 0.05$). Statistical processing is carried out using STATISTICA software (Stasoft, Tulsa, OK).

3.3 Results and discussion

3.3.1 Vegetative parameters of Single-cropping cultivars

According to the ANOVA analysis (Table 10), The interaction year x cultivar significantly influences all the parameters considered. The year x treatment interaction shows no significant differences for all parameters studied except for the leaves number. Same situation occurs for the interaction cultivar x treatment considering the branch crown number. Interaction of cv x year x treatment show significant differences in plant height, leaves number and leaf width.

Table 10: Two-ways analysis of variance (ANOVA) for the vegetative parameters **= significant interaction with $p<0.01$; *= significant interaction for $p<0.05$; n.s.= not significant interaction.

Parameter	Branch Crown number	Inflorescences number	Plant height	Leaves number	Leaf height	Leaf width
Year (a)	**	NS	**	**	NS	**
Cv (b)	**	**	**	NS	**	**
Treatment (c)	NS	NS	**	**	**	**
Year x Cv (a)x(b)	**	**	**	**	**	**
Year x Treatment (a)x(c)	NS	NS	NS	*	NS	NS
Cv x Treatment (b)x(c)	*	NS	NS	NS	NS	NS
Year x Cv x Treatment (a)x(b)x(c)	NS	NS	*	**	NS	**

<u>Branch crowns number</u>

The water regime had no effect on the plant branch crowns of the cultivars. For this parameter, a statistical difference was observed only among cultivars when grown in trials at W100 and W60, where “Cristina” shows statistically lower number of branch crown than “Romina” (Table 11).

Inflorescences

Also, for this parameter, the only statistical differences were detected among cultivars in the different treatments, with “Sibilla” having a statistically lower number of floral axes than “Cristina” in W80 (Table 11).

Table 11: Effects of water availability on branch crowns number, inflorescences number in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher’s LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically for Fisher’s LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Branch crowns number**			**Inflorescences number**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
“Cristina”	4.0±1.6 bc	4.3±1.4 abc	3.9±1.3 c	12.9±3.7 ab	13.3±3.3 a	12.0±4.3 abc
“Romina”	4.9±2.3 a	4.8±2.0 ab	5.0±2.3 a	12.0±3.5 abc	12.6±2.9 abc	12.0±3.7 abc
“Sibilla”	4.9±2.0 a	4.2±1.9 abc	4.4±1.9 abc	11.4±4 bc	11.4±4.1 c	11.2±3.7 c
AVERAGE	**4.6±2 NS**	**4.4±1.8 NS**	**4.4±1.9 NS**	**12.1±3.7 NS**	**12.4±3.5 NS**	**11.7±3.9 NS**

Plant height

The 3 cultivars revealed similar plant development at the different water treatments; in optimal conditions (W100), “Romina” and “Sibilla” showed the highest plants, followed by “Cristina”, and this trend is maintained for all the water reduction treatments. At 20% of water reduction (W80), “Cristina”, “Romina” and “Sibilla” were not statistically affected in respect to their control treatments. A 40% of water reduction (W60) caused a statistically significant plant height reduction compared to W100 for all the varieties (Table 12). The average values of plant height for each treatment points out a development decrease of 8% in 60W compared with 100W. The susceptibility of water reduction is more evident in the above-ground portion reduction than in the root system as confirmed by Klamkowski and Treder (2008); plant transfers the photosynthesized products to increase the number of roots, length, volume and dry weight (Kumar et al. 2012), allowing the plant to survive. This result confirms the necessity of an appropriate monitoring of the water need in order to restitute the appropriate amount for the beneficial effects of irrigation on strawberry plant growth (Krüger et al. 1999).

Leaves number

Leaves number is an additional parameter used to analyze the plant’s development. This parameter, under full irrigation, shows similar results among the cultivars used in the study. As a general trend, the average number of leaves statistically decreases with the diminution of

water supply; especially at W60, the reduction of leaves number compared to W100 is 10% in "Romina", 8% in "Cristina" and 6% in "Sibilla" (Table 12). This decrease is not significant for "Cristina" and "Sibilla" but significant for "Romina".
This study is in line with the results of Yuan (2004) and Grant et al. (2010), in which the plant leaves increased with increasing amount of irrigation water.

Table 12: Effects of water availability on plant height and leaves number in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Plant height**			**Leaves number**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Cristina"	32.6±4.4 d	31.4±4.3 d	28.1±3.6 e	25.5±7.2 ab	24.6±5.3 abc	23.5±7.1 bc
"Romina"	39.1±3.1 a	39±3.0 a	37±3.1 bc	25.1±5.9 ab	25.4±5.3 ab	22.5±4.8 c
"Sibilla"	39.1±5.1 a	38.2±6.3 ab	36.2±6.5 c	26.4±5.0 a	24.7±6.1 abc	24.7±5.4 abc
AVERAGE	**36.9±5.3 A**	**36.2±5.8 A**	**33.8±6.1 B**	**25.7±6.1 A**	**24.9±5.6 AB**	**23.6±5.9 B**

Leaf length

The lower water supply seems to negatively affect the leaves development (Table 13). In fact, the leaf length follows a decreasing trend with the increase of water restriction, but differences in each cultivar are not statistically significant. The only exception is for "Cristina" W60, whom leaves result significantly shorter than "Cristina" W100. Therefore, this parameter does not seem to be strongly influenced by the drought stress.

Leaf width

By taking into account leaf width, "Romina" shows the statistically highest value in each of the treatments. "Romina" is not statistically affected by water restriction, while Cristina and "Sibilla" show a significant lower value of leaves width at W60 in respect to W100 (Table 13). From these results is clear that the impact of water stress on plant vegetative development is strictly related to the genotype and that at different genetic background correspond a different effect of water stress on the main parameters of plant development.

Table 13: Effects of water availability on development of leaves in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Leaf length (cm)**			**Leaf width (cm)**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Cristina"	8.9±0.7 d	9±0.9 de	8.4±0.9 e	7.9±0.9 c	7.8±0.9 c	7.2±1.0 d
"Romina"	9.5±1.1 abc	9.2±1.0 bcde	9.2±1.1 cde	9.4±1.0 a	9.4±1.1 a	9.2±1.2 a
"Sibilla"	9.6±1.1 ab	9.8±1.2 a	9.3±1.0 bcd	8.4±0.9 b	8.1±1.2 bc	8.0±0.8 c
AVERAGE	**9.3±1.0 A**	**9.4±1.1 A**	**9.0±1.1 B**	**8.6±1.1 A**	**8.4±1.3 A**	**8.2±1.3 B**

3.3.2 Vegetative parameters of Remontant cultivars

According to the ANOVA analysis, the cultivar influences the plant response to all the parameters studied (p<0.01). The treatment affects significantly the branch crown number, plant height and leaves number. The interaction cultivar x treatment determines a non-significant effect for the size of the leaves (Table 14).

Table 14: Two-ways analysis of variance (ANOVA) for the vegetative parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	**Branch Crown number**	**Inflorescences number**	**Plant height**	**Leaves number**	**Leaf height**	**Leaf width**
Cv (a)	**	**	**	**	**	**
Treatment (b)	**	NS	**	**	NS	NS
Cv x Treatment (a)x(b)	**	*	**	*	NS	NS

<u>Branch crowns number</u>

Branch crowns number seems to be influenced by the higher reduction of water supply.
Also, development of branch crown is inhibited as demonstrated by previous studies of Sharp (1996) and Spollen et al. (1993).
"Monterey" capacity to produce branch crowns results to be affected by the water stress treatments, showing a significant decrease at W60 than full irrigation (W100) (Table 15).

<u>Inflorescences number</u>

Inflorescences number per plant is quite low in the different periods even at full irrigation, and the reduction of this parameter can have a direct effect to the total yield. Also in this case, "Monterey" shows a significant reduction of the inflorescences number at W60 in respect to W100 (Table 15).

Table 15: Effects of water availability on branch crowns number, inflorescences number in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Branch crowns number**			**Inflorescences number**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Albion"	2.3±0.8 cd	2.6±1 b	2.3±0.7 cd	2.2±2.4 bc	2.5±2 ab	2±2.7 cd
"Monterey"	2.6±0.9 bc	2.2±0.8 d	2.3±0.8 cd	2.7±3 a	2.5±2.6 ab	2.3±2.3 bc
"S. Andreas"	3.2±0.8 a	3.1±1 a	2.7±0.7 b	2±3.2 cd	1.8±2.7 d	2±3.4 cd
AVERAGE	**2.7±0.9 A**	**2.7±1 A**	**2.4±0.8 B**	**2.3±1 NS**	**2.2±1.1 NS**	**2.1±1 NS**

Plant height - leaves number and size

The height of the plant and the number of leaves are reduced at increasing of water stress treatments. These results were previously described in Yuan (2004) and Grant et al. (2012).
In the study of Kumar et al. (2012) the reduction of development mainly affected the aerial system of the plant, such as leaf area and total vegetation compared to the root growth, with the following reduction of traspiration. Therefore, the plants habit is more compact with less water supply.
"S. Andreas" is the most vigorous, with the highest value of plant height, number and size of the leaves, among the cultivars, for all treatments. Regarding the plant height and the leaves number, "S. Andreas" seems to be more sensitive to the W60 treatment (Table 16). "Monterey" does not respond to the water stress, highlighting only at W80 a lower plant height. In "Albion", the plants result smaller than the other varieties, with a particular negative influence of W60. Also the leaves number is significantly reduced by the W60 treatment. The leaf size for each cultivar is not affected by different treatments (Table 17).

Table 16 Effects of water availability on plant height and leaves number in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Plant height (cm)**			**Leaves number**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Albion"	20.3±1.0 e	20.7±0.9 e	19.3±1.1 f	15.2±4.9 cd	17±6.9 bc	13.6±4.2 d
"Monterey"	23.5±1.1 bc	21.8±1.1 d	23.3±1.0 c	16.5±5.7 bc	16.1±5.2 c	15.7±4.7 c
"S. Andreas"	25.4±1.1 a	24.1±1.3 bc	24.3±1.0 b	20.3±6.8 a	18.2±6.4 b	16.0±4.3 c
AVERAGE	**23.1±3.6 A**	**22.2±2.8 B**	**22.4±3.5 B**	**17.4±6.2 A**	**17.1±6.2 A**	**15.1±4.5 B**

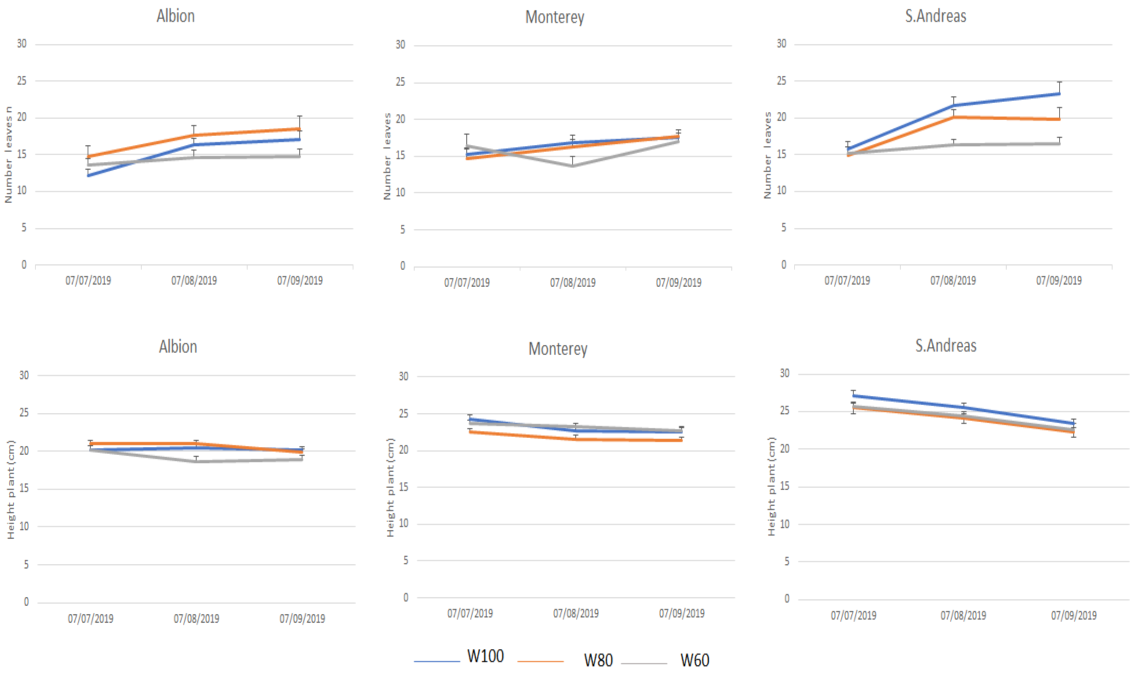
Table 17: Effects of water availability on development of leaves in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Leaf length (cm)**			**Leaf width (cm)**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Albion"	7.3±1.1 bc	7.3±1 bc	7.4±1.0 b	6.7±0.8 bc	6.9±1.3 ab	6.8±0.8 bc
"Monterey"	7.0±1.1 cd	6.8±1.1 d	7.0±1.0 cd	6.7±1.1 bc	6.5±0.9 c	6.7±1.0 bc
"S. Andreas"	7.9±1.2 a	7.9±1.3 a	7.8±0.9 a	7.2±1.0 a	7.2±0.8 a	7.0±1.0 ab
AVERAGE	**7.4±1.2 NS**	**7.3±1.2 NS**	**7.4±1.1 NS**	**6.9±1.1 NS**	**6.9±1.2 NS**	**6.8±1.0 NS**

Trend of leaves number and plant height during water reduction

The development of the plant in terms of leaves number and plant height was monitored in a time interval during the months of water reduction (Figure 17). It is to note note that the trend of the leaves number was growing for the three cultivars examined, particularly at W100 and W80. W60 shows a constant leaf emission response. Contrarily, the plant height tended to decrease slightly for all treatments. Among the varieties, "S. Andreas" seems to be the related to such trends.

Figure 17: Development of number leaves and plant height during water reduction (from July to September 2019) for Albion, Montery, S. Andreas.



3.3.3 *Qualitative parameters of Single-cropping cultivars*

The interaction year x cv had a significant effect on all the parameters considered (p<0.05). The interaction year x treatment and cultivars x treatment showed no significant differences on any of the parameters studied. Same situation was observed for the interaction year x cultivar x treatment, except for the sugar content which was influenced by the interaction of the three parameters (p<0.01) (Table 18).

Table 18: Two-ways analysis of variance (ANOVA) for the qualitative parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	Sugar Content	Titratable acidity	Firmness	Brightness L*	Redness a*	Yellowness b*	Chroma
Year (a)	**	**	**	**	**	**	NS
Cv (b)	**	**	**	**	**	**	**
Treatment (c)	**	*	NS	*	*	**	**
Year x Cv (a)x(b)	**	**	**	**	**	**	**
Year x Treatment (a)x(c)	NS	NS	NS	NS	NS	NS	NS
Cv x Treatment (b)x(c)	NS	NS	NS	NS	NS	NS	NS
Year x Cv x Treatment (a)x(b)x(c)	**	NS	NS	NS	NS	NS	NS

Sugar Content

For each irrigation treatment, “Sibilla” shows the highest fruit SS content, followed by “Romina”, and finally “Cristina” with the lowest value. Comparing the different irrigation regimes, a reduction in water supply value corresponds to an increase in SS fruit content, with a significant difference between W100 and W60 (Table 19). In fact, “Sibilla”, “Romina” and “Cristina” show an increase of around 0.5°Brix at W60 in respect to W100. This result points out the effect of water stress on increasing fruit soluble sugars content in all genotypes. The results here presented find support in previous studies by Terry et al. (2008) and Bordonaba and Terry (2010), which demonstrated the positive water stress effect on the concentration of sugar and on sugar/acid balance, which are considered important parameters by the consumer. In these works, a reducing water irrigation caused an increase in sugar content, but different cultivars responded at different extent. However, another study by Adak et al. (2017) differs from our findings, resulting in a solid soluble content decrease under deficit irrigation conditions.

Titratable acidity

Another important fruit quality descriptor is the titratable acidity. “Cristina” and “Romina” values are close to each other and have less acidity than “Sibilla”, which has the highest value in the first two treatments (Table 19). Differences gets less evident in W60, with the three varieties being statistically similar. According to our study, the increase in acidity appears to be affected by the decrease in water input. This result is particulary due to the behavior of “Cristina”, which increased the acidity of the fruit with reduced amounts of water, while “Romina” and “Sibilla” remain stable in the three water regimes. The results of those two last cultivars are consistent with the results found in Adak et al. (2017), where the acidity of fruit does not change under water deficit conditions.

Table 19: Effects of water availability on sugar content, titratable acidity in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically differet for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Sugar Content (Brix°)**			**Titratable acidity (meqNaOH/100g fruit weight)**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Cristina"	6.6±0.4 f	6.9±0.6 f	7.1±0.8 ef	10.4±1.1 d	10.9±1.1 bcd	11.3±1.6 abc
"Romina"	7.6±0.8 de	7.7±1 cd	8.1±1.1 bcd	10.7±0.7 cd	10.7±1.1 cd	11.1±1.0 abc
"Sibilla"	8.2±0.9abc	8.6±1.2ab	8.8±1.3 a	11.5±1.0 ab	11.7±1.1 a	11.7±1.1 a
AVERAGE	**7.5±1.0 B**	**7.7±1.2 AB**	**8±1.3 A**	**10.8±1.0 B**	**11.1±1.1 AB**	**11.4±1.3 A**

Firmness

"Sibilla" stands out for its utmost firmness, followed by Romina and by Cristina at last, in all the irrigation regimes (Table 20). However, the water stress seems to not influence the fruit firmness in any of the tested genotypes. Moreover, those results are in opposition with Adak et al. (2017), which demonstrates a reduction of fruit soluble solids, titratable acidity and firmness as response to increasing in water stress; while it concludes, as our case, that all these parameters are influenced by the genotype.

Table 20: Effects of water availability on firmness in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Firmness (g/cm^2)**		
Treatment	**W100**	**W80**	**W60**
"Cristina"	271.7±42.9 c	286.1±42.8 c	275.5±31.7 c
"Romina"	342.8±38.6 b	355.2±24.1 b	367.2±32.4 b
"Sibilla"	410.6±84.0 a	409.3±94.1 a	415.5±90.0 a
AVERAGE	**341.7±81.3 NS**	**350.2±78.8 NS**	**352.7±81.8 NS**

Fruit color (Brightness L*, Redness a*, Yellowness b*, Chroma)

Fruit color is an important factor for the consumers' acceptance. As well as the previous parameters, also this one is higly influenced by the genotype even more than the water treatment. The color of "Cristina" has the lowest values of L*, a*, b* and Chroma, in response to all treatments (Tables 21 and 22). Higher values of all parameters related to fruit color are detected for “Sibilla”, followed by “Romina”. The average values of color parameters remain stable at all water supply treatments, without significant differences. However, “Sibilla” is the most sensitive variety to changes in water supply, showing a significant decrease of L*, a*, b* and Chroma at W80 and W60 in respect to W100. The color of “Cristina” and “Romina” fruit is unaffected by the water treatment. These results are partially in line with Adak et al. (2017), that proved that the water reductions do not influence the color characteristic (L*, Chroma, a*). In Bordonaba and Terry (2010), the Chroma values were lower in fruits subjected to water stress. These results have been partially confirmed by our study: the Chroma values of “Romina” and “Cristina” did not change with the water stress with respect to control, but “Sibilla” significantly increased its darkness color with water reductions by 20% both at W80 and W60 in respect to W100 (Table 22).

Table 21: Effects of water availability on L*, a* and b* in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Brightness L***			**Redness a***			**Yellowness b***		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Cristina"	38.1±2.5 cde	37.8±2.2 de	37.6±2.2 e	38.6±2.2 d	38.3±2.1 d	38.1±1.9 d	23.1±3.1 d	22.9±3.2 d	22.1±2.9 d
"Romina"	39.5±2.4 bc	38.3±2.4 cde	39.2±2.4 bcd	41.2±0.7 c	40.5±0.7 c	41±1.1 c	27.9±2.2 bc	26.5±2.1 c	27.6±1.9 bc
"Sibilla"	41.7±2.6 a	40.4±2.5 ab	40.0±2.6 b	43.6±0.6 a	42.6±1.0 b	42.5±1.0 b	30.6±2.3 a	28.6±2.8 b	28.2±2.4 b
AVERAGE	**39.7±2.9 NS**	**38.9±2.6 NS**	**38.9±2.6 NS**	**41.1±2.5 NS**	**40.5±2.2 NS**	**40.5±2.3 NS**	**27.2±4 NS**	**26±3.6 NS**	**26±3.7 NS**

Table 22: Effects of water availability on fruit chroma in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD).

Cultivar	**Chroma**		
Treatment	**W100**	**W80**	**W60**
"Cristina"	45.1±3.4 e	44.7±3.3 e	44.2±2.9 e
"Romina"	49.8±1.4 cd	48.5±1.4 d	49.5±1.7 d
"Sibilla"	53.3±1.5 a	51.4±2.2 b	51±2.0 bc
AVERAGE	**49.4±4.1 NS**	**48.2±3.6 NS**	**48.2±3.7 NS**

3.3.4 Qualitative parameters of Remontant cultivars

According to the ANOVA analysis (Tab. 23), the cv effect is significant ($p<0.05$) for all the parameters considered. The treatment interferes on sugar content, firmness and yellowness. Finally, the interaction between treatment and cultivars affects all the parameters except the sugar content, the titratable acidity and the firmness.

Table 23: Two-ways analysis of variance (ANOVA) for the qualitative parameters **= significant interaction with $p<0.01$; *= significant interaction for $p<0.05$; n.s.= not significant interaction

Parameter	**Sugar Content**	**Titratable acidity**	**Firmness**	**Brightness L***	**Redness a***	**Yellowness b***	**Chroma**
Cv (a)	**	**	**	**	**	**	**
Treatment (b)	**	NS	**	NS	NS	**	NS
Cv x Treatment (a)x(b)	NS	NS	NS	**	**	**	**

<u>Sugar Content - Titratable acidity</u>

Fruit of all cultivars showed an increase in soluble solids content in relation to the lower water supply (Table 24). Many studies reported similar findings (Terry et al, 2007; Bordonaba and Terry, 2010). While the acidity is not affected in any of the varieties.

The varieties “Monterey” and “Albion” stand out for their very high sugar content, which increases respectively by 1.2° Brix and 1° Brix at W60, in respect to W100. “S. Andreas” shows a lower sugar content in all treatments in comparison the other varieties; however, it also shows a significant increase of soluble solids content with the lower irrigation supply. The lower water restituted to plant at W60 probably causes also a lower amount of water inside the fruit, allowing a greater sugar concentration.

In each irrigation treatment, “Albion” shows the highest acidity, followed by “Monterey” and “S. Andreas”, with similar values.

Table 24: Effects of water availability on sugar content, titratable acidity in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Sugar Content (Brix°)**			**Titratable acidity (meqNaOH/100g fruit weight)**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Albion"	14.1±1.2 c	14.6±1.2 bc	15.1±1.3 ab	14.9±0.8 a	14.5±1.7 a	14.5±1.3 a
"Monterey"	14.5±0.8 bc	15.2±1.1 ab	15.7±1.4 a	13.2±1.1 b	13.3±1 b	13.3±1.2 b
"S. Andreas"	11.2±0.7 e	11.4±0.8 e	12.3±0.8 d	13.5±1.3 b	13.2±1.0 b	13.5±1.0 b
AVERAGE	**13.3±1.7 B**	**13.7±2 AB**	**14.3±1.9 A**	**13.9±1.3 NS**	**13.7±1.4 NS**	**13.8±1.3 NS**

Firmness

The fruit firmness already increases with a reduction of 20% than full irrigation (Table 25). These results are different to those previously obtained by Krüger et al. (2000), in which fruit firmness decreased with reduced water supply. In the present study, fruits of the three cultivars show a good firmness: "Monterey" (422.1 g), "Albion" (371.0 g), and finally "S. Andreas" (338.9 g) in optimal condition of irrigation regime (W100). The reduction of irrigation water causes an increase on fruit firmness in "Albion" and "S. Andreas", by 25 g and by 20 g respectively at W60. While "Monterey" maintains a constant firmness during the three irrigation treatments.

Table 25: Effects of water availability on firmness in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Firmness (g/cm^2)**		
Treatment	**W100**	**W80**	**W60**
"Albion"	371.0±94.2 c	391.9±88.9 b	395.6±91.4 b
"Monterey"	422.1±105.7 a	419.7±110.3 a	420.3±121.5 a
"S. Andreas"	338.9±78.2 d	361.4±100.1 c	359.1±105.5 c
AVERAGE	**377.3±99.4 B**	**391±102.9 A**	**391.7±109.6 A**

Fruit color (Brightness L*, Redness a*, Yellowness b* and Chroma)

"S. Andreas" shows higher values for the fruit color parameters (L*, a*, b*) in the full irrigation treatment, followed by "Albion" and "Monterey" (similar to each other). The response to water

stress is different according to the genotype: “S. Andreas” has lower values of L* and a* at W60 than at W100, in opposition to “Albion” and “Monterey” (Table 26).
In optimal condition of water supply, “S. Andreas” fruits seem to be brighter, in apparence, than “Albion” and “Monterey”, as demonstrated by the Chroma values (Table 27). At the same time, there is not any significant differences between fruit brightness of “Albion” and “Monterey”. The responses of the three genotypes to higher water stress are different. The color of “Albion” and “Monterey” fruits becomes brighter with increasing water stress, in contrast to “S. Andreas” (Table 26). Our findings are partially in accordance with those of Terry et al. (2007) and Bordonaba and Terry (2010), but in contrast with Adak et al. (2017), where water stress did not affect the fruit color parameters (L*, Chroma).

Table 26: Effects of water availability on L*, a* and b* in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Brightness L***			**Redness a***			**Yellowness b***		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Albion"	35.4±3.7 c	35.7±3.5 bc	36.4±3.6 a	38.8±3.7 cd	39.3±3.2 bc	39.7±3.5 b	19.7±5.7 bc	20.4±5.4 bc	21.5±5.6 a
"Monterey"	35.3±4.1 c	35.4±3.6 c	35.6±4 bc	38.6±4.3 d	39.5±3.9 b	39.4±4.0 b	18.8±6.6 d	19.5±5.9 cd	19.9±5.8 bc
"S. Andreas"	36.4±3.8 a	36.1±3.5 ab	35.6±3.6 bc	40.5±3.7 a	39.8±4.0 b	39.6±4.1 b	20.5±5.7 b	20.3±5.4 bc	20.1±5.2 bc
AVERAGE	**35.7±4.0 NS**	**35.7±3.7 NS**	**35.9±3.9 NS**	**39.3±6 NS**	**39.5±5.6 NS**	**39.6±5.6 NS**	**19.7±6.0 B**	**20.1±5.6 AB**	**20.5±5.7 A**

Table 27: Effects of water availability on fruit chroma in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Chroma**		
Treatment	**W100**	**W80**	**W60**
"Albion"	43.7±5.5 cd	44.4±5 bc	45.4±5.3 a
"Monterey"	43.2±6.5 d	44.2±5.8 bc	44.4±5.9 bc
"S. Andreas"	45.6±5.7 a	44.8±5.9 ab	44.5±5.9 bc
AVERAGE	**44.1±6 B**	**44.5±5.6 AB**	**44.7±5.7 A**

3.3.5 Productive parameters of Single-cropping cultivars

According to the ANOVA analysis (Tab. 28), the interaction year x cultivar influences significantly ($p<0.01$) the precocity index, average fruit weight, total production and waste, as well as the commercial production ($p<0.05$). Both year and cultivar in relation to treatment did not affect the productive parameters. Considering the interaction between year, cultivar and treatment, only the average fruit weight ($p<0.01$) and waste ($p<0.05$) are affected.

Table 28: Two-ways analysis of variance (ANOVA) for the productive parameters **= significant interaction with $p<0.01$; *= significant interaction for $p<0.05$; n.s.= not significant interaction

Parameter	Precocity Index	Average Fruit Weight	Commercial Production	Total Production	Waste
Year (a)	**	**	**	**	**
Cv (b)	**	**	**	**	**
Treatment (c)	**	*	**	**	NS
Year x Cv (a)x(b)	**	**	*	**	**
Year x Treatment (a)x(c)	NS	NS	NS	NS	NS
Cv x Treatment (b)x(c)	NS	NS	NS	NS	NS
Year x Cv x Treatment (a)x(b)x(c)	NS	**	NS	NS	*

Precocity Index

Both the Precocity Index and Average Fruit Weight do not show significant differences among water supply trials. In optimal conditions of water supply (W100), “Romina” proves to be a medium-early variety (IP = 129), “Sibilla” an intermediate variety (IP = 134) and “Cristina” a late variety (IP = 146) (Table 29). These ripening periods denote important statistical differences among varieties, and those differences are maintained in the three treatments. For all varieties, a higher water stress (W60) causes an early ripening of the fruit (2 days in “Sibilla”, about of 1 day in “Cristina” and “Romina”), but the effect is not significant.

Average Fruit Weight

Among the tested cultivars, “Cristina” has the statistically highest average fruit weight at all the irrigation treatments (Table 29). Comparing the control test with the thesis of less water supply (W60), similar trends were detected for all the cultivars. In fact, in the three cultivars, the fruit weight slightly decreases, although those changes are not significant. Those results are partially in agreement with the works of Kirnak et al. (2003), Bordonaba and Terry (2008), Grant et al.

(2010), and Adak et al. (2017), where it is demonstrated that the fruit weight decreases with less water supply. Anyway, in our case, changes are not statistically significant.

