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**EFFECT OF TRAINING SYSTEMS AND PRUNING METHODS
ON FRUIT QUALITY IN APPLE**

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Abstract

The introduction of dwarfed rootstocks in apple crop has led to a new concept of intensive planting systems with the aim of producing early high yield and with returns of the initial high investment. Although yield is an important aspect to the grower, the consumer has become demanding regards fruit quality and is generally attracted by appearance. To fulfil the consumer's expectations the grower may need to choose a proper training system along with an ideal pruning technique, which ensure a good light distribution in different parts of the canopy and a marketable fruit quality in terms of size and skin colour. Although these aspects are important, these fruits might not reach the proper ripening stage within the canopy because they are often heterogeneous. To describe the variability present in a tree, a software (PlantToon®), was used to recreate the tree architecture in 3D in the two training systems. The ripening stage of each of the fruits was determined using a non-destructive device (DA-Meter), thus allowing to estimate the fruit ripening variability. This study deals with some of the main parameters that can influence fruit quality and ripening stage within the canopy and orchard management techniques that can ameliorate a ripening fruit homogeneity. Significant differences in fruit quality were found within the canopies due to their position, flowering time and bud wood age. Bi-axis appeared to be suitable for high density planting, even though the fruit quality traits resulted often similar to those obtained with a Slender Spindle, suggesting similar fruit light availability within the canopies. Crop load confirmed to be an important factor that influenced fruit quality as much as the interesting innovative pruning method "Click", in intensive planting systems.

Key words: *Malus domestica Borkh.*, Slender Spindle, Bi-axis, PlantToon®, fruit variability, crop load, light distribution.

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1. Introduction – A general overview

Apple orchards have undergone radical changes in the last decades, towards obtaining a high production over a short period of time. The introduction of dwarf rootstocks, new varieties and the need to produce as soon as possible modify the design of new orchards (Crassweller and Smith, 2007). Large free standing trees have been substituted by dwarfed trees with a requirement of a frame to support intensive planting systems. The increase in tree density leads to a return of the high initial investment as soon as possible.

The positive relation between yield and tree densities have been studied by many authors (Ferree, 1980; Wertheim et al., 1986; Palmer, 1988). For example 3000 trees/ha have been suggested as an optimal planting density for an intensive orchards to result in greater profitability (Robinson, 2011). High planting density orchards enhance labour efficiency and reduce the average orchard lifespan to *ca* fifteen years (Wagenmakers, 1991). Moreover, differences in managerial capacity such as training and pruning techniques can cause large variations in crop yield (Wagenmakers, 1991). It is important to achieve a balance between vegetative growth and cropping in high density planted orchards in the 3rd and 4th years for a stable production of quality fruits (Choi et. al., 2009). Fruit thinning is mandatory for maintaining an annual profitability in apple orchards (Robinson, 2011).

These intensive planting systems over the years increase in their foliage and therefore it is not only the high yield that may be important but also the quality of the fruit. Robinson et al. (1991) found that even small conic shape trees may have poor illumination in the denseness of the branches and foliage within the small canopy. It is known the light is very important not only for the improvement of fruit quality but also because might influence the tree vigour and the productivity for the next years (Rom, 1991). Light distribution depends on various inter-related factors. For instance the intrinsic architectural pattern of the cultivar itself is one of the factor affecting it (Sansavini and Corelli-Grappadelli, 1992). A second factor is the planting systems (Jackson, 1980) which includes both orchard design (planting distances, row orientation) and training of the canopy by pruning and bending procedures (tree shape and height). (Willaume et al., 2004).

Based on what has been discussed above, this thesis intends to analyze some main factors which could influence fruit quality in the apple orchard. The investigation of fruit variability within the canopy helps not only to understand better how fruit quality is distributed, but also to select the cultural management techniques that are able to ameliorate the quality of the fruit. The topological position is a determinant effect on fruit quality (Farhoomand et al., 1977; Barritt et al., 1987) and the measurement of the light levels in the different parts of the canopy could help to understand how the light is intercepted by the fruit located in different position of the tree. In-depth study on

innovative training systems and or alternative pruning methods will also aid in enhancing apple fruit quality in the orchard, which are discussed below in details .

1.1. Training systems and pruning methods affecting fruit quality

Fruit quality is influenced by many factors which are all inter-connected. Training systems are one of those which influence both yield and fruit quality (Robinson et al., 1991; Palmer, 1997) and are related to environment, tree densities (Robinson et al., 1991; Hampson et al., 2002; Licznar-Maanczuk, 2006) and pruning methods (Tustin, 2000; Özkan et al., 2009). The latter, moreover influences the shoot position, shoot type (Wunsche et al., 1996; Willaume et al., 2004; Stephan et al., 2008) and bud development (Ventura et al., 2005). Training system is defined as a method of manipulating the tree structure and canopy geometry to improve light interception and distribution, aiming to optimize fruit quality and yield (Costes et al., 1999; Caruso et al., 2003). Many kinds of training systems were adopted over the years with the intention of increasing production and maintaining a good fruit quality.

Among all the tree shapes, Spindle is still one of the most used and adaptable training systems adopted in high density plantings. Slender Spindle was developed by Wertheim in 1968 and designed for the greater biological and management efficiency by allowing all management operations to be done from the ground. The width of the canopy is less than 2 meters, which allows a better canopy exposure. These training systems due to their conical shape and reduced space distance between rows and trees might result in a lower fruit quality especially in the inner and lower parts of the tree due to the effect of shading (Corelli and Sansavini, 1989).

Other systems such as the “Thin vertical” shaped canopies were introduced with an aim to increase light exposure from both sides, while “inclined V” systems were introduced to increase light interception in the field (Robinson and Lakso, 1989). Ferree (1980) reported that the “Palmette hedgerow” had a better light penetration into the canopy than the Slender Spindle or the “Pyramid hedgerow” trees. Even the “Trellis “ introduced in the United States resulted to produce good fruit quality with a restriction to maintain the width under a limited measure to avoid a decrease in fruit quality. In fact, Ferree et al. (1989) found Palmetta hedgerow to have similar light transmission to that of the Slender Spindle only if the grower maintains a narrow width. As a result the Palmette tends to increase the growth of its top branches and increases shade in the bottom part. This leads to weaker branches in the lower part of the tree and in turn poor fruit quality.

Another recent addition to these training systems is the Bi-axis. It is similar to Palmette, but is formed by two leaders, which develop along the row. These have short lateral branches and unlike

the Spindle they measure almost the same from the bottom to the top. Thus this type of training system allows to control the vigour of the plant due to the presence of the two axes, also enhancing fruit light exposure (Dorigoni et al., 2011).

Given that high yield and fruit quality come from good light distribution and high light interception in the canopy, high tree densities planting combined with thin canopy depths such as Slender Spindle, and Vertical trellis are known to provide good results (Robinson et al., 1989). Good management operations such as pruning allow high yield and good quality thus maintaining tree vigour and growth control.

Pruning has been studied for centuries and still is one of the most important factor together with training in the apple orchards to improve yield and fruit quality (Özkan and Kücükler, 2009). One of the main objectives of pruning is to select fruiting branches to provide good light distribution thus improving fruit quality. In addition, chemical and manual thinning help to regulate the final yield.

Many pruning methods were introduced in the last three decades along with the M9 dwarf rootstock in intensive planting systems (Robinson, 2011). Pruning is tailored according to space distance, training systems, rootstocks and cultivars. Large planting distances require a long cut pruning as proposed by Lespinasse (1977). He developed the concept of renewal pruning through the “Vertical Axis” system. This allows to take advantage of the natural growth habit of the tree and to minimize the shading problem of permanent upper tier branches.

Robinson in 1987, introduced the operation of removing vigorous branches in the top of the tree rather than shortening back and keeping them as permanent scaffold branches. This resulted to be helpful for the tall spindle system to maintain a conic shape, which could otherwise be difficult as the trees age. The large branches should be removed completely with a bevelled cut so that a small stub of the lower portion of the branch remains. From this stub a flat weak replacement branch often grows. This operation allows the canopy with young fruitful branches shorter than the bottom branches giving the tree a conic shape. This strategy has been employed in many high density systems such as vertical axis (Lespinasse, 1980), Slender spindle, Tall spindle (Robinson et al., 2006), Super Spindle (Weber, 2000) and Y-trellis (Robinson et al., 1993) to maintain good light distribution over the life of the tree.

1.2. Fruit quality parameters

The fruit quality is measured by several parameters starting from fruit size to fruit over-colour among others (Schotzko, 1985), especially the colour is important since the consumer is

attracted by the fruit appearance. New redder and earlier colouring varieties and clones are introduced nowadays to fill up the consumers needs (Barritt, 1999). The traditional bicolour cultivars such as Gala and Fuji are now classified as “red skin colour varieties”. The market is demanding year by year a higher percentage of skin over-colour, which ranges between 60% - 70 % and for some cultivar even 80% - 90%.

What is important to the growers and to the market is the profit that is obtained in the end. Chemical and hand thinning are applied in the field to reduce the crop load to obtain a large size apple for market demands and consumers (William, 1985). It is known that a heavy crop tends to obtain smaller fruit sizes with poor fruit quality in terms of soluble solid contents (SSC), acidity, flesh firmness and over colour (Greene et al., 1989; Johnson, 1992 and 1994; Gottfried, 2000).

Fruit size and particularly fruit over colour are visual parameters but often are not represented the real fruit ripening stage at harvest time (Morgan et al., 1984; Tustin et al., 1988). Fruit maturity stage at pick time is a very important factor that determines storage-life and final fruit quality. In fact, fruits picked before of after the ideal date of maturity, are more susceptible to postharvest disorders (Kader, 1999). To ensure the highest fruit quality at the end of long term controlled atmosphere (CA) storage, apples must be harvested when mature but not when fully ripe (DeLong et al., 1999).

The maturity index used is the starch pattern, soluble solid contents and firmness and the choice of the initial harvesting date is often based on a compromise between these indices, to ensure the best eating quality to the consumer and provide the needed flexibility in marketing (Kader, 1999). The Streif Index was introduced as a quality parameter with an intention of estimating the optimum harvest time. It comprises three parameters: firmness, soluble solid contents and starch index (Streif, 1996). Other indices such “Perlim” and “Thiault” were introduced for apple quality evaluation with an attempt to pay the growers on the base of the internal quality traits.

All these parameters are obtained through destructive methods and need the removal of a small amount of fruit, which sometimes may not represent the whole fruit stage maturity within the canopy trees. Several attempts have been made to develop portable instruments with sensors that detect volatile production by fruits as a way to detect maturity and quality. Near-infrared detectors (vis-NIR) have great potential for non-destructive estimation of sugar content (Kader, 1999) and the DA-Meter device (I_{AD} – Index of Absorbance Difference- Costa et al., 2009), has been lately employed to monitor the fruit ripening stage in order to establish accurately the harvest time.

Fruit texture, a complex but comprehensive description of fruit quality is defined by “crispness”, “juiciness”, “hardness”, “firmness” and “mealiness” the key drivers of consumer preferences (Harker et al., 2003). To date a novel equipment is available and suitable for a complete dissection

of the texture components, and its potentiality has been recently presented also for apple by Costa et al. (2011; 2012). The introduction of these recent innovative methods could help not only to predict (Nyasordzy et al., 2013) the right moment for harvest time avoiding the fruit destruction but also they could be helpful to established in the future the right payment reward to pay off the grower when apples are graded.

1.3. Tree architecture design

Fruit positions within canopy tree have been studied by many researchers by using geometric parameters, spatial coordinates and databases. Literature abounds with sufficient information on trees in relation to canopy shape using 3D architecture (Sinoquet and Rivet, 1997; Godin et al., 1999; Sonohat et al., 2006). 3D representation of fruit species in relation to fruit quality were proposed by Smith et al. (1992 and 1994) and Costes et al. (1999). These models were used in many training systems in relation to light interception in several species (Génard and Baret, 1994; Buwalda et al., 1994; Potel et al., 2005; Sinoquet et al., 2007; Stephan et al., 2008; and Monney et al., 2012) and in relation to microclimate in the tree (Saudreau et al., 2009).

In this study PlantToon® software has been adopted to design the tree architecture of two training systems to study the positions of the fruits located within the canopy. The design not only helped locate fruit position but also was valid in understanding the fruit quality. In addition the statistical packages “R” and “Statistica” were employed to graphically represent the tree and its fruits.

2. Aims of the thesis

Fruit quality as a result of two different training systems and two pruning techniques are the main objectives of this study. As mentioned above fruit quality is of utmost importance to both the buyer and the producer. Therefore, this study dealt with the best fruit quality in its diversity of scientific vocabulary, expressed both as traditional parameters such as fruit size and over colour or by innovative parameters such as texture and I_{AD} (Index of Absorbance Difference) using novel software to design tree in 3D the tree architecture and the fruit position. Each of the following objectives has been meticulously studied within hypothesis-based experiments carried out over three years.

1. The influence of training system on apple fruit quality
2. 3D digitizing tool to determine fruit quality distribution within the canopy
3. Fruit quality variability within the canopy tree
4. The effect of pruning on fruit quality
5. Effects of light availability and training systems on apple fruit quality

2.1. Layout of the thesis

The thesis presents chapters 1 and 2 as introductory information and literature review to the five articles presented in chapters 3 to 7. Among the five manuscripts, one has already been published while the remaining are in the process. The manuscripts have maintained the general order of a scientific publication: Title, Introduction, Materials and Methods, Results and Discussion and Conclusions, Literature Cited, Figures and Tables.

Finally, the thesis concludes with General Conclusions and proposals of further research.

3. The influence of training system on apple fruit quality

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Keywords: Slender Spindle, Bi-axis, fruit distribution, PlantToon®, texture

3.1. Abstract

A comparison between the two training systems Slender Spindle and Bi-axis was performed on two main cultivars, such as Galaxy Evolution and Pink Lady® (Rosy Glow). Tree architecture description and fruit position in the canopy were monitored during the fruit maturation and ripening using a software developed to design tree architecture and fruit position on the tree, named “PlantToon®”. Fruit were monitored during the growing season using a non-destructive device, the DA-Meter. Finally, fruit were harvested, graded and analyzed for major quality traits. In addition, fruit texture was also assessed employing a novel texture analyzer equipped with an acoustic envelop detector device. Fruit quality was dependent upon cultivar, training system and position in the canopy. “Bi-axis Galaxy Evolution fruit” showed higher size in the lower level as compared to Spindle ones. Fruit size in Pink Lady® (Rosy Glow) was significantly higher in Spindle as compared to Bi-axis while fruit colour was not affected. “Bi-axis Galaxy Evolution fruit” showed differences as related to layer level as compared to Slender spindle, while no differences were observed in Pink Lady® (Rosy Glow) . In general, fruit harvested from “Bi-axis” resulted more homogeneous with regards to “Spindle” training system.

3.2. Introduction

The choice of training system is part of the whole orchard project and management, influencing planting distances, light interception and finally the success of the orchard in terms of obtained fruit quantity and quality (Robinson et al., 1991; Palmer, 1999). It is therefore essential to evaluate the training system not simply on the basis of the total yield but also on the fruit quality traits which instead remain actually confined to the definition of empirical parameters such as average fruit weight and standard quality traits (soluble solids content, acidity, flesh firmness and starch). In a modern fruit system management these descriptors should be now integrated with other parameters enabling a better description of the real differences induced by the training system. For instance, new modeling systems allowing an accurate description of the tree architecture are now available providing useful information and ultimately guidance on the most appropriate management cultural techniques to be adopted (pruning and fruit thinning in particular).

Other non-invasive instruments, in particular vis-NIR and the DA-Meter, have been recently employed to monitor the fruit ripening stage in order to establish accurately the harvest time as well as to highlight the ripening differences that might exist between apples located in different part of

the canopy. The combination of these two innovative systems can be considered a “decision support system” tool for a pre- and post-harvest management. It is known, in fact, that the definition of the ripening stage reached by the fruit at harvest is extremely important since it influences the storage length as well as the fruit quality at consumption. The best moment for harvest relies instead on the definition of some quality parameters which, although practical and rapid to assess, require the destruction of the fruit examined, and do not always express precisely the average fruit quality especially when heterogeneous. These determinations should be integrated with other nondestructive strategy nowadays available (vis-NIR, I_{AD} – Index of Absorbance Difference, etc.), able to assess the ripening homogeneity (Costa et al., 2009).

For a more comprehensive and exhaustive description of fruit quality, a better investigation of fruit texture should be also performed. Texture represents one of the principal factors defining fruit quality, together with appearance, flavour and nutritional properties and apple fruit textural characteristics are defined as “crispness”, “juiciness”, “hardness”, “firmness” and “mealiness” resulting the key drivers of consumer preferences (Harker et al., 2003). Crispness, in particular, has been largely recognised as the key attribute affecting consumer acceptability (Hampson et al., 2000). To date a novel equipment is available suitable for a complete dissection of the texture components, and its potentiality has been recently presented also for apple by Costa et al. (2011; 2012).

The present study was carried out to perform a comparison between Spindle, the most common training system for apple, and the Bi-axis, recently suggested as a valid alternative (Dorigoni et al., 2011). This comparison was carried out implementing novel modeling strategies and non-destructive devices for the fruit ripening determination as well as other quality parameters important for consumer’s satisfaction such as fruit texture.

3.3. Materials and methods

Trials were carried out in Trentino-Alto Adige, Northern Italy in the experimental farm of the Edmund Mach Foundation (San Michele all’Adige, Trento, Italy) with two apple cultivars, Galaxy Evolution and Pink Lady® (Rosy Glow). Both cultivar were trained as Slender Spindle and Bi-axis.

Training System

The Slender Spindle has a conical shape, formed by one trunk and both lateral temporary shoots and scaffold branches bearing fruit, although in some years fruit quality and appearance can be heterogeneous (Palmer, 1999). In fact, fruit located in the middle and lower canopy position

might intercept a lower amount of light as compared to upper levels as well as the fruit situated externally in the canopy might reach higher over-colour than those situated internally (Unuk et al., 2012). The Bi-axis (patented under the trademark Bi-baum®; Musacchi, 2008b), instead, consists of two trunks with a U shape developed along the rows allowing a preferable light penetration and, at the same time, enabling an easier shoot growing control.

The Galaxy Evolution orchard was established in 2005 at a planting distance of 3.45 x 1.00 m for Spindle (2900 trees/Ha) and 3.45 x 1.20 m for Bi-axis (2400 trees/Ha). The Pink Lady® (Rosy Glow) orchard was established in 2008 at a planting distance of 3.50 x 0.80 m (3570 trees/Ha) for both training systems. Standard cultural management techniques were used for both varieties. Chemical and hand thinning were performed in order to obtain 7 fruit/cm² trunk cross sectional area (TCSA) for Galaxy Evolution and 8 fruit/cm² TCSA for Pink Lady® (Rosy Glow). Eight plants for each cultivar (four for each training system) were selected in the central part of the block for the data collection.

Data Collection

Plant description and digital visualization was achieved with the use of the PlantToon® modelling software, an empirical architectural model based on the reconstruction of the three-dimensional (3D) tree structure based directly on the raw data collected in the orchard. PlantToon® was developed by Magnanini et al. (2010) using a single canvas for the 3D structure of the plant. On a single tree, the spatial position of each fruit as well as the entire canopy structure, was measured using a custom-made “woody stick-compass system” for two trees per each cultivar. Three parallel areas of equal size (upper, middle and lower layers) were identified within the canopies, related to their height from the ground. Length, direction (°N) and horizontal projection were measured on each segment of the trunk, as well as on branches and limbs, from the ground to the top of the canopy, following the insertion sequence and the direction changes of each element, as previously described by Sinoquet and Rivet (1997). The collected Cartesian coordinates were written down in the PlantToon® database and also the punctual position of each fruit was catalogued.

During the summer and at harvest, fruit monitored for maturation and ripening, expressed as I_{AD} (index of absorbance differences), was assessed by the DA-Meter device, a portable vis/NIRs. The I_{AD} is calculated as absorbance difference between two wavelengths (670 and 720 nm) and correlates with ethylene emission.

The fruit of the trees used for the PlantToon® plant architecture reconstruction were harvested, and assessed for weight (g), size (mm), over colour (%), fruit shape (index), length (mm) and internal

fruit quality measured. Additional 15 fruit were collected from the neighbouring trees from each layer and analysed at harvest and after 2 months of storage.