Table 29: Effects of water availability on precocity index and average fruit weight in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Precocity Index (days)**			**Average Fruit Weight (g)**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Cristina"	146.3±2.6 a	145.6±2.8 a	145.2±2.7 a	30.2±2.0 a	30.2±1.7 a	29.8±3.4 a
"Romina"	129.6±3.1 cde	128.1±3.4 e	128.2±3 de	17.9±1.7cd	16.6±1.5 d	16.5±0.8 d
"Sibilla"	134.0±3.6 b	132.5±4.4 bc	132.1±4.2 bcd	20.0±1.3 b	20.1±0.5 b	19.0±1.4 bc
AVERAGE	**136.6±7.8 NS**	**135.4±8.4 NS**	**135.2±8.1 NS**	**22.7±5.8 NS**	**22.3±6.0 NS**	**21.8±6.3 NS**

Commercial, Total Production and Waste

The different water supply treatments lead to significant differences in cultivars productive parameters (commercial production, total production) (Table 30). The reduction in commercial and total production per plant is directly proportional to the decrease in the water regime.
The loss of yield is influenced by the amount and times of irrigation. In fact, Nauman (1964) showed a decrease in flowering and production due to abundant irrigation in August, before the beginning of the short-day flower induction.
The total and commercial weight loss shows no statistical relevance between W100 and W80, while between W100 and W60 a significant decrease was detected. Those results do not agree with Nezhadahmadi et al. (2015), which show that different water supplies do not affect the plant yield. However, the commercial production is strongly related to the genotype effect. "Cristina" shows the greater commercial production at each treatment, resulting statistically higher than "Romina" and "Sibilla", which in turn are similar to each other. The trend of commercial production significantly decreases moving from the W100 toward the W60 thesis, with a decrease of 24% in "Cristina", 19% in "Romina" and 17% in "Sibilla". The same trend is registered in each variety for the total production. The loss of production at W60 is of 19% in "Cristina", 17% in "Romina", and 13% in "Sibilla", in respect to W100. Therefore, the genotype remains the main factor for controlling plant yield capacity at water stress conditions. "Cristina" appears to be the most susceptible to a great water reduction amount. In fact, the significant difference of total production at W100 between "Cristina" and "Romina", becomes not significant at W60. Overall, a reduction in irrigation caused a more marked reduction in

fruit productions in cultivar with a high yield potential. But in the case of "Cristina", even if with a reduced yield at W60, it remains always with a higher value in comparison with the other two cultivars. Observations on the yield reduction and fruit weight loss as response to water stress are also presented in Serrano et al. (1992), Klamkowski and Treder (2008), Grant et al. (2010), and Adak et al. (2017). In Kirnak et al. (2003), the reduction of water does not lead to yield loss. Finally, the amount of fruit waste (damages, small size and rot fruit) does not seem to be influenced by the reduction of water supplies nor by the genotype (Table 31).

Table 30: Effects of water availability on commercial production and total production in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Commercial Production (g/plant)**			**Total Production (g/plant)**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Cristina"	755,1±110,1 a	694,0±143 a	573,8±151,8 b	856,2±121,2 a	809,9±138,6 ab	689,6±162,9 cd
"Romina"	505,6±53,3bcd	494,3±66,6 bcd	409,8±51,1 d	695±51,4 c	712,0±75,9 bc	578,6±45 d
"Sibilla"	555,6±54,9 bc	465,5±69,8 cd	461,1±36 cd	809,8±76,9 ab	707,4±78,1 bc	703,9±57,5 bc
AVERAGE	**605,5±132,6 A**	**551,3±140,3 AB**	**481,6±113,6 B**	**787±108,2 A**	**743,1±107,3 A**	**657,4±112,6 B**

Table 31: Effects of water availability on waste in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Waste (g/plant)**		
Treatment	**W100**	**W80**	**W60**
"Cristina"	101,1±22,8 e	115,9±57,6 ed	115,8±24,2 ed
"Romina"	189,4±24,1 bc	217,7±38 abc	168,8±32,7 cd
"Sibilla"	254,2±50,2 a	241,9±100,2 ab	242,8±67,2 ab
AVERAGE	**181,5±72,4 NS**	**191,8±86,6 NS**	**175,8±68,5 NS**

Total production and average fruit weight were monitored during the months subjected to different water treatments (Figure 18-19) during 2017 and 2018. It can be seen in both years that the total production of "Romina" and "Sibilla" shows a growing trend characterized by a clear "productive peak" and by greater fluctuations than "Cristina". In the second year the peaks of production are also visible in "Cristina".

In the first year, the maximum production of “Romina” (early variety) and “Sibilla” (early medium variety) were in the second week of May, while for “Cristina” (late variety) from the end of May.

In the following year, “Romina” showed a production trend like the previous year, while “Sibilla” postponed the date of maximum harvest of about a week. While, “Cristina” confirmed the dates of maximum production, with a more evident decrease in the next phase.

In the first year, from the beginning of production to the second week of May, “Romina” (W80) was characterized by better productivity than other treatments, while this result was less clear in “Sibilla”. “Romina” and “Sibilla” (W60) achieved production values similar to W80 and W100 trials only in the second week of May. “Cristina” cultivated at the W100 treatment showed a higher yield than W80 and W60. In the following year, the largest production seems to be linked to the increase in water supply.

The average fruit weight of “Romina” showed a constant value, while “Sibilla” slightly decreased, in both years. “Cristina” in the first phase of production reaches 60g of AFW, decreasing then strongly and stabilizing on 20g. This trend indicates an inverse correlation to the production. The treatments did not seem to influence the average fruit weight of the three cultivars.

Figure 18: Development of average fruit weight and total production (Romina Sibilla, Crsitina) during 1°year test (2017)

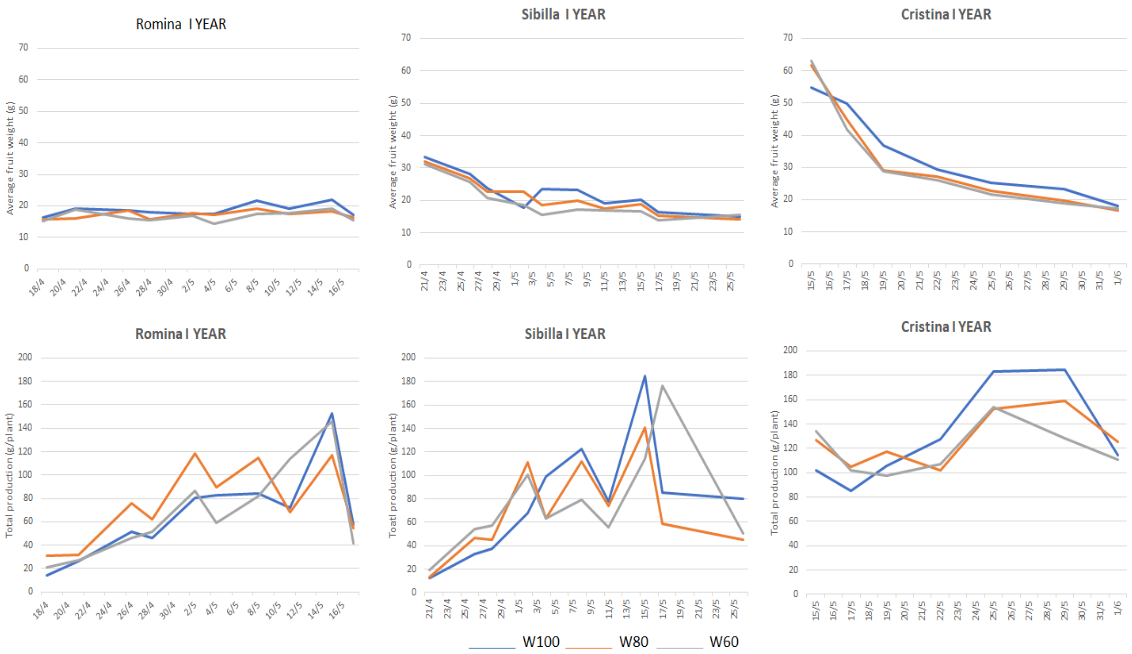
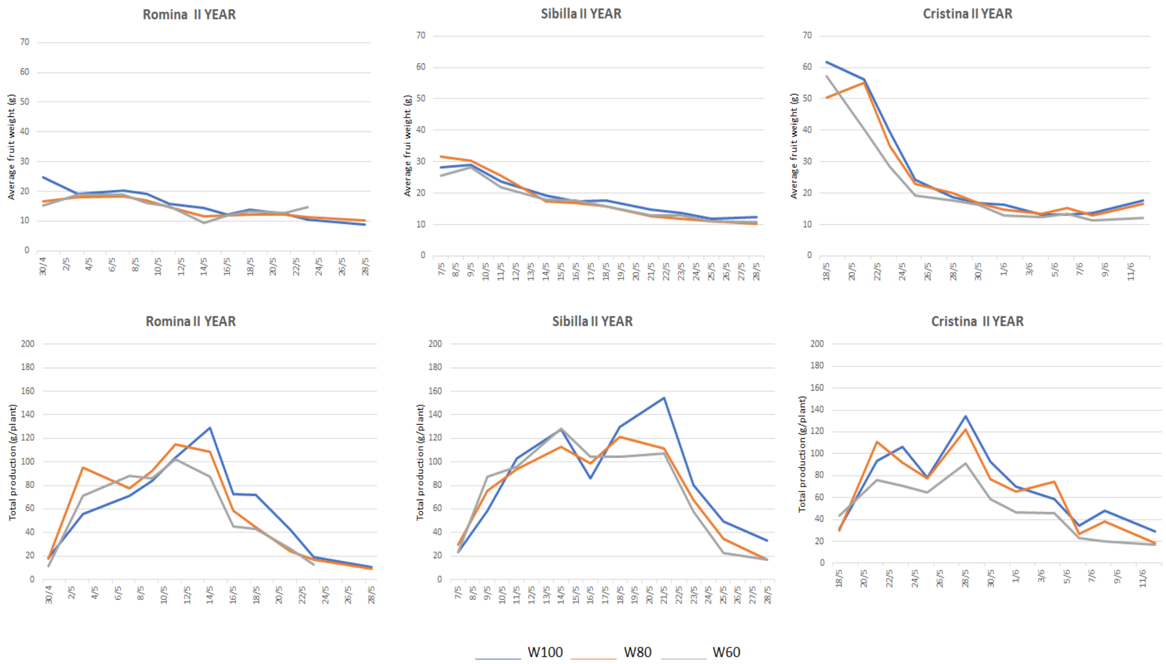


Figure 19: Development of average fruit weight and total production (Romina Sibilla, Crsitina) during 2°year test (2018)



3.3.6 Productive parameters of Remontant cultivars

The analysis of variance shows that the cv affect the plant response to all the measured parameters, except the precocity index, while the treatment affects all the productive parameters considered (Table 32). The interaction between cv and treatment does not show statistical differences for any of the parameters studied.

Table 32: Two-ways analysis of variance (ANOVA) for the productive parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	**Precocity Index**	**Average Fruit Weight**	**Commercial Production**	**Total Production**	**Waste**
Cv (a)	NS	**	**	**	**
Treatment (b)	**	*	**	**	*
Cv x Treatment (a)x(b)	NS	NS	NS	NS	NS

The three cultivars ripen simultaneously under the full irrigation trial (W100). The fruits ripen earlier moving from W100 to W60, in a significant manner (Table 33). The average difference of harvest time is 5 days before (W80) and 9 days before (W60) in respect to control thesis (W100). One interesting point is the anticipated fruit maturation of all cultivar in relation the water restriction. In particular, for "S. Andreas", W60 plants anticipated the harvest of about 12 days, in "Monterey" of about 10 days, and finally in "Albion" of about 6 days, in respect to control (W100). The variation of average fruit weight in relation to treatments is negligible. The cultivars under non-limited water conditions (W100) have originated fruits of relatively low average weight, in line with the data on the vegetative performances (Table 33).

In detail, "Monterey" and "S. Andreas" show a decrease of the average fruit weight with reduction of 20% in irrigation rate, but at W60 this parameter returned similar to W100. This study partially agrees whit Kirschbaum et al. (2003) and Yuan et al. (2004) which stated that an irrigation reduction causes a decrease of berry weight.

Table 33. Effects of water availability on precocity index and average fruit weight in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Precocity Index (days)**			**Average Fruit Weight (g)**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Albion"	214.2±2.4 ab	209.4±1.4 abcd	208.6±4.5 bcd	11±0.1 cd	10.5±0.5 d	11.1±0.4 cd
"Monterey"	214.6±3.4 ab	209.7±0.1 abc	204.4±2.7 cd	12.2±1.8 abc	10.5±0.9 d	12.1±1.2 abcd
"S. Andreas"	215.1±6.3 a	211.1±4.1 ab	203.6±2.8 d	13.4±0.8 a	11.6±1.4 bcd	12.9±0.5 ab
AVERAGE	**214.6±3.8 A**	**210.1±2.3 B**	**205.5±3.8 C**	**12.2±1.4 A**	**10.9±1 B**	**12.0±1.0 A**

"Albion" is the only cultivar that shows statistical reduction in commercial production (-37%) at W60 compared to W100 treatment (Table 34). However, also the other two cultivars decrease the commercial production at W60 in respect to W100 (-34% for "S. Andreas", -21% for "Monterey"), but without statistical significance. For all cultivars, the average commercial production/plant decreases at reduced amount of irrigation water. The total production/plant shows similar trends to the commercial production. At W60, the total production in "Albion" is reduced of about 60g, in "Monterey" of about 54g, and in "S. Andreas" of about 55g, in respect to W100. Among cultivars, "Monterey" shows the highest commercial and total production, resulting significantly better than "S. Andreas", for all treatments.

In general, a proper irrigation results to increase in the total plant yield: this result is aligned with the literature (Renquist et al., 1982; Savè et al., 1993; Krüger et al., 1999; Kirschbaum et al., 2003; Kim et al. 2009). The different amount of production among cultivars at the same water condition demonstrates that an efficient water use is genotype-dependent (Martínez-Ferri et al., 2016).

As already seen, the plant total and commercial production are higher in W100 treatment than in the water restriction treatments. In the same way, the amount of fruit waste is highest at W100, while is remains similar between the other two treatments (Table 35).

"Monterey", which showed higher total and commercial production, appears to have also a higher amount of fruit waste than "S. Andreas", especially in treatment W100 and W60, but always maintaining the highest values of commercial fruit yield (Table 34).

Table 34: Effects of water availability on commercial production, total production in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Commercial Production (g/plant)**			**Total Production (g/plant)**		
Treatment	**W100**	**W80**	**W60**	**W100**	**W80**	**W60**
"Albion"	162.9±24.8 ab	122.6±26.2 bcd	103.1±15.9 cd	204.8±26.1 ab	164.5±26 bc	144.7±31.7 cd
"Monterey"	181.2±42.3 a	174.0±44.2 a	142.3±17.1 abc	241.8±32.4 a	206.3±50.8 ab	187.9±15 bc
"S. Andreas"	122.1±11.4 bcd	79.2±26.5 d	80.9±22,0 d	159.4±14.9 bc	107.5±26.8 d	104.5±19.2 d
AVERAGE	**155.4±36.4 A**	**125.3±50.2 AB**	**108.8±31.4 B**	**202.0±42 A**	**159.4±53.3 AB**	**145.7±41.3 B**

Table 35: Effects of water availability on fruit waste in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significan. Values are expressed as means of one year (2019) ± standard deviation (SD)

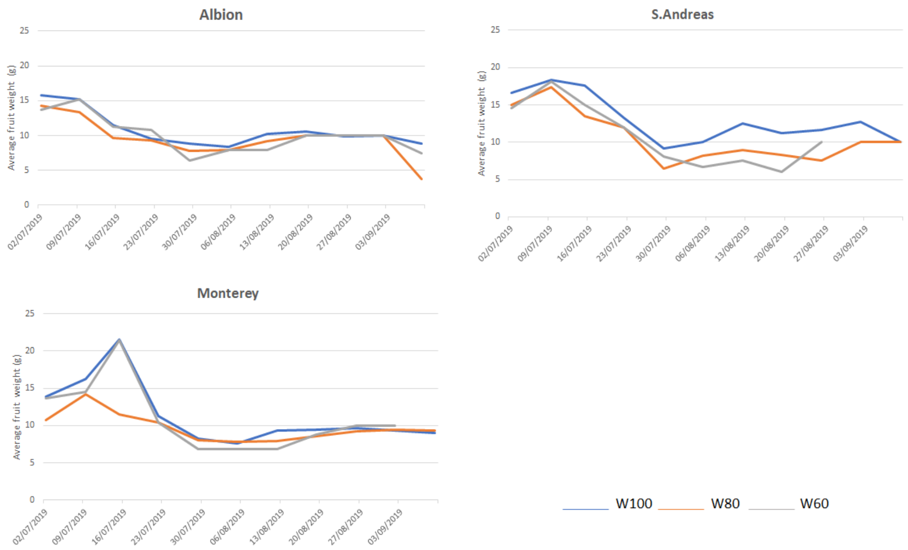
Cultivar	**Waste (g/plant)**		
Treatment	**W100**	**W80**	**W60**
"Albion"	41.9±1.3 bc	41.9±7.0 bc	41.6±19.0 bc
"Monterey"	60.5±10.7 a	32.3±7.0 bcd	45.6±3.5 ab
"S. Andreas"	37.3±16.5 bcd	28.3±1.0 cd	23.6±3.1 d
AVERAGE	**46.6±14.5 A**	**34.2±7.8 B**	**36.9±14.1 AB**

Total production and average fruit weight were monitored during the months subject to different water treatments (Figure 20-21) in 2019. The productions of "S. Andreas", "Albion", and

“Monterey” showed similar trends, decreasing since the second week of August. “Monterey” was characterized by greater oscillations. In general, the W100 treatment allowed a more constant production. The average weight of the fruit of each cultivar decreased with the progression of the harvest. The gap is more evident for the “Monterey” cultivar.

Figure 20: Development of Total Production (Albion, S. Andreas, Monterey) from July to September 2019

Figure 21: Development of Average Fruit weight (Albion, S. Andreas, Monterey) from July to September 2019



3.4 Conclusion

The objective of this study was to determine the effects of different water supplies on strawberry plant vegetative, productive and fruit quality parameters, considering six different strawberry

cultivars. The vegetative parameters are the most negatively affected, with a significant reduction of plant height, leaves number, length and width in response to water stress.
Among the Single-cropping cultivars, "Sibilla" seems to be less affected by plant height reduction; furthermore, it does not show significant variations for the other vegetative parameters. "Romina", followed by "Cristina", is strongly influenced by the water reduction, in terms of leaves number and plant height. "Cristina" exhibits an evident leaf length and width reduction. The remontant varieties "Monterey" and "S. Andreas" respond negatively to water reduction, decreasing the plant dimension already at reduction of 20% of water, in comparison to full irrigation. In "Albion", the negative consequences are visible only after a strong water stress (W60). The number of leaves was significantly reduced only in "S. Andreas", with the stronger water reduction trial (W60).
The productive parameters decrease as response to higher water stress for both Single-cropping and remontant cultivars. The only exception is "Sibilla", which presents similar yield values among different water trials. The qualitative parameters of all cultivars analyzed have been affected by the water reduction in a significant manner. For the Single-cropping cultivars, an increase in sugar and acidity, but not in firmness, was detected after water restriction. In "Cristina" the reduction in water leads to an increase of acidity, without consequences on fruit firmness and color, even at lower water supply (W60). "Sibilla" proves to be the most sensitive to water reduction, showing also a fruit color change, as the L*, a*, b* and Chroma decrease demonstrates. For the remontant varieties, the acidity remains stable at reduced water supply, but the fruit are richer in sugar and more consistent. "Monterey" and "Albion" show greater results of L* and a* in treatment with 40% less water than full irrigation, while "S. Andreas" behaved the opposite. This difference in the remontant cultivars at W60 is registered also for the Chroma, in which the fruits originated by "Albion" and "Monterey" become darker, while fruits from "S. Andreas" become clearer than W100. Summarizing, it is possible to affirm that all the three single cropping studied cultivars, already adapted to the cultivation environment, maintain regular plant development, yield and fruit quality at 80% of water supply. At a further reduction (60%), "Romina" and "Cristina" suffered a decrease of plant yield, while "Sibilla" presented lower quality fruits, in particular in terms of color (L*, a*, b* and Chroma).
By focusing on the performance of Remontant cultivars, it is possible to detect a negative impact already at W80, expecially on the plant height. In respect to Single-cropping cultivars, the remontant presents more difficulties to face the water restriction. This could be due to the different planting period of the two types of plants: the June-bearing, in fact, were planted in the August of the previous year in respect to start of irrigation trial, so they had several months

to expand their radical apparatus and to explore more soil volume. Differently, remontant cultivars were planted in the April of the same year of irrigation trial, so they had only few months to prepare the plant to face the water restriction. However, the response to water stress is strongly influenced by the genetic factor. The sensitivity of “S. Andreas” to less water input is evident during the vegetative growth and in productive parameters, as demonstrated by the reduction of average fruit weight and total production. “Albion” and “Monterey” show a constant production in W80 in comparison to W100. In general, remontant cultivars of this study could be adaptable to grow with 20% less water (-225 m^3/ha), causing not statistically significant differences in production. At the same time, there is an increase in fruit sugar and firmness. The results confirm also the important role of the genotype response as part of saving water use from growth to fruit ripening.

In conclusion, from the point of view of producers, the best strategy to increase the farm income is to adopt a moderate water reduction (80%) for both types of plants, because does not lead to excessive losses of yield, it allows to mantain the fruit quality and to achieve some water savings. If the company orientation is to improve the quality characteristic of the fruit instead, a strong water reduction could be evaluated, but it will negatively affect the plant yield. Furthermore, from this study emerged the possibility to use tensiometers, cheap and easy to use, for monitoring the soil water potential, an indicator for the irrigation schedule. This information could be useful for the introduction of procedures that facilitate the rational management of water resources.

3.5 Introduction – Spanish trial

The strawberries (*Fragaria* x *ananassa* Duch.) are one of the most consumed fruits in the world. In the overview of European production, Spain is classified as the first supplier with 360,416 tonnes/year, and in sixth position among the top 10 producers in the world (FAOSTAT). The Huelva region in Spain (southwestern coast of Spain) has conquered the primacy of Spanish strawberries production, thanks to the 6674 ha dedicated, yielding 340,471 tonnes during the season 2018/2019, increased by 2% compared with the previous campaign (andaluciainformacion.es). In consideration of the extended cultivation area, the optimization of water use and an accurate irrigation management for strawberry grow through agricultural strategies that find a compromise between production, quality and the environment is a primary target for the farmers of this region (Martínez-Ferri et al., 2014).

In fact, in areas of intensive horticultural production, such as the region object of this study, the irrigation water availability is limited and not sufficient to cover the growers' demand (Martínez-Ferri et al., 2016). For this reason, it is necessary to know the real needs of strawberry cultivars in order to determine their irrigation water-use efficiency, leading to a reduction of water demand (Martínez-Ferri et al., 2013, 2014). However, it is suggested to not apply a water shortage upper than 30% of the strawberry plant needs; this because a significant reduction of plant yield has been demonstrated for many cultivars (Martínez-Ferri et al., 2016).

The aim of this work is to assess the effect of variation of water supplies on strawberry production during crop cycle. Selections originating from UNIVPM D3A breeding program and "Rociera", a commercial variety widely cultivated in Spain, are compared to check performance on limited water condition. The genetic improvement can be used as a tool to obtain strawberries that adapt to limited water availability (Grant et al. 2010).

This study was carried out in commercial strawberry fruit production field in the province of Huelva, Spain.

3.6 Material and methods

3.6.1 Field trial and experimental design

The test was set at El Rocio, South-Spain, specifically in "la Laguna de la Yeguas".

The experiment was carried out from 16^{th} of October 2018 (planting date) until 15^{th} of May 2019. The soil analysis carried out at Ecosur laboratory describes a soil predominantly sandy 89.7%, with 5.1% of silt and 5.2% of clay (Table 37). The agronomic practices followed the standards for the organic certificated production. This production system is controlled by

Global G.A.P. (http://www.globalgap.org) and CAAE Agricultural ecologica (Comité Andaluz de Agricultura Ecologìca) (https://www.caae.es/). The strawberry plants were raised and grown under green house. The plants have been located at 25 cm from each other, along rows covered with polyethylene film (lenght 51 m). The distance between the center of two rows was 1.5 m (Figure 22).

Figure 22: General overview of field under experimentation

The study was carried out using fresh plants (bare rooted plants) of the Spanish cultivar "Rociera" and nine Ancona selections (rooted tips) resulting from the breeding program of D3A-Univpm. Ancona selection are produced by an Italian nursery company called Coviro Soc. Cons. a r.l. (Cervia, Italy) while Rociera by a Spanish nursery company Paseo de las Delicias, Sevilla (España), called Viveros Califoria. In this trial, three different irrigation programs were applied by using separate irrigation valves to allow 100%, 72% and 50% water supply (Figure 23). The different amounts of water were applied in the same month of the plantation. The first treatment W100 (100% water supply) was characterized by an irrigation volume of 5 l/h; the other treatment W72, consisting of 72% of water supply, provided 3.6 l/h, while W50 (50% of water supply) provided 2.5 l/h.

The watered time that the company has estimated corresponds to 268 hours. This value is representative of the complete cycle of the crop, from 20/10/2018 to 14/06/2019. On the light of this, and according to the guidelines from the Agenda del Regadío Andaluz Horizonte 2015 (Consejería de Agricultura y Pesca de la Junta de Andalucía, 2011), the total estimated amount of water used in the different trials are: 8889 m^3/Ha for 100% water supply (W100), 6431 m^3/Ha for 72% water supply (W72) and 4444.5 m^3/Ha for 50% water supply (W50), with a water saving of 2458 m^3/Ha and 4444.5 m^3/Ha for W72 and W50 trials, respectively.

Figure 23: Cultivars Rociera in three different treatments

The study was set according to a split plot design. The three treatments represent the main plots, and the 10 genotypes x 2 replicates were the subplots (Table 36). From each plot, 10 plants were selected for the productive and qualitative measurements; of those 10 plants, 6 were chosen for the vegetative parameters. The vegetative, productive, and qualitative parameters of "Rociera" and Ancona selections were evaluated to assess their adaptability to conditions of lower water supply.

Table 36: Experimental design Spanish trial

N° Main plot	**N° SubPlot**	**N° plants/plot**	**Total Plants**
(100%)	2(replicates)x10(genotype)	10	200
(72%)	2(replicates)x 10(genotype)	10	200
(50%)	2(replicates)x10(genotype)	10	200

Table 37: Soil analysis

Trial field	U.M.	Results	Judgment
PH	Unit Ph	7.75	High
Soil texture			Thick consistency
Sand	g/100g	89.7	
Silt	g/100g	5.1	
Clay	g/100g	5.2	
Phosphorus assimilable	mg/kg	130	excess
Potassium assimilable	mq/100g	0.55	normal
Organic Matter	g/100g	0.5	Low
Total orgainic N	g/100g	<0.05	deficient

3.6.2 Strawberry cultivar "Rociera" and Ancona selection evaluation

"Rociera" originated from research activity of Fresas Nuevos Materiales (F.N.M). This variety stands out for flavor and sweet taste of fruit, present until the end of the cycle. Moreover, the cultivar guarantees the highest level of homogeneity of color and size of fruits during the entire harvest period. All these variables, together with the high yield, allow a stable and profitable commercialization from the beginning up to the end of the harvest period. Finally, "Rociera" appears to have a good shelf life that allows it to compete with European seasonal fruit. For the trial, the plants of "Rociera" were planted on 16/10/2018, while all the Ancona selections were planted on 20/10/2018, and presented the main characteristics described in table 38. The breeding program of the Department of Agricultural, Food and Environmental Sciences (D3A) of the Università Politecnica delle Marche (UNIVPM) started in 1993 with the ambition to create new cultivars with an improved fruit quality, a high adaptability to heavy-chalky soils, resistance to soil bound diseases and late ripening. Parental genotypes of Ancona selections are suitable in the southern areas; this specific feature explains the choice of those selections for this trial.

Table 38: Brief description Ancona Selections

Code	Description	Planting Date
AN 14, 12, 59 (AN08,110,58 X BUDDY)	Fruit of high firmness, good and intense color, medium flavor.	20/10/2018
AN 13, 13, 55 (AN06,164,52 X PIRCINQUE)	Fruit with a good size, high production, medium firmness, few rotted fruits. Resistant to averse climatic conditions.	20/10/2018
AN 12, 44, 60 (AN08,113,53 X F. FORTUNA)	Early production, conic fruit, pale red color, medium firmness and good taste.	20/10/2018
AN 13, 16, 56 (AN08,113,53 X CRISTAL)	Fruit with matt color and conical elongated size. Good firmness and regular shape.	20/10/2018
AN 13, 16, 57 (AN08,113,53 X CRISTAL)	High production, medium firmness, good taste. Conical elongated size, red-orange fruit color.	20/10/2018
AN 14, 12, 58 (AN08,110,58 X BUDDY)	Fruit with regular size and conical elongated shape. Medium taste, uniform color.	20/10/2018
AN 12, 45, 53 (AN08,113,53 X PIRCIQUE)	Medium firmness and taste. Regular and conical elongated shape. Intense color. Good taste.	20/10/2018
AN, 13, 13, 62 (AN06, 164,52 X PIRCIQUE)	Fruit with good size and conical elongated shape. Medium taste and good color.	20/10/2018
AN14, 21, 61 (SABRINA X ROMINA)	Biconical fruit. Average size and good/intense color of fruit. High firmness.	20/10/2018

3.6.3 Analyzed parameters

To evaluate the effect of water reduction in the strawberry cultivation, it is essential to have a proper overview about the plants' response to various water supply strategies. Consequently, the vegetative, productive and qualitative parameters were measured during the entire harvest period that in the Spanish conditions is time-shifted then in Italy. E.g., the fruit harvest period in Huelva is earlier of about 2 months than the main productive period registered for Single-cropping cultivars on 2016/2017-2017/2018 studies in Italy (Figure 24). In Spain the total production is increasingly distributed over the harvest time, reaching a peak in March with 50% of production. While in Italy 80% of total production is concentrated in May. Probably one of the factors that have contributed to the early Spain's production is the temperature of March with about +5 °C of average temperature in respects to Italy.

Air temperature

The air temperature has been recorded at the cultivation site in the period from October 2018 to June 2019 (Figure 25).

Figure 24: Main productive period in Spain (2019) and Italy (2016/2017-2017/2018) expressed as the percentage of total production harvested in each month.

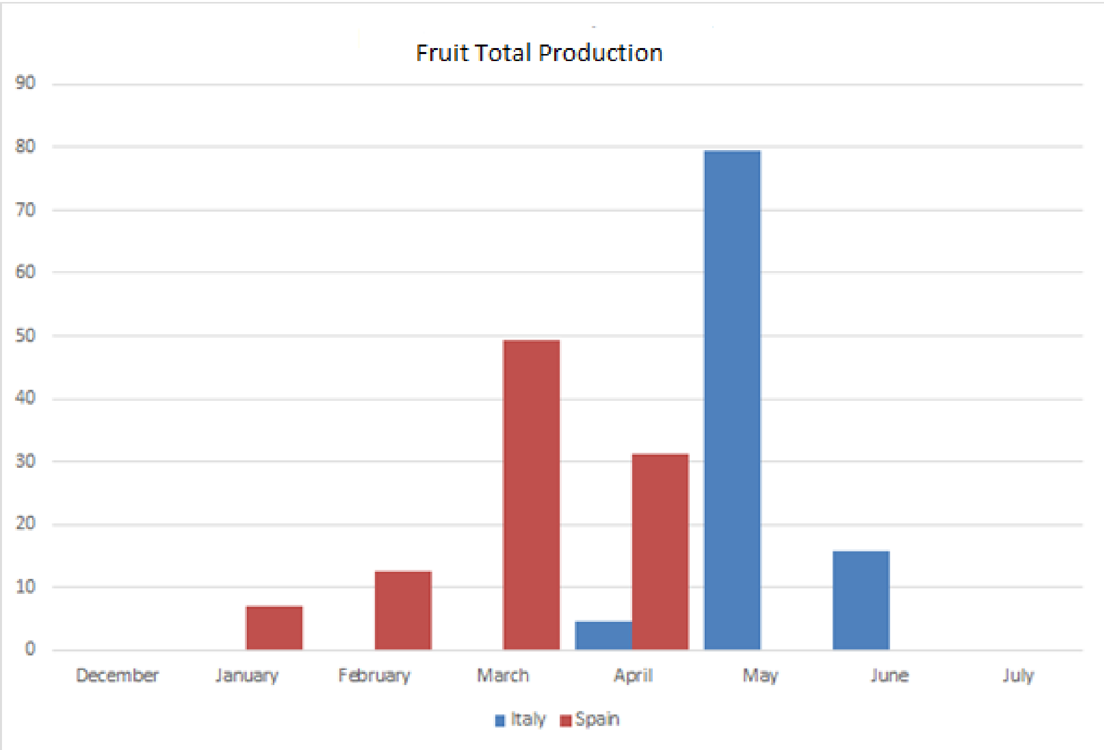
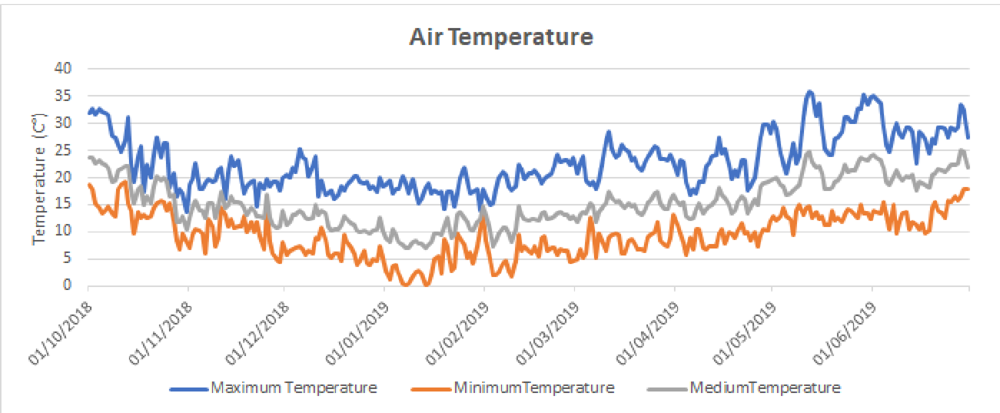


Figure 25 Air temperature from October to June 2019



Vegetative, productive, and qualitative parameters

The analysis and the methodologies used for vegetative, productive, and qualitative parameters assessment are the same applied applied to the water reduction trials for Single cropping and Remontant varieties (Chapter 3.2.4)

Vegetative Parameters

The development of the plants was monitored, through the measurement of the main vegetative parameters (plant height, number of leaves). The evaluations were performed once a month, on 06/02/2019, 06/03/2019, 06/04/2019. The vegetative parameters of each genotype were measured on 6 plants for each of three treatments (W100, W72, W50), with two replicates.

Productive Parameters

The harvest of the fully red strawberries has been set once a week from January to February 2019, while from February to April 2019 was performed twice a week (Table 39). The productive parameters of each genotype were measured on 10 plants per treatment (W100, W72, W50), with two replicates.

Table 39: Description of the date of fruit harvest

Month (Harvest Time)	**Date**
January 2019	9-14-17-21-24-29
February 2019	6-11-14-20-22-26
March 2019	1-5-8-12-15-19-22-26-29
April 2019	1-5-9

The analyzed parameters are:

- The Precocity Index, indicating the ripening period.
- The Average Fruit Weight (AFW) consisting a sample of 20 commercial fruits chosen in each harvest.
- The Commercial Production, corresponding to the weight of commercial fruits (healthy fruits with a diameter >22 mm).
- The Secondary production, corresponding to the waste fruits in the production cycle. Therefore, this parameter represents the small, deformed and rotten fruits.

- The total production, composed of global quantity of fruits (commercial production and fruit secondary production) obtained for each plot, during the harvest season.

Qualitative Parameters

Total Soluble Solids Content was measured by digital Pocket Refractometer-PAL-1 Atago (made in Japan) on up to 10 ripe fruits for each genotype at each harvest date.

3.6.4 Statistical analysis

Results for strawberry fruit vegetative, productive, and qualitative parameters are presented as mean ± standard error (SE) for each cultivar/irrigation treatment. One-way analysis of variance is used to test the differences between cultivar and irrigation treatments. Statistically significant means differences are determined with LSD test ($p \leq 0.05$). Statistical processing is carried out using STATISTICA software (Stasoft, Tulsa, OK).

3.7 Results and discussion

3.7.1 Vegetative parameters

According to the Anova analysis, the treatment affects both the plant height and the number of leaves (Table 40).

Table 40: One-ways analysis of variance (ANOVA) for the vegetative parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction.

Parameter	Plant height	Leaves number
Treatment (a)	**	**

Leaves number

The irrigation level in strawberry causes very significant changes in growth and development of plants. Numerous researches studied the effects of water reduction on morphological aspects of the plant and the variability of responses by genotype (Grant et al., 2010). The reduction of the number of leaves per plant, leaf size, and leaf longevity are the results of strong water limitation (Shao et al., 2007; Razavi et al., 2008). Other parameters studied in Grant et al. (2010) are reduced significantly by water deficit: transpiration rate, dry mass of leaves and roots, and leaf water potential. Contrarly, an increase of root/crown dry mass ratio are registered.
In Klamkowski and Treder (2008) symptoms of water suffering correspond to smallest size of the leaf, which leads to diminished photosynthesis.

The number of leaves is another important parameter to evaluate the plant growth, in limited water availability. This parameter has been monitored from February to April 2019 (Figure 26). In general, all the genotypes, at each treatment, have produced few leaves during the first month, but the trend of plant vegetative growth increased in the following months. It is evident that, going forward with the season, the majority of genotypes show a higher leaves number in W72 than in others thesis. During the reporting period, "Rociera" stands out in respect to Ancona selections for a higher number of leaves at W72.

In the first month, "Rociera" showed the highest leaves number (13.17 ± 0.10), followed by AN13,13,55 (12.5 ± 0.71) and AN12,45,53 (11.33 ± 1.32) in the W72 trial. Also, AN13,13,62 showed am high leaves number, but in thesis W50.

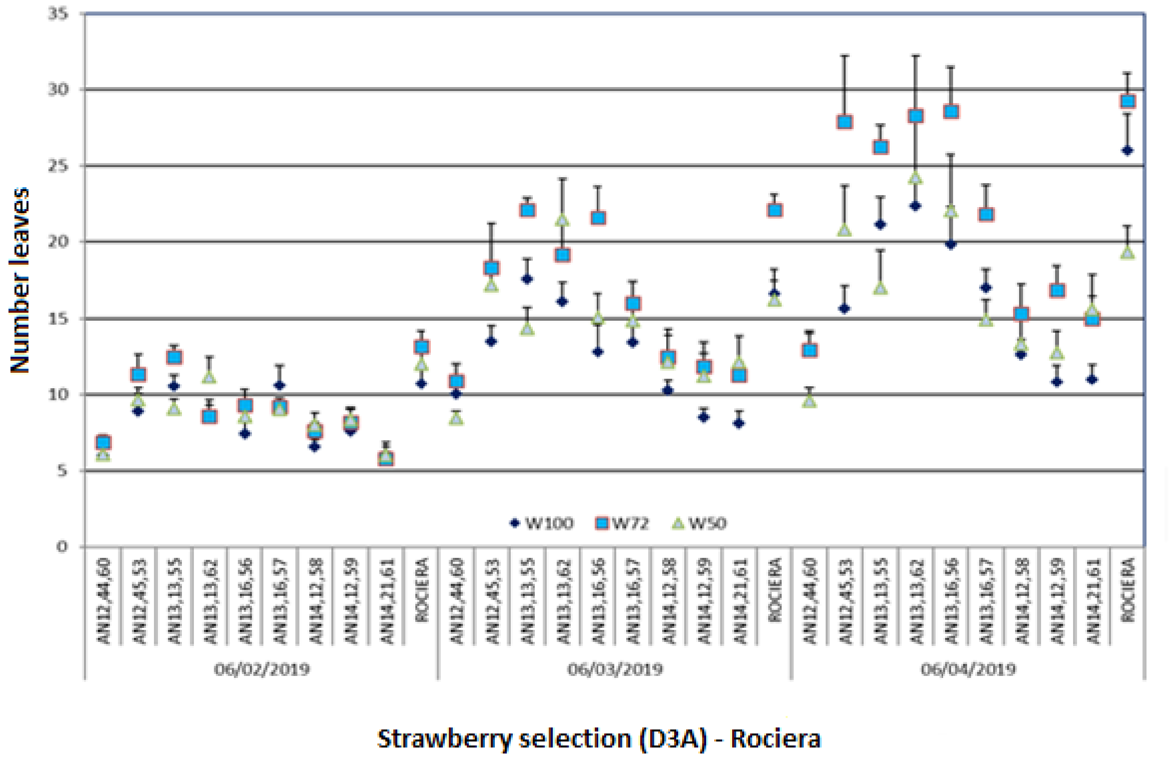
The previously mentioned selections showed a constant high leaves number also in the following month (March). In this case, also the selection AN13,16,56 is well developed in W72, with 21.58 ± 2.03 leaves/plant.

In the last month, AN13,16,56 (28.58 ± 2.87 leaves/plant), AN13,13,62 (28.27 ± 3.91), AN12,45,53 (27.91 ± 4.2) and AN13,13,55 (26.22 ± 1.42) stand out among the Ancona selection for the greater values of this parameters, while the highest leaves number was showed by "Rociera" (29.28 ± 1.76 leaves/plant), all of them at W72. In AN13,16,56, AN13,13,62, and AN13,13,55, the ratio of the total number of leaves among the three water treatments remained stable in respect to the measurement of March. Instead, in AN12,45,53 the gap among treatments increased, in advantage of W72.

If the W72 treatment incremented the leaves number for almost all the studied genotypes, the situation is different for the other two theses. Considering W100 and W50, the effect of genotype is interesting because in some genotypes the number of leaves is greater in the trial with less water amount supply: AN13,16,56 (W100: 19.83 ± 2.52 leaves/plant; W50: 22.08 ± 3.65 leaves/plant); AN13,13,62 (W100: 22.4 ± 1.51 leaves/plant; W50: 24.25 ± 4.33 leaves/plant); AN12,45,53 (W100: 15.67 ± 1.47 leaves/plant; W50: 20.83 ± 2.86 leaves/plant). Contrarily, "Rociera" (W100: 26 ± 2.4 leaves/plant; W50: 19.36 ± 1.68 leaves/plant) and other selections such as AN13,13,55 (W100: 21.17 ± 1.79 leaves/plant; W50: 17.00 ± 2.46 leaves/plant) showed a greater number of leaves in W100 than in W50.

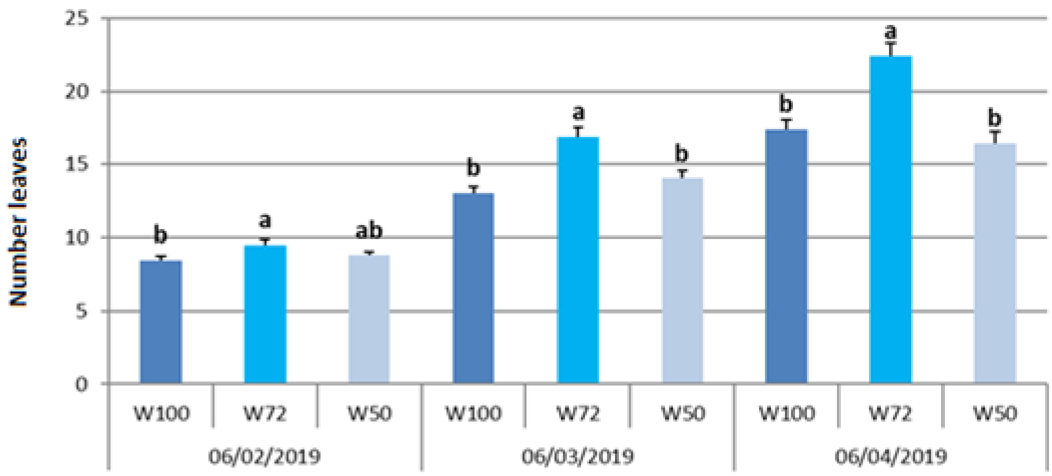
AN14,21,61, AN14,12,59, and AN12,44,60 stands out for their reduced number of leaves at each measurement period. Therefore, in April, the lowest leaves production for W100 was obtained by AN14,21,61 (11 ± 0.97 leaves/plant) and AN14,12,59 (10.83 ± 1.09 leaves/plant), while W72 and W50 was obtained by AN12,44,60, with 12.94 ± 1.07 leaves/plant and 9.61 ± 0.85 leaves/plant, respectively. The number of new leaves per plant with different water amount depends on the genotype and is also shown by Grant et al. (2010) through the 10 cultivars examined.

Figure 26: General overview of the number leaves of genotypes in responses to different amount of water (W100, W72, W50) during February, March and April 2019. The average number of leaves ± standard error (SE) for each cultivar/irrigation treatment are reported.



For a statistical comparison, the average leaves number for each treatment and for each month of measures was examined (Figure 27). These results showed that during the plants growth, until April 2019, W72 trial allows a higher leaves production in comparison to W100 and W50. In the first month, there is only a significant difference between W100 and W72; the difference of plants responses to water reduction is clearer after more months of different water supplies. In particular, in March, W72 (16.89 ± 0.61 leaves/plant) resulted in a significant higher number of leaves/plant in respect to W100 (13.01 ± 0.48 leaves/plant) and W50 (14.03 ± 0.54 leaves/plant), as well as in April (W72: 22.42 ± 0.88 leaves/plant; W100: 17.39 ± 0.69 leaves/plant; W50: 16.45 ± 0.80 leaves/plant). In the study conducted by Grant et al. (2010), the total number of new leaves is less under water-limited conditions. Shao et al. (2007) had previously shown the water deficit as a cause of decrease in the number of leaves. Instead, in our trial the decrease of the number of leaves is not explained by a low quantity of water (W50), because we have obtained similar results to the full irrigation trial (W100).

Figure 27: The average ± standard error (SE) n° of leaves of genotypes grown in different water treatments (W100, W72, W50) during three months of study. Average values for each parameter with same letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant.



Plant height

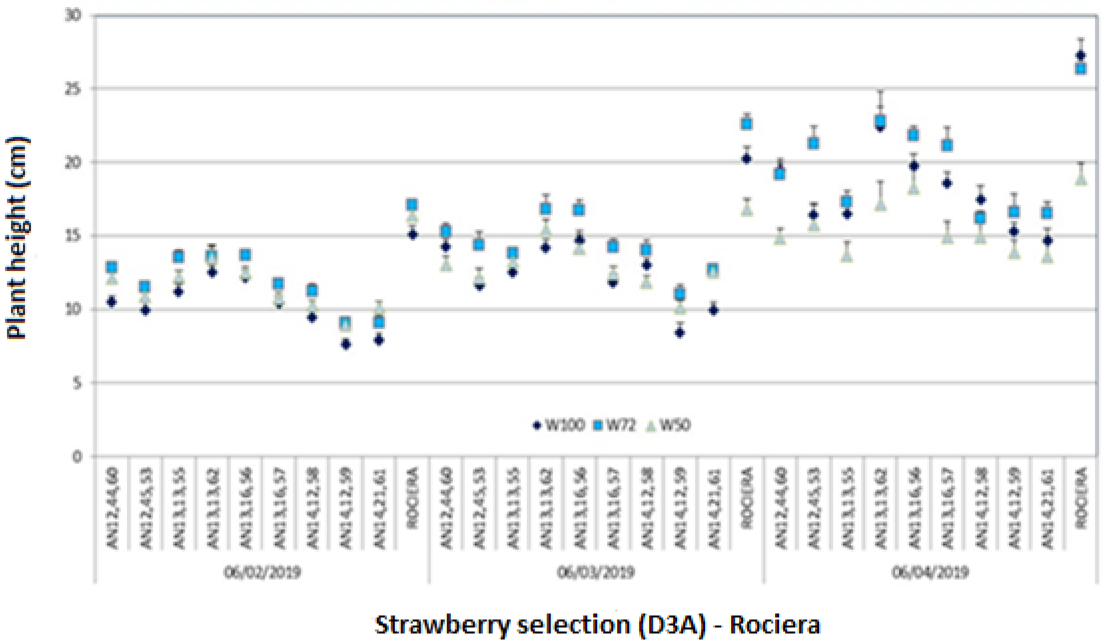
As for the leaves number, also the other indicator of the plant growth, the plant height, resulted higher in W72, in three months monitoring period (Figure 28). The plant height is closely linked to the quantity of photosynthesis and is very susceptible to the environmental conditions (Heschel and Rigions, 2005). The plant height is reduced under low water input as reported in Yuan (2004) and Grant et al. (2012).

In line with the results obtained for the leaves number, "Rociera" bare root plants revealed a stronger development than tip plants of the analyzed selections, for all measurements and for all the irrigation trials. In February, it showed a plant height of 15.08 ± 0.58 cm, 17.06 ± 0.37 cm, and 16.41 ± 0.32 cm at W100, W72 and W50, respectively. In March, it showed a plant height of 20.28 ± 0.75 cm, 22.58 ± 0.67 cm, and 16.83 ± 0.66 cm at W100, W72 and W50, respectively. Finally, in April, it showed a plant height of 27.28 ± 1.12 cm, 26.36 ± 0.63 cm, and 18.89 ± 1.03 cm at W100, W72 and W50, respectively. It is interesting to note the evolution of this parameter during the months of study. In all the genotypes analyzed, the plant growth constantly increased with the ongoing of the season at all the water treatments. Regarding the irrigation effect, it appears that at the first measurement the W72 and W50 treatments slightly increase the plant dimension in respect to control (W100). With the ongoing of the season, as demonstrated by the second and third measurements, it appears that the W72 trial continue to stimulate the plant development in respect to control, while W50 causes a plant height reduction in respect to W100, in particular at the third measurement.

In the first month of assessment, the selections with higher plant dimensions were AN13,13,55, AN13,13,62, and AN13,13,56, all of them at W72. This result is also in agreement with the previous analyzed parameter, giving that those three selections showed also a high leaves number of about 13 leaves/plant. This trend was maintained in the following months. In April, in fact, AN,13,13,62, and AN13,16,56 showed the higher plant heights among the selections, but also presented a high leaves number, with about 28 leaves/plant. In the last month, it is also possible to note the gap increase of plant development among water treatments.

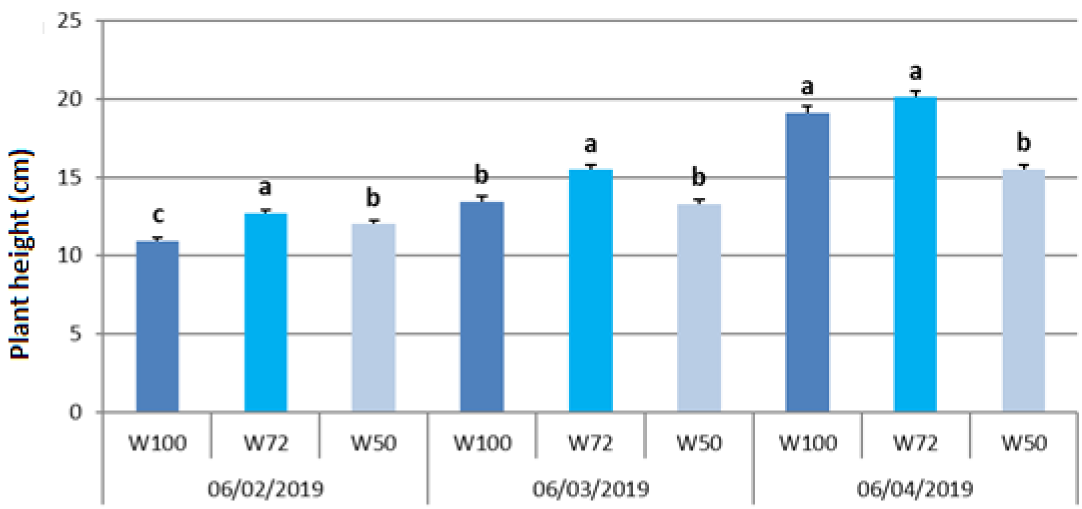
In general, it is possible to affirm that in the first month, W72 allowed a greater vegetative performance of the genotypes, in respect to W100 and W50. Furthermore, W50 has given slightly higher results than W100 for the plant height. In the following month, plants subjected to W72 trial showed the highest plant height, while for some genotypes, W100 showed better results than W50. Finally, in April, greater plant dimensions were reached again by the W72 trial, followed by W100 and then W50, which resulted with the smaller plants (AN13,13,55: 13.64 ± 0.91 cm; AN14,12,59: 13.88 ± 0.83 cm; AN14,21,61: 13.58 ± 0.90 cm.

Figure 28: General overview of the plant height of genotypes in response to different amounts of water (W100, W72, W50) during February, March and April. The average plant height ± standard error (SE) for each cultivar/irrigation treatment are shown.



For the statistical analysis, the average plant height for each water treatment, for each month of measurement, was considered (Figure 29). Interestingly, the period of growth has influenced the response of the plant to different water supply, especially to W100 and W50. In fact, during all the measurement periods, W72 has given the major vegetative vigour. Contrarily, the other treatments revealed opposite results according to the month of measurement. At the beginning, W100 gave smaller plant height values than W50, than they become similar in value during March, and finally W100 showed significantly higher plants than W50 and similar to W72.

Figure 29: The average plant height ± standard error (SE) of genotypes grown in different water treatments (W100, W72, W50) during February, March, April. Average values for each parameter with same letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant.



The possible explanation to those vegetative results is that the water requirement of strawberries plants is smaller than the usual amount given by the company (W100).

For both parameters, until March, W50 and W100 showed statistically similar results, indicating that the less growth of plant has been affected both by strong water reduction and full irrigation, respectively.

In April, the plants (W50) showed a cumulative number of leaves similar to W100 but with less growth in height, probably this parameter is influenced by water reduction. The decrease in height growth rates with increase in duration of low water input is demonstrated in Nezhadahmadi et al. (2015). In another study (Rizza et al., 2014), a limited water avability is a cause of lower plant height. These results provide concrete information on water use until April and underline the possibility to reduce the amount of irrigation water by 28% (W72), obtaining the great vegetative performance for all the analyzed genotypes.

The W100 water regime now used in most of the cultivation area of Huelva can be considered even excessive for the first period of cultivation. The reduced plant development observed for all genotypes during this period, in comparison with the other water regimes, can be induced by the effect of excessive water accumulation in the soil. The cultivation system needs to be revised in order to avoid this excessive application of water that beside the waste of water, it is also negative for the early stage development of the plants.

3.7.2 Productive Parameters

Anova analysis shows that the productive parameters are all influenced by the treatments, except the secondary production (Table 41).

Table 41: One-ways analysis of variance (ANOVA) for the productive parameters **= significant interaction with $p<0.01$; *= significant interaction for $p<0.05$; n.s.= not significant interaction

Parameter	Precocity Index	Average fruit weight	Commercial production	Secondary production	Total production
Treatment (a)	**	*	*	NS	*

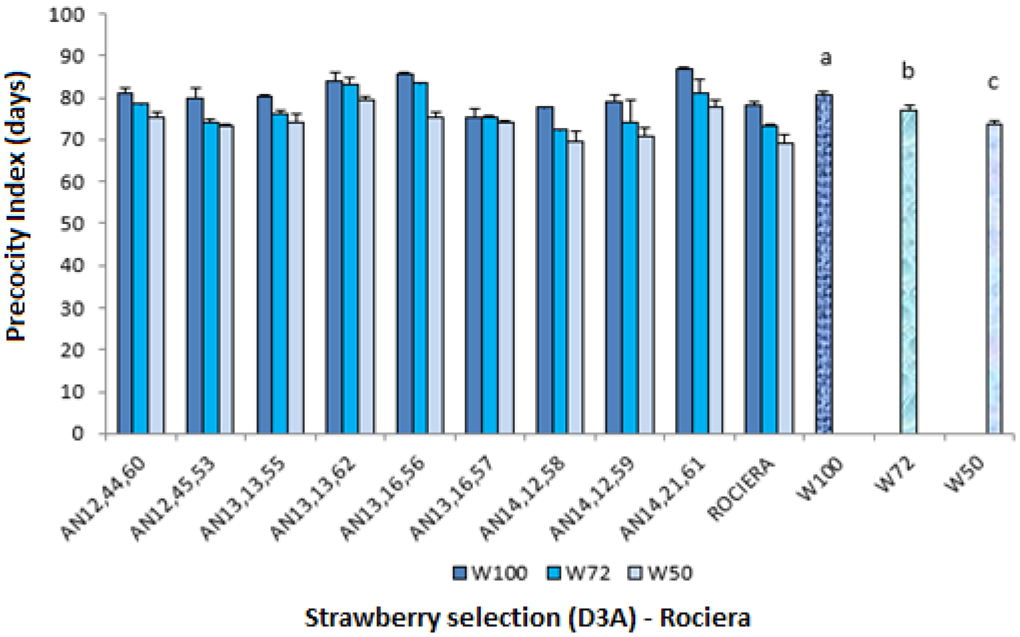
<u>Precocity Index</u>

The precocity index, together with high plant yield and average fruit weight and quality, and low percentage of second-class fruits, is an important parameter that can be taken into account in other to evaluate the interest of a new cultivar, in particular for the Spanish strawberry industry. In this study, the average precocity Index, in relation to the different treatments, resulted anticipated for plants cultivated at W50 (73.81 ± 0.74 days), followed by W72 (77.08 ± 0.90 days), and finally by W100 (80.77 ± 0.74 days). The average gap among the earlier W50 treatment and W70 and W100 treatments were four and seven days, respectively (Figure 30). Flowering times and fruiting are affected by the irrigation levels (Kumar et al., 2012). The results obtained from less irrigated plants show shorter times for the start of flowering and fruit maturing stage.

Clearly the genotype remains the main factor determining the earliness of a cultivar, but our results are evidencing how for this cultivation system it can be possible to increase the earliness of a genotype by applying lower water supply. This can be important for the Spanish production system mostly aiming to the earliest fresh strawberries on the market.

“Rociera” (69.17 ± 1.9 days), AN14,12,58 (69.3 ± 2.57 days), and AN14,12,59 (70.65 ± 2.26 days) have emerged for fruit’s early ripening in W50 thesis. Again, for the W72 thesis, the earlier genotypes were AN14,12,58 (72.28 ± 0.30 days), “Rociera” (73.41 ± 0.01 days) and AN14,12,59 (73.84 ± 5.58 days). Finally, in W100, the earlier genotypes were AN13,16,57 (75.39 ± 2.13 days), AN14,12,58 (77.65 ± 0.23 days) and “Rociera” (78.21 ± 0.71 days).

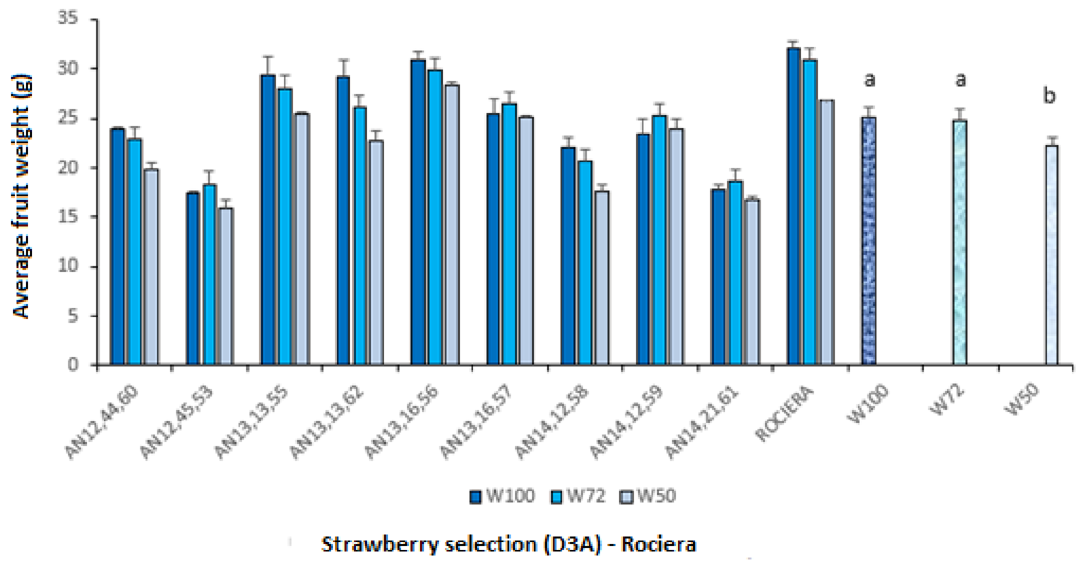
Figure 30: The average precocity index ± standard error (SE) for each genotype grown in different water treatment (W100, W72, W50). Average values for each parameter with same letter were not statistically different for Fisher’s LSD test (p<0.05). N.S.= not significant.



Average fruit weight

The average fruit weight is a fundamental parameter to be evaluated and is generally the most important for the producer when choosing the cultivar for the new plantation, because it is often an indicator of the plant productivity and therefore of the yield of the genotype. Particularly for this type of intensive strawberry production system, the higher fruit size is considered fundamental also to reduce picking costs, nowadays important because of the increasing labor costs. The susceptibility of the average fruit weight in connection to low water input is shown by a significant decrease in grams per fruit (Blatt, 1984; Kirnak et al., 2003; Kirschbaum et al., 2003). In the study of Kirnak et al. (2003), “Oso Grande” and “Camarosa” have produced a low fruit average weight with water deficit of 50%. In our study, the results of the average fruit weight are quite homogenous and describes good performance, despite the treatments (Figure 31). In fact, the fruit weight from plants irrigated at W100 (25.19 ± 0.96 g) was similar to those from W72 (24.7 ± 0.81 g), confirming that by reducing of 28% water supply, the fruit characteristics are not altered and maintain a high commercial value as for the control (W100). Only the thesis with very low amount of water (W50: 22.23 ± 0.79 g) negatively influenced the average fruit weight in a significant manner in respect to W100 and W72. The genotype effect is much stronger than the water treatment in determining the average fruit weight. Among the analyzed genotypes, "Rociera", AN13,16,56, AN13,13,55 and AN13,13,62 showed the best fruit weight in each treatment. But this last selection clearly showed a considerable fruit weight loss of 6% passing from plants treated with W100 to W50. The lowest weight is recorded for AN12,45,53 at each of the three treatments (W100: 17.42 ± 0.14; W72: 18.31 ± 0.30; W50: 15.90 ± 0.94).