In addition all the fruit harvested were also analysed for texture by using TA-XTplus Texture Analyzer equipped with an Acoustic Envelop Detector (AED) device (Stable MicroSystem Ltd., Godalming, UK). The combined acoustic-mechanical profiles data were processed by the software Exponent v.4 (Stable MicroSystem) provided with the TA-XTplus instrument. With the same software a macro instruction was also compiled to automate the parameter extraction from the force/sound curves. This equipment was used to profile both the mechanical and the acoustic profile after two months of storage. Out from the texture profiles, a set of texture parameters were digitally extracted and used as novel fruit quality descriptors (see Costa et al., 2011 for more details).

Statistical analyses were carried out using R package version 2.15 and 2.1.0. Duncan test was used for the calculation of the homogeneity within the variance. Multivariate statistical Principal Component Analysis (PCA) was computed with STATISTICA software v7.

3.4. Results and Discussion

Fruit Distribution

A 3D visualization of the plant architecture was provided by the PlantToon® software, allowing the digital identification of bearing shoot and the fruit position in the two training systems respectively (*Fig. 1*). The design faithfully describes the two systems showing the conic shape of the spindle and the two axis conformation of the Bi-axis. The uniformity of the bearing shoot all along the trees in both systems and the fruit position on the different part of the tree can be easily recognized. Galaxy Evolution fruit were uniformly distributed in the three identified layers of the tree (upper, middle, lower), while Pink Lady® (Rosy Glow) presented a lower number of fruit in the Spindle upper layer (data not shown). This might depend on the fact that the Galaxy Evolution cultivar (cv) tends to develop shoots and bear fruit in the upper part of the tree. On the contrary, Pink Lady® (Rosy Glow) is characterized by a higher number of stronger branches in the lower and middle layers.

Fruit Size

The analysis of the Galaxy Evolution fruit size, taking the “Bi-axis upper layer fruit size” as a reference value (75.7), reported that the two training systems do not influence the average fruit size, while fruit from lower and middle layers in Bi-axis were statistically smaller than the upper layer one. In spindle, only the “lower layer fruit” reached a smaller size (*Fig. 2a*).

In Pink Lady® (Rosy Glow) , instead, the training system played a role and, in fact, “Spindle fruit” reached a fruit size statistically higher than “Bi-axis ones” (reference value 78.3), in the upper and middle layers. As far as the layer is concerned, only the “Spindle lower layer fruit” were statistically smaller (*Fig. 2b*).

Fruit Ripening

Fruit ripening was determined for each fruit distributed over the different tree layers for both cv and training systems. The ripening stage, expressed as I_{AD} , is visualized in the 3D image by a different colour scale (white, grey and black), anchoring the ripening situation on the tree architecture and the three different layers (lower, middle and upper layers) (*Fig. 1*). At harvest the fruit ripening was differently affected by the training systems for the two varieties (*Fig. 3a and b*). In Galaxy Evolution fruit, in fact, the Bi-axis induced a more advanced ripening stage as compared to spindle and a more uniform distribution (*Fig. 3a*). “Pink Lady® (Rosy Glow) apples” showed an opposite trend, with the “Spindle fruit” a slightly more ripen, although the distribution was less uniform than “Bi-axis training system fruit” (*Fig. 3b*).

Fruit Over Colour

For skin colour, important differences were observed as affected by cultivar, training system and layers. In general, Galaxy Evolution fruit reached a more intense colour as compared to Pink Lady® (Rosy Glow) , and the fruit in the upper layer of both cultivars, in particular in Galaxy Evolution, were visually more intense than the “middle and the lower layers fruit”. More in details, examining the fruit colour with the statistical method described before, with the “Bi-axis upper layer fruit colour” took as reference value (84.7), it can be observed that the “Bi-axis Galaxy Evolution fruit” reached values always statistically higher than “Spindle fruit” (*Fig. 4a*), while no difference were detected in Pink Lady® (Rosy Glow) . In Pink Lady® (Rosy Glow), the fruit colour was less evident only in the lower layer (data not shown). At harvest “Galaxy Evolution Bi-axis fruit” had 85% of apples with more than 50% of over colour, while “Spindle fruit” reached only 57% with the same percentage (*Fig. 4b*).

Fruit Soluble Solid Content and Texture Analyses

For both cultivars “lower layer fruit” reached the lowest soluble solid contents (SSC), and “Pink Lady® (Rosy Glow) “middle layer fruit” also showed statistical lower values than upper layer fruit (data not shown). Fruit collected from the three layers of the two cultivars and the two training systems were also employed for a comprehensive texture analysis operated by a texture

analyzer. The data set was initially analyzed in Galaxy Evolution by the means of multivariate statistical approach, such as Principal Component Analysis (PCA). As reported in *figure 5a*, the PCA, computed by implementing the first two components (explaining together the 90% of the entire texture variability), clearly distinguished the projection over the hyperspace of the mechanical parameters with regards to the acoustic ones, validating the use of this technology to exploit new fruit quality traits, such as crispness response upon compression. The distribution of the texture behavior among the six layers over the PCA 2D plot distinguished the two training systems, highlighting that fruit from the Bi-axis were higher than those collected from the Spindle in terms of texture performance (*Fig. 5b*). Texture was also profiled on Pink Lady® (Rosy Glow), following the same experimental scheme, however for this cv the difference were not so clear as those observed for Galaxy Evolution (data not showed).

3.5. Conclusions

The objective of the present study was a comparison of fruit quality parameters between the Spindle and Bi-axis. The results obtained with the present research, although preliminary, underlined that other data than yield, average fruit weight and standard quality traits, must be examined to properly evaluate the effectiveness of a given training system, its adaptability to the environment and the influence on fruit quality. Thus, precision horticulture methods (such as modelling system and innovative devices able to describe plant architecture and fruit ripening homogeneity) should be added to the traditional ones as a modern evaluation tool to choose the most appropriate cultural methods to be used. In this study, the use of both PlantToon® model, DA-Meter and the TAXT for the two training system comparison, showed to be valuable and appropriate tools to fulfill the research expectations. In fact, the PlantToon® was easily used to represent the tree architecture, the fruit distribution and quality attributes variability as affected by the two training systems studied. The I_{AD} provided an objective and accurate evaluation of the fruit ripening induced by the training system, being also able to be friendly used in field conditions. The combined use of PlanToon® and I_{AD} can guide the tuning up of the main cultural management techniques (thinning, summer and winter pruning, etc.) as well as to eliminate outlier fruit to improve ripening homogeneity for a better management of the post-harvest. On the basis of the results and information achieved with the combined use of these two systems, the Bi-axis training system reached more interesting results than Spindle especially on Galaxy Evolution. In fact, with this cultivar, the fruit characteristic in term of size, skin colour and texture were superior with the Bi-axis training system to those obtained with the Spindle. The superior fruit quality of Galaxy Evolution was also confirmed by the texture analysis, which provided important indication about

the real prospect to measure crispness trait, the most appreciated fruit quality feature by consumers (Hampson et al., 2000; Harker et al., 2003) in particular for apple.

The use of precision horticulture methods (modeling system and innovative non-destructive devices) as well as the determination of other additional parameters characterizing fruit quality must be introduced for a proper evaluation of the training system representing an important “DSS tool” able to affect fruit quantity and quality parameters. In addition, these tools also allow the possibility to perform simulation to exploit the cultural techniques to be successfully verify in practical conditions.

Acknowledgements

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3.7. Figures

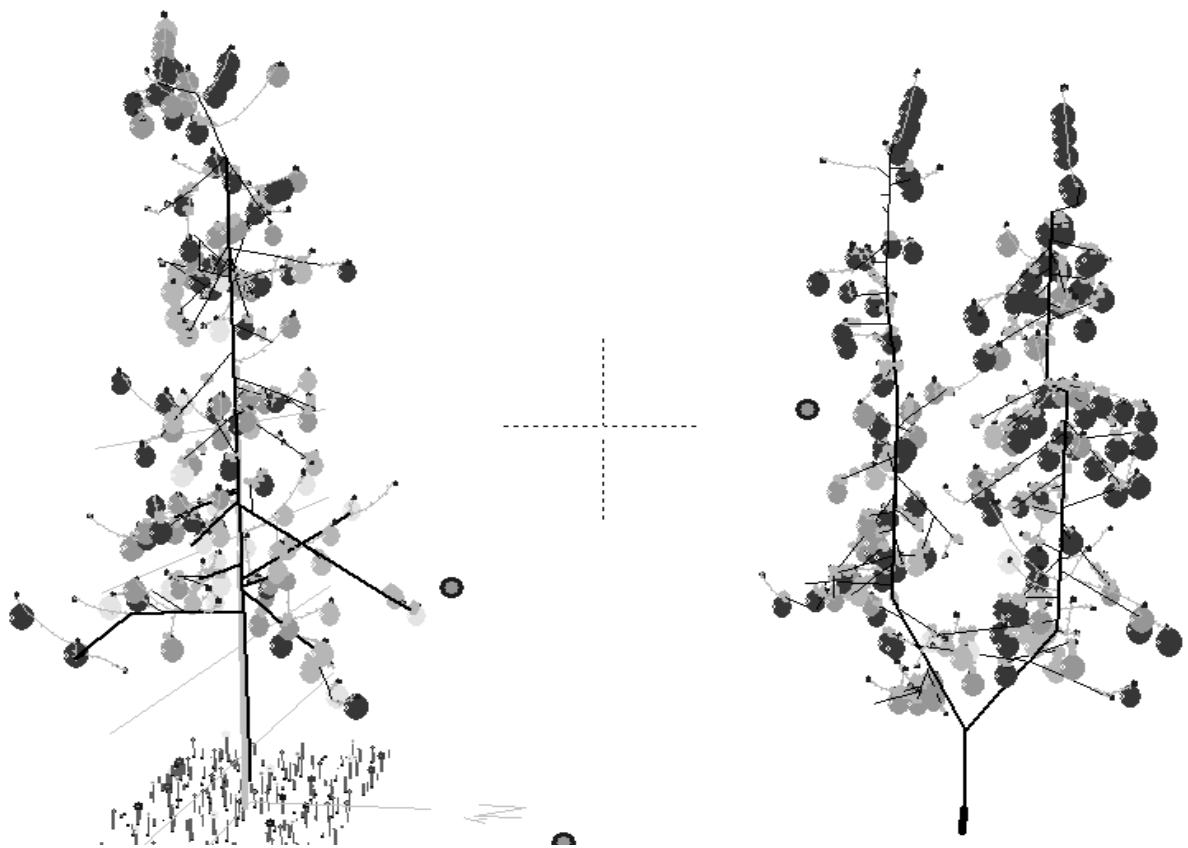


Fig. 1 a and b. PlantToon® 3D imaging of the plant architecture for the two training system: Slender Spindle (a) and Bi-axis (b).

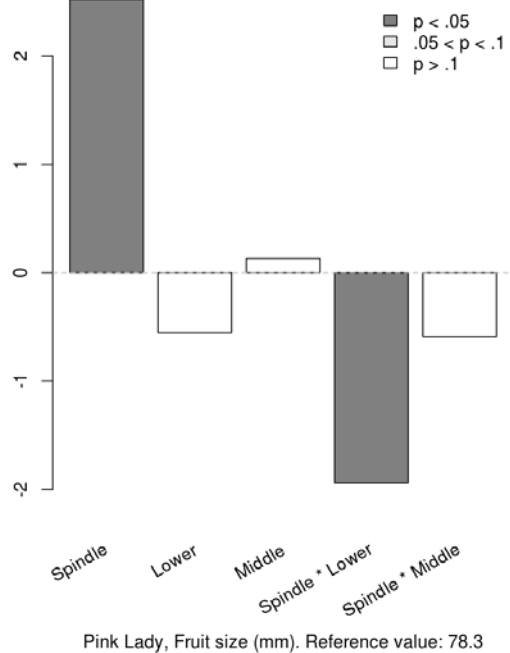
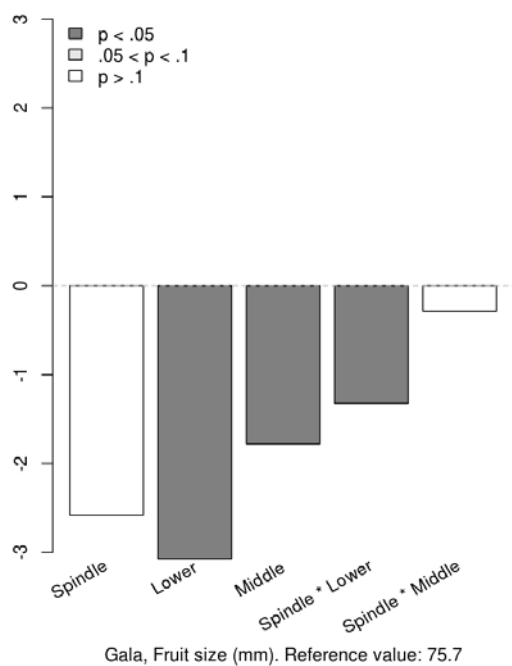


Fig. 2 a and b. Fruit size as affected by training system and tree layers. All the data are compared with the “Bi-axis upper layer fruit size” assumed as reference value 75.7 in Galaxy Evolution (a – left) and 78.3 (b – right) in Pink Lady® (Rosy Glow).

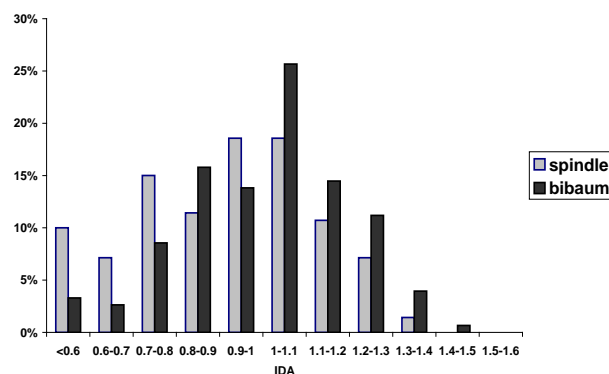
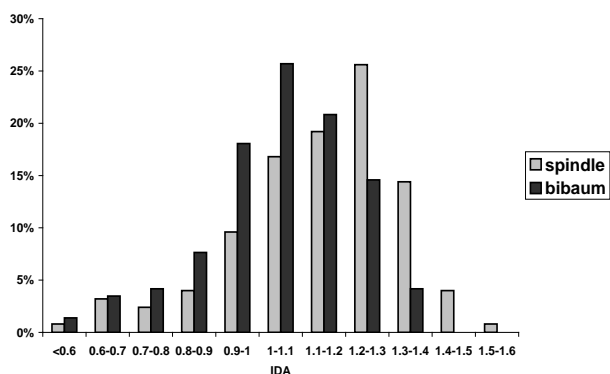


Fig. 3 a and b. Fruit ripening as affected by training system in Galaxy Evolution (a – left) and in Pink Lady® (Rosy Glow) (b – right).

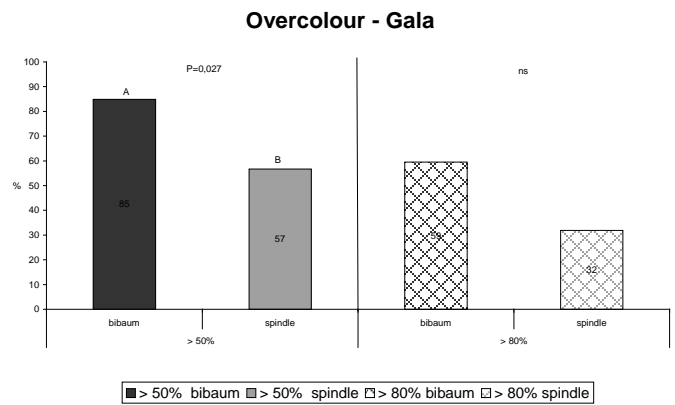
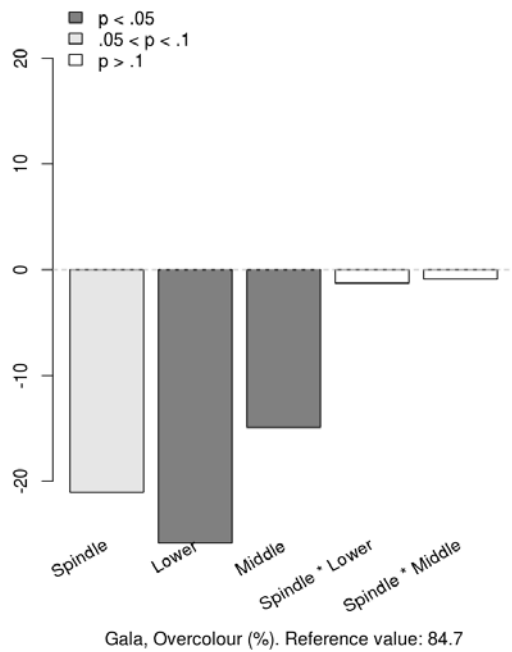


Fig. 4 a and b. Fruit overcolour as affected by training systems and tree layers. All the data are compared with the “Bi-axis upper layer fruit” assumed as reference value (84.7)(a – left). Percentage of fruits at harvest over 50 % and 80% of skin colour coverage (b – right).

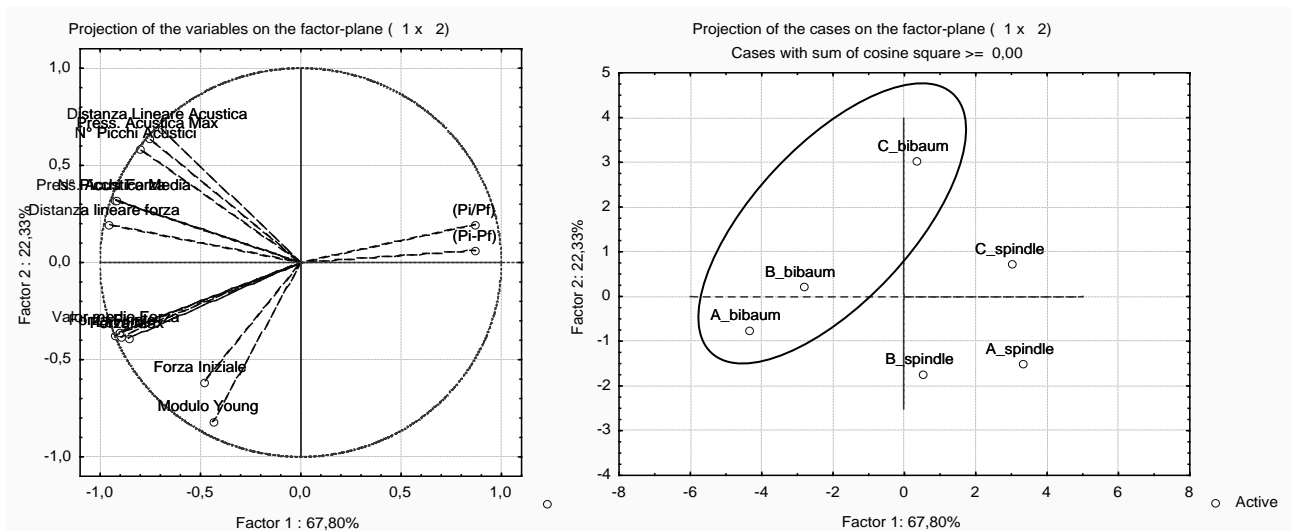


Fig. 5 a and b. In panel a (left) the projection of the texture parameters over the PCA 2D plot is shown. In panel b (right) the distribution of the texture behavior for the three layers per each training system is visualized.

4. 3D digitizing tool to determine fruit quality distribution within the canopy

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Keywords: Slender Spindle, Bi-axis, tree architecture, apple position, crop load.

4.1. Abstract

The “Slender Spindle”, one of the most used training system for apple, was compared with the “Bi-axis”, an innovative training system, to determine their effect on fruit quality and fruit distribution within the canopy on Gala cultivar. Two levels of crop loads were also introduced to observe their effects through chemical and manual thinning. Fruit position in the canopy was monitored during summer using different softwares to draw in 3D the tree architecture. Also, fruits were monitored during the growing season for their growth and maturation. At the end of the season fruits were harvested, graded and analyzed for their quality traits. Fruit texture was also assessed employing a novel texture analyzer equipped with an acoustic envelope device. Fruit position resulted to have a strong influence on fruit quality. The fruits in the layers were significantly different. In the upper part of the tree fruits showed a higher percentage of over colour and bigger fruit size compared to the middle and the lower part of the canopy. No significant differences were found between the two training systems. Crop load influenced fruit quality in both training systems in the same way on fruit size and over colour. Lower crop load treatment had higher percentage of over colour and bigger fruit size compared to the higher crop load. No differences in fruit ripeness were found between the two training systems and crop loads. Fruits in the lower part of the trees were less mature in both training systems. No differences in texture were found between the two training systems, the crop loads and the layers.

4.2. Introduction

Modern intensive orchards trained with Slender Spindle (SS) system grafted on the dwarfed M9 rootstock guaranty greater and early production (Barritt, 1989 and 1998). Also, plants provided by the nursery are already feathered trees and therefore are able to bear fruits in the first years (Van Oosten, 1978; Ferree and Rhodus, 1987).

The Slender Spindle with its conical and narrow shape is adaptable for intensive plantation (Wertheim, 1968) and trees fill up the available space very fast reaching earlier high yield efficiency and fruit quality (Robinson et al., 1991; Palmer, 1999). However, in this case, the branches may growth very quickly in the canopy leading to poor fruit colour and quality (Corelli and Sansavini, 1989). Sansavini et al. (1981) found a poor level of light in the lower part of the Spindle as compared to other systems like “Palmette” which has a flat shape tree. As a matter of fact fruits located in the middle and lower position have higher shade compared to upper levels and resulted in lower fruit quality (Robinson et al., 1991). Several studies have been carried out to show that

shading influences fruit quality (Jackson and Palmer in the UK, Ferree, Rom, Barritt and Robinson in the USA and Tustin and Warrington in New Zealand). Even fruits positioned externally are different compared to the fruits in the inner position and this results in fruits with different over colour, particularly for red-coloured variety such Gala (Unuk et al., 2012), parameter used by the cooperative to pay off the growers.

Bi-axis trees could represent a good alternative to the Slender Spindle using the same tree density. This system is patented under the trade mark Bibaum® and trees are obtained by a double chip budding technique in the nursery (Musacchi, 2008a, b). The two stems in Bi-axis give a U shape to the tree and developed along the rows allowing a good light penetration, improving skin over colour, and at the same time providing a good vigour control simplifying many cultural practices particularly winter pruning, limb bending in summer and harvesting (Dorigoni et al., 2006, 2010 and 2011).

Training systems and light interception in reference to fruit quality in apples within a canopy structure using a 3D model have been reported by Potel et al. (2005), Sinoquet et al. (2007), Stephan et al. (2008) and Monney et al. (2012). Other models, simulating spatio-temporal distribution of temperature in apples introduced interesting results (Saudreau et al., 2009). Similar studies have also been conducted in other fruits such as kiwis only to provide more in-depth understanding of how fruit quality is linked to plant architecture (Smith et al., 1992).

Taking into account the information and the precision that a three dimensional digitising description can provide especially in apples (Sinoquet and Rivet, 1997; Godin et al., 1999; Costes et al., 1999), this paper intends to develop this information that may be provided through the structural architecture and the position of the fruits related to fruit quality. For the purpose, two training systems the Slender Spindle and the Bi-axis were compared. Another element of difference in the study is the crop load, which has also been reported earlier in reference to fruit quality (Racskó, 2006), to fruit size (Assaf et al., 1982; Erf and Proctor, 1987; Forshey and Elfving, 1989) without the concept of the 3D architecture.

4.3. Materials & methods

The trial was carried out in Trentino province, Northern Italy in the experimental farm of the Edmund Mach Foundation (San Michele all'Adige, Trento, Italy) on cultivar Galaxy Evolution (Gala group), trained as Slender Spindle and Bi-axis.

Plant Material and Training Systems

The two types of trees were acquired from the same nursery and grafted on M9 rootstock using the chip budding technique for SS trees while Bi-axis plants were obtained by cutting back the leader in the nursery growing later the two axes. Trees were planted in 2005 at the space distance of 3.45 x 1.00 m for SS (2900 trees/Ha) and 3.45 x 1.20 m for Bi-axis (2400 trees/Ha). Six plants for each training system, were chosen with similar vigour and located in the same block of the orchard. Only trees from the centre of the block were used for the study in an effort to minimize the effect of sunlight that surrounds the orchard. The row orientation was about 9° NE.

Crop Load

The trees were managed according to commercial practices as integrated crop and pest management and thinned chemically and manually to have two different level crop load: three plants at 7 fruits/cm² and the other three plants at 8.5 fruits/cm² of trunk cross sectional area (TCSA) for each training system. The lower figure of fruit load (7) was set so as to include the generally accepted 7-8 fruits/cm² of TCSA considered as an optimized fruit load to balance the number of fruits per tree and individual fruit size (Roper, 1995). The upper figure (8.5) was chosen just to have a higher level of crop load in order to see the yield training system potentiality and the effect on fruit quality by the crop load.

Fruit Position and Measurements

The fruits were individually labelled after fruit set and mapped for subsequent identification. The spatial position of all fruits was measured using a custom-made “woody stick-compass system” (*Fig. 1*). The length, the direction (°N) and the horizontal projection were measured on each fruit from the ground to the top of the canopy. The collected Cartesian coordinates and the punctual position of each fruit were catalogued. Three parallel layers of equal size (upper, middle and lower) were identified within the canopies, related to their height from the ground. Data were used to draw a three-dimensional diagram using the statistical packages “R” and “Statistica” with an objective to digitally identify fruit distribution in the canopy (*Fig. 2*). The design precisely describes the two systems showing the conic shape of the SS and the two-axis conformation of the Bi-axis trees. During summer and at harvest, fruits were monitored for their growth with a manual callipers and maturation using the DA-Meter device, which by means of its absorbency (A670-A720) properties allows to measure the chlorophyll’s content in the fruit which is index of fruit’s ripening stage.

Fruit Quality Data Collection

Fruits were harvested on the same date, according to a given starch index, from three different heights within the tree previously demarcated into three different heights: upper, middle and lower positions. All Fruits were graded using Greefa equipment with a software that measures weight (g), size (mm), over colour (%), relative ground colour and shape (index) and length (mm) of each fruit.

Fruit Analyses

Fifteen fruits for each layer for each tree were sampled for the analyses. Ten of them were analyzed at harvest time using the Pimprenelle detecting soluble solid contents (Brix°), acidity (g/l malic acid), starch index and firmness. The rest (5 for each layer and tree) of the apples were stored in air conditioning cool storage two months and held at room temperature (approximately 20°C) for 24 hours before being graded and assessed for analyses. Each of these fruits were tested for texture using TA-XTplus Texture Analyzer equipped with an Acoustic Envelop Detector (AED) device (Stable MicroSystem Ltd., Godalming, UK) which helps detect both mechanic and acoustic profiles of the compress apple. Texture was measured on 4 discs of each apple of the similar shape and size.

Plant Measurements

Biometrical data such trunk cross sectional area (TCSA) 20 cm above graft union, height, width and breadth of the trees were recorded after harvest. At the end of the season, a sample of hundred leaves for each training system were collected and dried and weighted in order to calculate the mean of leaf area and weight for each leaf. Later, all the leaves for each tree were collected separately wrapping the plants with a hail net. Thus, leaves were dried in a oven and weighted in order to calculate, using the samples data estimated, the leaf area, the “Leaf Area Index” (LAI) and the dry matter for each tree of both training systems.

Statistical Analyses

We considered how training systems (Slender Spindle and Bi-axis) and layers (upper, middle and lower) affect fruit quality variables (Overcolour, Fruit size, I_{AD}). Apples from the same tree were considered correlated and therefore to estimate the quantity of interest we used a Generalized Linear Mixed Model with the variable “Tree” as random effect. Inference was carried out using Penalized Quasi-Likelihood (Schall, 1991; Breslow and Clayton, 1993; Wolfinger and O'Connell, 1993). To control the number of false positive we used the false discovery rate methodology (Benjamini and Hochberg, 1995); a test was deemed significant if the corresponding

adjusted p-value was lower than 0.05. Finally to assess differences in biometrical data, vegetative data and efficiency data we used two-sample Wilcoxon tests and we correct the p-values with Holm method.

The statistical analyses were carried out using R, version 3.0.2. The package MASS was used to fit the Generalized Linear Mixed Models.

4.4. Results and Discussion

Yield Efficiency

Significant differences in crop load were obtained as previously hypothesised (*Tab. 1*). The different crop loads were imposed tuning up the hand thinning intensity in order to find out the effect of crop load on fruit quality. Initial crop load which was set at 7 and 8.5 fruit/cm² after fruit set, changed to an yield efficiency of 6.5 and 8.1 fruit/cm² during summer, thus maintaining the difference between the two levels of crop load.

Leaf Area Index

The leaf area index was similar between treatments (*Tab. 1*). This is an important result, which influences fruit quality. It is known that yield is related to surface area (Winter, 1981) and the total number of leaves supplying carbohydrates to the fruits or the fruit / leaf ratio influence the carbohydrates content of the fruits as found by Corelli-Grappadelli et al. (1994) and Poll et al. (1996). The reduction in the number of fruits per tree, such as in the lower crop load level, could increase the leaf area per fruit resulting in an increase in the availability of assimilates in the remaining fruitlets corresponds to Palmer et al. (1991).

Fruit Position and Distribution

The fruits were differently distributed in the two training systems in the different parts of the tree layers (*Fig. 3*). Bi-axis had a more regular fruit number distributed along the canopy while SS trees showed a highest number of apple located in the lower level of the canopy. This might depend on the fact that the SS trees are characterized by a higher number of stronger branches in the lower layers (Wertheim, 1968).

Fruit Size

The growth of fruit size was affected similarly in both training systems during summer and until harvest showing a less growth by the fruits in the higher crop load treatments (*Fig. 4*). A reduction in fruit number has been associated with an increased fruit growth (Racskó, 2006). At

harvest Bi-axis produced similar fruit size compared to the SS trees. The crop load affected fruit size: in fact the lower crop load obtained bigger fruits compared to the higher crop load for both training systems (*Fig. 5a*). This confirmed the hypothesis of Roper (1995) who considered 8 fruit/cm² the maximum limit to obtain a ideal crop load in order to obtained a good fruit quality. Another close call comes from De Salvador et al. (2006) who confirmed the higher the crop load the smaller the fruit size. Also, another factor that might have influenced in spite of the same vegetative data in the two crop loads could be the ratio between the number of fruits and LAI (Wu et al., 2005). In fact, Palmer et al. (1991) showed that the mean fruit weight at harvest was linearly dependent on leaf area per fruit. Tree layer affected fruit size in both training systems (*Fig. 5b*). The upper layer obtained bigger fruit size followed by the middle and then the lower layer. This could be assumed to have a light effect, sustained by several (Morgan et al., 1984; Tustin et al., 1988). *Figures 6a* and *6b* highlight the fruit size distribution in the canopy in the two training systems.

Fruit Over Colour

Bi-axis trees performed similarly to SS trees as regards to percentage of fruit skin colour. Instead, crop load affected significantly skin colour in both training systems (*Fig. 7a*). The higher crop load resulted significantly less in the percentage of skin colour. This suggests that more the number of fruits within a canopy, the more the amount of shading is present. Morgan et al. (1984) suggested that fruit over colour was positively correlated with the level of exposure to sunlight. Linking shading to the layers the results obtained in this study showed that the upper layer with the highest percentage of fruit skin colour followed by the middle and then the lower part of the canopy for both training systems (*Fig. 7b*), supported by other studies (Jackson et al., 1971; Robinson et al., 1983). It underlines the fact that shade and the penetration of global radiation within the canopy are key factors for fruit coloration. Warrington et al. (1996) showed that the light environment at the fruiting sites is more important for fruit colour and quality and not the canopy system itself. The same result was also obtained under netting by Dussi et al. (2005) who found less skin colour in the bottom part of the tree due to shading. The upper part has better light exposure resulting in better over colour as found by Unuk et al. (2012). *Figures 8a* and *8b* highlight the over colour distribution in the canopy in the two training systems.

Fruit Analyses

The training systems and the crop load didn't affect fruit ripening stage at harvest. Only the fruit position affected fruit ripeness. Fruits on the bottom part of the tree resulted with a lower ripening stage compared to the other layers (*Tab. 2*). This is may be due to the effect of shading

which is higher in the lower part of the canopy and delays fruit ripening stage (Robinson et al., 1983). Analogous results were found by Dussi et al. (2005) under netting, who confirmed that the height of the canopy significantly affected fruit ripening. Texture didn't show any statistical significant differences between the two training system and crop load treatments and fruit positions.

4.5. Conclusions

The 3D construction of the plant architecture and the fruit positions within the canopy provided data to confirm fruit quality in the two training systems. Two important parameters fruit size and skin over colour were selected among many others to examine fruit quality. Also because, nowadays, as far as economics are concerned, the growers and the market use fruit size together with skin colour to evaluate fruit.

Fruit position confirmed to affect more fruit quality than the training systems per se. In fact, no differences were found between the two training systems. Both tree shapes have compact and narrow trees, which may guarantee similar light distribution within the canopies. Instead the layers showed differences with the obvious of the upper position to have better quality fruits in terms of size and colour and higher fruit ripening stage.

Other studies showed that Bi-axis had better performance in fruit quality compared to Spindle trees mainly in vigorous cultivars such as Fuji. This study instead showed no differences between the two training systems, probably due to the less vigorous cultivar such as Gala maintaining a compact shape in SS as in Bi-axis.

Another important factor that did have an effect is the crop load, confirming its influence on fruit quality. A mere increase of 30-40 apples per tree influenced the size and the colour of both training systems. This factor of crop load was included to test if the innovative Bi-axis could have had a major potential yield without altering fruit quality. As a matter of fact both the systems resulted to be similar.

Other than thinning, operations such as the introduction of alternative pruning methods could enhance fruit quality in lower part of the tree. This will ensure an increased light distribution through the canopy thus improving fruit light exposure, which can guarantee a better fruit skin over colour.

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4.7. Figures



Fig. 1. The woody stick-compass system used to measure the fruit positions. Data were used to draw a 3 dimensional diagram using the two different software (R and Statistica) with an objective to monitor the fruit position and its development on the tree.

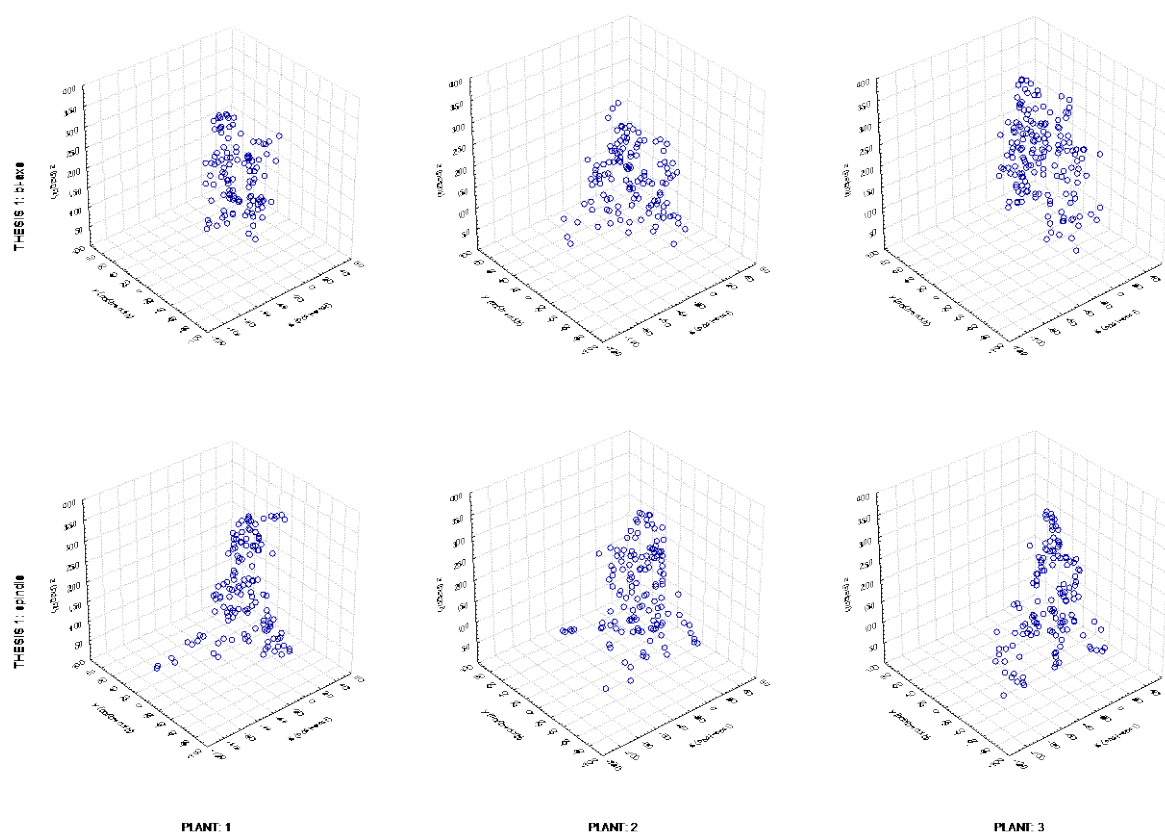


Fig. 2. 3D visualization example of the plant architecture provided by the Statistica software which allowed the digital identification of the fruit position in the two training systems in lower crop yield treatment.

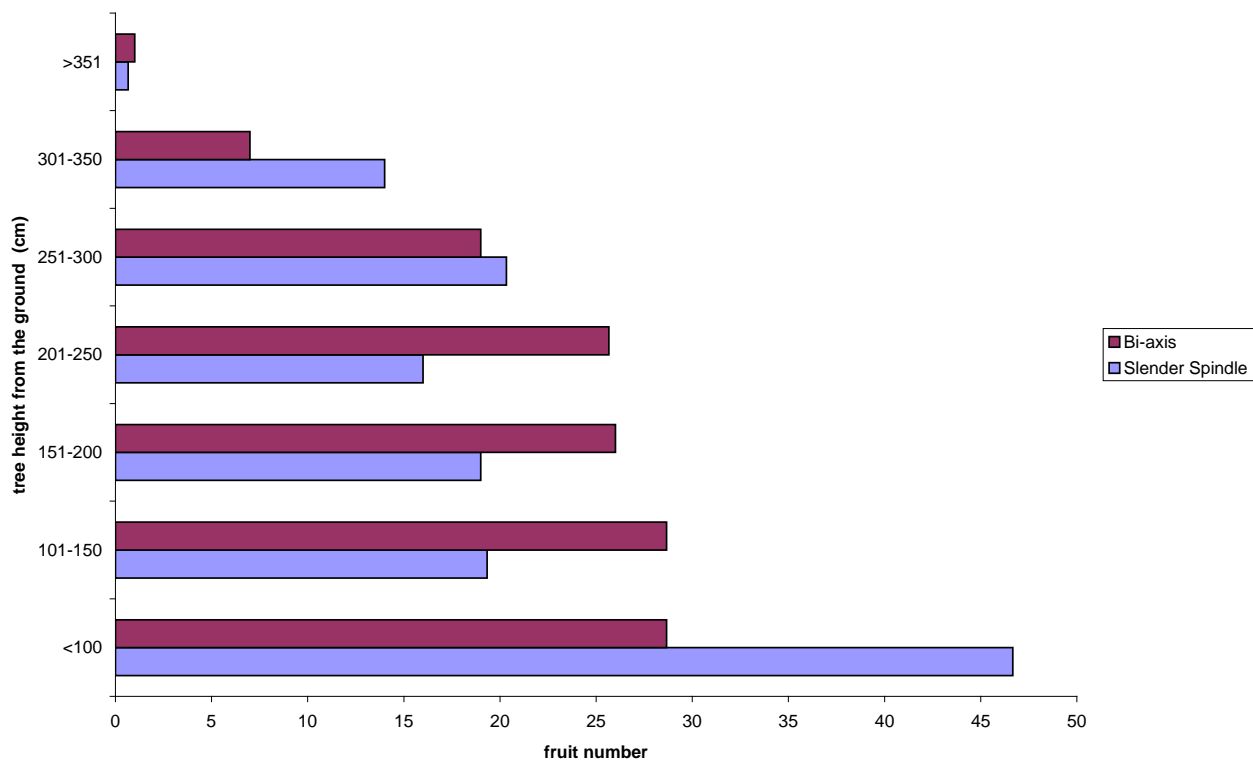


Fig. 3. Fruit number distribution in the canopy layers in the lower crop load treatments.