Figure 31: The average fruit weight ± standard error (SE) for each genotype grown in different water treatment (W100, W72, W50). Average values for each parameter with same letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant.



Commercial production

The good effectiveness of water in terms of commercial production coincides with great yield potential, wich is predefined by flower initiation obtained in the previous autumn (Grant et al., 2010). The low availability of water can accelerate the vegetative growth and lead the plant to the formation of flower buds. Early fruit with small size can reduce the commercial production/plant. Thus, proper use of water during the flower initiation phase can contribute to a good future yield. In many studies (Mcneish et al., 1985; Peñuelas et al., 1992; Grant et al., 2010) was reported that reduction in yield occurred under conditions of not well watered soil.

In our study, differently from the Average Fruit Weight, the commercial production/plant is significantly influenced by the amount of water given. In general, the plants cultivated under the W72 thesis showed a production ratio significantly higher than plants cultivated at water regimes W100 and W50, which are statistically similar to each other.

This average difference in commercial production per plant could be quantified as -92 g for W100 and -109 g for W50 compared to W72, respectively (Figure 32). For all the tested genotypes, the greatest production is found for the plants subjected to the treatment with 28% less water amount (W72).

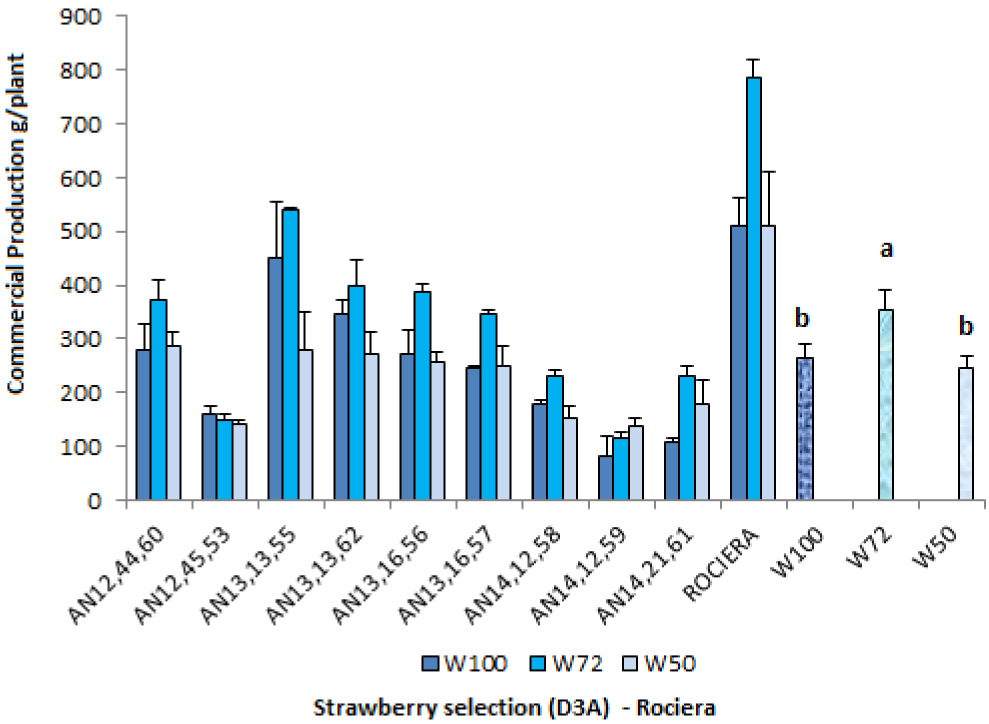
As for the previous parameters, “Rociera” positively stands out, with 784.0 g/plant, followed by three Ancona selections: AN13,13,55 (539.76 g/plant), AN13,13,62 (396.46 g/plant) and AN13,16,56 (389.0 g/plant). Also in this case, the difference observed with “Rociera” can be determined by the different type of plants used at the plantation stage.

As for the Average Fruit Weight, also for this parameter different genotypes respond differently to the irrigation water amounts, allowing to check the most sensitive plants to water reductions. In respect to W72, “Rociera” produced 273 g/plant less at both W100 and W50 theses. With greater water reduction (W50), the AN13,13,55 selection showed a strong susceptibility, with substantial production decrease of 295 g compared to W72 but with not significant difference among W100 and W72; the selection AN13,16,56 resulted less susceptible, with a reduction of “only” 133 g in respect to W72.

The selections that in April achieved very low levels of production (below 300 g/plant), even at W72, were AN14,12,58, AN12,45,53, AN14,21,61, and AN14,12,59.

A possible explanation for the great difference in commercial production between Rociera and Ancona selections could be found on the different types of plants used for the plantation; in fact, for the Ancona selections bare rooted plants were used, while for “Rociera” rooted tops plants were used. For the same reason, in the vegetative stage the development of “Rociera” plants was stronger compared to the selections, regardless of the water treatment considered.

Figure 32: The average commercial production ± standard error (SE) for each genotype grown in different water treatment (W100, W72, W50). Average values for each parameter with same letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant.



Secondary production (discarded fruits)

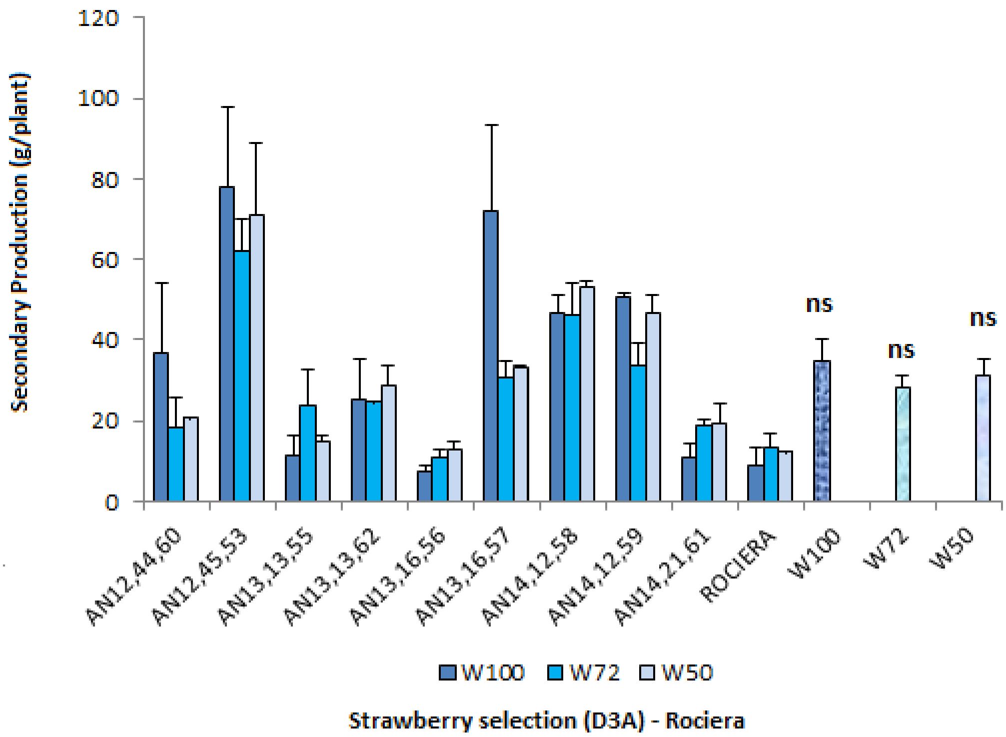
The average secondary fruit production of the tested genotypes showed no significant differences among water treatments (Figure 33). This finding is confirmed also by Martínez-Ferri et al. (2016), which showed that the not-marketable production in strawberry plants (fruit size<10 g) are not influenced by water treatment and it represented no more than 28% of the total production. According to the genotype, Ancona selections have produced a higher amount of discarded fruit, mostly due to lower size fruit, in comparison to the commercial cultivar "Rociera". Also for this parameter the main variation between cultivar and breeding selections can be related to the different type of plants.

Some selections presented a high amount of discarded fruit, mostly due to the low size of the fruit, in respect to the total production achieved: AN14,12,59 had the 38% of discarded fruit on the total production, followed by AN12,45,53 with 32% and AN13,16,57 with 30%.

The best selections with the highest total production (at thesis W72) are the same with the most relevant commercial production (again at W72); this result was determined by the low impact of the % of discarded/small fruit on the production. In particular, the amount of waste was quantified as 6% of the total production for AN13,13,62, 4% for AN13,13,55, and 3% for

AN13,16,56. “Rociera” showed only the 1% of secondary production in respect to the Total Production. This result confirms the potentiality of the cultivar but also the positive effect of the bare root fresh plants on plant yield and fruit size.

Figure 33: The average fruit secondary production ± standard error (SE) for each genotype grown in different water treatment (W100, W72, W50). Average values for each parameter with same letter were not statistically different for Fisher’s LSD test (p<0.05). N.S.= not significant.



<u>Total production</u>

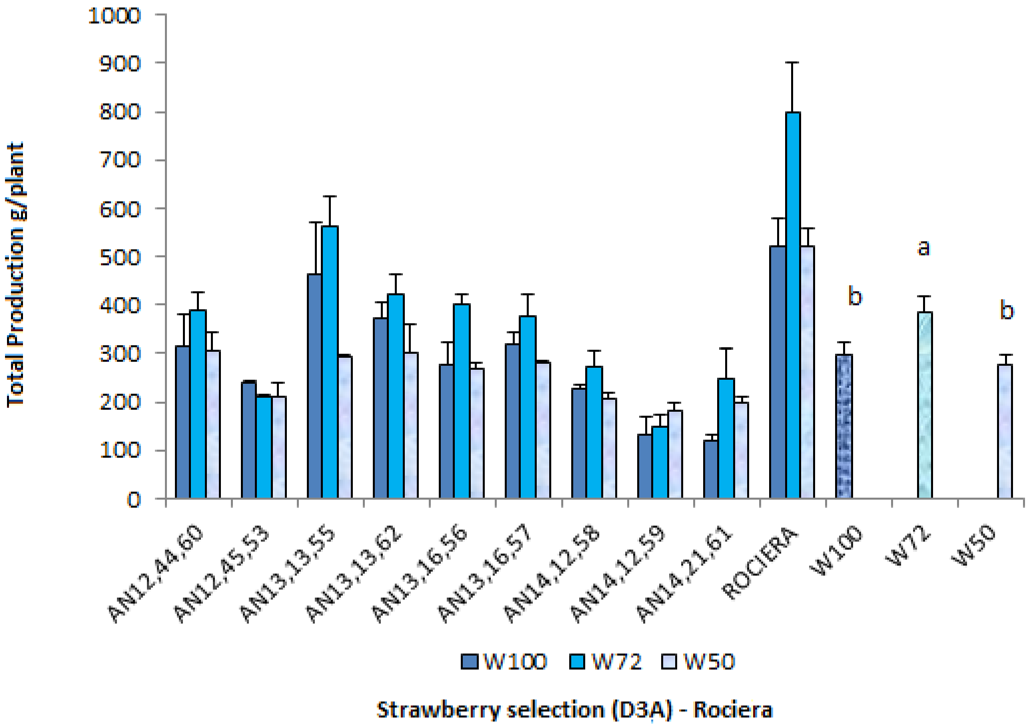
In the literature (Renquist et al., 1982; Save et al., 1993; Krüger et al., 1999; Liu et al., 2007) the irrigation improved total and commercial production, and the number of berries.

The average total production registered for the tested genotypes in our study is in line with the results for the commercial production.The plant yield registered during the harvest season (until April) seems to be positively influenced by the irrigation trial, with plants cultivated at W72 regime showing significant better results compared to full irrigation (W100) and treatment with 50% of decrease in water (W50), which result statistically similar to each other (Figure 34). Therefore, the average total production decreases by 86 g in W100 and by 106 g in W50, in comparison to W72. The volumes of water administreted in Lozano et al. (2016), similar to our study, have determined a negligible effect on the strawberry’s yield.

In Khan et al. (2017), the plant yield and fruit quality are influenced by physiological and biochemical agents that are sensitive to water limit conditions. Many research activities show how water deficit leads to a reduction in yield (Bordonaba and Terry, 2010; Nezhadahmadi et al., 2015; Kachwaya et al., 2016; Adak et al., 2017). Reduction yield of 36% is given by medium water stress (Save et al., 1993).

In our study, the maximum total production has been reached by “Rociera” with 797.5 g/plant, followed by selections AN13,13,55 (with 563.72 g/plant) and AN13,13,62 (421.3 g/plant). Taking into account the treatments, the productive loss of “Rociera” is of 36% for both W100 and W50. For the above-mentioned selections, the W100 negatively influenced the production of AN13,13,62 (-18%) and AN13,13,55 (-12%), as well as W50 (-48% and -29%, respectively). The behavior of “Rociera” bare-root fresh plants is really of high interest because demonstrates how the full water management, in particular in the early stage of plant development, is having a negative effect on plant yield, while the most accurate management of water supply during the early period and during the entire picking season can have really a positive effect in inducing a significant increase of yield (+36%) while saving the 28% of water, that correspond to -2458 m^3 of water per ha.

Figure 34: The average fruit total production ± standard error (SE) for each genotype grown in different water treatment (W100, W72, W50). Average values for each parameter with same letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant.



3.7.3 Qualitative parameters

Anova analysis shows that the soluble content is not affected by the water treatments (Table 42).

Table 42: One-ways analysis of variance (ANOVA) for the qualitative parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	Soluble Sugar Content
Treatment (a)	NS

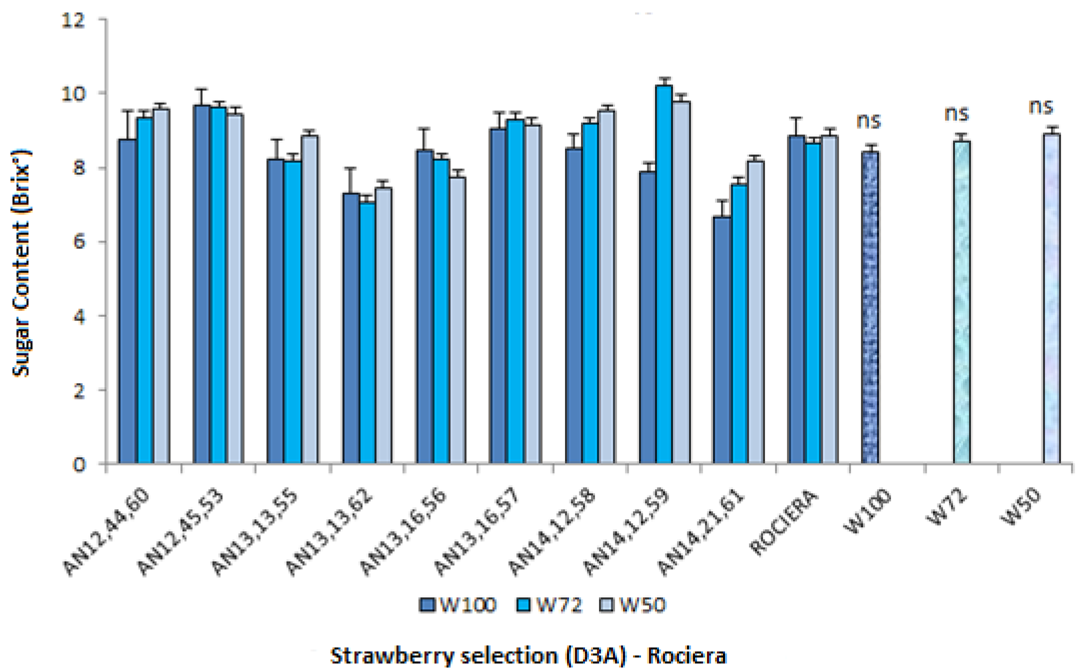
Soluble Sugar Content

If the vegetative and productive parameters are particularly appreciated by the industries, the customer satisfaction depends mostly on qualitative parameters of fruit, as Soluble Sugar (SS-°Brix) and Total Acidity. The sugar content of fruit does not seem to be influenced by the irrigation amount, differently to what happened for vegetative and productive parameters (Figure 35). In literature, is reported that sweeter berries are originated by plant subjected to strong water reduction (Terry et al., 2007; Bordonaba and Terry, 2008). In stressed plant, greater concentration of monosaccharides (fructose and glucose) are found, thus berries result sweeter (Nora et al., 2012).

Considering all the genotypes analyzed, fruit SS ranges from 6.7 °Brix (AN14,21,61) up to 10.2 °Brix (AN14,12,59). Only this latter genotype seems to be strongly influenced by different water irrigation amounts, with a reduced value at W100 (7.91 °Brix), then increased up to 10.2 °Brix at W72.

Differently from the other parameters, "Rociera" did not differed from the other selections in term of fruit SS values but remained stable at changing the water treatment. Among selections, AN13,16,57 and AN12,45,53 had similar high and stable values of fruit SS. While higher values were detected for AN14,12,59 when treated with W72 treatment, thus confirming this water treatment as the best for improving fruit quality of this genotype.

Figure 35: The average sugar content (°Brix) ± standard error (SE) for each genotype grown in different water treatment (W100, W72, W50). Average values for each parameter with same letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant.



3.8 Conclusion

The main purposes of this project can be summarized in: 1) Optimization of the plant performance, in terms of vegetative, productive and qualitative parameters through the

appropriate water supply; 2) Individuation of genotypes that maintain good yield and quality with reduced irrigation amount. The added value of this experimental research is to meet the demand for economic and environmental sustainability of the strawberry intensive cultivation.
The first important result of the study is the demonstration the current cultivation protocol used for "Rociera" bare-root fresh plants is using too much water, in particular in the early stage of development, affecting the yield potential of the cultivar. The W72 treatment tested in this trial resulted much more appropriate for the cultivation of "Rociera", resulting in 32% increase of plant yield. This benefit combined with the save of water use and labor work for irrigation results with a high economic benefit for the company at a reduced environmental impact.
"Rociera" (W72) in the last month of trial, is the most vigourous genotype and is charactherized by the highest commercial production and average fruit weight. In addition, "Rociera" (W50), strongly anticipates the harvest time.
The positive impact of the 28% reduction of water use (W72) was detect also for the breeding selections and under this trial, some selections had a plant vegetative and yield performance near to "Rociera", even if the different type of plant used (fresh and plug plants) can have a high impact in inducing a different vegetative performance independently to the genotype.
As expected, the different breeding selections showed a wide variability in response to the irrigation treatments. Among those, AN13,13,55 resulted of high interest for the vegetative development and yield, almost reaching "Rociera" values, at a quite high and stable fruit quality. This selection presented also a very interesting average fruit weight, as well as AN13,16,56, showing values very close to "Rociera". AN13,16,56 presented also an interesting vegetative development with similar values to the Spanish cultivar, as well as AN12,45,53 (very interesting also for the sugar content). AN14,12,59 and AN14,12,58 resulted the most interesting for the high soluble solids content. This study could represent a good starting point for an optimal water management, which could allow water saving, preserving economics and environmental aspects.

4 RESEARCH LINE II: IDENTIFYING STRAWBERRY CULTIVARS WITH REDUCED NITROGEN UPTAKE

Abstract

Genotype, planting date, mulch type, temperature, fertilizers and production systems strongly influence the strawberry yield and quality (Anttonen et al., 2006). Therefore, an adequate supply of essential nutrient can significantly improve plant growth, fruits quality and their nutritional values (Marschner, 2011). On the light of this, my research activity was mainly focused on the evaluation of the plant vegetative development, the production and the fruit quality of strawberry genotypes fertilized with different amount of nitrogen (N100, N80, N60), in a protected crop. The study consisted of two trial conducted during the 2016-17 and 2017-18 cycles for Single-cropping ("Cristina", "Romina", "Sibilla") and 2018-19 cycle for Remontant ("Albion", "Monterey", "S. Andreas") cultivars. Nitrogen rates were 113-90-68 Unit N/ha for the first year, 118-97-76 Unit N/ha for the second year of single cropping. Nitrogen amounts were 108-87-65 Unit N/ha for the remontant cultivars. The reduction of nitrogen did not affect the height of the plant for single cropping, while resulted in a greater development for Remotant. Not significant differences were detected for the total production and average fruit weight for both types of plants. Remontant cultivars had a lower concentration of sugar at N60, while stable values were detected for single cropping varieties. The ripeness of the fruits is delayed with administration of N80 for all the remontant genotypes. The results obtained could be of interest for the farmers, indicating the optimal amount of nitrogen to apply to maximize the plant performances and the fruit quality, and to guarantee environmental sustainability.

4.1 Introduction

The vegetative, productive and qualitative parameters are influenced by nutritional status of strawberry plant (Yavari et al., 2009; Maathuis et al., 2013). Adequate levels of nitrogen (N), phosphorous (P) and potassium (K) are essential for proper plant growth and development (Boyce and Matlock, 1966; John et al., 1975). The amounts of fertilizer supply for strawberries in Italy are mainly based on farmers' experience and sensitivy and there is often an abuse of some nutrients. Plants that are efficient in absorption and utilization of nutrients, greatly enhance the efficiency of applied fertilizers, reducing cost of inputs and preventing losses of nutrients to the ecosystems (Tagliavini et al., 2004; Baligar et al., 2007). Therefore, the studies on the vegetative, productive and qualitative plant responses at different nutrient inputs are necessary to achieve correct supply of nutrients. It is important to define amount of nutrients to obtain the maximum performance of the plant with a low environmental impact.

Nitrogen is one of the essential mineral nutrients for proper growth and higher biomass yield of the plant (Bacon, 1995). It plays a vital role in the biochemical and physiological functions of the plants, contributing substantially to an increase in plant yield and fruit quality (Saadati et al., 2013; Leghari et al., 2016; Muhammad et al., 2018). Strawberries grown in open field (Hochmuth and Albregts, 1994) or in green house (Papadopoulos, 1987) have low nitrogen request. Contrarily, strawberries are susceptible to excessive N rates, especially during the fruits formation, when many problems could raise, such as a rapid fruit growth, delayed ripening, lower yields, an increasing acidity and fruit water content (Stadelbacker, 1963; Voth et al., 1967; May and Pritts, 1993). These factors lead to a reduction of fruit shelf-life, because softer fruits are more susceptible to diseases. Instead, the N deficiencies show yellowing of the older leaves and limited runners' production. Therefore, nitrogen supply needs to be closely monitored and balanced with other nutrients. The total rate of N required will vary with variety and growing system. The aim of this work is to compare six varieties on the different responses to nitrogen input reduction, through the evaluation of vegetative, productive, and qualitative characteristics. Therefore, this trial is focused on three (early, intermediate, and late) Single-cropping varieties ("Romina", "Sibilla", and "Cristina"), during the first two years, and three Remontant varieties ("Albion", "Monterey", and "S. Andreas") in the third year.

4.2 Material and methods

4.2.1 Field trial

The site, the experimental design and the management of the trial were the same of the first research line of my thesis (Chapter 3.2.3), but in this trial three different amounts of nitrogen instead of three different water regimes were applied.

The trial for the identification of strawberry cultivars with reduced nitrogen uptake was conducted for three years, of which the first two cycle of cultivation (2016/2017-2017/2018) were dedicated to Single-cropping cultivars, while in one cycle of cultivation (2019) Remontant varieties were tested.

4.2.2 Plant material

Single-cropping strawberry cultivars under evaluation in the 1nd and 2nd years of trial

The varieties object of study during the first two cultivation cycles (2016/2017 and 2017/2018) were "Romina", "Sibilla" and "Cristina". The accurate description of each variety has been already provided in the previous chapter (Chapter 3.2.2). The plant material is composed of "cold stored plant", category A+. In the first year, the planting took place on 28/07/2016 in open field, covered with a plastic tunnel on 24/02/2017 and the fruits were harvested in spring 2017. The same experiment was run for another cycle (2017/2018): planting (26/07/2017) and coverage of the green house (21/02/2018).

Remontant strawberry cultivars under evaluation in the 3nd year of trial

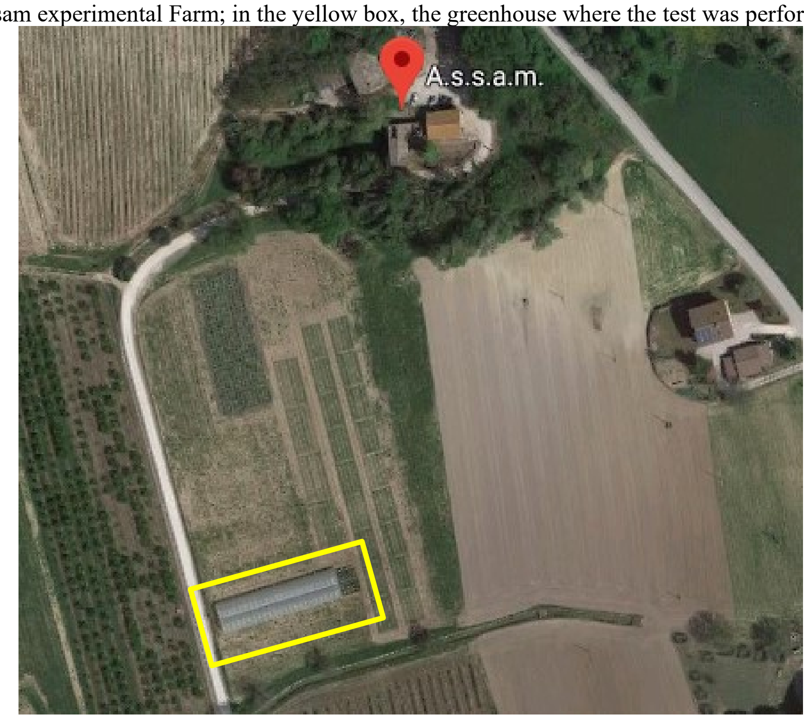
In the season 2019, the Remontant varieties "Albion", "S. Andreas", and "Monterey" were analyzed. The plants used were cold stored (category A+ for "Albion" and "Monterey", category A++ for "S. Andreas"). The varieties were planted on 24/04/2019 directly under green house and fruit collected in summer 2019. Also in this case, the accurate description of varieties has been made in the previous chapter (Chapter 3.2.2).

Single cropping and Remontant were produced by nursery, called Coviro Soc. Cons. a r.l. (Cervia, Italy). This is an agricultural nursery company that sells certified plants for the fruit production field.

The plants were placed in 3 different rows, each of 54 meters length, each row corresponding to a different amount of nitrogen supply (100% N - 80% N - 60% N). The experimental design was realized according to the split plot model, with 3 different levels of nitrogen supply (main plots) and 3 varieties (sub-plots). Two lysimeters were installed at each of three rows with the different nitrogen treatments. The lysimeters were positioned in the soil between two plants, at different depths (15 cm and 35 cm). These depths corresponded to the area of roots exploration and below. Lysimeters were used to sample soil circulating solutions; then these samples were analyzed through ion chromatography for detecting anionic and cationic species content (Figures 36 and 37).

Figures 36-37: The measurement site and lysimeter probes.

Figure 38: Assam experimental Farm; in the yellow box, the greenhouse where the test was performed is indicated



During the three years of the nitrogen input reduction test, a rotation of the plantation was applied, so that every year the field trial was placed in the same farm, but in different places in respect to the previous years' cultivation (Figure 38).

Nitrogen Fertilization amounts

Total nitrogen application during the first two years of trials (from August to June), when the Single-cropping cultivars have been object of study, have been maintained as homogeneous as possible between the two years (Table 43). The nutrient equilibrium has been obtained with 10-52-10 (N-P-K) from August until March, and 20-20-20 (N-P-K) from April to June. The third year of the trial (April to September) has been focused on Remontant varieties. The nutrition supply has been formulated in this way: 10-52-10 (N-P-K) from April to July, and 20-20-20 (N-P-K) from July to September. The total amounts of N fertilization for the N100 trial were decided according to the guidelines from the Delibera 786 of 10/07/2017 (Marche Region).

Table 43: The Unit N/Ha used during the cycle of crop (1° year-2° year-3° year) in the three different nitrogen trials (N100, N80, N60).

NITROGENOUS FERTILISATION			
YEAR	**N100 (Unit N/Ha)**	**N80 (Unit N/Ha)**	**N60 (Unit N/Ha)**
1°Single cropping	113	90	68
2° Single cropping	118	97	76
3° Remontant	108	87	65

4.2.3 Analyzed parameters

Water analysis

The soil circulating solution available to the plants has been sampled and analyzed for the first year of trial. This investigation took place in 3 main phases:

- Solution sampling. Before to start the laboratory analyses, it was necessary to perform a delicate sample process. Its accuracy could significantly influence the analytical results. Knowledge of correct practices of sampling and type of most suitable container is fundamental. The sample collected should be representative of the soil area to investigate. The solution samples have been picked up at two different depths of the soil (15 cm and 35 cm), through the lysimeter previously placed. Thanks to the low-grade depression created inside the lysimeter (through manual vacuum pump), the soil solution was retrieved. The ceramic tip allowed to pass the water for later sample extraction. The sampling was carried out after the operation of fertigation. For the Single-cropping cultivars, the fertilization was repeated at the same dates during the two years of trial. In the period of nitrogen shortage, three samplings per month were performed.

- The EC (Electrical Conductivity) of the soil solution samples was measured through Wtw 340 conductivity meter. The conductivity is used to measure the ionic concentration and activity of the solution. The more salt, acid or alkali are present in a solution, the greater is its conductivity. The unit of measurement of EC is S/m, often also S/cm.
- The last analysis was conducted through ion chromatography or IC, an analytical technique for the selective ion separation and determination. This technique is based on a liquid-solid chromatographic interaction, in which the liquid (mobile phase or eluent) containing the solution to analyze interact with a solid phase (stationary phase or column) allowing the separation of the individual components. The sample is injected through an injector in the mobile phase, which flows constantly, then enters in the column. The analytes present in the sample cross the column at different speed, according to their interaction with the inner membrane of the column, then pass through the detector at different times. The analysis of chloride, bromides, nitrate and sulphate were realized with ion chromatographer Dionex ICS1000 connected to a laptop.