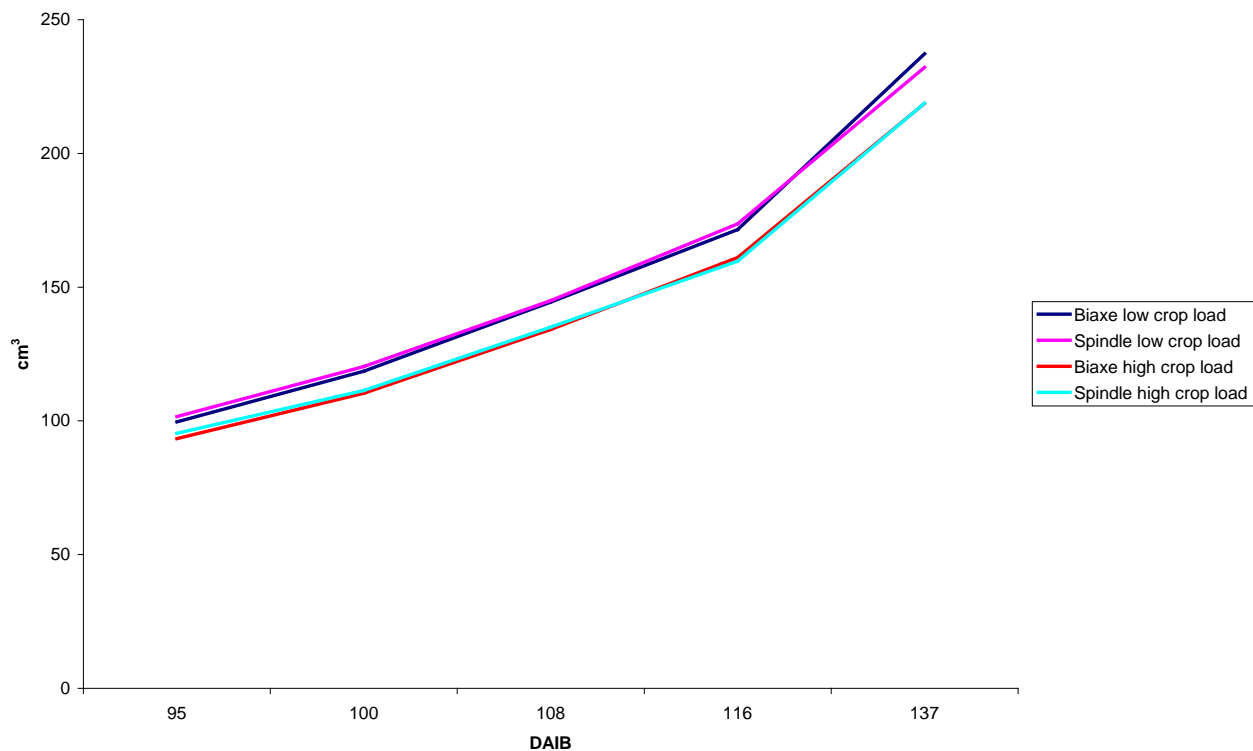


Fig. 4. Fruit growth over the season of all the treatments.

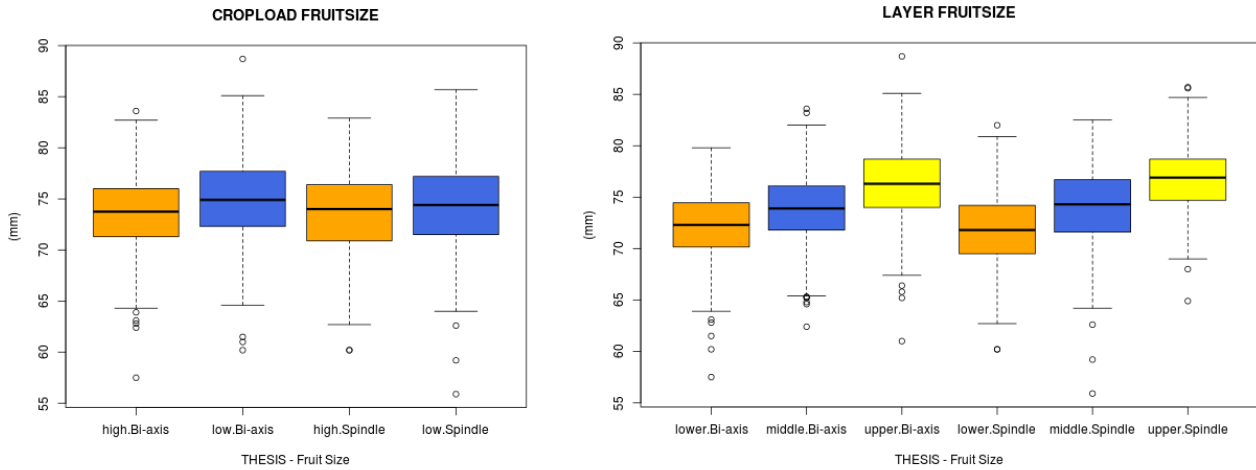


Fig. 5. a) Fruit size at harvest time comparing the four treatments (left). The adjusted p -value for the training systems comparison is not significant. The adjusted p -value for the crop loads comparison is: < 0.05 for both training systems. b) Fruit size at harvest time comparing the tree layers for both training systems (right). The adjusted p -value for the tree layers comparison is: < 0.001 for the comparison between the upper and lower layers and < 0.001 for the comparison between the upper and middle layers.

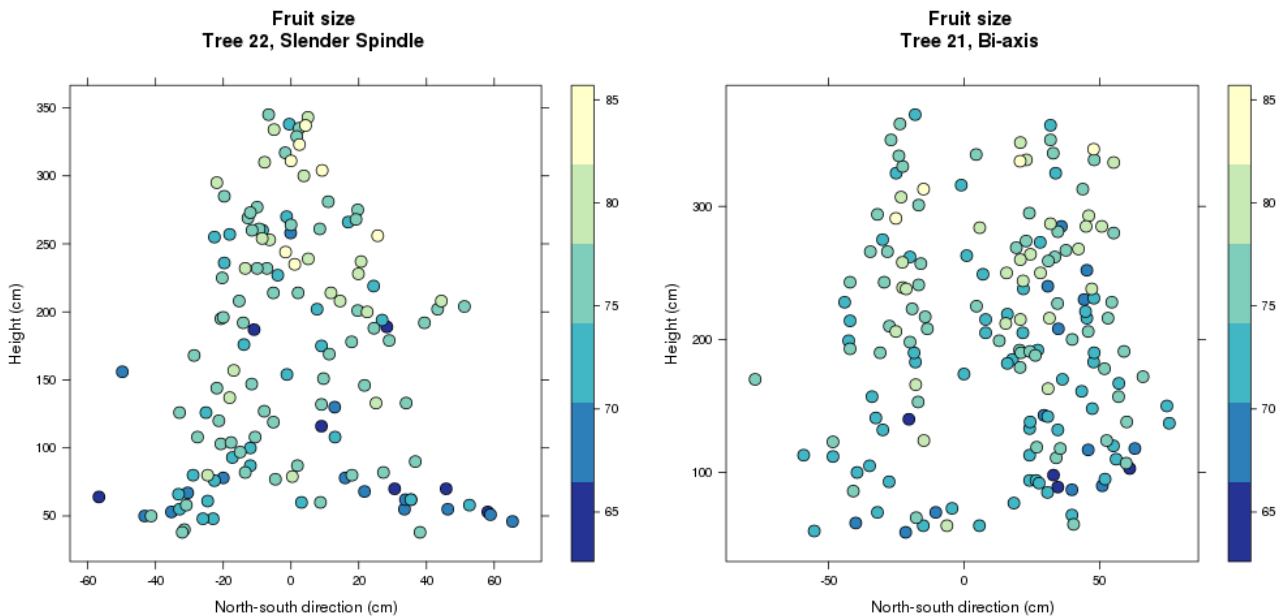


Fig. 6. a) Fruit size distribution within the canopy tree of the Slender Spindle (left). b) Fruit size distribution within the canopy tree of the Bi-axis (right). The gradient colour shows the differences in fruit sizes: from dark (smaller fruits) to fair (bigger fruits). The negative numbers on the x axis represent the south and the positive numbers the north direction.

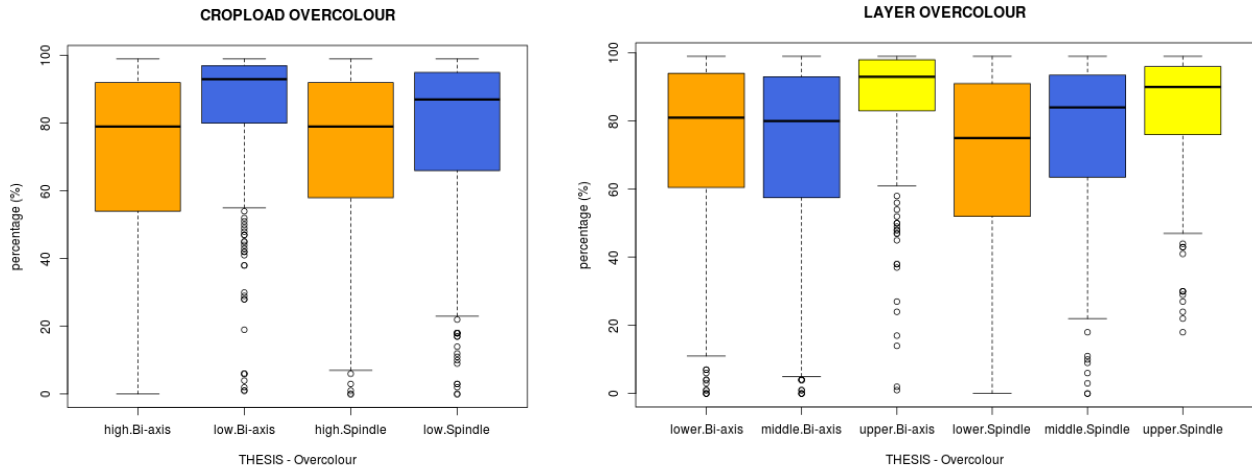


Fig. 7. a) Fruit overcolour at harvest time comparing the four treatments (left). The adjusted p -value for the training systems comparison is not significant (ns). The adjusted p -value for the crop loads comparison is: < 0.01 for both training systems. b) Fruit overcolour at harvest time comparing the tree layers for both training systems (right). The adjusted p -value for the tree layers comparison is: < 0.001 for the comparison between the upper and lower layers and < 0.001 for the comparison between the upper and middle layers.

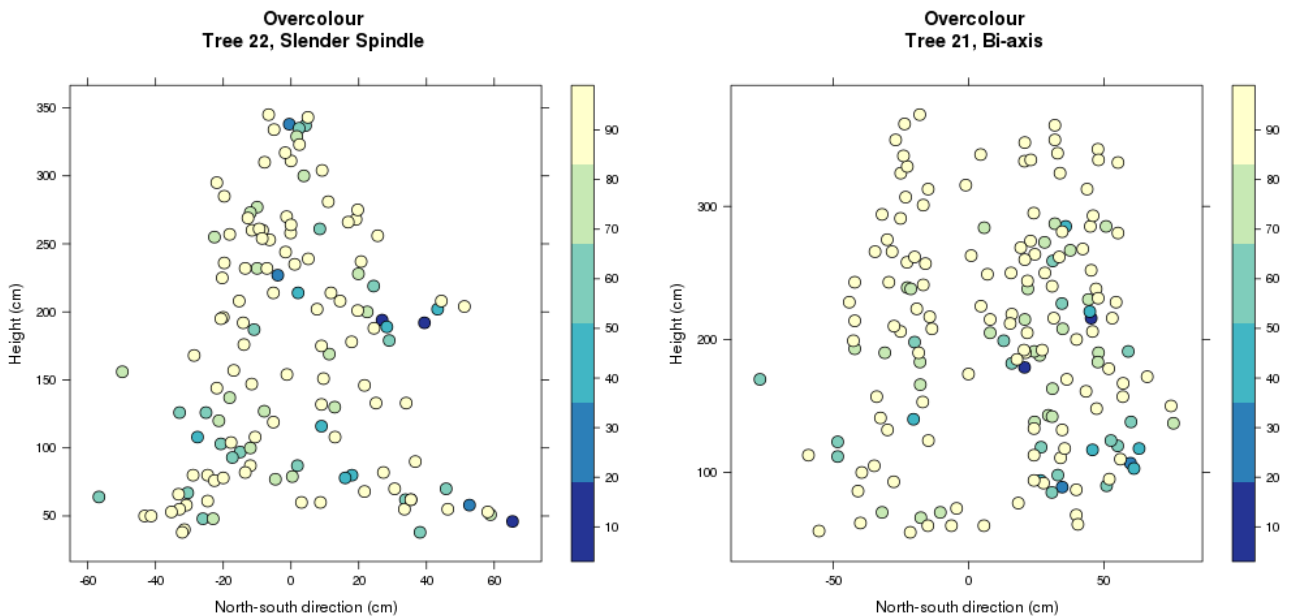


Fig. 8. a) Fruit overcolour distribution within the canopy tree of the Slender Spindle (left). b) Fruit overcolour distribution within the canopy tree of the Bi-axis (right). The gradient colour shows the differences in percentage of skin overcolour: from dark (lower %) to fair colour (higher %). The negative numbers on the x axis represent the south and the positive numbers the north direction.

4.8. Tables

Table 1. Yield Efficiency and Vegetative data (not significant indicated as ns).

<i>Thesis</i>	<i>Yield Efficiency (fruits /cm²)</i>	<i>Leaf area (m²)</i>	<i>Dry Matter (g)</i>	<i>LAI</i>
Slender Spindle Low crop load	6.4 a	5.22	577	3.29
Bi-axe Low crop load	6.5 a	5.47	611	3.97
Slender Spindle High crop load	8.1 b	6.19	647	2.80
Bi-axe High crop load	8.1 b	5.66	634	4.59
<i>p-values</i>	<i>p<0.05</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table 2. Fruit ripening stage data: adjusted p-value (in bold the significant p-values).

<i>Environment traits</i>	<i>Comparison</i>	<i>Estimated effect</i>	<i>p-values</i>
I _{DA} (index)	Bi-axis Slender vs Spindle	-0.0632	0.2146
	High crop load vs Low crop load	-0.028	0.5598
	upper vs lower	0.0805	0.0112
	upper vs middle	0.0613	0.0552

5. Fruit quality variability within the canopy tree

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Keywords: king flower, flower cluster, length of flowering, bud wood age, texture.

5.1. Abstract

The aim of the present research focuses on the infra-canopy fruit quality variability related to the flowering time and the age of the bud wood in apple trained to Spindle system. The experiment was carried out on adult “Gala” apple trees grafted on M9 rootstock. Three trees were chosen for the experiment and on these all the flower clusters were labelled just before bloom. The initial and final date of the “king flower” opening were recorded and for each of these, fruit diameter and ripening stage were monitored during the growing season. Also, the age of the wood carrying each flower cluster was recorded. At harvest, fruit size, over colour and quality traits were assessed. The results pointed out that fruit variability within the canopy is very large. Flower opening time has a strong influence on fruit size and ripening stage. The first flowers to open are immediately characterized by a higher growth rate of fruits. At harvest these fruits reach a bigger dimension, an early ripening stage and lower flesh firmness confirmed also by the mechanical parameters found in the texture. Moreover, the short flowering length also induced an early ripening. In addition the bud wood age has an effect on fruit quality and the flowers carried on old wood set larger size fruits, characterised by an earlier ripening stage and lower flesh firmness at harvest. No differences neither in over colour nor in soluble solids content (SSC) in fruits produced by different flower clusters and bud wood ages were found.

5.2. Introduction

Several studies demonstrated that there is a large variability in fruit quality traits within the canopy trees in many species (Smith et al., 1994; Forlani et al., 2002; Lewallen and Marini, 2003; Barry et al., 2004; Basile et al., 2008). This variability has been reported also in apple crop (Volz et al., 1993; Skrzyński and Streif, 1996; Broom et al., 1998; Costes et al., 1999).

Fruit trees can be considered as a population of organs that compete for resources (Grossman and DeJong, 1994). They behave as sinks for carbohydrates and this complex system of source-sink relationship can influence the growth rate and fruit quality (Corelli Grappadelli et al., 1999). The latter is also influenced by several factors, particularly by fruit growth rate within the canopy (Basile et al., 2007) such as source proximity (Corelli Grappadelli and Coston, 1991), leaf-to-fruit ratio (Wu et al., 2005), fruit growth competition (Black et al., 2000) and fruit/shoot competition (Caruso et al., 1997). Among these factors bud wood age, type of flower cluster and their position have a relevant influence on apple fruit quality.

Fruit on two-year spurs and one-year terminals are generally larger at commercial harvest than those on one-year laterals and spurs older than three years (Volz et al., 1994). Fruit growth rate from two-year spurs was observed to be greater as compared with those from one-year laterals, although relative growth rates were similar (Volz, 1992).

Wood age influences fruit ripening also. Volz et al. (1993) found that internal ethylene concentration and starch pattern index scores were greater in fruits from 2-year old spurs indicating that ripening and starch hydrolysis began earlier for fruits from one-year old laterals. Spur age has an intermediate role for nutrients and influences fruit quality within the canopy. Apples borne from old spur (three-four years old) showed a more rapid decrease in flesh firmness, acidity and sugar content than fruits from young bud wood age after a period of cold storage (Skrzyński and Streif, 1996). In addition to spur age, even spur position could have an influence on fruit quality. Fruit near the end of the lateral branches and to the top of the central leaders were found by Farhoomand et al. (1977) with lower ethylene production at harvest than fruit within the canopy. This may be due to the fact that fruit within the canopy developed sooner and initiated ethylene production before the other fruits (Sfakiotakis and Dilley, 1973). The positional effects on red colour and soluble solids were similar for both wood ages and did not reflect fruit maturity differences (Volz et al., 1993).

Apical or king flower in apple cluster usually are the first to develop and bloom and also has a greater sink potential producing a bigger fruit compared to lateral flowers (Dennis Jr., 1996; Miranda et al., 2005). This flower dominance could be overtaken by the lateral flower if the king flower is removed by thinning (Ferree et al., 2001). Flower buds in the axils of leaves on one-year-old wood differentiate and open late and develop fruits considerably smaller than those from early blooming ones (Greene, 1996). Fruits from late blooming flowers produce less ethylene, exhibit a lower starch index and develop superficial scald during storage. This indicates that apple cultivars with a prolonged bloom period should be harvested at least twice (Tomala, 1999).

Given that various studies have reported individual findings, this study aims to investigate on the effect of time of flowering and bud wood age on fruit quality in Gala checking the variability within the canopy.

5.3. Materials and methods

The trial was carried out in Trentino-Alto Adige, Northern Italy in the experimental farm of the Edmund Mach Foundation (San Michele all'Adige, Italy) on Annaglò cultivar (Gala group), trained as Spindle.

The trees were acquired from a single nursery and grafted on M9 rootstock using the chip budding technique. Trees were planted in 2006 at a distance of 3.20 x 1.00 m (3125 trees/Ha). Three plants uniform in vigour and located in the same block of the orchard were chosen.

A sample of 388 flower clusters were individually labelled on different kinds of wood age spread throughout the canopy trees before initial bloom stage. This helped ensure identification during the growing season and harvest analysis (*Fig. 1*). After flowering lateral flowers were manually removed in order to leave the king flower on the tree. By the end of fruit set 228 flower clusters labelled remained.

The trees were managed according to commercial practices as integrated crop and pest management and thinned manually to a single fruit per spur to have a similar level crop load: seven fruits / cm² trunk cross sectional area (TCSA).

Data Collection

Initial, final date and length of flowering of king flower were recorded (*Fig. 2*). Each flower cluster was categorized according to the wood age on which they developed.

Fruit size was monitored during the growing season and at harvest time fruits were graded using Greefa equipment with a software that measures weight (g), size (mm), over colour (%), relative ground colour and shape (index) and length (mm) of each fruit.

Labelled fruits were monitored for their maturation using the DA-Meter device (TR Turoni srl, Forlì, Italy), which by means of its absorbency (A670-A720) properties allows measuring the chlorophyll content in the fruit. All fruits were analysed at harvest time detecting soluble solid contents (Brix°), acidity (g/l malic acid), starch index and firmness (kg/cm²). Soluble solid contents (SSC) was detected by a digital refractometer (Atago CO. LTD, Tokyo, Japan) while acidity was determined by titration analysis (Crison instruments SA, Barcelona, Spain). Fruit firmness was measured using a digital fruit firmness tester (TR Turoni srl, Forlì, Italy) with a 11 mm probe (held on a stand). For each fruit, determinations were performed on the two opposite fruit chucks and averaged for a single value. Each of these fruits were tested for starch content (Lugol index) and texture using TA-XTplus Texture Analyzer equipped with an Acoustic Envelop Detector (AED) device (Stable MicroSystem Ltd., Godalming, UK) which helps detect both mechanic and acoustic profiles of the compress apple. Texture was measured on 4 discs of each apple of the similar shape and size.

Statistics Analysis

To assess the influences of “Initial Flowering Date”, “Length of Flowering” and “Wood Age” on the features of interest a Generalized Linear Mixed Model was fitted for each feature using penalized Quasi-Likelihood (Schall, 1991; Breslow and Clayton, 1993; Wolfinger and O'Connell, 1993). Apples from the same tree were considered correlated and therefore the variable “Tree” was considered a random effect. Multiplicity corrections (false discovery rate, Benjamini and Hochberg, 1995) were adopted to control the number of false positives. A test was deemed significant if the corresponding adjusted p-value was lower than 0.05.

Statistical analyses were carried out using R, version 3.0.2. The package MASS was used to fit the Generalized Linear Mixed Models.

5.4. Results and Discussion

Fruit Size and Volume

Time of flower opening had a strong influence on fruit size until harvest time. The first flowers which bloom showed a higher fruit growth rate as compared to the other fruits borne later (*Fig. 3a*). Similar results were detected by Lakso et al. (1989), where a large fruit size occurred when a rapid fruit growth occurred early in the season. At harvest these fruits resulted statistically bigger than those coming from flowers that open later (*Fig. 3b*). In fact Greene (1996) hypothesized that flowers, which open late in the season produced generally smaller fruits. His results pointed out that the spur that bloom early and terminal and lateral shoots that bloom later respectively producing different fruit sizes at harvest. Similarly, Dennis (1996) found that terminal flowers developed bigger fruits than lateral flowers because the latter were formed later. The length of flowering didn't influence fruit size (data not shown).

Bud wood age, which also has a strong influence on fruit quality, showed that flowers carried on old wood set fruits of bigger size at bloom time and during the season (*Fig. 4a*) and at harvest time (*Fig. 4b*). This confirms previous results obtained by Volz et al. (1993) in two-year spurs which were larger than those from one-year lateral buds for Braeburn, Granny Smith and Gala. Similar results were obtained earlier (Jackson, 1970; Calleson, 1988) for other cultivars. Jackson (1970) found that the average weights and diameters of individual fruits borne on young wood (one-year old) were 23% and 8% less than the means of those borne on older wood.

Fruit Ripening

The initial flowering date influenced fruit maturity. Fruits developed from flowers that opened first resulted riper than those that originated from flowers that bloomed later at pre-harvest

time (*Fig. 5a*). Even at harvest these fruits confirmed to be more mature than fruits with later initial flowering dates (*Fig. 5b*). Tomala (1999), found that fruit variability in a canopy tree was related to differences in bloom time. In this trial, fruits developed from late blooming flowers produced less ethylene, exhibited a lower starch index and developed superficial scald during storage, suggesting that apple cultivars with a prolonged bloom period should be harvested at least twice. Length of flowering also affected fruit maturity. Fruit with shorter period of flowering resulted more mature at harvest than fruits with long period of flowering (*Fig. 6a*). This is probably explained by the fact that longer period of flowering is characterized by late opening flowers (*Fig. 6b*).

Bud wood age influenced fruit maturity: fruit borne from young wood were characterized by a lower ripening stage as compared to fruit present on old bud wood (*Fig. 7a*) (Volz et al., 1993). In fact, Farhoomand et al. (1977) found that wood age may exert local ripening effect on ripening fruit stage. It seems that balance of naturally occurring growth regulators derived from vegetative shoot tissues near the fruits strongly influences the time of ripening by delaying initiation in individual fruit while the fruit is attached to the tree.

Fruit Over Colour and SSC

Fruit skin colour and SSC were not affected by the flowering date, length of flowering and bud wood age (data not shown) confirming previous results (Volz et al., 1993). Morgan et al. (1984) and Tustin et al. (1988), realized that red colour and SSC variation within the canopy is mostly dependent on differences in light levels than differences in fruit maturity.

Firmness and Texture

Fruits developed from flowers that open late and those present on young wood showed higher flesh firmness values as compared to the rest of the fruits (*Fig. 8a and 8b*). This is explained by the fact that the majority of late opening flowers are on young wood (*Fig. 7b*). Similar results pointed out that apples borne from old spur (three-four years old) declined more in flesh firmness, acidity and sugar content than fruits from young bud wood age after a period of cold storage (Skrzyński and Streif, 1996). Length of flowering didn't affect flesh firmness (data not shown). Texture showed that fruits developed by late opening flowers resulted with higher mechanical parameters, confirming their lower ripening stage at harvest (data not shown). No significant differences were found in the acoustic parameters.

5.5. Conclusions

Fruit variability within a canopy tree (*Fig. 9*) could be influenced by many factors. This study considered the flowering time and bud wood age as factors influencing fruit quality. Spindle training system is formed by a vertical trunk and many lateral branches and a variable number of secondary branches. These limbs are defined as “complex branches” and are different in wood age. This trial showed that wood age influenced fruit quality and is one of the main factors to cause fruit variability within the canopy tree. Also the initial flowering date and length of flowering have shown to influence fruit quality and they are often connected with bud wood age. In fact, normally the flower open earlier on the oldest wood while those present on the lateral flowers on terminal and young wood showed late flowering time and as a result fruit ripening time is also delayed.

It is well known that fruit maturity at harvest influence storage and shelf-life. Consequently achieve homogeneous fruit quality in field will have consequences on the whole fruit chain and shelf life. Fruit quality homogeneity allows to avoid many picks and fruit storage will be improved if fruits reach an ideal fruit ripening stage. Generally fruit are harvested on the base of their skin colour, which is misleading since skin colour can not be considered exhaustive to define precisely maturity. Skin colour is mostly influenced by fruit canopy position and light exposure than fruit ripening.

Finally as far as fruit thinning and pruning/fruit quality relationship is concerned, fruit thinning from one-year laterals should ensure a uniform population of fruit present on the tree canopy at harvest and after storage and different kinds of pruning methods could improve quality and increase apple quality homogeneity: branch renovation may increase light penetration in the inner lower and middle part of the spindle canopy tree.

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5.7. Figures



Fig. 1. Labelled flower clusters.

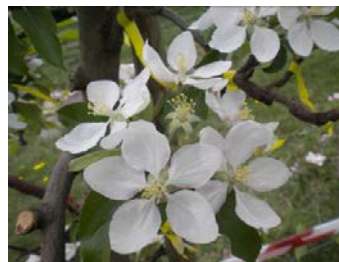


Fig. 2. Initial flowering date (above); all open flower stage (centre) full bloom stage(bottom).

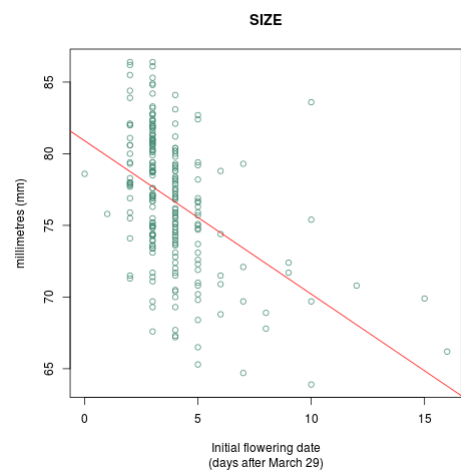
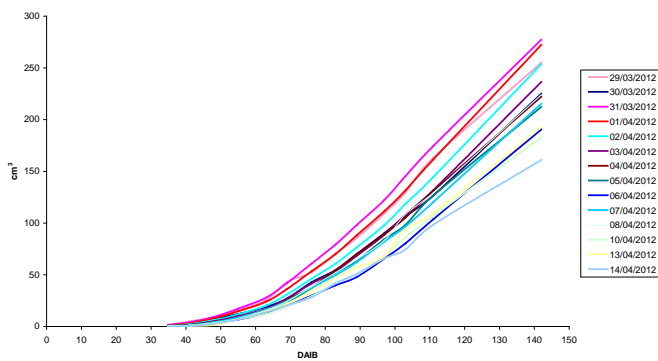


Fig. 3 a and b. Volume trend (cm^3) of the fruit borne from flowers with different initial flowering date (a - left). Average fruit size borne from different initial flowering date flowers at harvest time. The adjusted p -value is: $< 10^{-7}$ (b - right).

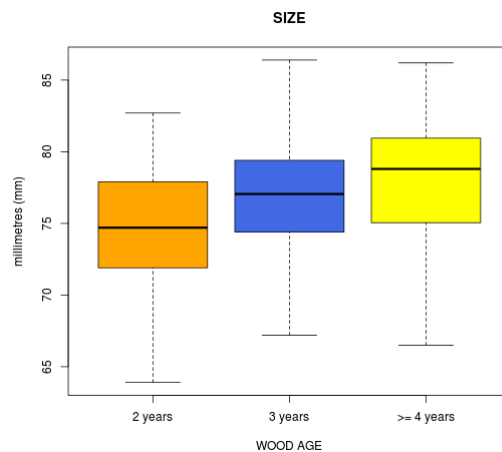
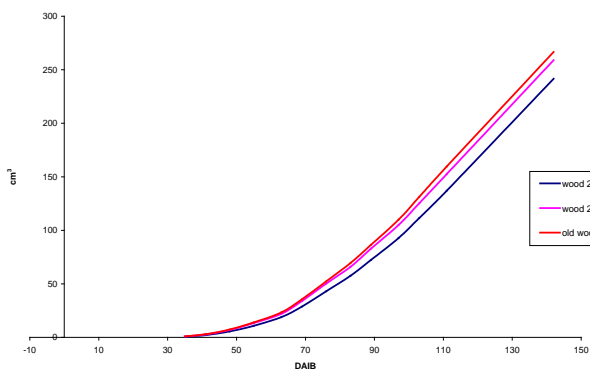


Fig. 4 a and b. Volume growth (cm^3) of the fruit borne from different bud wood ages during summer period (a - left). Average fruit size (mm) of fruit borne from different bud wood ages at harvest time. The adjusted p-value is: < 0.05 (b - right).

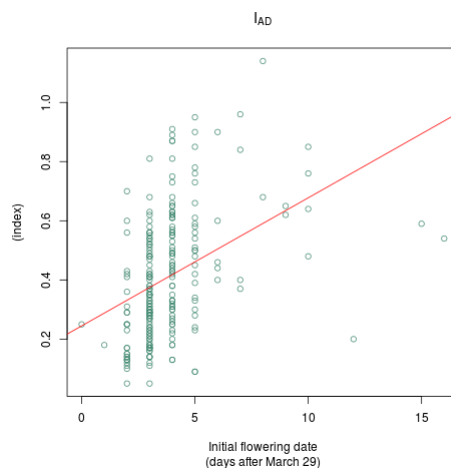
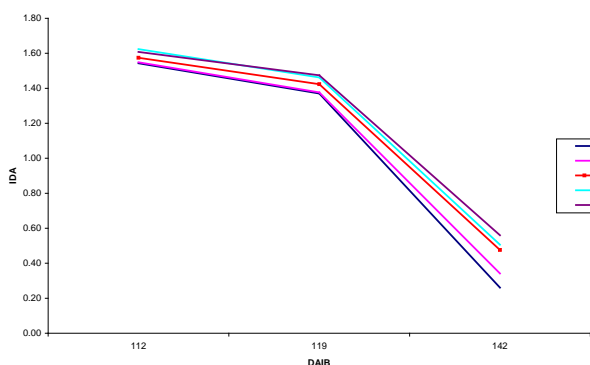


Fig. 5 a and b. Fruit ripening stage (I_{AD}) reached by fruit borne from different initial flowering date flowers at pre-harvest time (a - left). Fruit ripening stage (I_{AD}) reached by fruit borne from different initial flowering date flowers at harvest. The adjusted p-value is: $< 10^{-5}$ (b - right).

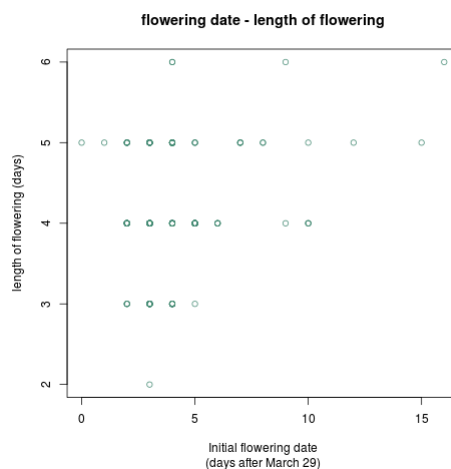
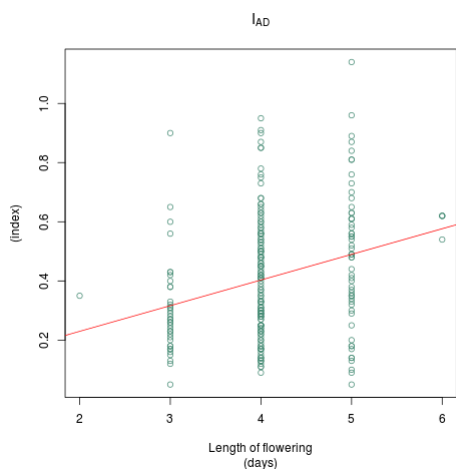


Fig. 6 a and b. Fruit ripening stage (I_{AD}) reached by fruit borne from different length of flowering date flowers at harvest. The p value is: < 0.05 (a - left). Length and Initial date of flowering comparison (b - right).

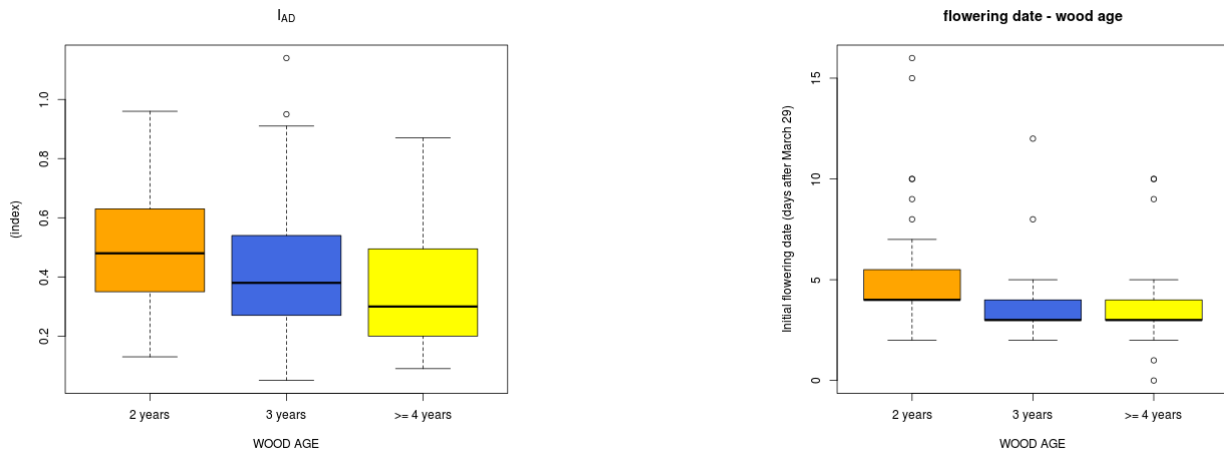


Fig. 7 a and b. Fruit ripening stage (I_{AD}) reached by fruit borne from bud inserted on different wood age at harvest. The p value is: < 0.05 (a - left). Initial date of flowering and bud wood age comparison (b - right).

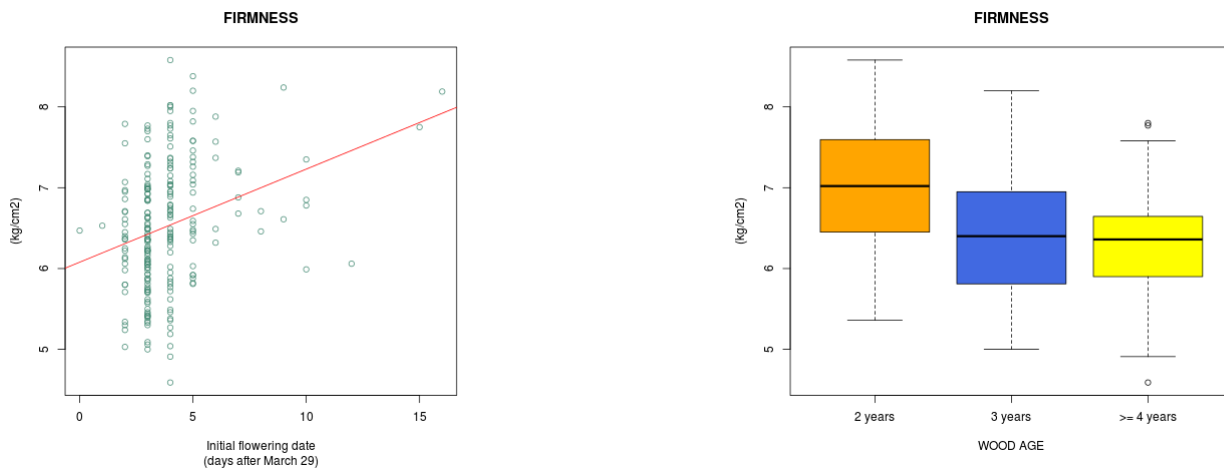


Fig. 8 a and b. Fruit firmness (Kg/cm^2) of fruit borne from flowers characterized by different initial date of flowering. The adjusted p value is: < 0.05 (a - left). Fruit firmness (Kg/cm^2) of fruit borne from bud of different bud wood. The p value for the comparison of 2 year wood with 3 years wood is < 0.001 while for 2 years versus 4 years old is $< 10^{-5}$ (b - right).



Fig. 9. Branch flower clusters variability (left); starch index variability at harvest (centre); fruit variability within a canopy tree at harvest (right).

6. The effect of pruning on fruit quality

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Keywords: training system, “Click”, branch age, texture.

6.1. Abstract

A comparison between long (rich) and short (poor), pruning methods known as “standard” and “Click” respectively, was performed on cv Annaglò (Gala group) trained as Spindle system. At harvest, fruits were divided according to branch age. Fruits were harvested, graded and analysed for main quality traits. In the first year, fruits carried by young shoots reached a bigger size, a better over colour, a higher soluble solid contents (SSC) and an earlier ripening stage as compared to fruits carried by old shoots. Even though the Click method had a higher fruit number on young branches, determining a significantly lower fruit size compared to the “standard” pruning method. Fruits of the trees pruned with the “standard method” reached higher soluble solid content and earlier ripening at harvest. No significant differences were found between the two pruning methods as far as over colour percentage, firmness and texture are concerned.

The data collected in the second year confirmed that, independently from the pruning method, the younger the bearing wood the better were the fruit size and skin colour.

6.2. Introduction

It has been established that the combination of the “Spindle” training system and the use of “M9” rootstock represent an ideal formula to get an early and abundant yield in apple (Robinson, 2007a). Orchards high density planted realized with feathered trees grafted on M9 reduced the unproductive period and allow early production already in the second and third year (Van Oosten, 1978; Ferree and Rhodus, 1987; Robinson and Stiles, 1990), fulfilling the goal to reach in a short span of time high efficiency (Robinson et al., 1991) and greater yields per hectare, and allowing to recover the initial high investment cost of the “high planting density” (HPD) orchard (Green, 1991; Warner, 1991). As far as the Spindle system is concerned, this training system was always considered suitable for the HPD orchards, although some inconveniences have been found in some cultural situations. In general, the “Spindle” is a training system where lateral fruiting branches develop from a main central leader axis; usually “laterals” are replaced over the years by pruning, but some of them, as scaffold basic branches, may remain permanent. Generally, these “laterals” have the same age as the trunk leader and bear a high number of fruits, which represent a high percentage of the total apple tree yield. This could be a limiting factor in the spindle system

specially where the limited space between branches might be filled quickly leading to increasing shade causing apple fruit quality decrease (Corelli and Sansavini, 1989).