  In detail, the samples are previously filtered at 0.22 micron, then injected in carbonate and bicarbonate buffer mobile phase flow and pumped through two different types of anion exchange columns (pre-column and column). The anions are separated on the basis of their different affinity to active resin sites. Then, from the end of the column, separated ions flow through a suppressor that converts every anion in the corresponding acid form. This allows a more accurate measure of the ions through the conductivity cell (the detector). The signal revealed by the detector is now sent to a computer, to obtain the chromatogram. The unknown anions are qualitatively identified comparing their chromatographic retention times peaks with those of known anion standards solutions. Then, the quantitative determination is so possible through the comparison between the measured area of the anion peak sample with calibration curve obtained by the area of standard anion concentration in mg/l.

Vegetative parameters

The analytical methodology used in this trial are the same indicated for the first research line (Chapter 3.2.4).

The vegetative parameters were measured to evaluate the effect of different nitrogen amounts: n° branch crowns/plants, n° inflorescences /plant, n° and size of leaves/plants, plant height. One measure was performed during the nitrogen trial period for each year of trial (27/04/2017 first year single cropping - 30/04/2018 secod year single cropping - 7/08/2019 first year remontant). Measurements were made for 8 plant in each subplot included in the three main plots (treatment). Each subplot is replicated 3 times for each variety. The values obtained in two years for single-cropping cultvars (2017-2018) were averaged.

Productive parameters

The analytical methodology used in this trial are the same indicated for the first research line (Chapter 3.2.4).

The main productive parameters were evaluated for the different nitrogen regimes: precocity index (IP), total and commercial production, waste, average fruit weight (AFW). During the period of the greater production, two harvests were performed each week (Tables 5 and 6). The productive data of each cultivar (total, commercial and waste production) are expressed as average plant production (the plot production for each harvest is divided by the number of plants present in the plot). Then, these values are summarized for all harvest until the end of the season. Starting from the third harvest, twenty uniform fruits for size and ripeness degree were collected from each plot, for three consecutive harvests, for the qualitative analyses.

Qualitative parameters

The analytical methodology used in this trial are the same indicated for the first research line (Chapter 3.2.4).

The main qualitative parameters were evaluated for the different nitrogen regimes: Sugar content, Titratable acidity (TA), Fruit color: L* (luminescence), a* (red tone), and b* (yellow tone), Chroma index, Firmness.

4.2.4 Statistical analysis

Results for strawberry fruit vegetative, productive, and qualitative parameters are presented as mean ± standard deviation (SD) for each cultivar/nitrogen treatment. Two-way analysis of variance is used for Single-cropping cultivars to test the differences among cultivation years, cultivar, irrigation treatment, and corresponding interaction. Two-way analysis of variance is used for Remontant cultivars to test the effect of cultivar and treatment, and corresponding interaction. Statistically significant means differences are determined with Fisher (Least Significant Difference, LSD) test ($p \leq 0.05$). Statistical processing is carried out using STATISTICA software (Stasoft, Tulsa, OK).

4.3 Results and discussion

4.3.1 Water analysis

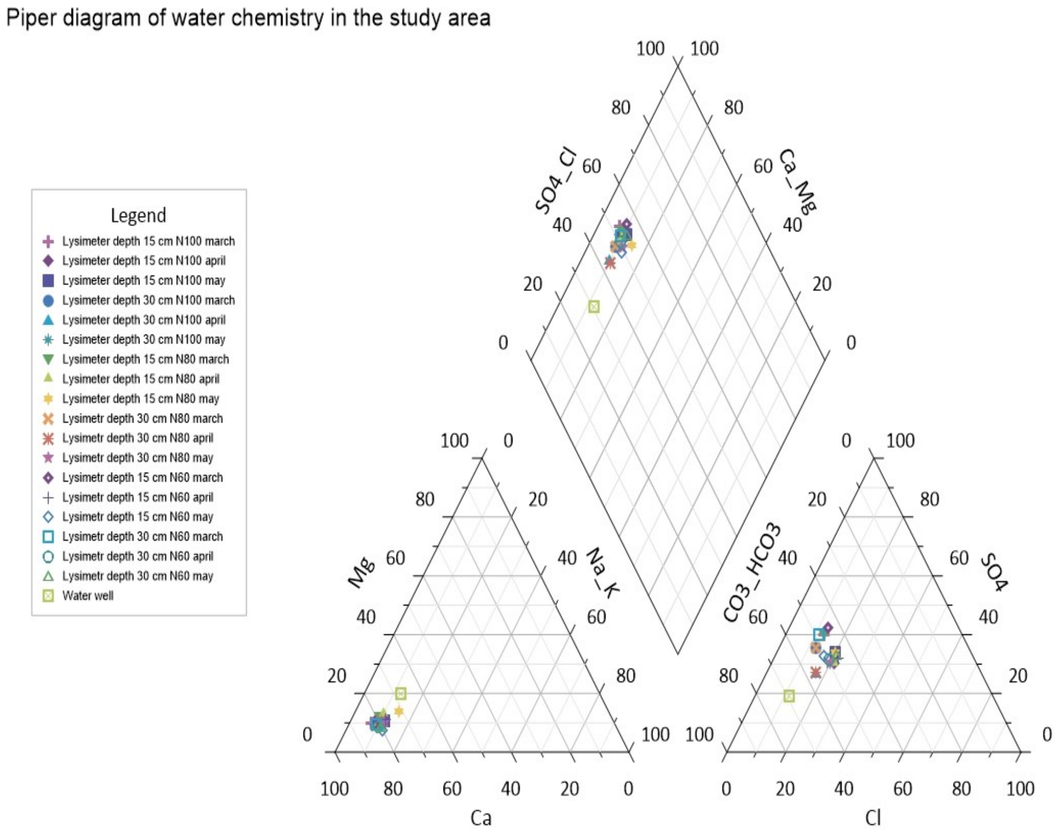
The analysis of irrigation water reveals some soluble salts, in function of the water source. The suitability of water to a specific purpose depends on the types and amounts of dissolved salts. Some of the dissolved salts or other constituents may be useful for crops. However, the quality or suitability of waters for irrigation purposes is usually assessed in terms of the presence of undesirable constituents, and only in limited situations the irrigation water is assessed as a source of plant nutrients. Some of the dissolved ions, such as NO_3, are useful for crops (Roy et al., 2006). The most important characteristics that determine the quality of irrigation water are: pH; total concentration of soluble salts assessed through EC; relative proportion of Na to other cations such as Ca and Mg, indicated as the sodium adsorption ratio (SAR); concentration of B and other elements that may be toxic to plants; concentration of carbonates and bicarbonates in relation to the concentration of Ca and Mg, indicated as residual sodium carbonate (RSC); content of anions such as chloride, sulphate and nitrate (Xu et al., 2019).

The piper diagrams were created to show the relative abundance of common ions, that are present in water samples (Figure 38). This graphic allows to show more samples from the same area, grouping them in terms of chemical composition. The piper is composed of three components:

- Ternary Diagram, on the lower left, that represent cations (Mg, Ca, Na + K)
- Ternary Diagram, on the lower right, that represent anions (Cl, SO_4, HCO_3 + CO_3)
- Diamond Diagram, in the middle, that is a combination of previous diagrams.

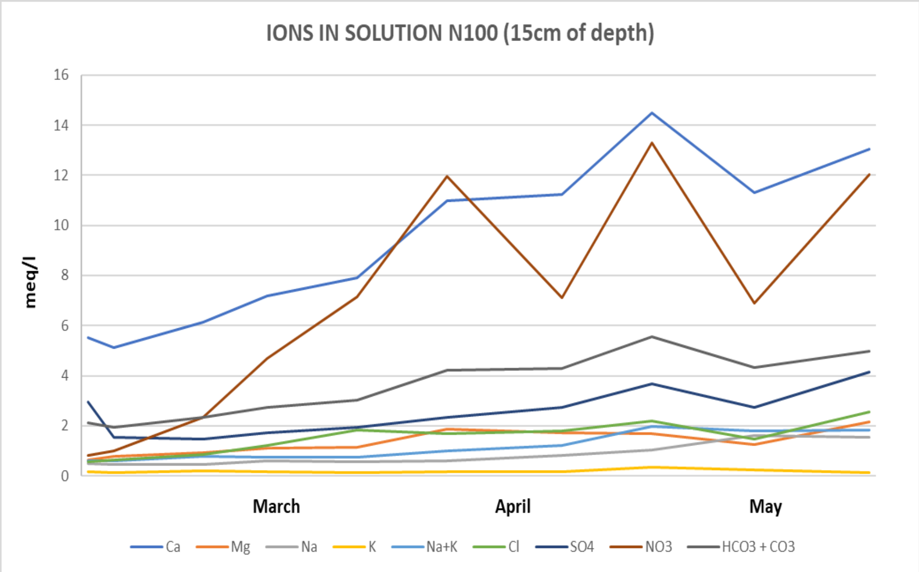
The concentration of each ion is expressed on percentage (meq/l). Percentages indicated in the graph are related to the soil circulating solution samples taken from lysimeter placed in field for the trials of N100, N80, N60 at two different depths (15 cm and 35 cm) and to the water taken directly from well (Figure 39). The water samples composition tends to the calcium bicarbonate, while water well is lightly towards bicarbonate. According to the Diamond diagram, the majority of lysimeter water is in the quadrant around 60% calcium sulfate. The variations in the chemical ratio characteristics among treatment and different depths of sampling are not shown.

Figure 39: Piper diagram of water chemistry in the study area.



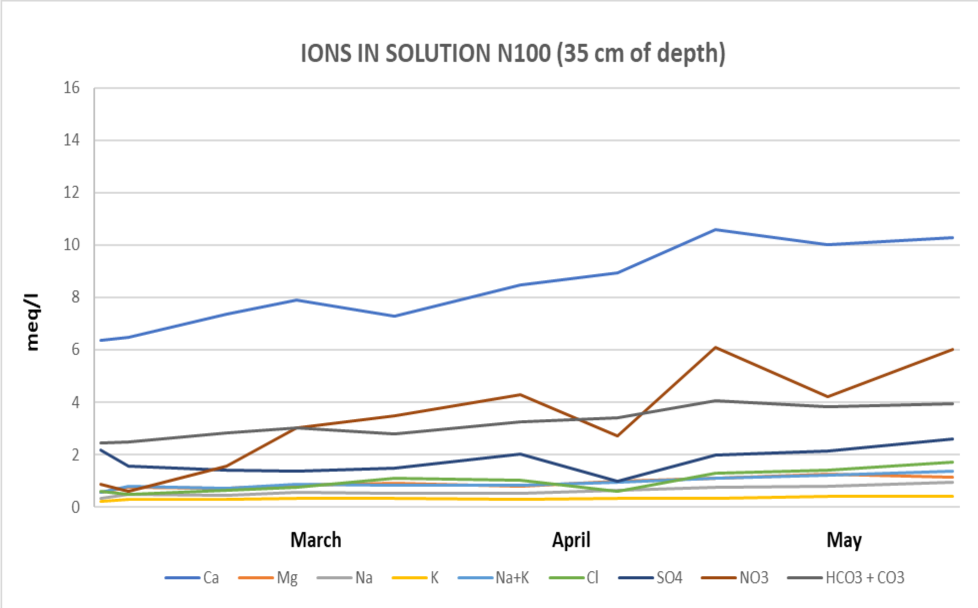
On the following graph (Figure 40) the trend of the ionic concentration, specifically cations (Ca, Mg, Na, K, Na + K) and anions (SO_4, NO_3 HCO_3 + CO_3), in the soil circulating solution for the treatment N100, at depth of 15 cm, is reported. From the second week of March toward April and May, it is possible to find a light increases of ionic concentration, due to fertigation. The more evident trend is for Calcium and Nitrate: Calcium ranges from 5.1 meq/l to 13.2 meq/l, with a monthly average of 7.4 meq/l in March, 11.5 meq/l in April, and 12.16 meq/l in May. Nitrates range from 1.94 meq/l to 12.04 meq/l, with a monthly average of 1.94 meq/l in March, 9.88 meq/l in April, and 9.46 meq/l in May. Those trends may be related to the phases of plant development: the stronger uptake of these element from the plants occurs on March, while is stable in the following months. The sharp peaks appearing in the graphic are due to a big lag time between treatments. This is clear in the nitrates level of 10th and 20th of April, respectively of 11.96 and 7.10 meq/l. The same trend was registered between 26th of April and 5th of May, with respectively 13.29 and 6.88 meq/l of nitrates. The concentration of the other elements appears to be quite linear during the trial. Magnesium ranges from 0.65 to 2.15 meq/l; Sodium from 0.45 to 1.67 meq/l; Potassium from 0.13 to 0.36 meq/l; Chloride from 0.56 to 2.56 meq/l; Sulphate from 1.45 to 4.40 meq/l, and finally Bicarbonate from 1.95 to 5.05 meq/l. Only the last two ions showed slightly evident fluctuations.

Figure 40: Ions in solution N100 (15 cm of depth)



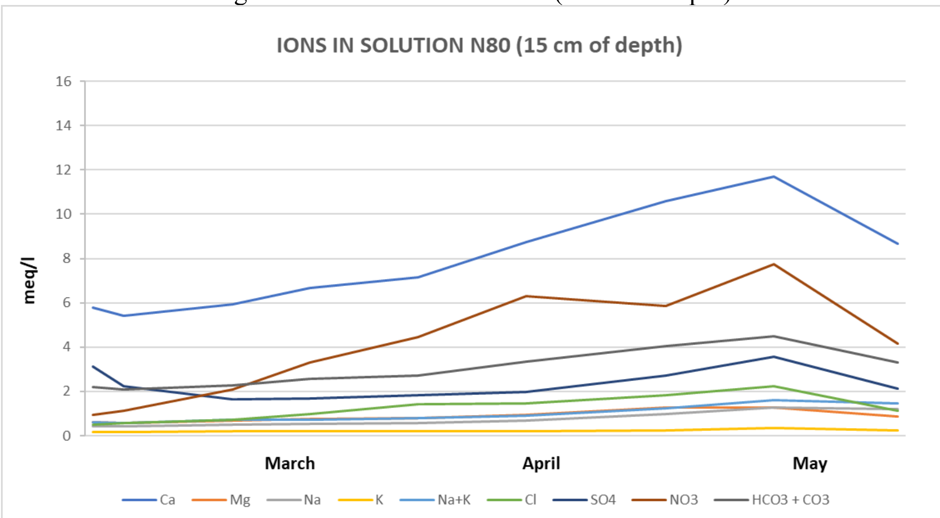
The trend of ionic concentration of soil circulating solution for the same trial, but at depth of 35 cm, showed results similar to those obtained at 15 cm, but at lesser extent (Figure 41). In particular, Calcium and Nitrate ions resulted prevalent again, with a similar but more linear trend than at 15 cm. Calcium ranges from 6.34 to 10.02 meq/L, with a monthly average of 6.46 meq/l in March, 8.82 meq/l in April, and 10.14 meq/l in May. Nitrates range from 0.60 to 6.07 meq/l, with a monthly average of 1.37 meq/l in March, 4.14 meq/l in April, and 5.10 meq/l in May. The concentration of the other elements appears to be quite linear during the trial. Magnesium ranges from 0.56 to 1.22 meq/l; Na from 0.33 to 0.99 meq/l; Potassium from 0.22 to 0.40 meq/l; Chloride from 0.50 to 1.67 meq/l; Solphate from 0.98 to 2.60 meq/l, and Bicarbonate from 1.63 to 4.04 meq/l.

Figure 41: Ions in solution N100 (35 cm of depth)



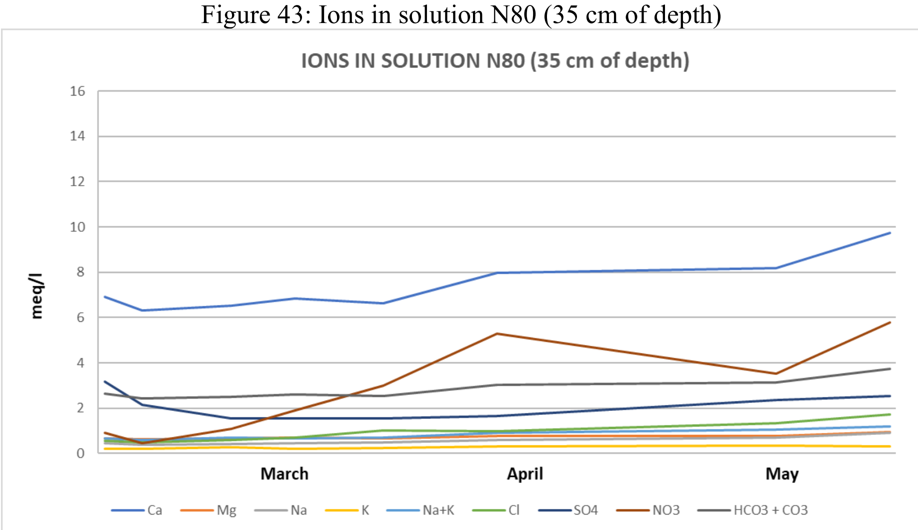
Considering the ion concentration in the soil circulating solution for the treatment N80, at depth of 15 cm (Figure 42), a decrease in overall concentration values compared to N100 is evident. In particular, the lower amounts start from March. The value of calcium and nitrates concentration remains the most relevant. Ca ranges from 5.41 to 11.71 meq/l, with a monthly average of 5.94 meq/l in March, 9.54 meq/l in April, and 8.68 meq/l in May. Nitrates range from 0.95 to 7.74 meq/l, with a monthly average of 1.87 meq/l in March, 6.08 meq/l in April, and 4.16 meq/l in May. There are also sharp peaks similar, but of a smaller entity, to those obtained at N100, in correspondence of 10th and 20th of April (6.28 and 5.86 meq/l), and of 26th of April and 5th of May (7.74 and 4.16 meq/l). The concentration of the other elements appears to be quite linear during the trial, with less variations in comparison to trial N100. Magnesium ranges from 0.59 to 1.29 meq/l; Sodium from 0.41 to 1.26 meq/l; Potassium from 0.17 to 0.34 meq/l; Chloride from 0.49 to 2.24 meq/l; Sulphate from 1.66 to 3.58 meq/l, and Bicarbonate from 2.07 to 4.48 meq/l. Only the last two ions showed slightly evident fluctuations.

Figure 42: Ions in solution N80 (15 cm of depth)



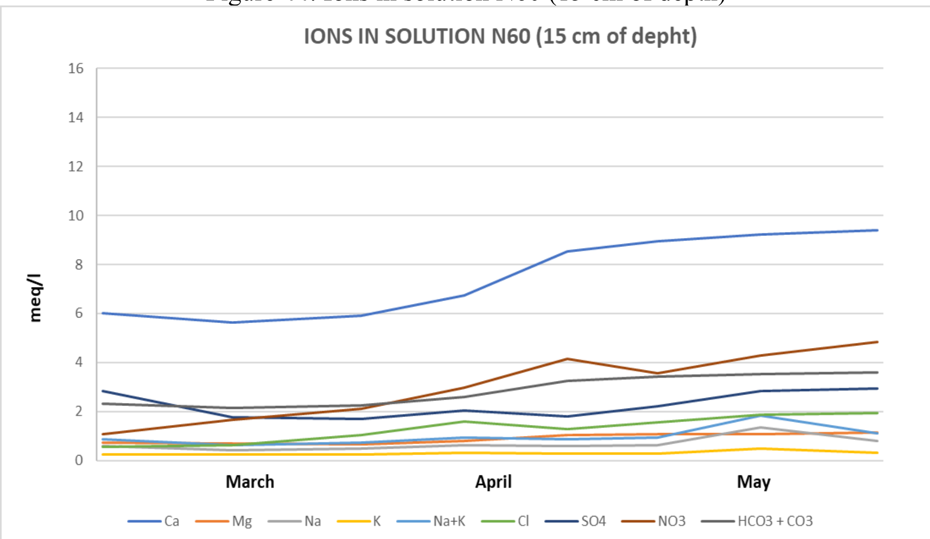
The trend of ionic concentration of soil circulating solution for the same trial, but at depth of 35 cm, is slightly lower than the previous graph (Figure 43). The fertilization seems to have less influence on circulating solution with increasing depth. The only evident variations were registered for Calcium and Nitrates. Calcium ranges from 6.33 to 9.73 meq/l, with a monthly average of 6.65 meq/l in March, 7.59 meq/l in April, and 8.95 meq/l in May. Nitrates concentration ranges from 0.45 to 5.78 meq/l, with a monthly average of 1.38 meq/l in March, 4.12 meq/l in April, 4.73 meq/l in May. A slight increase in concentrations was noticed after the end of April, for all element present at lesser extent. It is believed that this increase could be related to a post-fertirrigation migration of the elements. Magnesium ranges from 0.62 to 0.96 meq/l; Sodium from 0.39 to 0.90 meq/l; Potassium from 0.19 to 0.33 meq/l; Chloride from 0.50 to 1.72 meq/l; Sulphate from 1.55 to 3.16 meq/l, and Bicarbonate from 2.42 to 3.73 meq/l.

Figure 43: Ions in solution N80 (35 cm of depth)



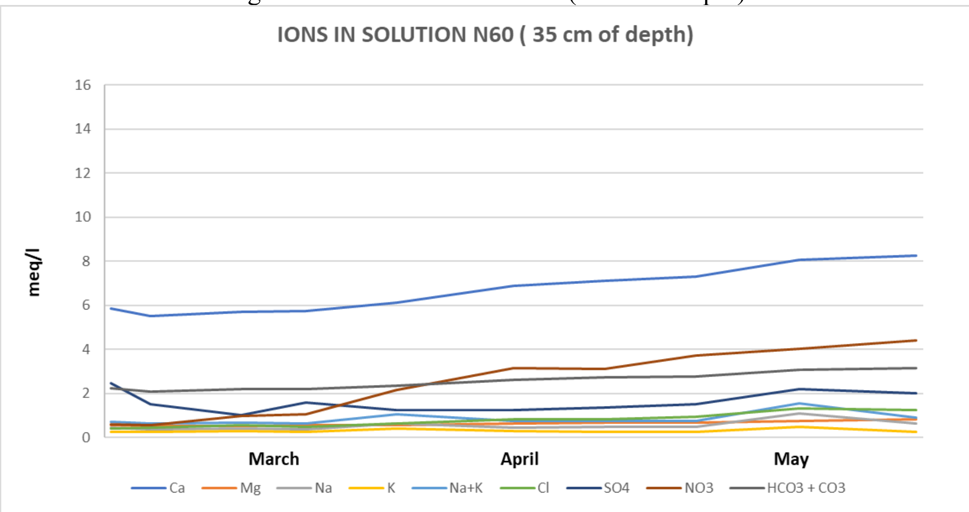
The analyzed soil circulating solution for the thesis N60 (at 15 cm of depth) shows a slightly lower amount of ionic concentrations, and with a more linear trend in comparison to N80 (Figure 44). Calcium ranges from 5.62 to 9.38 meq/l, with a monthly average of 5.82 meq/l in March, 7.53 meq/l in April, and 9.30 meq/l in May. Nitrates range from 1.09 to 4.85 meq/l, with a monthly average of 1.49 meq/l in March, 4.54 meq/l in April, and 6.83 meq/l in May. The range of concentrations of the remaining elements is almost linear: Magnesium varies from 0.68 to 1.16 meq/l; Sodium from 0.40 to 1.34 meq/l; Potassium from 0.23 to 0.49 meq/l; Chloride from 0.56 to 1.94 meq/l; Sulphate from 1.69 to 2.95 meq/l, and Bicarbonate from 2.15 to 3.59 meq/l.

Figure 44: Ions in solution N60 (15 cm of depth)



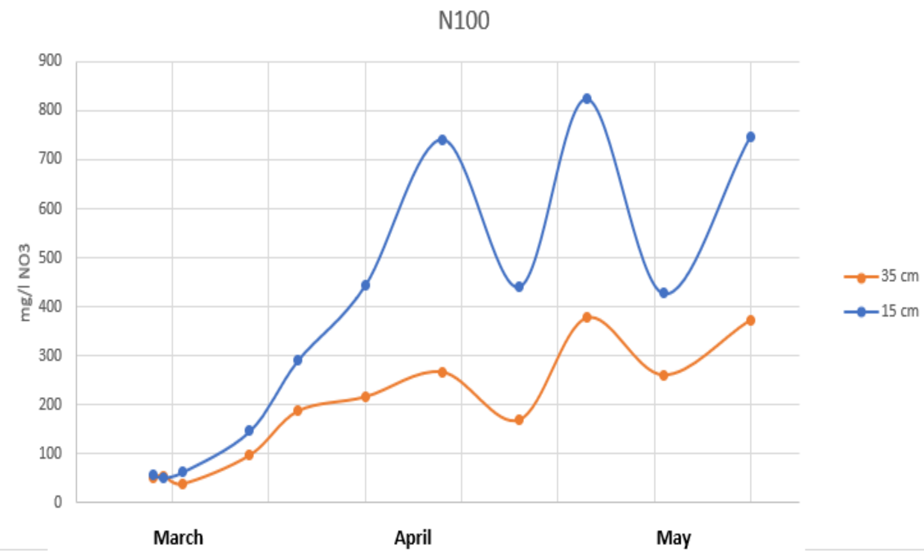
The trend of ion concentration in the soil circulating solution for the thesis N60 at 35 cm depth (Figure 45) shows lower values, as particularly evident for the Nitrate ion, in comparison to the same trial at 15 cm depth (Figure 44). Calcium ranges from 5.49 to 8.25 meq/l, with a monthly average of 5.68 meq/l in March, 6.84 meq/l in April, and 8.15 meq/l in May. Nitrate ranges from 0.59 to 4.43 meq/l, with a monthly average of 0.71 meq/l in March, 3.04 meq/l in April, and 4.12 meq/l in May. The other elements maintain an almost linear trend: Magnesium ranges from 0.51 to 0.82 meq/l; Sodium from 0.37 to 1.08 meq/l; Potassium from 0.25 to 0.49 meq/l; Chloride from 0.42 to 1.33 meq/l; Sulphate from 1.04 to 2.46 meq/l, and Bicarbonate from 2.10 to 3.16 meq/l.

Figure 45: Ions in solution N60 (35 cm of depth)



The graphs below (Figures 46, 47, 48) report the NO_3 concentrations availability in the trials N100, N80, N60 at different soil depths (15 cm/35 cm). The Figure 46 shows that the NO_3 concentration into the soil solution analyzed at 15 cm depth is higher than at 35 cm; there is a general positive trend for NO_3 concentrations at both depths during the months of trial. Inflection points related to 20/04 and 5/05 should be due to a wide timing interval that separates two successive fertigations.

Figure 46: Evolution of NO_3 amount in N100 (mg/l) March-April-May 2017.



The Figure 47 shows that at the same depth (15 cm), N80 and N100 thesis have a halving of the nitrogen concentration; the reason can be attributed to the lower nitrogen supply at N80, according to the trial; moreover, the maximum reduction of NO_3 availability can be detected in N60 (Figure 48). Comparing the three theses, it can be noticed a reduction in the NO_3

availability from thesis N100 to N60 for both depths. In Table 44 it can be observed a drop of -31% for the nitrate value (mg/l NO_3) from N100 to N80 (15 cm), and a decrease of 44% from N100 to N60 (15cm). The same trend is observed between N100 and N60, but at smaller extent, in 35 cm experiment.

Figure 47: Evolution of NO_3 amount in N80 (mg/l) March-April-May 2017

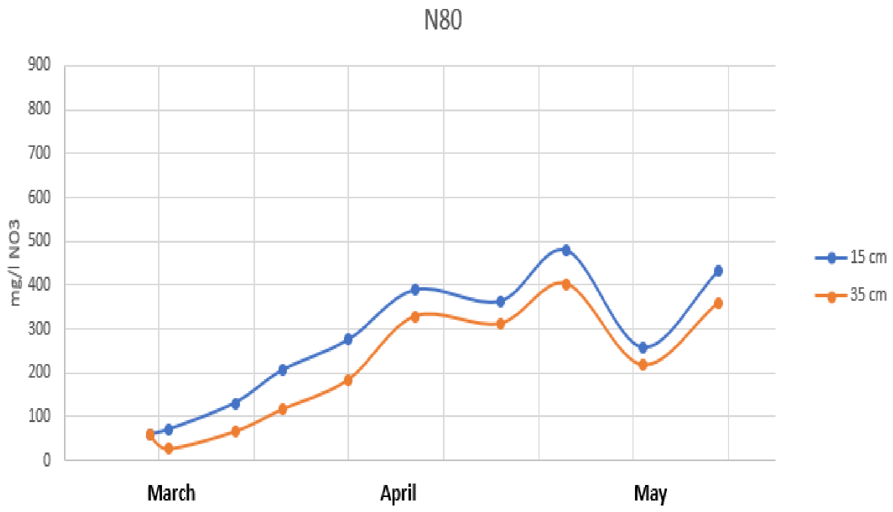


Figure 48: Evolution of NO3 concentration in N60 (mg/l) March-April-May

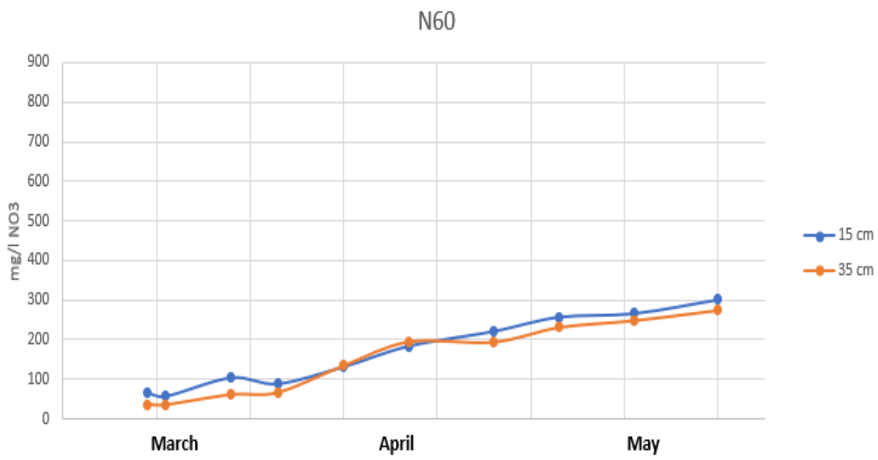


Table 44: Mean values (NO_3 mg/l) in N100, N80, N60 (15-35 cm)

Treatment	**mg/l NO_3**	
Depth	**15 cm**	**35 cm**
N100	384.33	189.59
N80	266.13	207.35
N60	167.69	147.81

Strawberries negatively respond to the salt stress in terms of growth and yield, so it is a salinity sensitive species (Karlidag, 2009). The hydric stress, due to the electrical conductivity (ECs) of the saturated soil extract, is a factor which contributes to reduce the number of leaves, the leaf area, the shoot dry weight, the number of crowns, the yield and the fresh weight of the fruit (Pirlak and Esitken, 2004; Awang et al., 2015). In the study of Barroso and Alvarez (1997), the leaves of strawberry cultivars did not develop symptoms of toxicity for EC value lower than 2000 µS/cm. In the study of HA-Joon et al. (2011), the optimal EC value was detected at 1000 µS/cm instead of 2000 µS/cm; the compared parameters were fruit length, diameter, weight and plant yield. Moreover, dry branch crowns and dry roots in the 1000 µS/cm experiment were heavier than 2000 µS/cm.