Hence, pruning could solve or limit the problem (Robinson et al., 1997; Barritt, 2000) being one of the most important and immediate cultural management techniques to influence fruit quality and yield (Özkan and Kücükler, 2009). In fact, pruning might model the canopy allowing light interception, yield efficiency and fruit quality improvement without increasing vegetative growth potential (Robinson et al., 1991; Tustin, 2000).

“Long pruning” method was developed in France with the aim to better control plant vigour and yield (Lespinasse, 1977 and 1980). This method allows the development of vigour branches from the central leader stem and avoids strong basic scaffold branches and weak branches on the top. (Lauri, 2009). A similar method was adapted to the climatic conditions of Trentino region, becoming one of the most used training systems in this region. The system performs quite well, although the “shading” occurring in the Spindle formation has not been properly addressed. Branching renewal strategy through pruning was reported by Warrington et al. (1995) and Robinson et al. (1997) aiming to allow better light penetration in the canopy.

“Click”, a short pruning method, could be one of the solutions to improve light penetration in the canopy allowing a better return bloom and improves fruit quality and yield. This technique requires continue branch renovation with the final aim to improve fruit quality homogeneity within the canopy. The heading back cut of the leader and of the basic scaffold branches proposed in this method could promote a better flower buds formation on one-year old branches as observed by Mohammadi et al. (2013). This technique was created in The Netherlands and Belgium apple growing areas to ameliorate light penetration in the canopy, essential in these latitude-growing areas. The renewal pruning increases photosynthesis, promotes shoots growth, and improves yields and fruit weight in apple (Tustin et al., 1988; Warrington et al., 1995; Li MingXia et al., 2011). Renovation of limbs promotes fruiting shoots for early cropping, and contemporarily overcome alternate bearing (Ventura and Sansavini, 2005). On the other hand, Mitre et al. (2010) observed that fruit quality decreased in trees with branches of different ages. In addition, the cylindrical compact shape of the tree forces the plant to stay in the established space (Dallabetta et al., 2013). Nevertheless, the maintenance of a tall narrow tree in a proper space increases the fruit quality in the lower part of the canopy (Robinson et al., 2006).

The aim of this study is to verify the effect of “Click”, an innovative pruning method to ameliorate fruit quality in apple “HPD” orchards trained as Spindle.

6.3. Materials and methods

The trial was carried out in Trentino-Alto Adige, Northern Italy in the experimental farm of Edmund Mach Foundation (San Michele all'Adige, Italy) at 210 m a.s.l.. Annaglò was the Gala group clone selected for the trial grafted on M9 rootstock. The trees were trained as Spindle and pruned according to two methods: “long cut standard” and “short cut Click”. Orchard was established in 2006 at 3125 trees/Ha planting density (3.20 x 1.00 m) for the “long cut standard” pruning method and at 3906 trees/Ha (3.20 x 0.80 m) for the “short cut Click” one. Four trees of similar vigour and crop load in the first year and ten trees in the second year were chosen for each pruning method. The trees were managed according to commercial practices as integrated crop and pest management.

Pruning Methods

The “standard pruning method” consists in growing a central leader and lateral branches without heading cuts. From the lateral branches small secondary shoots develop forming complex branches. Branches are renewed periodically if they become too vigorous, while basic scaffold lateral branches are permanent.

The “Click” pruning technique consists in heading back the leader and the basic scaffold branches on new wood at the second-third-bud level. The lateral branches are continuously renewed in order to obtain fruiting branches of 2-3-4 years only. Old lateral branches are removed when too old or too big ($> 1/3$ or $1/2$ of the diameter of the central leader) by cutting the head leaving a stub to facilitate shoot renovation.

Biometrical Data

Biometrical data such as trunk cross sectional area (TCSA) 20 cm above graft union, height, breadth and depth of the trees were recorded after harvest.

Fruit Data Collection

In both years, all the fruits were individually labelled and mapped for subsequent identification during harvest and post-harvest analysis. Fruits were harvested on the same day and divided in two categories according to the bearing wood age (young and old branches). Two-four years old chains form the young branches while shoots form older branches are over four years. All the harvested fruits were graded using Greefa equipment measuring weight (g), size (mm), over colour (%) and length (mm) of each fruit.

Fruit Analyses

Only in the first year, a sample of 20 fruits for each plant was randomly chosen. These fruits were monitored for their maturation using the DA-Meter. This device measures on intact fruits the Index of Absorbance difference (@ 670 and 720 nm), which correlates with the flesh chlorophyll content decrease, allowing monitoring the evolution of ripening in planta. Ten fruits of the 20 selected were also analysed at harvest for standard parameters (soluble solid contents, expressed as Brix° and firmness, expressed as kg/cm², using the “Pimprenelle”). The remaining apples were stored in normal atmosphere for two months and kept at room temperature (approximately 20°C) for 24 hours before being graded and analysed again. Soluble solid content was detected by a digital refractometer while acidity was determined by titration analysis. Fruit firmness was measured using a digital fruit firmness tester (TR Turoni srl, Forlì, Italy) provided with a 11 mm probe. For each fruit, determinations were performed on the two opposite fruit chucks. Each of these fruits were tested for starch content (Lugol index) and texture using TA-XTplus Texture Analyser equipped with an Acoustic Envelop Detector (AED) device (Stable MicroSystem Ltd., Godalming, UK) which helps detect both mechanic and acoustic profiles of the compressed apple. Texture was measured on 4 discs of each apple of similar shape and size. No analyses were made on the fruits in the second year of the trial.

Statistics Analysis

To assess the influence of “thesis” and “wood age” on the features of interest a Linear Mixed Model with thesis and wood age as independent variables were fitted for each feature. Apples from the same tree were considered correlated and therefore the variable “tree” was considered a random effect. Tests of hypothesis within such models were performed following the approach of Hothorn et al. (2008). Multiplicity corrections (false discovery rate, Benjamini and Hochberg, 1995) were adopted to control the number of false positives. A test was deemed significant if the corresponding adjusted p-value was lower than 0.05.

To analyse the biometrical data two-sample Wilcoxon tests were used. Finally to test the difference between the percentage of young and old wood the data were additive-log-ratio transformed and then a t-test was applied (Aitchison, 1986).

Statistical analyses were carried out using R, version 3.0.2. The package nlme (Pinheiro et al., 2013) was used to fit the Linear Mixed Models. The package multcomp (Hothorn et al., 2008) was used to perform hypothesis testing from the fitted Linear Mixed Models.

6.4. Results and Discussion

Yield Efficiency

Trees pruned according to both pruning methods resulted similar in crop load and yield efficiency at harvest (*Tab. 1*). The similar crop load was also imposed tuning up the chemical and hand thinning intensity in order to avoid the effect of crop load on fruit quality.

In 2013 the yield was higher in trees pruned with the standard method but the yield efficiency resulted similar due to the bigger trunk cross sectional (*Tab. 1*) as compared to Click pruned trees, confirming that the pruning severity decrease the vigour of trees (Barden et al., 1989).

Biometrical Data

Trees pruned with the standard method resulted significant bigger in volume compared to trees in “Click” method (*Tab. 2*). The width also was lower in the “Click” pruned tree, probably because of the shorter distance between the trees (80 cm) as compared to the standard method, where trees were 100 cm apart (Dallabetta et al., 2013).

Branch Distribution

The trees pruned with the Click method resulted in the first year of trial with a significantly higher percentage (62%) of young branches compared to the standard pruning method (38%). Even in 2013, the percentage of younger branches resulted to be significantly higher in the Click (82%) than in the standard method (57%) (*Tab. 3*).

This difference confirms the typical trait of the Click method, which renews periodically the older branches over the years, and gives rise to a higher number of new branches distributed within the canopy tree. Even though the standard system featured lower percentages, the increase from 38% to 57% in the second year allowed a relatively good quality of fruits.

Fruit Size

Fruits carried out by young branches, in 2012, reached bigger size as compared to those carried by old branches in both pruning methods (*Fig. 1*) confirming previous achievement obtained in similar experiment (Warrington et al., 1995) who found a 8% of increased in fruit size renewing branches. More in details, the “standard” pruning technique produced bigger size fruit as compared to “Click” (*Fig.1*). This was also confirmed by the fruit size commercial classes distribution (*Fig. 2*). This result can be explained considering that trees pruned with the standard method have bigger canopy volume as compared to “Click” ones and therefore although the yield efficiency resulted similar, the “standard” pruned trees could produce a higher number of fruits. In fact, Palmer (1992)

observed that fruit weight is linearly dependent on leaf area / fruit ratio and the consequent the assimilate amount available for the fruitlets.

The bigger fruit size in the younger branches was also confirmed in 2013. However, the average fruit size of the trees pruned with the two methods was not significant (*Fig. 3*). This is an interesting result considering that the “Click” tree volume is smaller. This may be due to the increased percentage of renewed branches (82%) in “Click”, which produce bigger fruits.

Fruit Over Colour

In 2012, the fruits carried by young branches were significantly higher over coloured as compared to those carried out on old branches. No statistical differences were found between treatments (*Fig. 4*). These data were confirmed in 2013 (*Fig. 5*) and a possible explanation might be related to the relevant percentage of renewed branches. In addition Annaglò is a naturally very coloured clone. This result is in agreement with the results found by Dallabetta et al. (2013) in a similar experiment.

Fruit Ripening

Also fruit ripening stage was affected by the branch age. Fruits from young branches resulted riper than fruits on old branches (*Fig. 6*) and this was also confirmed by the high soluble solid contents (*Fig. 7*). This could be possibly explained considering that renewed branches are better exposed to light and have an homogeneous wood age while old branches in the standard system bear fruits on different wood age (Mitre et al., 2010).

The standard method also induced an higher ripening stage and soluble solid contents as compared to the “Click” method fruits (*Fig. 6 and 7*). This is again probably due to the fact that trees pruned with the standard method have a bigger canopy volume and could bear a higher fruit number than trees in the Click method.

Texture Analyses and Firmness

No statistical differences between the pruning methods and the kinds of branch ages were found. Also fruits firmness was not statistically significant neither at harvest nor after two months of cold storage (data not shown).

6.5. Conclusions

The objective of this study was to study the relationship between pruning methods (“standard” and “Click”) and fruit quality. The latter was introduced to specifically improve fruit quality in an intensive spindle training system. Branch renovation that was observed in both the pruning methods, although to a different extent, it improved fruit size and skin colour, two of the most important parameters for the market. HPD systems need to renew periodically their lateral branches to promote new fruiting limbs and to improve light penetration within the canopy trees.

The standard pruning, proposed in this trial, reached higher yields and good vigour control. The growth of complex branches increased the tree volume and this might create shading conditions if this aspect is not regulated by a continuous renewal of branches.

Instead, the “Click” method allowed a more intensive branch renovation, and determined a fruit quality improvement within the canopy. This is important for HPD spindle orchard where the available space is limited (less than a meter between trees). Branch renovation in the Click method also involves the basic scaffold branches (*Fig. 8*) allowing a better light penetration also in the lower part of the canopy. This maintains the canopy size within its original planting distance (*Fig. 9*). The continuous branch renovation could help to improve fruit quality homogeneity within the canopy thus avoiding many picks at harvest.

Therefore, this paper recommends the Click pruning method for intensive plantation particularly for bicolour cultivar, like Gala, to maintain a high percentage of fruit skin colour and at the same time a marketable fruit size.

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6.7. Figures

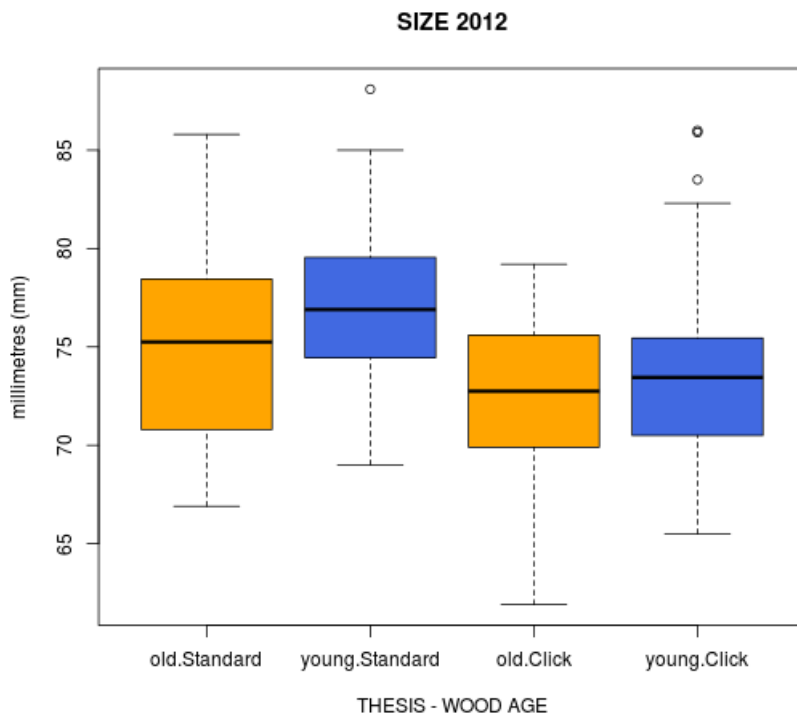


Fig. 1. Average size (\varnothing mm) of the fruits carried by old and young braches in the two different pruning methods (standard and Click) in year 2012. The adjusted p -value for the comparison between old and young branches fruits is: $< 10^{-4}$ for both treatments (Click and standard methods). The adjusted p -value for treatment comparison is: < 0.05 .

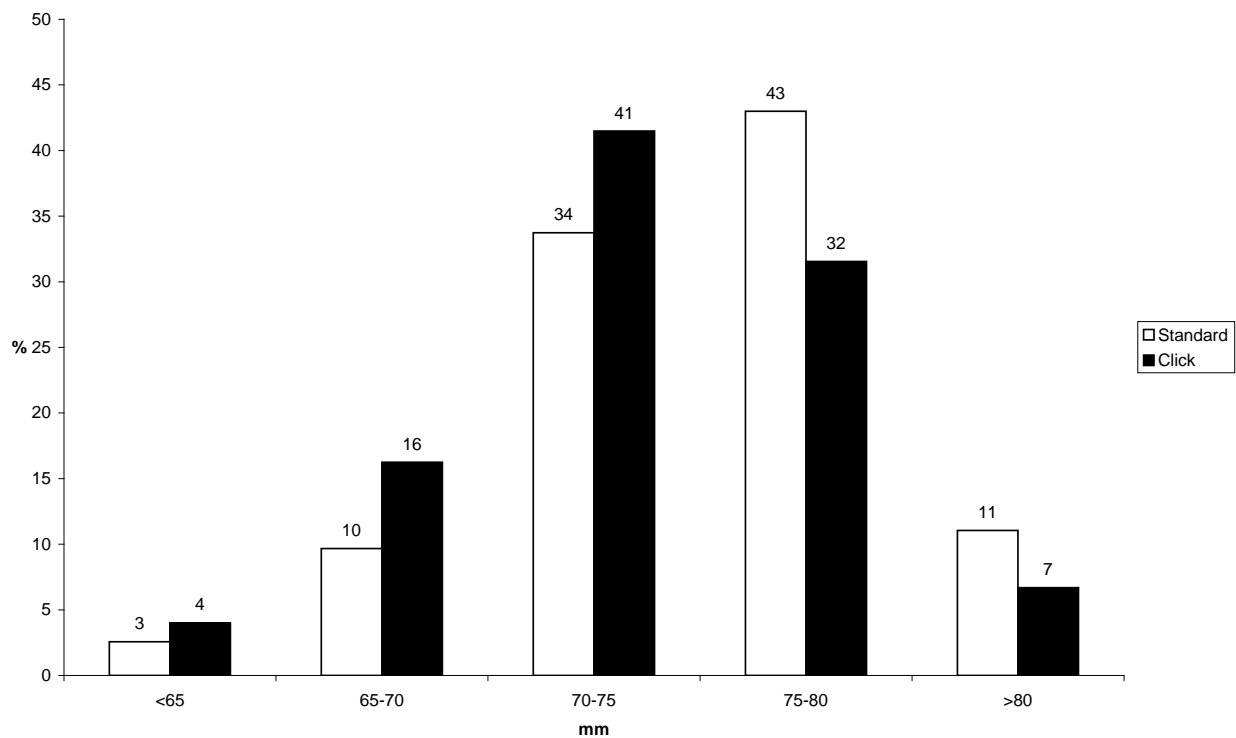


Fig. 2. Fruit size classes distribution (year 2012).

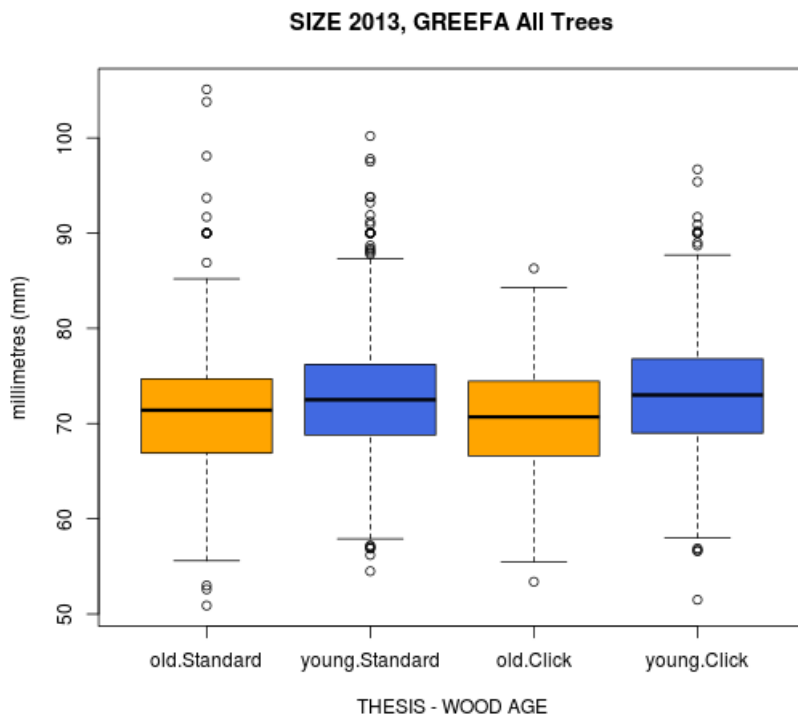


Fig. 3. Average size (\varnothing mm) of fruits carried out by old and young braches in the two different pruning methods (standard and Click) in year 2013. The adjusted p -value for the comparison between old and young branches fruits is: $< 10^{-6}$ for both treatments (Click and standard methods). The adjusted p -value for the treatment comparison is not significant (ns).

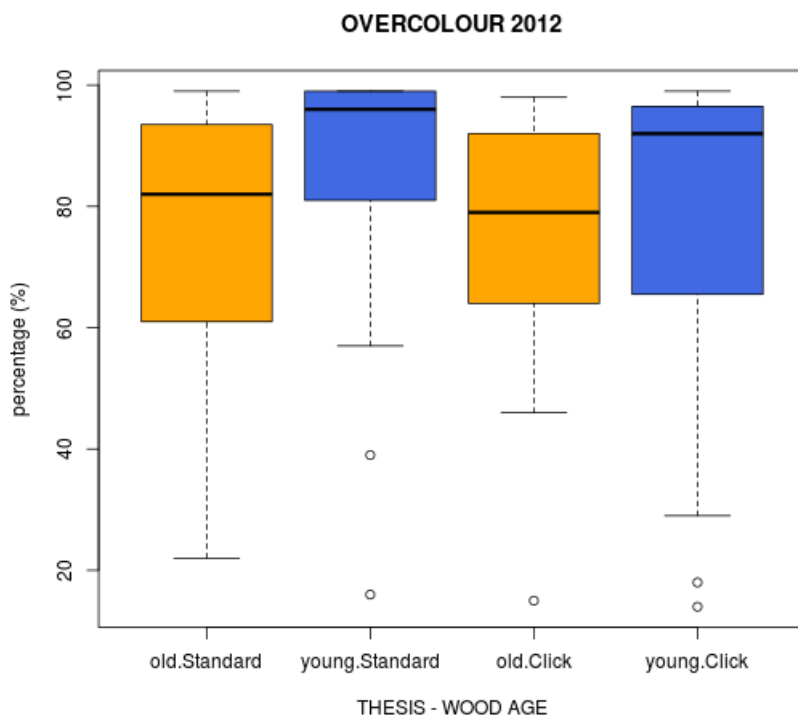


Fig. 4. Average over colour (%) of fruits carried by old and young braches the in two different pruning methods (standard and Click) in year 2012. The adjusted p -value for the comparison between old and young branches fruits is: $< 10^{-6}$ for both treatments (Click and standard methods). The adjusted p -value for treatment comparison is not significant (ns).

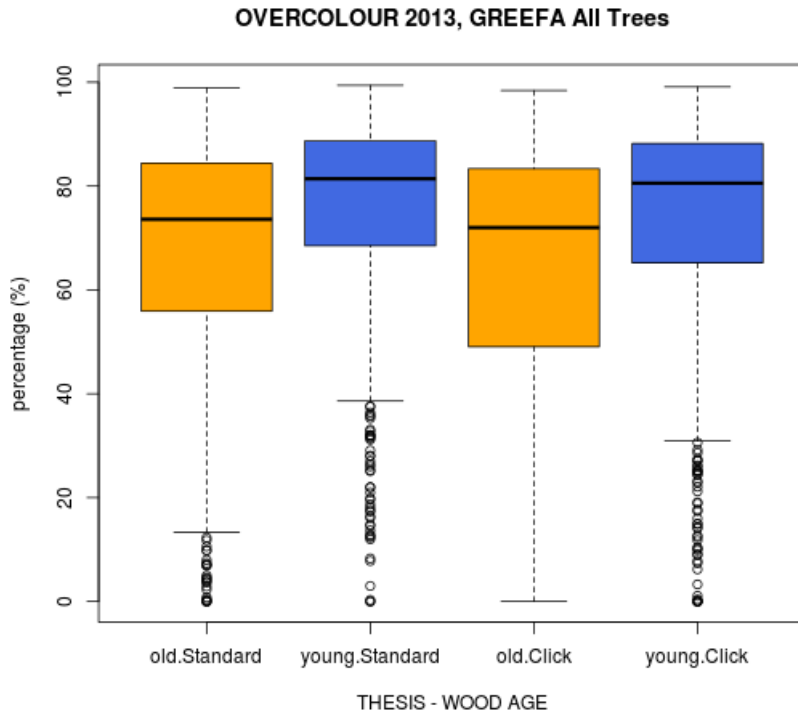


Fig. 5. Average over colour (%) of fruits carried by old and young braches in two different pruning methods (standard and Click)in year 2013. The adjusted p-value for the comparison between old and young branches fruits is: $< 10^{-6}$ for both treatments (Click and standard methods). The adjusted p-value for treatment comparison is not significant (ns).

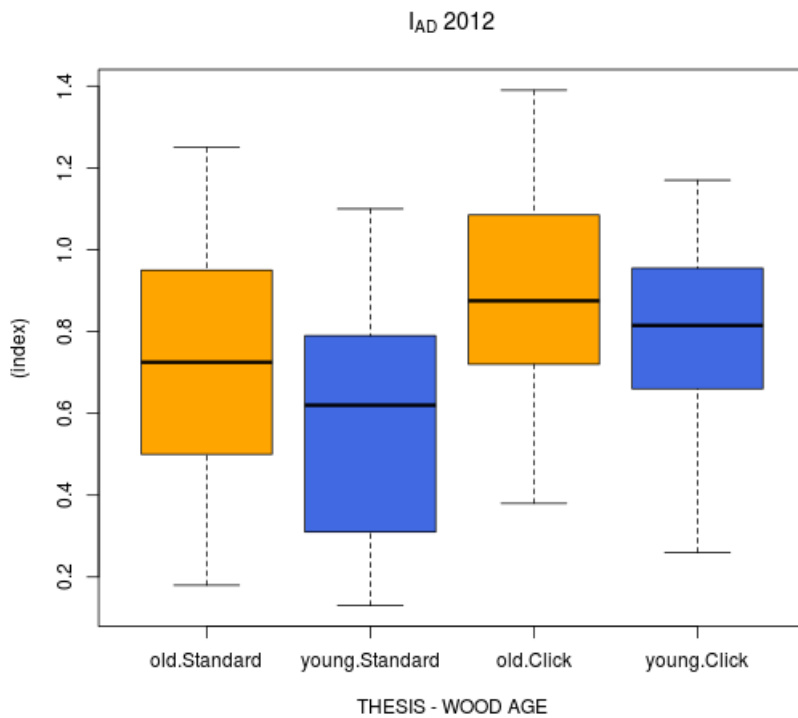


Fig. 6. Ripening stage (IDA) at harvest time of fruits carried out by old and young braches in the two different pruning methods (standard and Click)in year 2012. The adjusted p-value for the comparison between old and young branches fruits is: < 0.05 . The adjusted p-value for treatment comparison is: < 0.05 .

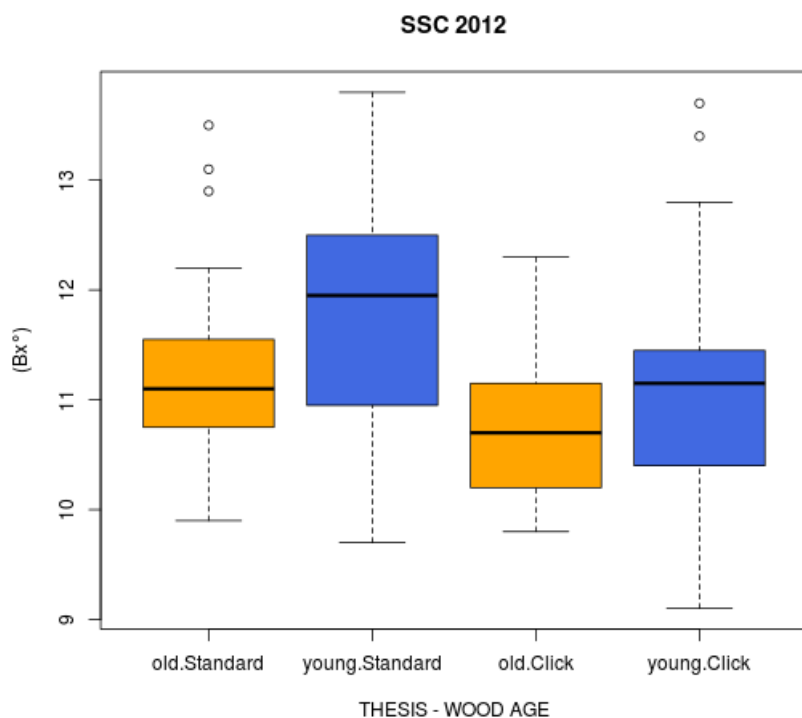


Fig. 7. Soluble Solid Content (Brix°) at harvest time of fruits carried out by old and young branches in the two different pruning methods (standard and Click) in year 2012. The adjusted p-value for the comparison between old and young branches fruits is: < 0.01 The adjusted p-value for treatment comparison is: < 0.05.



Fig. 8. Renewed basic branches in Click method.



Fig. 9. Trees pruned with Click in winter time.

6.8. Tables

Table 1. Yield, Trunk Cross Sectional Area (TCSA) and Yield Efficiency in 2012 and 2013. The *p*-values not significant are indicated as *ns*.

Year	Thesis	Yield (Kg/tree)	TCSA (cm ²)	Yield Efficiency (kg/cm ²)	Fruit number/ tree	Yield Efficiency (fruits/ cm ²)
2012	Standard	24.8	20.0	1.45	127	7.4
	Click	23.8	18.3	1.44	131	7.9
	<i>p-values</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>
2013	Standard	25.2	20.3	1.30	145	7.3
	Click	21.2	17.6	1.20	120	6.8
	<i>p-values</i>	<i>p</i> <0.05	<i>p</i> <0.10	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table 2. Biometrical data in 2013. The *p*-values not significant are indicated as *ns*

Thesis	Height (cm)	Width (cm)	Depth (cm)	Plant Volume (m ³)
Standard	387	162	167	2.73
Click	383	127	162	2.06
<i>p-values</i>	<i>ns</i>	<i>p</i> <0.01	<i>ns</i>	<i>p</i> <0.01

Table 3. % of young branches in the canopies. The *p*-values not significant are indicated as *ns*.

Thesis	% of Young branches (year 2012)	% of Young branches (year 2013)
Standard	38	57
Click	62	82
<i>p-values</i>	<i>p</i> < 0.05	<i>p</i> < 0.001

7. Effects of light availability and training systems on apple fruit quality

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Keywords: *Malus domestica*, ceptometer, fisheye photography, Slender Spindle, Bi-axis, fruit position.

7.1. Abstract

The aim of this study is to correlate light availability and fruit quality traits in apple fruits (*Malus domestica* Borkh.) as a result of their positions within the canopy in two training systems: Slender Spindle (SS) and Bi-axis. The experiment was carried out on nine-year old trees of Gala cultivar grafted on M9 rootstocks. Three trees were chosen for each training system and twelve fruits from each tree were selected in different canopy positions and labelled after fruit set. During the growing season the light availability on each labelled fruit was measured using two different methods. A ceptometer bar, equipped with an external sunshine sensor, was used six times during the growing season and, in addition, hemispherical pictures were taken with a digital camera and fish-eye lens to investigate the radiative regime. At harvest, fruit size, over colour and quality traits were assessed for each monitored fruit. The results indicate a large variability of fruit light availability within the canopy depending on fruit positions. The upper and outer fruit positions for both training systems resulted significantly higher in terms of light compared to the lower and inner positions. The percentage of fruit skin colour and the soluble solid contents (SSC) are influenced by the canopy layers. Fruits in the upper tree positions showed a higher percentage of over colour and higher SSC, while no differences were found in the inner and outer positions. Fruit position didn't affect fruit size, ripening stage, flesh firmness and texture. No differences were found between the fruits in the two training systems for both light availability and quality traits.

7.2. Introduction

The Trentino province is an important apple growing area and represents 25% of the total apple production of Italy. Apple cultivars are grafted for the majority on dwarfed M9 rootstock and trees are trained mainly with Spindle and Slender Spindle systems. These trees are formed by a vertical leader trunk and lateral horizontal weak branches which are much shorter at the top than at the bottom giving to the plants a conical shape (Buler and Mika, 2009).

Recently an alternative kind of tree has been introduced in the orchards patented under the trade name "Bibaum®" (Musacchi, 2008). This tree is formed by two axis which develop along the row giving the plant a flat shape. The lateral branches are shorter than those of the Spindle, thus providing apparently a better light canopy penetration and improving fruit light exposure (Dorigoni et al., 2011). Both training systems are well adapted to high planting density and full canopy can be

achieved by the end of the third year (Robinson, 2011; Dorigoni et al., 2011) fundamental in light interception and distribution within the canopy tree. Generally, small trees show less shade within the canopy, but once the tree density increases, poor illumination could occur even in these planting systems (Robinson et al., 1991; Barritt, 2000). As the orchard ages, conical shaped trees like spindle may have problems to maintain both good fruit light exposure and quality in the lower part of the canopy (Robinson, 2011). Spindle planted in less space could also affect fruit quality increasing the denseness of the branches and foliage (Robinson et al., 1991). If canopy thickness is > 3 ft, light levels can decline to $< 30\%$ of full sunlight, resulting in poorer flower bud initiation and development, fruit set and quality (Barritt and Rom, 1987). Moreover Sansavini et al. (1981) observed Spindle in vigorous conditions planted in intensive systems, which obtained poor fruit quality and colour compared to Palmette hedgerow system, a similar shape to Bi-axis tree. Planar canopies have been introduced even in the past with the aim to improve light penetration within a canopy (Lakso et al., 1989a; Palmer, 1997).

Relationship between canopy shape, training systems and light interception has been studied over the years by many researchers (Palmer, 1977 and 1981; Palmer and Jackson, 1977; Jackson, 1980 and 1981; Wünsche et al., 1996; Wei et al., 2004; Willaume et al., 2004). Wagenmakers (1995) concluded that greater the capacity of the canopy tree in capturing light, the greater is the maximum potential assimilation. Fruit productivity and total dry matter is strongly dependent on potential light interception obtained by any orchard system (Jackson, 1980; Palmer, 1989; Robinson et al., 1991). High planting density, such as those studied in this experiment, have smaller and compact trees which are more efficient in light interception than bigger trees in low density systems (Robinson, 2000). Canopy light interception and light distribution has shown to influence fruit quality particularly for colour and bicolour cultivar (Jung and Choi, 2010; Unuk et al., 2012).

Fruit positions within the canopy were studied in-depth in this research, and there is evidence of relationship between fruit quality and light exposure (Jackson, 1970; Robinson et al., 1983; Wünsche et al., 1996; Warrington et al., 1996). Insufficient light transmission across the canopy may reduce fruit size, colour and soluble solid contents (SSC) (Robinson et al., 1991; Palmer et al., 1992; Unuk et al., 2012). Earlier results first reported by Tustin et al. (1988) showed that the fresh fruit weight is linearly correlated by the photosynthetic photon transmission flux (PPF) measured in the fruiting zone within the canopy tree. Later, Warrington et al. (1996) found similar results in Granny Smith cultivar, realizing that light environment at the fruiting sites has a stronger effect on fruit size and quality than the canopy system itself. Moreover, fruit SSC was found to be strongly influenced by leaf light exposure in the immediate area around the fruit (Robinson et al., 1983; Tustin et al., 1988; Wünsche et al., 1996). Fruit colour was positively correlated with PPF but not

always with fruit maturity (Tustin et al., 1988). In fact, Farhoomand et al. (1977) found that external positioned fruit may be colourful but unripe concluding that there are other factors such as fruit position and bud wood age that may influenced fruit ripening stage. Thus the estimation of total light interception in an orchard is necessary to understand the differences on yield and fruit quality (Wünsche et al., 1995), considering a combination of factors such as latitude, slope, aspect, row orientation, trellis type, and canopy management (Zorer and Policarpo, 2006).

Several methods have been used to detect light interception (Jackson, 1980; Palmer, 1987; Barritt et al., 1991). Direct methods measure the solar light intensity transmitted through the canopy as compared to the incoming radiation (Wünsche et al., 1995). This approach is based on the Beer-Lambert law, which considers the total amount of radiation intercepted by a canopy layer dependent on incident irradiance measured as total, direct, and/or diffuse radiation and optical properties using line quantum sensors or radiometers.

Other indirect methods involve the fisheye or hemispherical photography, based on photographs acquired through a hemispherical (fisheye) lens from beneath the canopy oriented towards the zenith and then processed by proper image analysis software (Anderson, 1971; Lakso, 1980a,b; Frazer et al., 1999). This method was first used in horticulture by Smart (1973) in vineyards and then applied to other fruit species (Lakso and Musselman, 1976; Ferree and Lakso, 1979; Lakso, 1980a,b; Robinson and Lakso, 1989a; Schechter et al., 1990).

The aim of this work was: (i) to investigate light interception and distribution within the canopy as a result of different training systems; (ii) to assess light regime and quality of apple fruits in different parts of the canopy using both direct and indirect methods, and, finally, (iii) to find out correlations between light availability and fruit quality properties.

7.3. Materials and methods

Trials were carried out in Trentino province, Northern Italy (46° 11' 18" N; 11° 06' 11" E) in an apple orchard of the experimental farm of the Fondazione Edmund Mach (San Michele all'Adige, Trento, Italy) using the cultivar Galaxy Evolution, trained to SS and Bi-axis.

Plant Material

The cultivar Galaxy Evolution (Gala group) was grafted on M9 rootstock using the chip budding technique for Slender Spindle (SS) trees while Bi-axis trees were obtained by cutting back the already grafted leader in the nursery by growing later into two axes. Trees were planted in 2005 at a spacing distance of 3.45 x 1.00 m for SS (2900 trees/Ha) and 3.45 x 1.20 m for Bi-axis (2400 trees/Ha) between rows and tress respectively. The row orientation was about 9° NE.

Three plants for each training system were chosen with similar vigour. Only plants from the centre of the block were used for the study in an effort to avoid edge effects in terms of light conditions. Trees were chemically and manually thinned to achieve similar crop load and yield efficiency (7 fruits/cm² trunk cross sectional area - TCSA).

Training Systems

The SS is a narrow tree formed by a central axe leader from which lateral fruiting branches develop giving the plant a narrow conic shape.

The Bi-axis training system, patented under the trademark Bi-baum® (Musacchi, 2008), instead, consists of two trunks developed along the rows allowing a preferable light penetration and, at the same time, enabling an easier shoot growing control.

Fruit Selection

Three parallel layers of equal size (upper, middle and lower) were identified within the canopies, according to their height from the ground. Twelve fruits, four from each layer (lower, middle and upper) for each tree were chosen to make a total of 72 fruits. In each layer two fruits were located in the outer position and two in the inner position facing east and west. Fruits were individually labelled and mapped for subsequent identification during summer data collection, harvest, and post-harvest analysis.

Light Interception Measurements

Fisheye photography

Light transmitted to the fruit position was evaluated using a fisheye photography technique. First, the labelled fruits of each tree for both training system were sampled according to a given starch and DA-meter index. After fruit removal, an hemispherical photograph was acquired at the same position facing the levelled lens upwards to the sky (*Fig. 1*) with a digital camera (Coolpix 4500, Nikon Corporation, Tokyo, Japan) equipped with a fish-eye additional lens (FC-E8, Nikon Corporation, Tokyo, Japan) (*Fig. 2*). The images were taken under diffused light conditions to avoid high light reflection and scattering due to direct sunbeam. The camera was placed on a pole equipped with a bubble level in order to keep the camera levelled and oriented according to the row direction (9 ° NE).

The hemispherical images were processed with the free software Gap Light Analyzer 2.0 (Frazer et al., 1999; Zorer et al., 2013). The procedure began with the configuration, which included the definition of the latitude, and eventually slope and aspect of the site, number of angular sectors to

divide the hemisphere, day or time interval for the calculations of the sun path, and transmitted radiation. 12 Zenith and Azimuth 48 regions to obtain 7.5° sky regions were used. The blue channel, usually the best one to separate the canopy from the sky (Frazer et al., 1999; Nobis and Hunziker, 2005) was used for all the image analyses performed. A threshold value was then set manually for the separation of canopy and sky elements, producing a binary black and white image, corresponding to pixels with no and 100% transmittance, respectively. The following calculation returned the percentage of transmitted direct, diffuse and global radiation on shooting position, corresponding to the removed fruit. The output of the image processing is reported in *Fig. 3 & 4*. Calculations of radiative regime were calculated for the period from the 1st to 23rd August, when the harvest was performed. A longer period was not taken into consideration due to the fact that the canopy vegetation might undergo a change.

Ceptometer

The ceptometer (SunScan-SS1 Delta-T Devices Ltd.) equipped with a sunshine sensor (BF3 Delta-T Devices Ltd.) was used (*Fig. 5a & b*) as a photosynthetic photon flux (400 to 700 nm) line sensor, that integrated readings of 64 light sensors placed at 1 cm interval along a 100 cm long probe reading. The probe was covered by a black tape to allow only 4-5-6 sensors for the readings, which have been calibrated to the fruit size over the growing season until the harvest time (*Fig. 6a & b*). A total of six readings were taken over summer for the purpose starting from mid-June until harvest in August. Each of the six readings consist in five readings at five different times of the day per day for each fruit, two from the East side, one from above and two from the West side of each fruit were taken. A total of 24 fruits, 12 fruits in a SS tree and 12 fruits in a Bi-axis tree were considered for the measurement. The two trees were the same where the fish eye was also employed. Out of the six readings the last three were utilised for statistical analysis because a comparative measurement between the two instruments was possible due to the same period. A bubble level on the ceptometer was used to keep the probe horizontal to the ground. All readings were taken under the open sky conditions. The fraction of PAR available on each single fruit was calculated by the ratio between average PAR measured by the 4-5-6 photodiodes and the total incident PAR, detected by the external BF3 sunshine sensor. The individual Photosynthetically Active Radiation (PAR) readings and the corresponding incident direct and diffuse PAR, measured by the BF3 sunshine sensor above the canopy, have been recorded by a laptop connected to the ceptometer probe. The percentage of transmitted PAR is derived from the ratio between the average percentage of transmitted PAR and the percentage of incident PAR.