In our study the electrical conducivity of the water during April and May 2017 at 15 cm of depth has the higher values for the three nitrogen trials. These results agree with the nutrient concentration explained above. In N100 and N80, the maximum registered value is 1400 µS/cm (Figure 49-50), while in N60 this value is lower, slightly exceeding 1000 µS/cm (Figure 51). The trends are similar, but with lesser extent, for the water sampled at 35 cm of soil depth.

Figure 49: Water electrical conductivity µS/cm (N100)

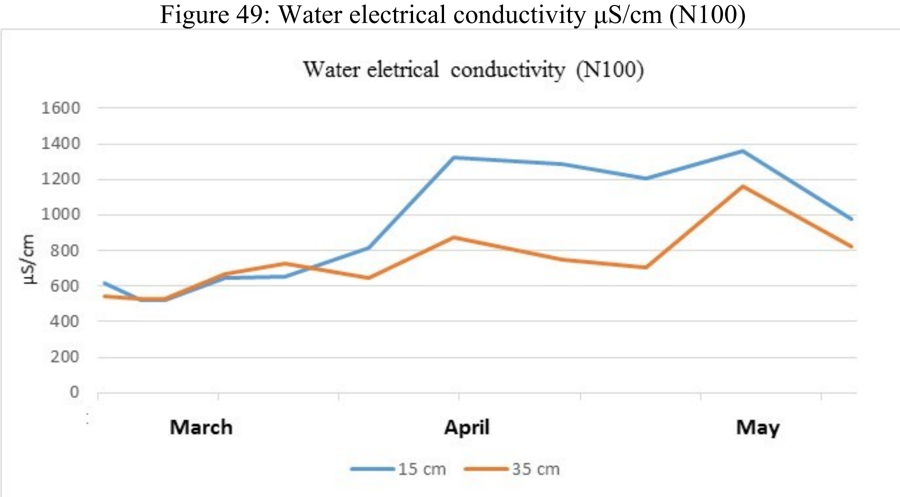


Figure 50: Water electrical conductivity µS/cm (N80)

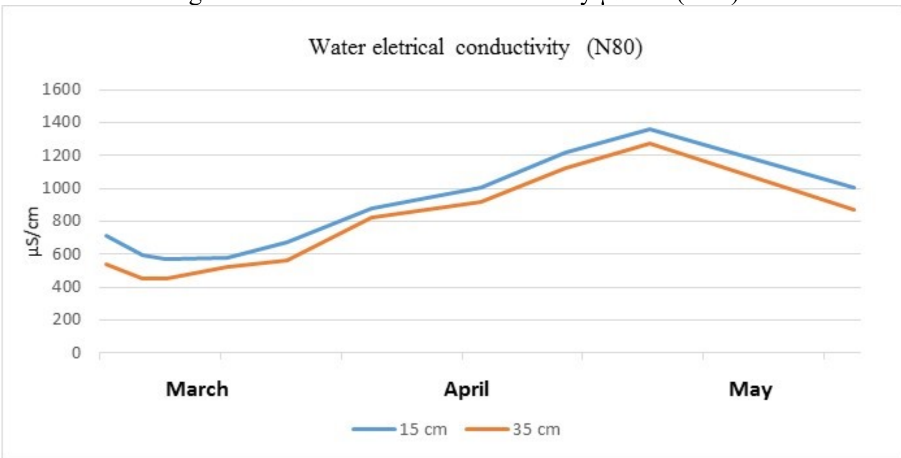
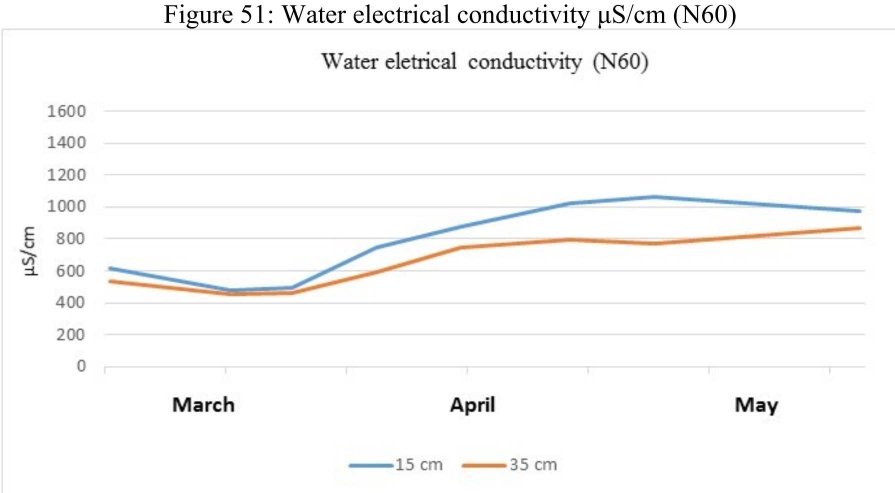


Figure 51: Water electrical conductivity µS/cm (N60)



4.3.2 Vegetative Parameters of Single-cropping strawberry cultivars

The ANOVA analysis shows that the interaction year x cv determines significant differences for all parameters studied ($p<0.01$). The interaction year x treatment shows no significance except for the number of branch crowns. Cv x treatment do not affect any of the parameters considered. The leaf width shows to be influenced by the interaction among year x cv x treatment (Table 45).

Table 45: Two-ways analysis of variance (ANOVA) for the vegetative parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	Branch crowns	Inflorescences number	Plant height	Leaves number	Leaf height	Leaf width
Year (a)	**	NS	**	*	NS	**
Cv (b)	**	NS	**	NS	**	**
Treatment (c)	*	NS	NS	NS	NS	NS
Year x Cv (a)x(b)	**	**	**	**	**	**
Year x Treatment (a)x(c)	**	NS	NS	NS	NS	NS
Cv x Treatment (b)x(c)	NS	NS	NS	NS	NS	NS
Year x Cv x Treatment (a)x(b)x(c)	NS	NS	NS	NS	NS	*

Plants height and leaves number describe the vegetative growth phase of plant cycle. In all the nitrogen trials, “Romina” shows a higher plant height compared to “Cristina” in a significant manner. In maximum nitrogen condition (N100), the values are similar between “Sibilla” and “Romina”; in N80 and N60, “Romina” resulted significantly higher also than “Sibilla” (Table 46). The growth of plants, in terms of height, is not affected by different nitrogen input for all the tested varieties.

As regard the number of leaves, only “Sibilla” shows a significant decrease of this parameter with a decreasing amount of nitrate fertilization (N60) in respect to the control trial (N100). The study of Odongo et al. (2008) explains how the vegetative growth of crops depends on the high amount of nitrogen in organic fertilizers and the soil organic matter; in fact, the content of soil organic matter hides the effect of mineral nitrogen intake, the principal macro-element for the growth of the plants (Kirschbaum et al., 2010). In studies of Venâncio et al. (2013) and Medeiros et al. (2015), the variation of nitrogen value did not produce modifications on the plant height value; on the other side, an increase of nitrogen supply led to a higher number of leaves. Our trial, unlike the previous presented studies, took place in a soil with low values of total nitrogen concentration (0.90 g/kg) and organic matter (11.9 g/kg). In this case, the effects of fertilization practices are not masked by the soil properties. In similar conditions, González and Acuña (2008) reported the effect of fertilizers on strawberry performance giving important results, such as a decrease of number and size of leaves with less amount of fertilizer.

Table 46: Effects of nitrogen on plant height and leaves number in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant.
Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Plant height (cm)**			**Leaves number**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Cristina"	33.3±3.2 c	34.2±3.5 c	33.3±4,0 c	25.4±5 ab	24.6±4,0 ab	24±6.1 ab
"Romina"	38.9±2.9 ab	40.2±3,0 a	40.2±2.6 a	24.1±7.1 ab	25.8±6.2 ab	24.4±6.2 ab
"Sibilla"	38.5±7.6 ab	37.9±5.9 b	38.3±5.6 b	26.2±5.6 a	25.1±6.7 ab	23.6±4.3 b
AVERAGE	**36.9±5.6 NS**	**37.4±5 NS**	**37.3±5.1 NS**	**9.7±1.1 NS**	**9.6±1 NS**	**9.7±1.3 NS**

For both the branch crowns number and the inflorescences number, the nitrogen treatment did not showed any significant effect on the studied cultivars (Table 47). The only recognized differences are due to the genotype effect: for the branch crowns number, "Cristina" showed a lower value than "Romina" and "Sibilla", being significantly lower only at N60 in respect to them. For the inflorescences number, nor the nitrogen treatment nor the genotype affect the plant performances (Table 47). Previous studies (Sønsteby and Heide, 2009) have shown that the effect on the number of inflorescences and flowering crowns is related to fertilization time before and at the beginning of the SD period. An increasing amount of calcium nitrate solution applied to plants of "Korona" cultivar a week after the first short day determined a duplication of the number of inflorescences compared to short day control, while no effect was found with the application of 2 weeks before.

In our study, the nitrogen fertilization supply took place at the end of August (<13 hours daylight): the differentiation of the amount, but not of the timing, of fertilization, resulted in an unchanged number of inflorescences among cultivars and treatments. In fact, in Lieten (2004) the largest flower numbers and plant yield of "Elsanta" variety was observed with the suspension of fertilization under declining day length (end of August) and the restart in September-November.

Table 47: Effects nitrogen on branch crowns number, inflorescences number in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Branch crowns number**			**Inflorescences number**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Cristina"	4.1±1.4 bc	4.2±1.4 abc	3.6±1.2 c	11.9±3.5 ab	12.1±3.2 a	11.3±3.3 ab
"Romina"	4.8±2 ab	4.9±2.3 a	4.8±2 ab	11.1±2.8 ab	11.4±2.9 ab	10.6±3.4 b
"Sibilla"	4.8±2.1 ab	4.6±2.1 ab	4.4±1.7 ab	11.3±2.7 ab	10.9±3 ab	11.8±3.4 ab
AVERAGE	**4.5±1.9 NS**	**4.6±2 NS**	**4.3±1.8 NS**	**11.4±3 NS**	**11.5±3.1 NS**	**11.3±3.4 NS**

As for the branch crowns and the Inflorescences number, also for the leaves dimension (length and width) any significant effect of nitrogen treatment was found. The only differences are due to genotype effect: for the leaves' length, "Sibilla" and "Romina" show the highest values and are statistically similar, while "Cristina" show significantly lower dimension than both of them at N100 and N60. For the leaves' width, "Romina" stands out for its significantly highest values at all nitrogen treatments (Table 48).

Table 48: Effects of nitrogen on development of leaves in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Leaves length (cm)**			**Leaves width (cm)**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Cristina"	9.2±1 c	9.3±0.9 c	9.2±1.6 c	8.2±1 cd	8.3±0.9 bcd	8.1±1 d
"Romina"	9.8±1.1 ab	9.6±1.1 bc	10±1ab	9.9±1.3 a	9.6±1.3 a	10±1 a
"Sibilla"	10.1±1.1 a	9.8±1 ab	9.9±1 ab	8.6±1.3 bc	8.6±0.7 bc	8.7±1 b
AVERAGE	**9.7±1.1 NS**	**9.6±1 NS**	**9.7±1.3 NS**	**8.9±1.4 NS**	**8.8±1.1 NS**	**8.9±1.3 NS**

4.3.3 Vegetative Parameters of Remontant Strawberry cultivars

Considering the Anova analysis, the cultivar effect is determinant for all the analyzed parmeters, except the leaf width. The significant effect (p<0.01) of the treatment occurred only for the height of the plant, while the interaction treatment x cultivar significantly affected only the number of branch crowns (p<0.05) (Table 49).

Table 49: Two-ways analysis of variance (ANOVA) for the vegetative parameters **= significant interaction with $p<0.01$; *= significant interaction for $p<0.05$; n.s.= not significant interaction.

Parameter	Branch Crown number	Inflorescences number	Plant height	Leaves number	Leaf height	Leaf width
Cv (a)	**	**	**	**	**	NS
Treatment (b)	NS	NS	**	NS	NS	NS
Treatment x Cv (a)x(b)	*	NS	NS	NS	NS	NS

From Table 50, the strawberry nitrogen reduction positively affects the vegetative development, in particular the plant height, as reported in N60. Considering the three varieties, “S. Andreas” is the most vigorous and maintains this characteristic in all three treatments, increasing significantly its height in N60 (about 2.5 cm in respect to N100). “Monterey” is not influenced by different nitrogen amounts. Even if “Albion” shows a less growth than the other two varieties, it is evident a height increase of about 2 cm with less nitrogen supply (N60) in respect to N100. The different genotype responses may be due to different capacity to uptake the nitrogen, which has an essential role in the cell division increment and in the plant growth improvement (Khalid, 2013). Moreover, it seems that the different treatments do not affect the number of leaves. Also, from this parameter, it is clear that “S. Andreas” is the most vigorous cultivar, followed by “Monterey” and “Albion” that are statistically similar to each other (Table 50).

Table 50: Effects of nitrogen on plant height and leaves number in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher’s LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher’s LSD test ($p<0.05$). N.S.= not significant Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Plant height (cm)**			**Leaves number**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
“Albion”	19.5±2.3 g	20.3±2.3 fg	21.2±2.4 ef	16.5±6.9 b	16.7±4.9 b	16.2±5.5 b
“Monterey”	22±2.3 de	22.6±3 cd	22.9±2.9 bcd	16.4±5 b	17.5±5.4 b	16.1±6.3 b
“S. Andreas”	23.5±3.7 bc	23.6±3.5 b	26±2.8 a	21.7±6.7 a	22.2±5.5 a	22±6.4 a
AVERAGE	**21.7±3.3 B**	**22.2±3.3 B**	**23.4±3.4 A**	**18.2±6.7 NS**	**18.8±5.8 NS**	**18.2±6.7 NS**

Both branch crowns number and inflorescences number are not significantly affected by the nitrogen application (Table 51). Only few exceptions could be detected in the branch crowns number. In fact, “Monterey” presents at N80 a significant higher amount of branch crowns than N60; contrarily, “S. Andreas” shows a significant higher number of branch crowns at N60 in respect to N80. The number of branch crowns of “S. Andreas” is significantly higher than

"Albion" for all the nitrogen treatments, highlighting a genotype effect for this parameter. The interpretation of the responses in relation to fertilization is complex because in studies such as Abbott (1968), Breen et al. (1981) and Guttridge (1985), growth stimulating nutrients have stimulated plant growth and potential flowering sites but inhibited flower formation.
Regarding the inflorescences number, "Albion" and "Monterey" are statistically similar at all the nitrogen application, and both are significantly higher than "S. Andreas" at all the nitrogen trials (Table 51).

Table 51: Effects nitrogen on branch crowns number, inflorescences number in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Branch crowns number**			**Inflorescences number**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Albion"	2.7±1.0 cde	2.4±0.8 ef	2.6±1.1 def	2.8±1.3 a	2.7±1.0 a	2.7±1.2 a
"Monterey"	2.5±0.9 def	2.7±0.9 cd	2.3±0.9 f	2.7±1.2 a	2.7±1.2 a	2.6±1.2 ab
"S. Andreas"	3.3±1.1 ab	3±0.7 bc	3.3±1.0 a	2.1±1.1 c	2.2±1.1 bc	2.1±1.1 c
AVERAGE	**2.8±1 NS**	**2.7±0.8 NS**	**2.8±1.1 NS**	**2.5±1.2 NS**	**2.5±1.1 NS**	**2.5±1.2 NS**

Finally, both parameters related to the leaf development are not influenced by the nitrogen treatment. Interestingly, the leaves width does not show any significant difference also among the studied cultivars. However, it is possible to find an influence of the genotype on the leaves' length: at N100 and N80, "S. Andreas" shows the significantly highest values, while at N60 it is similar to "Albion". "Monterey" always showed the worse result, even if at N100 is statistically similar to "Albion" (Table 52).

Table 52: Effects of nitrogen on development of leaves in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Leaves length (cm)**			**Leaves width (cm)**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Albion"	7.3±1.0 cd	7.7±0.9 bc	7.9±1.1 ab	6.8±1.0 ns	7.0±1.1 ns	7.4±1.1 ns
"Monterey"	7.1±1.1 d	7.1±1.2 d	7.0±1.2 d	7.5±6.1 ns	7.6±7.1 ns	6.5±1.1 ns
"S. Andreas"	8.1±1.1 a	8.2±1.0 a	8.2±1.2 a	7.2±1.0 ns	7.5±1.0 ns	7.3±1.2 ns
AVERAGE	**7.5±1.1 NS**	**7.7±1.1 NS**	**7.7±1.3 NS**	**7.2±3.6 NS**	**7.4±4.2 NS**	**7.1±1.2 NS**

4.3.4 Qualitative Parameters of Single-cropping strawberry cultivars

According to the ANOVA analysis (Table 53), the interaction year x cultivar shows a significant effect for all parameters studied, except for titratable acidity. The interaction year x treatment or cultivars x treatment or year x cultivar x treatment does not show significant effects for any of the parameters considered.

Table 53: Two-ways analysis of variance (ANOVA) for the qualitative parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	**Sugar Content**	**Titratable acidity**	**Firmness**	**Brightness L***	**Redness a***	**Yellowness b***	**Chroma**
Year (a)	**	NS	**	**	**	NS	NS
Cv (b)	**	*	**	**	**	**	**
Treatment (c)	NS	NS	NS	NS	NS	NS	NS
Year x Cv (a)x(b)	**	NS	**	**	**	**	**
Year x Treatment (a)x(c)	NS	NS	NS	NS	NS	NS	NS
Cv x Treatment (b)x(c)	NS	NS	NS	NS	NS	NS	NS
Year x Cv x Treatment (a)x(b)x(c)	NS	NS	NS	NS	NS	NS	NS

The genotype is the main source of fruit quality, plant growth and yield variations (Rahman et al., 2014). Specifically, the genotype is the principal factor affecting the sugar content and titratable acidity of strawberry fruit (Moing et al., 2001; Lourdes et al., 2002; Kafkas et al., 2007; Shim et al., 2007). According with those findings, in our study “Romina” and “Sibilla” have the highest sugar content in all nitrogen trials (Table 54). The titratable acidity does not show significant differences among treatments for each analyzed cultivar. “Sibilla” present the main acidity content in test N80 in comparison to “Cristina” and “Romina”, that are similar each other. Therefore, the lower nitrogen supply applied does not seem to affect the soluble solids content and titratable acidity of fruits (Table 54). These results appear in contrast to the work of D’Anna et al. (2012), according to which at the lowest doses of nitrogen supplied to the plant corresponds a higher soluble sugar content.

Table 54: Effects of nitrogen on sugar content and titratable acidity in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Sugar content (°Brix)**			**Titratable acidity (meqNaOH/100g fruit weight)**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Cristina"	6.6±0.5 c	6.4±0.6 c	6.4±0.5 c	10.9±1abcd	10.4±1 d	10.9±1.7 abcd
"Romina"	7.6±0.6 ab	7.2±0.5 b	7.5±0.7 ab	11.1±0.8 abcd	10.6±0.7 cd	10.6±0.8 bcd
"Sibilla"	7.8±0.8 a	7.9±0.7 a	7.6±1.1 ab	11.5±1.4 a	11.4±1.8 ab	11.3±1.6 abc
AVERAGE	**7.3±0.9 NS**	**7.2±0.8 NS**	**7.2±0.9 NS**	**11.2±1.1 NS**	**10.8±1.3 NS**	**10.9±1.4 NS**

Our study also proves that the fruit firmness and Chroma index differences are due to the genotype but not to the nitrogen dose used in this trial (Table 55). D'Anna et al. (2012) and Cardeñosa et al. (2015) studies, that showed an increase in fruit firmness with nitrogen input reduction, are in contrast with our results. According to other reports in literature, the excess of nitrogen could cause uneven ripening of the fruit, which result to be soft and with poor taste (Van der Boon, 1961). Accordingly, the optimum nitrogen decrease should determine a better fruit firmness. In our study, it has been registered a good fruit firmness in all nitrogen treatments, for each variety. "Sibilla" shows the greatest firmness at N100 (414.2 g), followed by "Romina" (356.9 g) and "Sibilla" (292.8 g), all of them significantly different. This trend is confirmed at all the nitrogen trials.

Color is an important qualitative attribute for food and fruits, influencing the choices and preferences of consumer (Pathare et al., 2013). In all the nitrogen treatments, the chromatic index of skin color does not change in a significant manner. The only significant differences are detected among the varieties, with "Sibilla" having the darkest fruits, followed by "Romina" and "Cristina".

Table 55: Effects of nitrogen on firmness and chroma in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Firmness (g/cm²)**			**Chroma**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Cristina"	292.8±58.3 c	297±43.7 c	298.8±59.5 c	44.1±3.6 c	43.8±3.4 c	44.3±3.3 c
"Romina"	356.9±64.9 b	348.9±47.8 b	352.8±51.9 b	48.9±1.5 b	49.4±1.8 b	49.4±1.9b
"Sibilla"	414.2±104.3 a	425±82 a	415.5±102.5 a	52.1±2.4 a	52.1±2.3 a	50.6±6.7 ab
AVERAGE	**354.7±91.9 NS**	**356.9±79.5 NS**	**355.7±87.6 NS**	**48.3±4.2 NS**	**48.4±4.3 NS**	**48.1±5.2 NS**

4.3.5 Qualitative Parameters of Remontant strawberry cultivars

According to the Anova analysis, the cultivar influences the plant response to all the parameters considered (P<0.01). Treatment statistically affects firmness (p<0.01), redness and Chroma (p<0.05). Interaction cultivar x treatment significantly affects firmness, redness, yellowness and Chroma (p<0.01) (Table 56).

Table 56: Two-ways analysis of variance (ANOVA) for the qualitative parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	**Sugar Content**	**Titratable acidity**	**Firmness**	**Brightness L***	**Redness a***	**Yellowness b***	**Chroma**
Cv (a)	**	**	**	**	**	**	**
Treatment (b)	NS	NS	**	NS	*	NS	*
Cv x Treatment (a)x(b)	NS	NS	**	NS	**	**	**

Sourness and sweetness are the two most important qualitative factors of strawberry (Azodanlou et al., 2004; Mitcham, 2004; Shim et al., 2007). Remontant cultivars show higher content of sugars, if compared with Single-cropping cultivars (Ruan, 2013). The significant difference in sugar content between remontant and single-cropping cultivars depends on changes of temperature and photoperiod (Lee et al., 2005; Ruan et al., 2011). In the Table 57, the influence of genotype on sugar and acidity for remontant cultivars is shown; the fruits of "Monterey" show the highest concentration of sugar (14.4 °Brix), followed by "Albion" (13.6 °Brix) and "S. Andreas" (10.9 °Brix), at N100 trial. The different nitrogen availability has determined a significant sugar content reduction (from N100 to N60) in "Monterey" and "S.

Andreas", with almost 1 °Brix of difference. The titratable acidity is not influenced by the different nitrogen amounts. "Monterey" and "S. Andreas" show similar values of acidity, while "Albion" registered significantly higher values than the others.

Table 57: Effects of nitrogen on sugar content and titratable acidity in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Sugar content (°Brix)**			**Titratable acidity (meq NaOH/100g fruit weight)**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Albion"	13.6±1.2 b	14.0±1 ab	13.9±1.1 ab	14.2±3.4 ab	14.7±0.9 a	15±1.4 a
"Monterey"	14.4±1 a	13.9±1.3 ab	13.6±1.2 b	13.1±1 c	13.2±0.9 c	13.6±0.7 bc
"S. Andreas"	10.9±0.9 c	10.9±0.9 c	10.1±1.1d	13.1±0.9 c	13.6±0.9 bc	13.5±1.2 bc
AVERAGE	**13±1.8 NS**	**12.9±1.8 NS**	**12.5±2 NS**	**13.4±2.1 NS**	**13.8±1.1 NS**	**14±1.3 NS**

A good food texture is one of the consumer's favourite parameter; it is an important quality factor and it is evaluated by hand when the fruit is touched and by mouth when the fruit is eaten (Kopjar et al., 2008; Stokes, 2013;). In our study, the statistical data analysis shows that the nitrogen mineral amount influences each variety firmness. In trial N100, "Monterey" has a higher fruit firmness (400 g) than "Albion" (368.7 g) and "S. Andreas" (323.7 g). In the other nitrogen trials, those gaps become smaller, and "Albion" and "Monterey" show similar fruit firmness each other, while "S Andreas" always presents the lowest values (Table 58). The effects of nitrogen trials on fruit firmness are evident at different extent for the different cultivars: in "Albion", N60 treatment caused a significant firmness reduction, while N80 trial produces fruits with the highest firmness values, but similar to N100; for "Monterey", the highest firmness value is at N100, while N80 and N60 produces fruits with a significantly lower firmness than N100. As result of a 20% nitrogen reduction (N80), "S Andreas" is characterized by its greatest fruit firmness.

The fruits color plays an important role in the market; in fact, the consumers, at the purchase moment, base their choice on the fruit aspect (Pădureț et al, 2017). In our study, in optimal nitrogen fertilization condition (N100), the fruit aspect indicates similar data among the three varieties (Table 58). A different result can be observed for the minimum nitrogen supply (N60); "San Andreas" shows a significant better value in respect to N100 and also in respect to the other cultivars at N60; then "Albion" maintain the same fruit color at the three nitrogen trials, resulting significantly better than “Monterey” at N60; this variety is the only that is negatively influenced in a significant manner by the lowest nitrogen supply (N60) in respect to N100.

Table 58: Effects of nitrogen on firmness and chroma in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher’s LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher’s LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Firmness (g/cm²)**			**Chroma**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
“Albion”	368.7±96.2 bc	375.6±87 b	359.9±93.6cd	44.6±5.3 b	44.6±5.3 b	44.8±4.9 b
“Monterey”	400±97.9 a	370.3±91.5 bc	363.7±88.5 bc	44.2±6.2 b	44.6±6.2 b	43.1±6.5 c
“S. Andreas”	323.8±78.2 e	347.3±86.1 d	330.2±89.6 e	44.6±5.9 b	46±5.8a	45.8±5.9 a
AVERAGE	**364.1±96.3 A**	**364.4±89 A**	**351.3±91.7 B**	**44.5±5.8 B**	**45.1±5.8 A**	**44.6±5.9 AB**

4.3.6 Productive Parameters of Single-cropping Strawberry cultivars

The Anova results indicate that all the production parameters are statistically influenced (p<0.01) by the interactions year x cultivar. Considering the interaction year x treatment, the statistical relevance occurs for the precocity index and the average fruit weight. Interaction cv x treatment does not show influence on the parameters examinated. Finally, the interaction year x cv x treatment significantly affects the average fruit weight and fruit waste amount (Table 59).

Table 59: Two-ways analysis of variance (ANOVA) for the productive parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	**Precocity Index**	**Average Fruit Weight**	**Commercial Production**	**Total Production**	**Waste**
Year (a)	**	**	**	**	*
Cv (b)	**	**	**	**	**
Treatment (c)	**	**	**	**	**
Year x Cv (a)x(b)	**	**	**	**	**
Year x Treatment (a)x(c)	*	**	NS	NS	NS
Cv x Treatment (b)x(c)	NS	NS	NS	NS	NS
Year x Cv x Treatment (a)x(b)x(c)	NS	**	NS	NS	*

The Precocity Index and Average Fruit Weight values does not show significant differences among the three nitrogen treatments (Table 60). The control of "Cristina" showed a delayed fruit maturation by 2 days in respect to the trial with lower nitrogen apply (N60). "Romina" confirmed an early variety behaviour, followed by "Sibilla" (medium-early variety) and finally "Cristina" (late variety). Regardless the amount of nitrogen supplied to the plants, "Cristina" has an average fruit weight greater than "Romina" (of about 13-15 g) and "Sibilla" (of about 10-13 g). These results show that lower doses of nitrogen do not affect the ripening period nor the average weight of the fruit, which are important parameters in the farmer's choice of a cultivar (Table 60).

Table 60: Effects of nitrogen on precocity index and average fruit weight in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Precocity index (days)**			**Average fruit weight (g)**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Cristina"	146.7±2.6 a	148.2±1.4 a	148.5±1.9 a	32.4±3.3 a	31.2±3.9 a	33.4±2.3 a
"Romina"	132.5±3.7 c	132.1±2.4 c	132.3±2.1 c	18.8±2.6 b	18.3±2.9 b	18.3±2.2 b
"Sibilla"	136.9±3.6 b	136.7±3.9 b	136.9±3.3 b	21.3±2.3 b	21.4±3.2 b	20.7±2.1 b
AVERAGE	**138.7±6.9 NS**	**139±7.4 NS**	**139.2±7.4 NS**	**24.1±6.6 NS**	**23.7±6.5 NS**	**24.1±7.1 NS**

The statistical analysis does not confirm a difference for the commercial nor the total production among the different nitrogen supplies (Table 61). Similar findings were already observed on the study from D'Anna et al. (2012). In our study, the commercial production originating by plant with the lowest dose of nitrogen shows a slight downward trend of -7%, compared to N100. Taking into consideration the varieties in thesis N100, "Cristina" appears to be commercially more productive, followed by "Sibilla" and then "Romina". This rank is maintained for all the nitrogen trials, although the reduction of this element provides a not significant decrease of production for all the three varieties. Total production does not suffer any significant decrease with a reduction in nitrogen supply. In all the fertilization trials, also in this case, "Cristina" stands out for the most abundant total production compared to the other two varieties. In the thesis N100, "Cristina" produces 201.6 g/plant more than "Romina", and 71.7 g/plant more than "Sibilla".

Despite the highest commercial and total production, “Cristina” presented the lowest amount of waste/plant among the studied cultivars, at all the nitrogen amounts, demonstrating its good productive features also at low amounts of nitrogen supply (Table 62).
The amount of waste in “Romina” and “Sibilla”, at N100, resulted higher than the amount registered by “Cristina”, of 88 and 153.1 g/plant, respectively. A similar trend was maintained at all nitrogen levels. Is important to underline that, according to our study, there is not a significant effect of nitrogen fertilization reduction on the amount of waste fruits in the three studied cultivars. Other studies on reduction of nitrogen reported similar results for each cultivar, thought there is a trend of the amount of unsellable fruits in relation to lower nitrogen supply.

Table 61: Effects of nitrogen on commercial production and total production in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Commercial production (g/plant)**			**Total production (g/plant)**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Cristina"	783.1±114.9 a	744.9±124.4 ab	710.5±170.1 abc	869.4±125 a	847.5±159.7 a	818.5±205.3 ab
"Romina"	529.9±95.1 de	533.7±70.6 de	496.4±60.1 e	667.8±104.4 cd	702.9±87.6 bcd	665.7±59.4 d
"Sibilla"	635.8±51.2 bcd	609.6±34.6 cd	600±39.3 de	797.3±44.5 abc	798.9±57.8 abc	807.8±63.5 ab
AVERAGE	**649.6±136.9 NS**	**629.4±120.2 NS**	**602.3±134.6 NS**	**778.1±125.5 NS**	**783.1±120.7 NS**	**764±140.5 NS**

Table 62: Effects of nitrogen on waste in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of two years (2017-2018) ± standard deviation (SD)

Cultivar	**Waste (g/plant)**		
Treatment	**N100**	**N80**	**N60**
"Cristina"	101.1±22.8 e	115.9±57.6 ed	115.8±24.2 ed
"Romina"	189.4±24.1 bc	217.7±38 abc	168.8±32.7 cd
"Sibilla"	254.2±50.2 a	241.9±100.2 ab	242.8±67.2 ab
AVERAGE	**181.5±72.4 NS**	**191.8±86.6 NS**	**175.8±68.5 NS**

4.3.7 Productive Parameters of Remontant strawberry cultivars

The analysis of the variance highlights that average fruit weight, commercial and total production are strongly dependent on cv, while the precocity index only on treatment. Finally, the interaction cv x treatment does not show significant effects for any of the parameters considered (Table 63).

Table 63: Two-ways analysis of variance (ANOVA) for the productive parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction

Parameter	Precocity Index	Average Fruit Weight	Commercial Production	Total Production	Waste
Cv (b)	NS	**	**	**	NS
Treatment (c)	**	NS	NS	NS	NS
CvxTreatment (c)x(b)	NS	NS	NS	NS	NS

Considering the average values of the three nitrogen trials, the precocity index shows an advance of production in N80 of 4 days. The three varieties appear to have a very close maturation period among them. In the N60 trial, "S. Andreas" with 221 days is the latest, followed by "Monterey" (219 days) and finally "Albion" (218 days) (Table 64).

It is important to highlight the early ripening in N80 trial for all the cultivars compared to N100 thesis, with a 6-days advance in "S. Andreas", 4 days in "Monterey" and almost 2 days in "Albion". "S. Andreas" and "Monterey" present significant differences between the N60 and N80 trials, differently from "Albion". This can be confirmed by the harvest time changes for the studied cultivars. Summarizing, it appears that a moderate nitrogen reduction (N80) could contribute to anticipate the harvest, but further reduction of mineral nitrogen (N60) determines a non-statistically significant delay in respect to control (N100).

Passing to the average fruit weight, it is clear that the nitrogen reduction does not affect in a significant manner this parameter in any of the studied cultivars; these results are in line with the study of Miltiadis et al. (2016). "S. Andreas" registers the highest average fruit weight, significantly higher than "Albion" at N100 and N80, and significantly higher than "Monterey" at N60 (Table 64).

Table 64: Effects of nitrogen on precocity index and average fruit weight in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p < 0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p < 0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Precocity index (days)**			**Average fruit weight (g)**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Albion"	218.6±1.9 ab	217.2±1.3 ab	218.8±3.5 ab	11.1±0.5 c	11.1±0.3 c	12.4±1.1 abc
"Monterey"	214.2±3.7 bc	210.1±1.6c	219±3.1ab	12±0.4abc	11.5±0.6c	11.7±0.7bc
"S. Andreas"	219.7±4.9ab	213.7±4.9bc	221±5.1 a	13.2±0.5 ab	13.2±2 ab	13.4±0.9 a
Average	**217.5±4.1 A**	**213.7±4.1 B**	**219.6±3.6 A**	**12.1±1 NS**	**11.9±1.4 NS**	**12.5±1.1 NS**

Other productive parameters like commercial and total production do not show significant differences among nitrogen supplies to the plants, even if an interesting increasing trend with a reduction of mineral nitrogen could be observed (Table 65). The absence of a significant effect of nitrogen reduction on plant yield was also found in the work of Miltiadis et al. (2016).
"Albion" has the highest values of commercial and total production in two nitrogen trials (N100 and N80), although without significant differences among cultivars and between the two-nitrogen amounts. In the thesis N60, for both productive parameters, "Monterey" stands out for the biggest amount of production, resulting significantly better than "S. Andreas". It is very interesting to note a significant increase of 89.3 grams for commercial production and 104.4 g for the total production of "Monterey" at N60 in respect to the control (N100). Also "Albion" and "S. Andreas" show an increase of both parameters in thesis N60, but without statistical significance in respect to N100. In Guttridge (1985), lower yield is explained as consequence of an excess of nitrogen that previously inhibited flower formation. Finally, nor the reduction of nitrogen supplies, nor the genotype effect influence the amount of waste (Table 66).

Table 65: Effects of nitrogen on commercial production and total production in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

CULTIVAR	**Commercial production (g/plant)**			**Total production (g/plant)**		
Treatment	**N100**	**N80**	**N60**	**N100**	**N80**	**N60**
"Albion"	194.9±26.3 abc	188.9±24 bc	208.1±49.2 ab	238.5±25.6 bc	235.6±16.9bc	249.6±56.3 ab
"Monterey"	166.6±31.5 bc	175.5±19.4 bc	255.9±29.7 a	211.3±32.7 bc	225.8±46.2 bc	315.7±16.8 a
"S. Andreas"	140.9±66.4 c	144.1±22.1 c	154.4±26.5 bc	172.9±74 c	184.2±31.4 bc	195.7±22 bc
AVERAGE	**167.5±45.5 NS**	**169.5±27.5 NS**	**206.2±54.2 NS**	**207.6±51.1 NS**	**215.2±37.6 NS**	**253.7±60.8 NS**

Table 66: Effects of nitrogen on waste in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (SD)

Cultivar	**Waste (g/plant)**		
Treatment	**N100**	**N80**	**N60**
"Albion"	43.6±6.6 ab	46.7±8.6 ab	41.5±7.7 ab
"Monterey"	44.7±1.4 ab	50.4±27.1 ab	59.8±16.1 a
"S. Andreas"	32±8.3 b	40.1±16.4 ab	41.3±5.7 ab
AVERAGE	**40.1±8.1 NS**	**45.7±17 NS**	**47.5±13.1 NS**

4.4 Conclusion

The objective of this study was to verify any changes in vegetative, productive and qualitative responses of single cropping and remontant cultivars at different nitrogen supplies. The purpose of this project is the achievement of a positive tradeoff between a good plant performance and a low environmental impact. The most interesting results obtained can be disclosed and applied by the growers working in open field conditions, in soil conditions with low organic matter and total N levels. The Single-cropping varieties, “Cristina”, “Romina” and “Sibilla”, do not show significant changes in plant height as response to reduced nitrogen inputs. Only “Sibilla” is slightly sensitive, with lower plant height. Contrarily, in the Remontant varieties, “Albion”, “Monterey” and “S. Andreas”, the plant height increases after the input reduction. Analyzing these results, it seems that a decreasing in nutritional intake, from about 100 Units/ha (N100) to about 70 Units/ha (N60) of nitrogen, does not negatively affect the plant development.

It is interesting to note that the medium nitrogen input (N80) has a delaying effect on Precocity Index in all Remontant varieties. On the other hand, “Cristina” in lower nitrogen supply (N60) slightly anticipates its production. The total production is one of the mainly productive parameters. It has not been noticed any significant difference for this parameter among Single-cropping plant with lower nitrogen rates. For the Remontant varieties, there is a slight increase of total production in trial N60, but is not evident any significant difference, except for “Monterey”. In general, for both Single-cropping and Remontant cultivars, the average fruit weight is not affected by nitrogen reduction.

Regarding the qualitative parameters, the sugar content value remains stable in fruits from the Single-cropping cultivars for all nitrogen supplies. Differently, in Remontant varieties, there is a reduction in the fruit sugar content with a lower nitrogen fertilization. This reduction is significant in “Monterey” and “S. Andreas”. All the varieties, at each nitrogen trial, maintain high values of fruit titratable acidity. “Cristina”, “Romina” and “Sibilla” keep a medium fruit firmness at the 60% nitrogen supply (N60); while, for the Remontant cultivars, firmness of “Monterey” fruits is negatively affected by the reduction of nitrogen supply. Chroma Index of Single-cropping varieties is not influenced by different nitrogen amounts. Among Remontant varieties, an important role is played by genotype: in fact, the brightness of the fruit is influenced by less nitrogen supply at different extent, according to the variety. “S. Andreas” increases its fruit darkness with less amount of nitrogen, “Albion” is not affected by different nitrogen amounts, while “Monterey” fruits become brighter with decreasing nitrogen.

From these data, it seems clear that there is a marked interaction between the genotype and the adaptation to the different nitrogen fertilization amount. The three Single-cropping varieties appear to have a similar rustic and resistant behavior, reacting to the nitrogen reduction almost in the same way. Differently, for the Remontant varieties, “Monterey” slightly increases production in N60, but reduces its fruit sugar content and firmness. In the same condition, “S. Andreas” reduces the fruit sugar content, but the firmness remains similar to the control. “Albion” appears to be less influenced by the soil nitrogen condition.

5 RESEARCH LINE III: DIFFERENCES IN THE RESPONSE OF SINGLE-CROPPING AND REMONTANT STRAWBERRIES TO DIFFERENT AMOUNTS OF CHILLING HOURS

Abstract

Different strawberry varieties require an exposure to chilling hours to get over the dormancy and to guarantee the high production. Climate change may impact winter chill. This fact could reduce the normal process of plant growth, reproductive development and finally the yield. In view of this, it is important to monitor the behaviour of strawberry plants in order to know and address the problems related to inadequate chilling requirements. The proper number of cold hours is crucial for strawberry management. The effect of different chilling hours (1200, 700, 400, 0) has been evaluated through the study of Single cropping ("Romina", "Sibilla", "Cristina") and Remontant ("Albion", "Monterey", "S. Andreas") on vegetative (Plant height, Leaf petiole length, Number of leaves, Number of inflorescences) and productive (Precocity index, Average fruit weight, Commercial, Total and Waste production) parameters.

The results of the study showed that without chilling hours the plants were more compact in terms of plant height and leaf petiole length. On the other hand, a decrease of chilling hours determined the higher number of inflorescences. Interesting was the same harvest period of the single cropping cultivars at 0 hours, unlike at 1200 they were classified in early and late varieties. The remontant cultivars showed the same harvesting period at 0 and 1200 hours. Moreover, the effect of optimum range (0 to 400) of chilling hour is confirmed by the best yield results, that are genotype-specific.

5.1 Introduction

The chilling requirements regard the hours spent in the cold that creates internal changes essential to the strawberry plant to assure the production in the next productive season. Strawberry has an optimum chilling temperatures range of between -1 and 10 °C, depending on cultivar (Guttridge, 1985). In such conditions, the plant takes on a low and compact growth habit charachetized by shorter petioles and smaller leaflets (Åström et al, 2015). In the temperate regions, during the autumn and winter, the shoots remain dormant in response to short days and low temperatures and the plant store carbohydrates in the roots and crowns until to have accumulated an adequate number of chilling hours (Durner et al, 1984; Maas, 1986; Al-madhagi et al., 2018). After the chilling hours are satisfied, the plants restart normal development, generating well lengthened leaves and flower-trusses with active flowers. Many studies confirm that there is a positive correlation between greater amount of cold hours and vegetative vigor, such as number of leaves, length of petiole, together with the production of runners and narrowing of ripening window (Bringhurst et al., 1960; Piringer and Scott, 1964; Braun and Kender, 1985). It is important to know the optimum chilling requirements of a species, that improves the vegetative development and supports the bud differentiation (Durner and Poling, 1987). Studies show the improvement in size and quality of the fruit caused by a correct exposition to the cold, better than the same genotypes that were not subject to chilling (Hamann and Poling, 1996; Bringhurst et al., 1960). On the other hand, an excess of chilling determines negative effect such as flower induction delay (Durner and Poling, 1987), thereby affecting yield. The requirement of chilling is determined by the genotype and may vary in different species and varieties (Piringer and Scott, 1964; Durner and Poling, 1987; Darnell and Hancock, 1996). Although the short-day cultivars, suitable for colder areas, can also be cultivated in tropical areas, they will never reach their full activity because they lack the essential chilling period (Darrow and Waldo, 1934) for the vegetative stasis. The situation is different for day-neutral cultivars, which produce crowns and flower buds approximately 3 months after plantings, regardless the length of day and chilling hours (Bringhurst and Voth, 1980; Galletta and Bringhurst, 1990). In Nicoll and Galletta (1987) and Yanagi and Oda (1992), it is explained that it is complicated to rank genotypes as day-neutral or short day due to many variable factors as genotype, temperature and photoperiod. In the last two decades, the analysis of temperature in Europe confirms the presence of extreme values more frequently in comparison to the past (Lorenz et al., 2019). There is an increase in the minimum temperature, in most European locations including

Italy, with the average indicating warmer winters. A decrease in winter temperature variability is related to constantly higher temperatures on cold days. The future climate scenarios will be characterized by a decrease of amount of winter chilling. The aim of this work is to investigate the effect of different amounts of cold temperature on strawberry vegetative and productive parameters. This study conducted at UNIVPM, compares the plant performance of six strawberry cultivars (three June-bearing and three Day neutral) placed in a cold storage (4 °C) for a different number of cold hours (1200, 700, 400) and those placed in green house, in temperature not falling below 7°C (no cold hours), during the winter dormancy.

5.2 Material and methods

The experimental work took place in the green house of Università Politecnica delle Marche (Figure 52), Marche Region, Italy (latitude 43°60′522; longitude 13°50′297). In the end of July 2018, plants subjected to study were placed individually in 16-cm-high pots, with a diameter of 18 cm, with a soil mixture of peat (80%) and pumice (20%). The irrigation system consisted of capillary tubes, and watering time was managed by a timer set at 5 minutes of watering per day. The nutritional elements have been added through granular mineral fertilizer (Osmocote Exact Mine 5-6M Aicl). The fertilizer rate was of 4 g/l for each plot, given during the growing season. Each granule contains all the nutritional elements present in the formula (15-9-11+2MgO+TE-NPK (Mg)). The plant types were cold stored plants (A+). The studied cultivars were "Romina", "Cristina", and "Sibilla" (Single-cropping) and "S. Andreas", "Albion", and "Monterey" (Remontant). The vegetative material was purchased from the nursery Coviro (Ravenna-Italy), that produces and markets certified nursery material. The plants were grown following a randomized complete block design. 7 plants for each treatment (each plant represents one replicates) and variety were chosen, according to common features as uniform plant development and wellness. The treatments consisted in four different amounts of cold hours the plants were exposed to (1200, 700, 400, 0 hours).

All the plants were planted on 28/07/2018 and keep to open air environmental light and temperature conditions until the 15/10/2018 (Figure 53). After that, plants were moved in the warm greenhouse and subjected to temperatures > 7°C and average temperature =16.7°C. Plants subjected to 0 chilling hours were mantained here until the harvest period in the following spring. The plants of the treatments 1-2-3 (1200, 700, and 400 chilling hours, respectively) have been placed in a cold room at controlled temperature (4°C) and in total darkness, in sequential dates, and simultaneously removed on 21/01/2019, in order to reach the planned chilling hours, and subsequently placed back in warm green house (Table 67).

Table 67: Date of plant entrance into cold storage and green house. Total number hours at 4°C in different treatment

TREATMENT	DATE OF PLANT ENTRANCE INTO COLD STORAGE (4°C)	N° HOURS at 4°C	DATE OF PLANT ENTRANCE INTO WARM GREEN-HOUSE
1	30/11/2018	1200	21/01/2019
2	23/12/2018	700	21/01/2019
3	4/01/2019	400	21/01/2019
4	NOT STORAGE	0	15/10/2018

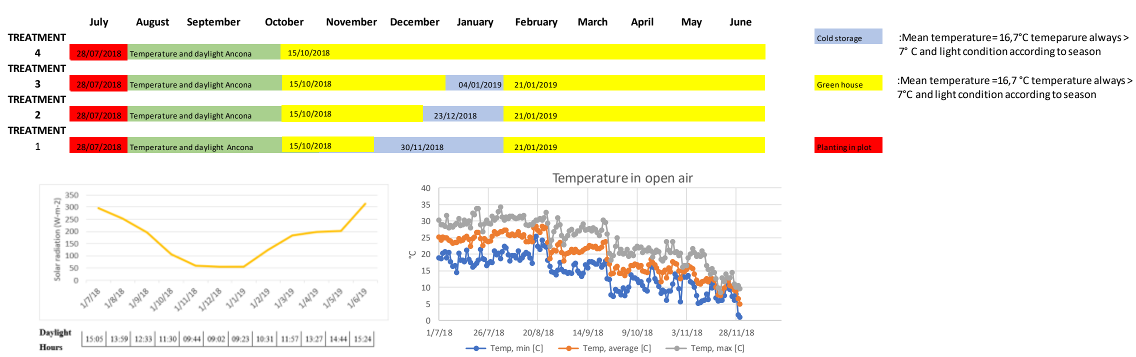
Plant height, length of leaf petiole, number and length of floral axis, precocity index, average fruit weight and yield (Commercial, Total and Waste production) were evaluated after the plants were subjected to cold treatments. All the vegetative parmeters were recorded on 28/03/2019 for all the plants. Plant height and length of the petiole were measured through the ruler, expressed in cm. Plant height was measured from the base of the branch crown to the apex of the primary leaves. Petiole length was measured from the base to the point where the three leaves of the trifoliate leaf join. The length of the inflorencesces was measured from the base of the branch crown to the calix of the primary fruit. Measurements of each cultivar/treatment were averaged. The fruits were handpicked from February to June (Table 68). Fruits were divided in: Commercial production (fruit suitable for marketing with diameter >22mm), waste (fruits cracked or rotten with diameter <22mm) and Total production, represented by total amount of harvest production (Commercial and Waste production). The description of the productive parameters has been already provided in the Chapter 3.2.4.

Table 68: Date of harvest from February to May 2019

Month (Harvest Time)	Date
February 2019	19
April 2019	19-22-23-30
May 2019	3-6-9-13-16-21-22-29
June 2019	5-12-19-29

Figure 52: Green house of University Politecnica delle Marche

Figure 53: General information on test setting and environmental data.



5.2.1 *Statistical analysis*

The STATISTICA Software (Stasoft, Tulsa, Ok) was used for the statistical analysis. Data were analyzed by two-way analysis of variance (ANOVA) to determine differences between cultivars, treatment and their interaction. Significant differences were detected by Fisher's Least Significant Difference (LSD) test, at $p < 0.05$. Results for strawberry vegetative and productive parameters are presented as mean ± standard error (SE) for each cultivar/treatment.

5.3 Results and discussion

5.3.1 Vegetative parameter of Single-cropping cultivars

According to Anova analysis, cv effect is significant for the plant response to all the parameters studied. The treatment significantly influences the number of leaves and inflorescences. The interaction cultivar x treatment significantly affects the plant height, the length of petiole and the number of leaves (Table 69).

Table 69: Two-ways analysis of variance (ANOVA) for the vegetative parameters **= significant interaction with $p<0.01$; *= significant interaction for $p<0.05$; n.s.= not significant interaction.

Parameter	Plant height	Leaf petiole lenght	Number of leaves	Number of inflorescences	Inflorescences lenght
Cv (a)	**	**	**	*	**
Treatment (b)	**	**	NS	**	NS
Treatment x Cv (a)x(b)	NS	NS	NS	**	*

<u>Plant height of Single-cropping cultivars</u>

The trend of the plant development and leaf petiole height is similar for the analyzed cultivars (Table 70). Results showed that temperature conditions, during the winter period, have an impact in terms of vegetative development of strawberry plants. In previous research, it was stated that low temperatures can influence the vegetative development, after a period of dormancy (Swartz and Powell, 1981; Lang, 1987). In our study, it is interesting to note that the gradual increase of cold hours has a positive effect on the plant development. The increase in vegetative growth due to chilling hours has been previously reported for other cultivars such as "Elsanta" (Tehranifar et al., 1998), "Sonata" and "Figaro" (Lieten, 2009), "Sachinoka" (Yamasaki, 2013), TFT BSU, "Pierre" and "Hawaiian" (Ledesma et al., 2017).

In the present study, the gap in terms of development between plants subjected to maximum chilling hours (1200 hours) and 0 hours is 8 cm (for plant height) and 5 cm (for leaf petiole height). It is to note that "Romina" and "Sibilla", statistically similar, present the best height result in relation to the greatest exposure to the cold. “Cristina”, with only 21 cm of plant height, remains more compact.

The minor number of hours spent in the cold corresponds with a very low growth. This behavior is more evident for “Romina” and “Sibilla”, with the latter most susceptible (less 10 cm of plant height at 0 hours than 1200), followed by “Romina” (less 8 cm of plant height at 0 hours than 1200). Also “Cristina” suffers lack of chilling, but at lesser extent.

Leaf petiole length of Single-cropping cultivars

Similar results to plant height were obtained for the leaf petiole length (Table 70). Lieten (2009) showed that the petiole and inflorescences lengths improve thanks to the highest chilling amount for “Sonata” and “Figaro” cultivars. In Fabien et al. (1999), the petiole length was measured in growth chamber, after natural autumnal exposition, revealing a decrease in the development also depending on the genotype.

The decline of petiole length is proportional to the reduction of chilling hours. The most sensitive cultivar is “Sibilla”, with a reduction of 6 cm from 1200 to 0 hours, then “Romina” (5 cm reduction) and “Cristina” (4 cm reduction).

Number of leaves of Single-cropping cultivars

The average number of leaves produced per plant remains quite constant, regardless different chilling treatments (Table 70). The study of Ledesma et al. (2017) shows a bigger leaves production in plants grown without chilling hours, but this result is strongly influenced by the genotype, as some cultivars produce many leaves regardless the chilling treatment.

In our study, among the plants not exposed to chilling temperature (0 hours), only “Cristina” stands out for to greater vegetative development in respect to the other chilling treatments. A similar result was previously reported by Asadpoor and Tavallali (2015), where “Camarosa” showed the best vegetative performance with high temperatures, in tropical and subtropical climate. This adaptability is genotype-dependent because other short-day cultivars, in the same conditions, have reduced their vegetative parameters (leaves number, length and width).

Number of Inflorescences of Single-cropping cultivars

The highest average number of inflorescences has been registered at 0 hours of cold storage (Table 71). There are no variations between 700 and 400 chilling hours trials. However, in values related to 1200 hours and 0 hours, a significant difference has been registered. In particular, “Sibilla” shows an interesting increasing trend of inflorescences with the decrease of chilling hours, obtaining its maximum value at 0 chilling hours, significantly better than all the other chilling treatments. Contrarily, “Cristina” shows its better values at 700 and 400 chilling hours, significantly higher than inflorescences of 1200 and 0 hours. The correlation between the reduction in number of inflorescences and duration of chilling is found in Yanagi and Oda (1992) for a junebearer cultivar (“Hokowase”), in a study designed similarly to our trial. Madhagi et al. (2018) explained that the plants treated with 360-h of chilling (2/1 °C at total darkness) in the first season produced about 71.2 % more flowers in respect to control group (without chilling), while in the second season 750-h of chilling produced about +50.3 % of flowers. The plants without chilling have been planted directly in January, while the others after February, with an average temperature above 15 degrees. On the other hand, Kirschbaum (1998) described a relation between increasing chilling hours and reduction of number of flowers. Nevertheless, in Ledesma et al. (2017) this parameter was not affected by different chilling amounts (with plants placed in dark cold storage at 4-5 °C) in some cultivar evaluated. These results suggest the great importance of the quantity of chilling hour to create optimal conditions for the development and flower induction of plants.

<u>Inflorescences length of Single-cropping cultivars</u>

There is not any statistical difference in the average length of the flower axis among cultivars subjected and not-subjected to chilling treatment (Table 71). The best result has been registered for “Sibilla” (21 cm) in 1200 chilling hours. Only for this cultivar, a decreasing trend has been observed in terms of inflorescences length moving from 1200 to 0 chilling hours. “Romina” showed values of inflorescences length similar to “Sibilla”, but with an opposite trend: in fact, the reduction of chilling hours stimulates the elongation of floral axes, even if without any significant difference among the four treatments. Finally, “Cristina” generates very short floral axes at each of the chilling treatments. An increase in the inflorescence length with increasing chilling hours has been demostrated in the cultivar “Elsanta” by Lieten et al. (2006).

Table 70: Effects of temperature on plant height (cm), petiole leaf height (cm) and N° leaves in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (ES)

Cultivar	**Plant height (cm)**				**Leaf petiole length (cm)**				**N° leaves**			
N° cold-hours	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**
"Cristina"	21.1± 1.5cd	21.9± 0.9c	18.6± 0.7d	14.6± 0.6e	12.7± 0.6ef	13.6± 0.8de	11.4± 0.6f	8.8± 0.4g	30.4± 2.4bc	33.1± 2.9abc	31± 1.6bc	39.4± 3.0a
"Romina"	30.2± 0.7a	25.6± 0.6b	25.7± 0.7b	22.7± 1.6c	20± 0.9a	17.4± 0.6bc	17± 0.6bc	15.4± 0.8cd	27.4± 2.1bc	28.4± 3.3bc	26.9± 1.2c	30.1± 1.7bc
"Sibilla"	28.6± 0.9a	25.9± 0.7b	23.6± 0.8bc	19± 0.6d	18.8± 0.8ab	16.9± 0.7bc	15.8± 1c	12.8± 0.9ef	28.9± 1.6bc	33.4± 2.9ab	28.3± 1.5bc	27.6± 1.7bc
Average	**26.6± 4.9A**	**24.5± 2.6AB**	**22.6± 3.6B**	**18.8± 4.3C**	**17.2± 3.7A**	**16± 2.4AB**	**14.7± 3.1B**	**12.3± 3.4C**	**28.9± 5.3NS**	**31.7± 8NS**	**28.7± 4.1NS**	**32.4± 7.7NS**

Table 71: Effects of temperature on n° inflorescences and inflorescences length (cm) in different strawberry cultivars . Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (ES)

Cultivar	**N° Inflorescences**				**Inflorescences lenght (cm)**			
N° cold-hours	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**
"Cristina"	4.3± 0.9e	7.3± 0.7abc	7.4± 0.2ab	5.3± 0.6de	11.2± 0.9c	11.8± 1c	8.9± 0.5c	9± 0.5c
"Romina"	5.6± 0.8cde	4.1± 0.6e	4.9± 0.3de	6.4± 0.4bcd	17.4± 1.8b	17.3± 2.1b	17.9± 1b	21.7± 1.9ab
"Sibilla"	5± 0.4de	5.4± 0.7de	6.1± 0.6bcd	8.7± 0.8a	21.8± 0.4a	18.2± 1.3b	17.8± 0.6b	17.7± 0.9b
Average	**5.0± 2.0B**	**5.6± 2.1AB**	**6.1± 1.5AB**	**6.8± 2.2A**	**16.8± 5.4NS**	**15.8± 4.8NS**	**14.8± 4.7NS**	**16.2± 6.3NS**

5.3.2 Vegetative parameter of Remontant cultivars

Analysis of Anova shows that cv effect is significant only for the number of leaves, while treatments affect also the number of inflorescences. Interaction cv x treatment shows significant relevance for plant height (Table 72).

Table 72: Two-ways analysis of variance (ANOVA) for the vegetative parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction.

Parameter	Plant height	Leaf petiole lenght	Numbers leaves	Number inflorescences	Inflorescences lenght
Cv (a)	NS	NS	**	NS	NS
Treatment (b)	**	**	NS	**	NS
Cv x Treatment (a)x(b)	**	NS	NS	NS	NS

<u>Plant height of Remontant cultivars</u>

Another significant finding of this study concerns remontant varieties, in which the varying number of hours of exposure to the low temperatures produced different effects on the cultivars (Table 73). The plants subjected to low temperature condition generally improved the performance in terms of plants height, in particular with 700 chilling hours. Also in this case, results are slightly influenced by the genotype response to chilling exposition. “Albion” and “S. Andreas” show a smaller plant growth for the unchilled treatment (0 hours), while “Monterey” for 400 chilling hours. All the three cultivars show a significant reduction of plant height in the absence of chilling treatments (0 hours) in respect to 1200 and 700 hours.

<u>Leaf petiole length of Remontant cultivars</u>

The length of leaf petiole is subjected to the same trend of plant growth, with an average reduction of 4 cm from 1200 to 0 chilling hours (Table 73). As for the plant height, also for the petiole length the best values were recorded at 700 chilling hours, with “S. Andreas” having a significant greater value than “Albion” and similar to “Monterey”. From a statistical point of view, “S. Andreas” do not show any difference among the petiole length at different chilling treatments (1200, 700, and 400), while the control plants (0 chilling hours) have a significantly negative impact on the vegetative performance. A similar result was also obtained by “Albion”, while in “Monterey” a significant reduction of petiole length already appears at 400 hours.

Number of leaves of Remontant cultivars

The average production of leaves does not show variations related to the exposure to the different chilling treatments, even if each cultivar behaves in different manner to the chilling hours (Table 73). “Albion” presents a decreasing trend of leaves number with the decrease of chilling hours, but without any significant difference. “S. Andreas” increases its leaves number with decreasing chilling hours, up to 24.7 in 400 hours treatment, and then shows the lowest number at 0 hours, but without any significant difference among treatments. Finally, “Monterey” increases the leaves number with decreasing chilling hours, obtaining its maximum at 0 hours. However, the 400 hours treatment has a negative impact on this cultivar, showing significant lower value than 700 and 0 hours.

Number of Inflorescences of Remontant cultivars

As for the single-cropping, even for the remontant cultivars there is a greater number of inflorescences in correlation with the decrease in the number of hours at low temperature (Table 74). This trend shows a significantly lower number of floral axes at 1200 chilling hours in respect to the other three treatments, which are similar to each other. In contrast, in the study of Yanagi and Oda (1992) there is not significant effect of chilling on inflorescence production of two everbearers cultivars (“Ostara” and “Rabunda”).

Inflorescences length of Remontant cultivars

There seem to be lack of correlation between chilling hours and the length of the inflorescences. “S. Andreas” produces longer floral axes in plants placed in green house without chilling treatment (Table 74). Also “Monterey” showed longer floral axes at 0 chilling hours but, differently from “S. Andreas”, this parameter does not present any statistical difference among treatments. Finally, “Albion” have showed longer floral axes than “Monterey” and “S. Andreas”, but this difference was noticeable only at 700 chilling hours, which also represent the better performance for “Albion”.

Table 73: Effects of temperature on plant height (cm), petiole leaf height (cm) and n° leaves in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (ES)

Cultivar	**Plant height (cm)**				**Leaf petiole length (cm)**				**N° leaves**			
N° cold-hours	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**
"Albion"	27.4± 0.9cd	28.4± 0.6bcd	26.4± 1.2de	23.9± 1.1ef	18.7± 0.9bcde	19.5± 0.9bc	17.2± 1.1cde	16.3± 0.8def	17.4± 1.9bcd	16.9± 2bcd	16.4± 2.3bcd	16.1± 1.8bcd
"Monterey"	29.2± 0.9abcd	30.1± 1.1abc	23.9± 1.1ef	24± 1.3ef	19± 0.7bcd	20.5± 1.1ab	16.3± 1ef	16± 0.9ef	15.6± 1.6cd	19.9± 1.6abc	14± 0.9d	20.3± 2.2abc
"S. Andreas"	29.1± 1.1abcd	31.6,0± 1a	30.7± 1.3ab	21.9± 1.4f	20.3± 1.2ab	22.2± 0.8a	20.3± 0.9ab	14.4± 1f	21.4± 1.7ab	23.9± 2.2a	24.7± 2.1a	20± 1.8abc
Average	**28.5±2.6B**	**30±2.7A**	**27±4.2B**	**23.2±3.4C**	**19.3±2.5AB**	**20.7±2.7A**	**17.9±3B**	**15.6±2.4C**	**18.1±5NS**	**20.2±5.7NS**	**18.4±6.6NS**	**18.8±5.3NS**

Table 74: Effects of temperature on n° inflorescences and inflorescences length (cm) in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (ES)

Cultivar	**N° Inflorescences**				**Inflorescences lenght (cm)**			
N° cold-hours	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**
"Albion"	4.3± 0.8abc	5.6± 0.6ab	5.3± 0.6ab	4.7± 0.3abc	21.5± 1.8ab	24± 1.7a	20.3± 1.2ab	20.7± 1.1ab
"Monterey"	4.3± 0.5abc	5.1± 0.6ab	5.9± 0.5a	5.9± 0.3a	17.0± 1.6b	19± 1.1b	19.3± 2.1b	20.4± 1.1ab
"S. Andreas"	3.1± 0.3c	4.1± 0.6bc	4.6± 0.5abc	5.6± 0.8ab	18.8± 2.6b	17.6± 1.9b	17.7± 1.7b	24.1± 0.9a
Average	**3.9±1.6B**	**5±1.7A**	**5.2±1.5A**	**5.4±1.4A**	**19.1±5.4NS**	**20.2±4.9NS**	**19.1±4.4NS**	**21.7±3.1NS**

5.3.3 Productive parameters of single cropping cultivars

Anova analysis shows that the cultivar influences all the analyzed parameters, except the fruit waste, while the treatment is effective ony of commercial production; the interaction cv x treatment affects the precocity index, total production and fruit waste (Table 75).

Table 75: Two-ways analysis of variance (ANOVA) for the productive parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction.

Parameter	Precocity Index	Average fruit weight	Commercial Production	Total production	Waste
Cv (a)	**	**	**	**	NS
Treatment (b)	NS	NS	*	NS	NS
Cv x Treatment (a)x(b)	**	NS	NS	**	**

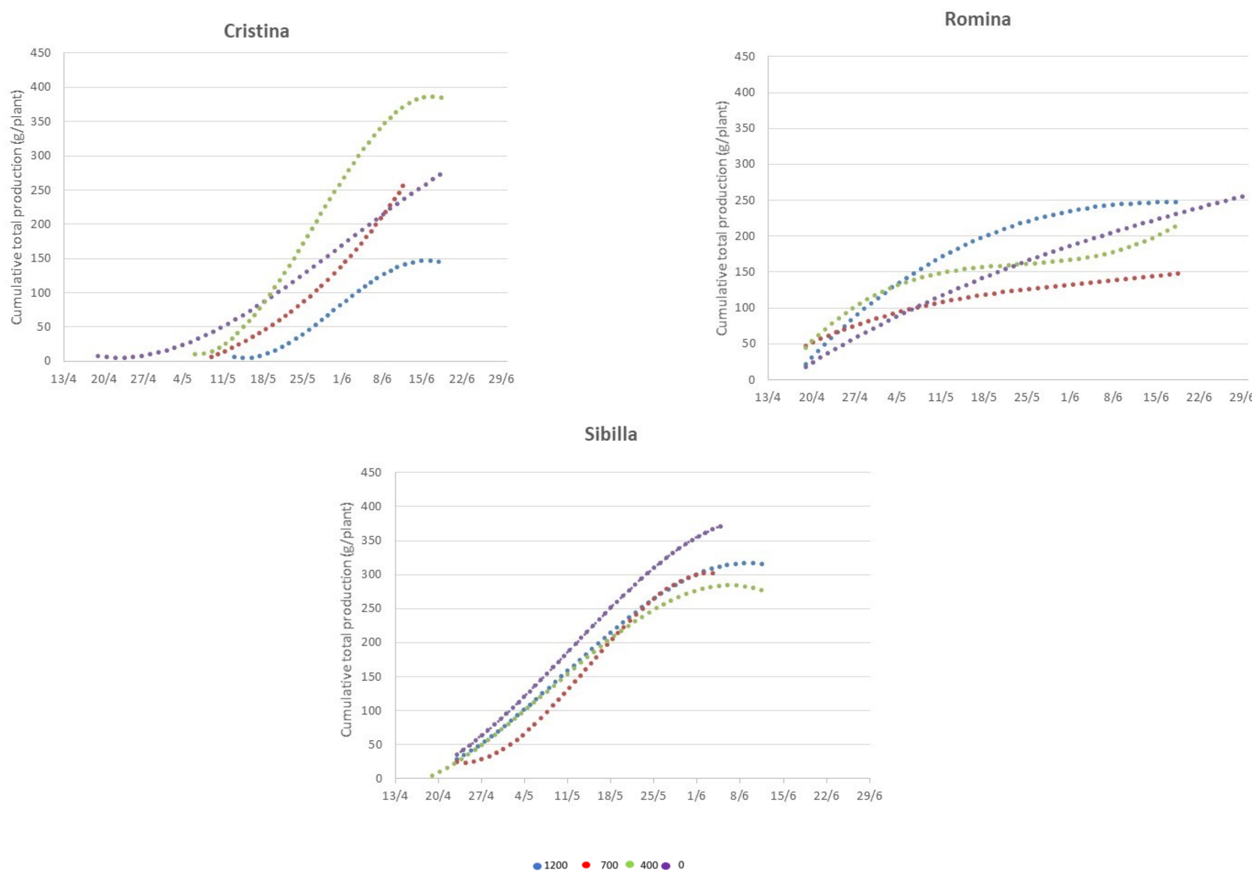
<u>Precocity Index of Single-cropping cultivars</u>

The "Romina" plants which were placed directly in the warm greenhouse without any chilling treatment show a harvest delay of 15 days in comparison to the plants exposed to 1200 hours of chilling (Table 76). In addition, the total cumulative production of the plant during the harvest period is lower. For both treatments, the harvest is more constant than in "Cristina". On the other side, "Cristina" plants enter in production 13 days before in the 0 hours treatment in respect to 1200 chilling hours. However, at treatment 1200, it shows a short time in total cumulative production per plant during the harvest period (Figure 54).

If collected after 0 chilling hours, both "Cristina" (late variety) and "Romina" (early variety) lose their characteristic ripening period. The only cultivar not affected by chilling hours in terms of ripening time is "Sibilla". In this regard, the 0-hours treatment differs from 1200 for the large and early harvest of the end production. The number of days necessary for obtaining fully ripen fruits is not regular, because for some varieties the chilling treatment does not causes any difference, while for others a faster fruit ripening of unchilled plants occurs (Ledesma et al., 2017).

These results could be also related to another parameter: the date of blossoming. Tehranifar et al. (1998) reported that increase in the chilling time causes earlier blossoming. Many authors have also noticed this correlation, together with the relation between the earlier opening of the first flower and the increase of the chilling period (Lieten, 1995; Tanino and Wang, 2008; Ledesma et al., 2017).

Figure 54: Cumulative total production (g/plant) of Cristina, Romina, Sibilla, during the harvest period.



Average fruit weight of Single-cropping cultivars

It is important to mention that the shift in the harvest time, due to different chilling treatments, does not significantly affect the average fruit weight (Table 76). The heaviest fruits belong to “Cristina” variety. “Romina” and “Sibilla” show statistically similar average fruit weight. Among the tested cultivars, “Cristina” has significantly highest average fruit weight in all chilling treatments, comprising 0 hours.

Commercial production of Single-cropping cultivars

The average commercial production of tested varieties differs significantly among chilling treatments (Table 77). For Single-cropping cultivars, some chilling hours were needed for maximum efficiency production. In our study, they showed the best productive performances when exposed to 0 or 400 chilling hours. In a study from Lieten (1995), the highest commercial production was obtained between 500 and 800 chilling hours

Each studied cultivar exposed to the chilling treatments shows specific features: “Romina” produces more commercial fruits in 1200 chilling hours, “Cristina” in 400 chilling hours and “Sibilla” in 0 chilling hours condition. The highest commercial production of this study was obtained by “Sibilla” subjected to 0 chilling hours.

Total production and waste of Single-cropping cultivars

Total production does not show significant differences among cold treatments, even if the trend is similar to that of commercial production, with higher values for 0 and 400 chilling hours (Table 77). The best productive performances were obtained by the different cultivars as follows: the highest value (383.1 g) for “Cristina” in 400 chilling hours, for “Sibilla” (369.3g) and “Romina” (251.9 g) in 0 chilling hours. The lower productions were registered by “Cristina” (150.3 g), “Romina” (152.7) and “Sibilla” (278.9 g) at 1200, 700 and 400 chilling hours, respectively.

The average of not-edible fruits, not suitable for marketing, does not show a statistical difference among chilling applications. There is a correspondence between largest plant production and higher amount of waste for all the cultivars.

Table 76: Effects of temperature on precocity index (days) and average fruit weight (g) in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (ES)

Cultivar	**Precocity index (days)**				**Average fruit weight (g)**			
N° cold-hours	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**
"Cristina"	156.0± 2.1a	154.4± 2.4a	149.5± 1.3ab	143.9± 1.5b	21.9±2.3a	19.9±1.8ab	22.4±1.2a	23.1±1.5a
"Romina"	127.4± 1.7c	127.3± 4.8c	132.2± 2.1c	142.0± 6.0b	16.1±1.2bc	13.6±0.7c	15.0±0.9c	16.5±2.1bc
"Sibilla"	133.0± 2.0c	133.5± 2.6c	131.9± 1.4c	133.2± 1.2c	16.8±1.3bc	16.0±0.3bc	17.2±1.6bc	15.1±1.1c
Average	**138.8±3NS**	**138.4±3.2NS**	**137.9±2.1NS**	**139.5±2.3NS**	**18.2±1.1NS**	**16.5±0.8NS**	**18.2±1NS**	**18.3±1.2NS**

Table 77: Effects of temperature on commercial production/plant (g) total production/plant (g) and waste (g) in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test (p<0.05). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test (p<0.05). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (ES)

Cultivar	**Commercial production (g/plant)**				**Total production (g/plant)**				**Waste (g/plant)**			
N° cold-hours	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**
"Cristina"	95.7±26.4cd	134.4±32.3bcd	205.7±20ab	170.6±35.8bc	150.3±42.4e	258.7±49.2cd	383.1±40.3a	262.6±39.1bc	54.6±17.8d	124.3±27.4ab	177.4±35.7a	92±20.8bcd
"Romina"	174.1±33.3b	91.1±20.0d	158.1±27.5bcd	145.6±28.2bcd	249±37.8cde	152.7±27de	221.3±33cde	251.9±39.5cde	74.9±16.1bcd	61.6±11.9cd	63.2±11.9bcd	106.3±20.3bcd
"Sibilla"	204.6±33ab	181.3±16.4ab	203.3±22.9ab	253.6±25.7a	317.2±50.6abc	298.2±25abc	278.9±24.7abc	369.3±38.7ab	112.6±22.5bcd	117±26abc	75.6±22.6bcd	115.7±19.9abcd
Average	**158.1±19.9AB**	**135.6±15.5B**	**189±13.9A**	**189.9±19.5A**	**238.8±28.5NS**	**236.6±23.8NS**	**294.4±23.6NS**	**294.6±24.5NS**	**80.7±11.7NS**	**100.9±14NS**	**105.4±18NS**	**104.7±11.3NS**

5.3.4 Productive parameters of Remontant cultivars

Anova shows that the cultivar effect is visible only for the precocity index, while the treatment influences the precocity index, the commercial production and the total production. The interaction cv x treatment has an effect only on precocity index (Table 78).

Table 78: Two-ways analysis of variance (ANOVA) for the productive parameters **= significant interaction with p<0.01; *= significant interaction for p<0.05; n.s.= not significant interaction.

Parameter	Precocity Index	Average fruit weight	Commercial Production	Total production	Waste
Cv (a)	**	**NS**	**NS**	**NS**	**NS**
Treatment (b)	*	**NS**	*	**	**NS**
Cv x Treatment (a)x(b)	**	**NS**	**NS**	**NS**	**NS**

<u>Precocity index and average fruit weight of Remontant cultivars</u>

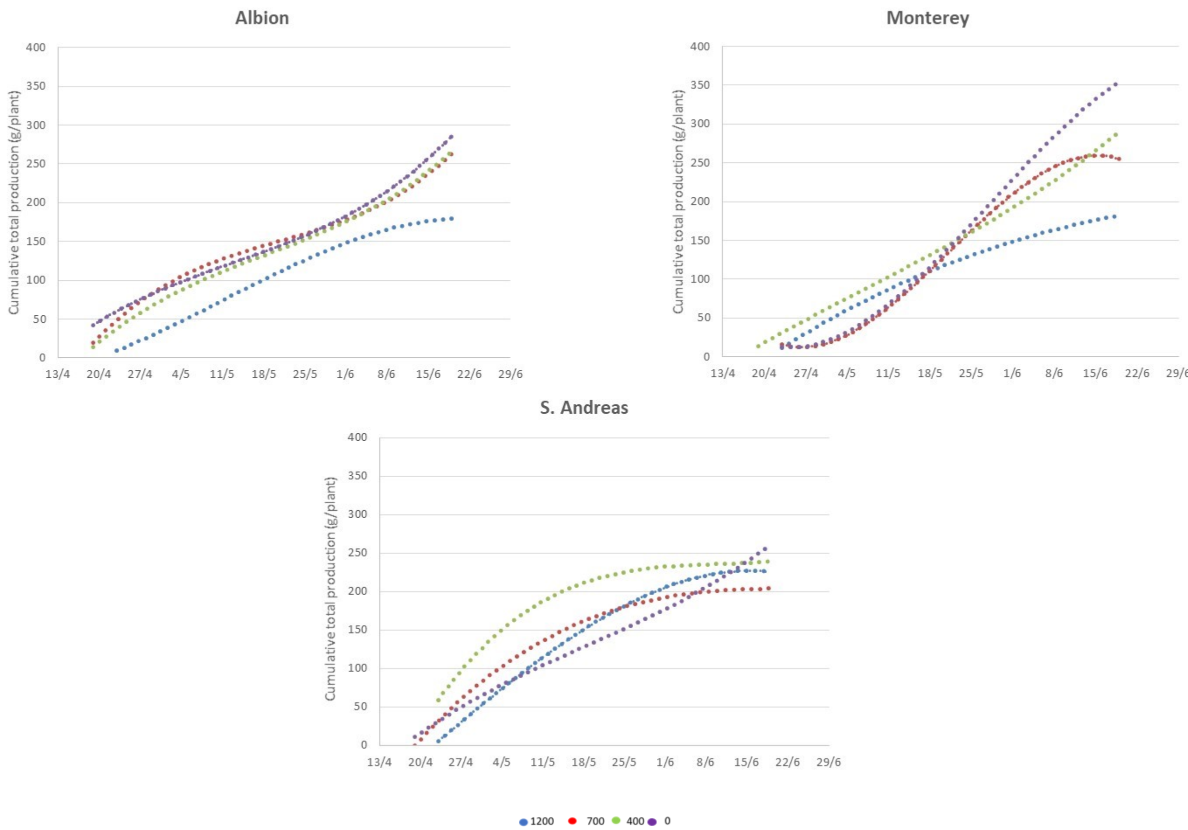
The statistical analysis allows to confirm an earlier average maturation time of fruit, quantifiable in about 9 days, of plants subject to 700 chilling hours (the earliest) in respect to the control (0 hours) (Table 79). In general, the relation between longer chilling time and earlier ripening has been respected by almost all the cultivars. “S. Andreas” shows its earliest harvest with 700 and then with 400 chilling hours, anticipating of 25 and 16 days the harvesting of 0 hours treatment. Plants subjected to those two treatments show a similar productive trend that reaches the maximum production in late May, with greater production for plant that received 400 chilling hours. While plants with 0 chilling hours assumes a linear productive growth until the end of June (Figure 54). The ripening period for “Albion” and “Monterey” remains almost unchanged among the chilling treatments.

Considering the cumulative production for “Monterey” plants during the harvest period, the 400 chilling hours differs from the other treatments for a linear increase in time. “Albion” shows similar trends (Figure 55).

However, it is to note that for “Monterey” the difference between plants with the earliest precocity index (1200 chilling hours) and the latest (0 chilling hours) is of 12 days, even if this difference is not significant. “S. Andreas” stands out because it is significantly earlier, with 400 and 700 chilling hours, than both the other cultivars.

The different chilling times do not affect to the average fruit weight (Table 79). Only “Monterey”, with 700 chilling hours, presents significantly bigger fruits than 1200 and 0 chilling hours. Other studies (Smeets, 1982; Yanagi and Oda, 1992) declare that without fulfilling the chilling requirement, the big-sized fruits are not produced.

Figure 55: Cumulative total production (g/plant) of Albion, Monterey, S. Andreas, during the harvest period.



Commercial production of Remontant cultivars

The average commercial production of remontant cultivars show a loss of 28% of yield from plants that have been subjected to the maximum amount of chilling hours (1200) in relation to plants without chilling treatment (0 hours) (Table 80). By going deeper in details, the results for commercial production are similar for the 0 and 400 chilling hours, then the production gradually fall with the chilling hours increase.

The data obtained from the assessment of single cultivar show that the behavior is quite similar: for both “Monterey” and “S. Andreas”, the maximum production was obtained with plants grown without chilling hours, while in Albion the highest amount of commercial strawberries is obtained with an amount of 700 chilling hours, significantly better than the 1200 chilling hours treatment.

Total production and waste of Remontant cultivars

The total production follows the same trend of the commercial yield, as already showed above, so that avoiding chilling hours has a positive effect on this parameter (Table 80). In particular, this trend is respected by all the three studied cultivars, and the 0 chilling hours treatment shows the best result for all of them. However, in “Albion” and “S. Andreas”, despite this trend, there are not significant differences among treatments. On the contrary, “Monterey” shows a clear significant difference between 0 and 1200 chilling hours treatments, with the latter causing a drop of production of 51% in respect to 0 chilling hours. Comparing the number of inflorescences present in Table 74 to the productive performance, it is evident that the lowest yield is associated to the lowest number of inflorescences. Asadpoor and Tavallali (2015) compared the productive parameters of short days and remontant cultivars in the mild tropical wheater. Some short days cultivars (“Camarosa” and “Queen”) and “Mark” (remontant variety) had the highest number of fruits compared with other short day (“Kordestan”, “Parose”) and remontant cultivars (“Selva”).

Finally, the average waste production does not seem to be influenced by chilling hours. The only exception is “Monterey”, that highlights a lower waste (-83%) of 1200 hours trial compared to 0 chilling hours trial (Table 80).

Table 79: Effects of temperature on precocity index (days) and average fruit weight (g) in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (ES)

Cultivar	**Precocity index (days)**				**Average fruit weight (g)**			
N° cold-hours	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**
"Albion"	139.0±2.9a	138.9±2.5a	141.1±1.7a	139.4±3.7a	17.7±0.6b	20.0±2.1b	21.2±1.6b	22.2±2.3ab
"Monterey"	133.7±2.8ab	141.0±2.5a	139.7±4.2a	145.9±2.1a	20.0±1.5b	26.8±2.7a	22.0±2.6ab	20.0±2.8b
"S. Andreas"	133.6±2.7ab	116.6±7.6c	125.4±2.2bc	141.8±3.6a	21.6±1.2b	21.0±1.8b	18.2±0.9b	21.2±1.1b
Average	**135.4±1.6AB**	**132.2±3.6B**	**135.4±2.2AB**	**141.5±2.1A**	**19.8±0.7NS**	**22.6±1.4NS**	**20.5±1.1NS**	**21.4±1.1NS**

Table 80: Effects of temperature on commercial production/plant (g) total production/plant (g) and waste (g) in different strawberry cultivars. Values with same lowercase letter for the same parameter were not statistically different for Fisher's LSD test ($p<0.05$). Average values for each parameter with same uppercase letter were not statistically different for Fisher's LSD test ($p<0.05$). N.S.= not significant. Values are expressed as means of one year (2019) ± standard deviation (ES)

Cultivar	**Commercial production (g/plant)**				**Total production/plant (g/plant)**				**Waste (g/plant)**			
N° cold-hours	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**	**1200**	**700**	**400**	**0**
"Albion"	138.9±29.5d	230.1±9.2abc	215.7±30abcd	210.7±22.7abcd	180.1±24c	261.9±7.4abc	261.1±39.1abc	271±20.8abc	41.2±12ab	31.8±9.4ab	45.4±13.8a	60.3±16.5a
"Monterey"	170±41.3bcd	204±29.4abcd	250.6±41.1ab	290.7±20.8a	181.1±44.1c	253.8±32.7abc	284.2±40.7ab	356.4±37.8a	11.1±5.4b	49.8±10.3a	33.7±10.9ab	65.8±17.3a
"S. Andreas"	173.1±33.7bcd	161±33.1cd	200.6±16.1abcd	208.1±21.8abcd	225.5±41.2bc	206.2±40.8bc	239.5±15.3abc	258.3±29.1abc	52.3±12.7a	45.2±10ab	38.9±10ab	50.2±17.5a
Average	**160.7±19.6B**	**198.4±15.7AB**	**222.3±17.5A**	**223.8±14.9A**	**195.6±21.1B**	**240.7±17.6AB**	**261.6±18.9A**	**280.9±17.5A**	**34.9±7A**	**42.3±5.7AB**	**39.3±6.5AB**	**57.1±9.9A**

5.4 Conclusion

Climate change is causing an average increase of global temperature, more variable weather and varying precipitation patterns. It is therefore essential to investigate and identify the strawberry cultivars that can give satisfactory vegetative and productive performance, even in different climatic conditions.

The negative effects caused by no chilling application have been manifested by plants through the reduction of the development, in terms of plant/leaf height, both for Single-cropping and Remontant cultivars. Other parameters such as N° leaves and length of inflorescences showed no significant difference among the different amounts of chilling hours at which plants were subjected. It was interesting to note that the optimum chilling hours amount changes according to the genotype. “Romina” and “Sibilla” have the maximum plant/petiole height after 1200 cold hours. While “Cristina” and Remontant cultivars need only 700 chilling hours. The opposite situation emerges for N° of floral axes, where decreasing chilling hours gave a greater production. Results also underlined a remarkable influence of chilling hours on the start of ripening. “Romina” and “Cristina” in the test with 0 chilling hours had a contemporary harvest, because “Cristina” was characterized by an early production, while “Romina” was delayed, in respect to 1200 chilling hours trial. This parameter in “Sibilla” was not affected. The Remontant cultivars evidenced an early harvest at 700 chilling hours in comparison to the trial without chilling hours. It is important to note how the same harvest period among “Albion”, “S. Andreas” and “Monterey” was kept for both 0 and 1200 cold hours.

Finally, our results suggest that Single-cropping total production is constant and higher even with the application of 0 chilling hours (“Sibilla” and “Romina”), while for “Cristina” at least 400 chilling hours are needed. The yield is reduced of about 19 % in the 700 and 1200 chilling hours trials. The most productive cultivar resulted “Sibilla”. The same trend was registered for Remontant varieties, with a greater production lost (-30%) in 1200 chilling hours trial in respect to 0 chilling hours trial. There is no significant difference among “S. Andreas”, “Albion”, and “Monterey”.

In conclusion, reduction of number of chilling hours brings the vegetative habitus more compact but stimulates good productive performance also from 0 up to 400 chilling hours applied.

6 CONCLUSIONS OF THE STUDY

Water, nitrogen and temperature are three of the main factors that influence the plant performance and adaptability. Be aware about the need to save natural resources, and to reduce agronomical inputs in order to preserve the natural environment, and to face the climate change, is leading to the realization of studies, like the present research, aimed to reduce and optimize the use of inputs in agriculture.

Besides the environmental and cultivation conditions, also the plant type, the genotype, the cultivation site and the year of study are factors influencing the plant performance, so they were also taken into account in this study.

- Regarding the reduction of irrigation water trial held in Italy, both the Single-cropping and the Remontant cultivars analyzed showed satisfying results for all the evaluated parameters up to a 20% water reduction. It is to note that the Remontant cultivars result slightly more susceptible to water stress than Single-cropping cultivars. Besides the genotype effect, this difference could be due to the different planting period of the two types of plants: the Single-cropping, in fact, were planted in the August of the previous year in respect to start of irrigation trial, so they had several months to expand their radical apparatus and to explore more soil volume. Differently, Remontant cultivars were planted in the April of the same year of irrigation trial, so they had only few months to prepare the plant to face the water restriction. However, in both cases, a reduction of water not higher than 20% of the optimal amount is suggested. In fact, a further reduction up to 40% of water amount led to a significant reduction in both plant vegetative and productive parameters, in particular for Remontant cultivars. Only the qualitative parameters result positively affected by the water stress, in particular for the fruits of the Remontant cultivars, but this increase of fruit quality is not sufficient to counteract the negative impact of water stress on plant development and yield. For all these reasons, the growers of the Marche Region are suggested to cultivate the varieties tested in this study with a 20% of water reduction, without losing plant performances and fruit quality, and allowing to save a good amount of water.

  For the trial held in Spain, some of the Ancona selections showed interesting vegetative, productive and qualitative results, similar to the well-adapted cultivar "Rociera", in particular with a slight decrease of irrigation water (72% of the normal irrigation amount). This reduced amount of water allowed to save about 2458 m^3/Ha of irrigation water that, for the average Spanish farms, it could be translated into a good environmental and economic advantage. Furthermore, this study has allowed to evidence that an excessive (100%) and a reduced (50%) amount of water both resulted in a worsening of plant vegetative performance (in particular plant height and leaves number), probably due to a stressing situation at the radical apparatus which led to a reduced plant development. Similarly, also the productive parameters resulted compromised, with a reduction of commercial production in both W100 and W50 trials in respect to W72. An interesting result was the anticipation of the ripening period in the water-less trials in respect to control: this is a very interesting parameter for the Spanish farms, which could become more competitive on the global market if they could be able to produce fruits earlier than the competitors. Concluding, some Ancona selections have demonstrated good adaptability to the Spanish conditions, resulting similar or even better, especially for the qualitative parameters, than "Rociera"; those selections could be suggested to the farmers of the Huelva region as a valid alternative to this cultivar. Only the production of "Rociera" resulted significantly higher than the selections, but this could be due to the difference of plant type used for the trial: the Ancona selections were rooted tips, that were less adapt to the planting period than the bare rooted plants of "Rociera".

- The nitrogen reduction trial revealed that the decrease of N fertilization could be applied on soil with low levels of organic matter and total N, as the soils of the area where the trial was performed. Regarding the results obtained in the nitrogen reduction trial, no great variations could be observed in general when the mineral fertilization with N is applied, for any of the analyzed parameters. In particular, for the Remontant cultivars, the plant height resulted higher with a reduced nitrogen supply, and the same plants showed an anticipation of the harvest period. Regarding the productive parameters, it stands out that the total production was not affected by the reduction of nitrogen fertilization in both of the strawberry types. For the qualitative parameters, the effect of the reduction of nitrogen is different according to the considered one, in particular for the Remontant strawberries. E.g., the sugar content in fruits of Single-cropping cultivars did not change with the nitrogen reduction, while in the Remontant varieties it was registered a decrease in the fruit sugar content, in particular for "Monterey" and "S. Andreas". Also for the fruit acidity values, the nitrogen effect was higher in Remontants cultivars, with "Monterey" showing softer fruits with less nitrogen fertilization.

  On the light of these considerations, it is possible to suggest that in the environmental conditions where the trial was performed, a slight reduction of nitrogen fertilization does not negatively affect both Single-cropping and Remontant cultivars studied. However, the plant adaptability to the reduction of mineral nitrogen is strongly influenced by the genotype, in particular in the Remontant varieties.

- The third aim of the study was to investigate the performance of Single-cropping and Remontant plants in response to different amounts of chilling hours. The study was organized in a way to give to the plants four different amounts of winter chilling hours (from 0 to 1200), that reflect different environmental conditions that could be found in many geographical areas. The absence of chilling hours negatively influenced many vegetative parameters (as the plant height and the petiole length), while others (as the floral axes number) were positively influenced by the reduction of chilling hours. One of the most influenced parameters was the precocity index, in particular in the Single-cropping cultivars: in fact, it stands out how two cultivar with completely different ripening period at 1200 chilling hours, as "Romina" (early cultivar) and "Cristina" (late cultivar), strongly modified their ripening time in absence of chilling hours, resulting contemporary in terms of harvest period. Also for the Remontant cultivars, it was possible to detect an effect of the chilling hours on the ripening period, with 700 chilling hours anticipating the fruit ripening, but this effect was less evident than in the Single-cropping cultivars. Regarding the production, the Single-cropping cultivars were not negatively influenced by the reduction of chilling hours and they can assure the same amount of total production in all the tested conditions, while the Remontant cultivars showed the optimal production with low amounts of chilling hours. In conclusion, the different amounts of chilling hours could affect at different extent many of the vegetative and productive parameters, both negatively and positively, in Single-cropping and Remontant cultivars. For this reason, the application of more or less chilling hours, when possible, should be managed by each grower according to the final aim of his farm.

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