Indirect light reflected by the ground and the canopy vegetation were also recorded. Two readings without black tape on the probe, two on the inner position and two on the outer position of the canopy, were taken with the sensor facing the ground for each test tree making a total of four readings.

Fruit Analyses

The fruits were harvested and assessed for weight (g), size (mm), over colour (%), fruit shape (index) and length (mm). Fruit ripening stage were measured by the DA-Meter device (TR Turoni srl, Forlì, Italy), a portable vis/NIRs, expressed as I_{AD} (index of absorbance differences). The I_{AD} is calculated as absorbance difference between two wavelengths (670 and 720 nm) and correlates with ethylene emission. Fruit internal quality was measured with traditional methods as soluble solid contents (refractometer mod. PR 101, 0-45% - Atago CO. LTD, Tokyo, Japan), firmness (penetrometer 11.1-mm-diameter probe - TR Turoni srl, Forlì, Italy) and titratable acidity (Crison instruments SA, Barcelona, Spain) mod. Crimson Compact Tritator versione D). In addition fruit were also analysed at harvest time for texture by using TA-XTplus Texture Analyzer equipped with an Acoustic Envelop Detector (AED) device (Stable MicroSystem Ltd., Godalming, UK). The combined acoustic-mechanical profiles data were processed by the software Exponent v.4 (Stable MicroSystem) provided with the TA-XTplus instrument. With the same software a macro instruction was also compiled to automate the parameter extraction from the force/sound curves. From the texture profiles, a set of texture parameters were digitally extracted and used as novel fruit quality descriptors (see Costa et al., 2011 for more detail).

Biometrical Data

Biometrical data such as trunk cross sectional area (TCSA) 20 cm above graft union, height, breadth and depth of the trees were recorded after harvest.

Statistical Analyses

We considered how training systems (Slender Spindle and Bi-axis), layers (upper, middle and lower) and positions (inner and outer) affect fruit quality variables (Over colour, Fruit size, I_{AD} , SSC and Firmness) and environment variables (Canopy Openness, Direct Radiation, Diffuse Radiation and Total Radiation). Apples from the same tree were considered correlated and therefore to estimate the quantity of interest we used a Linear Mixed Model with the variable "Tree" as random effect. Specifically, we used a Generalized Linear Mixed Model and penalized Quasi-Likelihood (Schall 1991; Breslow and Clayton, 1993; Wolfinger, and O'Connell, 1993). To control

the number of false positive we used the false discovery rate methodology (Benjamini and Hochberg, 1995); a test was deemed significant if the corresponding adjusted p-value was lower than 0.05. Principal Component Analysis (PCA) plots were used to visualize variability and relationships between the different variables (Jackson, 1991). Finally to assess differences in biometrical data we used two-sample Wilcoxon tests and we correct the p-values with Holm method.

The statistical analyses were carried out using R, version 3.0.2. The package MASS was used to fit the Generalized Linear Mixed Models.

7.4. Results and Discussion

Yield Efficiency and Biometrical Data

Trees in both training systems resulted to have similar yield efficiency at harvest (*Tab.1*), also because the trees were intentionally chemically and manually thinned soon after flowering to avoid the effect of crop load on fruit quality. SS trees resulted to have a similar canopy volume of the Bi-axis trees (*Tab. 1*)

Fruit Light Availability at Fruit Level

A significant ($t=0.05\%$) relationship between the percentage of transmitted PAR detected by the Ceptometer probe and the percentage of total radiation detected by the digital camera with fisheye at level fruit (*Fig. 7*) was observed.

Fruit light availability, measured by the camera using the fisheye, resulted significantly different within the canopy. Fruits in the outer and upper position of the canopy received the highest value of direct, diffuse and total radiation. The percentage of canopy openness were also higher in the outer and upper positions. No differences were found between the middle and lower positions of the canopy (*Tab. 2*). Fruit light availability resulted clearly different in the inner and outer position but no difference were found between the fruit in the two training systems (*Fig. 8a*).

Fruit Quality Traits

The percentage of fruit over colour resulted to be the most relevant factor influenced by fruit position in the three different layers (*Fig. 8b*). Fruit located in the upper layer resulted with higher percentage of over colour compared to the fruit located in the bottom level of the canopy (*Tab. 3*) thus indicating the amount of light available in the top strata (*Tab. 2*). No significant differences were found between fruits positioned in the lower and middle part and in the inner and outer part of the canopy and between the two training systems (*Tab. 3*). These findings tie with other studies

where the skin colour was positively correlated with the level of exposure to sunlight (Jackson, 1970; Morgan et al., 1984) and relative to fruit position (Wertheim et al., 1986; Yuri et al., 1996). Similar conclusions were reported even for apples grown under net conditions (Dussi et al., 2005). Temperature effects go beyond over colour and an increase in the concentration of anthocyanins occur in apples when exposed to adequate sunlight for 20 days before harvest (Arakava et al., 1985; Saure et al., 1990).

Fruit position affected SSC but not fruit size, even though both resulted to have an interaction between them (*Fig. 8b*). There has been a layer effect in both training systems but none as far as inner and outer positions are concerned (*Tab. 3*). No differences were found between the fruits in the lower and middle canopy position. However there was no difference between the two training systems (*Tab. 3*). The lower level in the canopy produced fruits with less SSC compared to the fruits in the upper position of the trees. Robinson et al. (1983) found 22% less SSC in shaded basic branches, even confirmed by Campbell and Marini (1992). Tustin et al. (1988) found that fruit SSC increases with increasing height in the canopy and was higher in the outer compared with the inner horizontal canopy position. This latter result was not confirmed in this study where fruits in the inner and outer positions were similar in terms of fruit SSC. Other studies do confirm (Barritt, 2000) that in areas of the canopy with poor light distribution, fruit of inferior quality is produced, particularly fruit of small size, poor colour and low SSC. Therefore sufficient light penetration in deeper layers of the canopy is essential (Jackson and Palmer, 1977; Robinson et al., 1983).

Fruit position and training systems didn't affect fruit ripening stage and firmness (*Fig. 8b and Tab. 3*). This confirmed the results obtained by Farhoomand et al. (1977) who found that fruit position in the canopy didn't affect fruit ripening stage. There were others factors such as wood age, which may exert local ripening effect on ripening fruit stage. It seems that balance of naturally occurring growth regulators derived from vegetative shoot tissues near the fruits strongly influences the time of ripening by delaying initiation in individual fruit while the fruit is attached to the tree. Fruit ripening stage and flesh firmness resulted not to be affected by light availability (*Fig. 8a*). This study confirms the results obtained by Seeley et al. (1980) and Ferree (1989) who reported no effect of light environment (or canopy position) on flesh firmness. Light environment and fruit position didn't affect fruit texture (data not shown).

7.5. Conclusions

Gala trees used in this study had relatively open canopy with similar light levels at interior positions. In fact, there were no differences in light fruit availability in the different parts of the canopy comparing the two training systems. Both training systems played an important role in light

interception and its distribution within the tree canopy. Well illuminated fruiting zone is important for high quality apple production. Significant relationship between fruit skin colour and SSC with light transmission across the canopy types indicate that the primary influence on fruit quality in apple crop is the light environment at the fruiting sites and not the canopy system in se. (Warrington et al., 1996). Both the trees resulted very compact in terms of volume due to their short lateral branches. It appears that light penetration into the canopy is more affected by the lateral shoot length rather than tree volume or width (Jung and Choi, 2010).

Primarily the fruit position in the layers was very important in connection with light availability to influence fruit over colour. Even though light is a fundamental factor, it explains only 40% of the variation in fruit quality characteristics according to Campbell and Marini (1992). For example one among the other contributing factors could be the “year effect”, that is why no differences in the quality traits were found between the two training systems. The climatic conditions characterizing the season 2013 were not ideal to enhance fruit colour in the Trentino province. The percentage of fruit skin colour coverage resulted low as compared to other years compromising fruit quality. When these climatic conditions occur it is difficult to reach a good over colour coverage and this may explain the lack of differences between the two training systems.

Due to the fact, that the lower layers don't get enough amount of light and therefore produce a lower fruit quality, other instruments such as proper pruning methods must be adopted to increase light penetration especially in the lower canopy layers to allow enough light and a better fruit quality.

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7.7. Figures



Fig. 1. Camera positioned in the fruit position.



Fig. 2. Photo camera with fisheye.

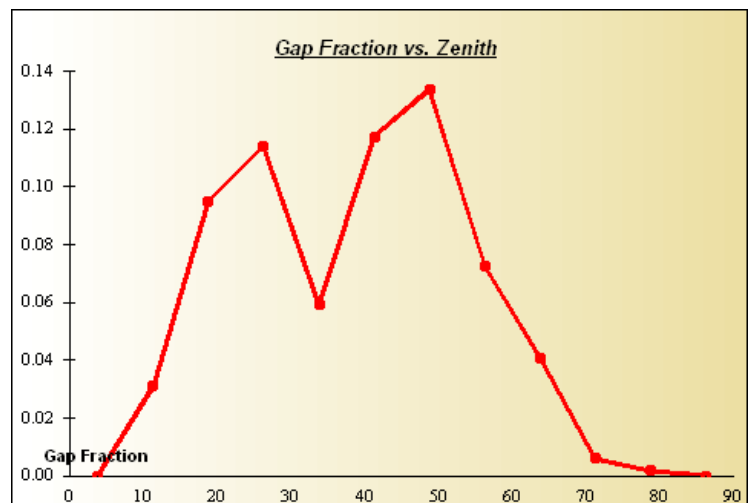
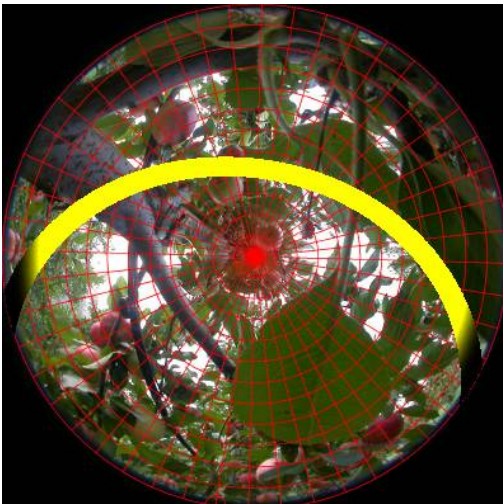


Fig. 3. Photo in the inner west lower position of the fruit in the canopy tree. 5.25% of canopy openness and 24.3 MJ m⁻² of total radiation at fruit level.

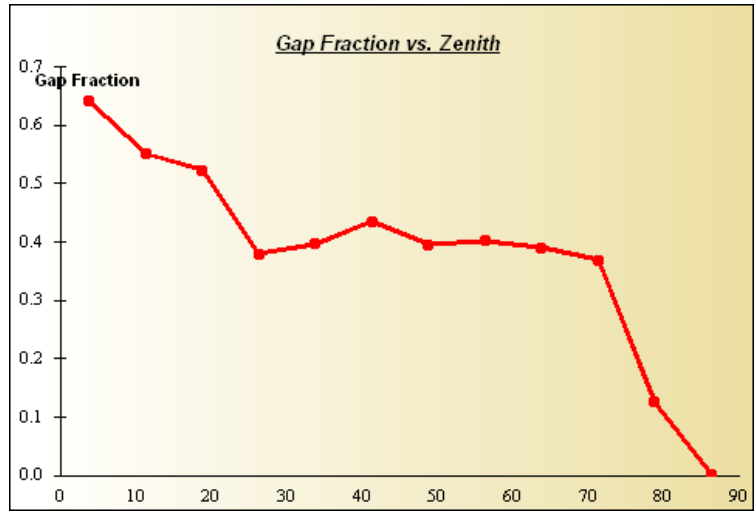
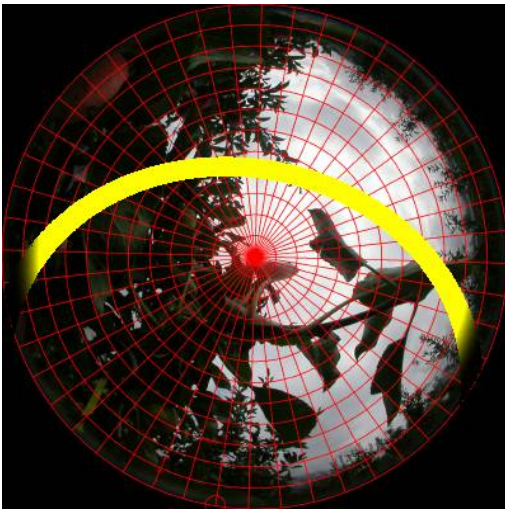


Fig. 4. Photo in the outer west upper position of the fruit in the canopy tree. 32.02% of canopy openness and 222.8 MJ m⁻² of total radiation at fruit level.



Fig. 5. a) SunScan-SS1 Delta-T Devices Ltd equipped with a sunshine sensor BF3 Delta-T Devices Ltd (left). b) Detail of the Sunshine sensor BF3 (right).



Fig. 6. a) Data collection using the ceptometer (left). b) Black tape used to allowed only 4-5-6 sensors for the readings (right).

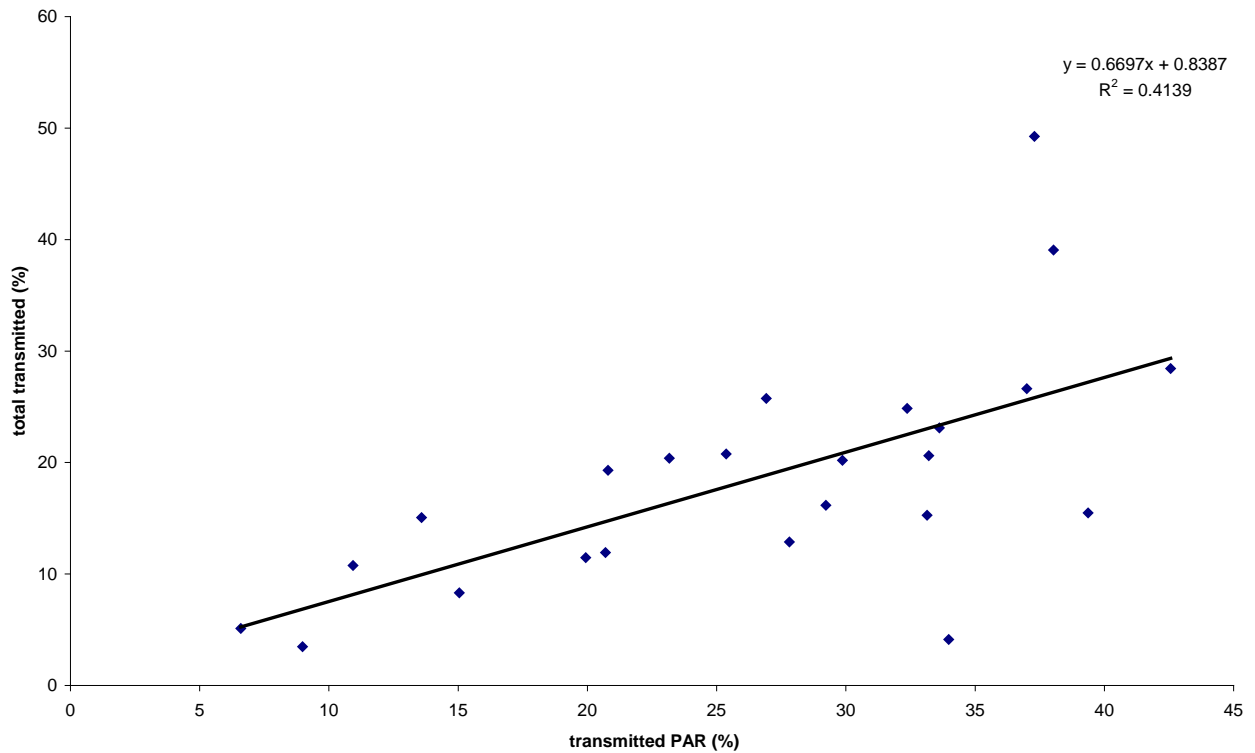


Fig. 7. Linear correlation between the percentage of transmitted PAR measured by ceptometer and sunshine sensor and % of total radiation calculated from hemispherical pictures.

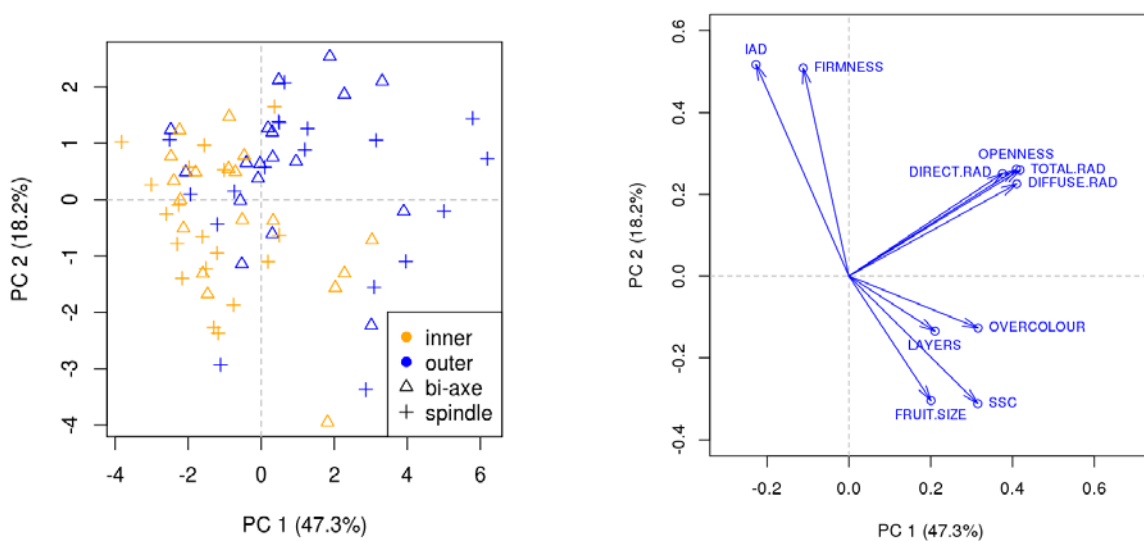


Fig. 8. a) In the panel the fruit light availability of both training systems in the inner and outer position of the canopy over the PCA 2D plot is shown (left). b) In the panel the projection of the quality parameters, tree layers and environment variables over the PCA 2D plot is shown (right).

7.8. Tables

Table 1. Biometrical data and Yield efficiency (not significant indicated as ns).

Thesis	Depth (cm)	Plant Volume (m ³)	Yield Efficiency (fruits/cm ²)
Slender Spindle	177	2.65	6.1
Bi-axis	130	2.03	6.1
<i>p-values</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

Table 2. Light radiation data detecting by camera with fisheye. In bold the significant *p-values*.

Environment traits	Comparison	Estimated effect	<i>p-values</i>
Canopy openness (%)	Bi-axis vs Slender Spindle	-0.5472	0.8351
	inner vs outer	7.5528	1.00E-06
	lower vs middle	2.4917	0.2461
	lower vs upper	5.7625	0.0016
Direct fruit radiation (MJ m ⁻²)	Bi-axis vs Slender Spindle	-0.8694	0.9810
	inner vs outer	63.2750	0.0003
	lower vs middle	13.1333	0.7348
	lower vs upper	47.5333	0.0320
Diffuse fruit radiation (MJ m ⁻²)	Bi-axis vs Slender Spindle	-2.9417	0.8799
	inner vs outer	49.6139	1.00E-06
	lower vs middle	10.8542	0.4950
	lower vs upper	26.8375	0.0320
Total fruit radiation (MJ m ⁻²)	Bi-axis vs Slender Spindle	-1.9083	0.9406
	inner vs outer	56.4583	4.20E-06
	lower vs middle	11.9875	0.5612
	lower vs upper	37.1792	0.0166

Table n.3. Quality traits data. In bold the significant *p-values*.

Quality traits	Comparison	Estimated effect	<i>p-values</i>
Over colour (%)	Bi-axis vs Slender Spindle	-9.2583	0.7677
	inner vs outer	11.7694	0.0981
	lower vs middle	1.1500	0.9406
	lower vs upper	17825	0.0320
Fruit size (Ø mm)	Bi-axis vs Slender Spindle	0.0083	0.9948
	inner vs outer	1.2139	0.4950
	lower vs middle	-0.1125	0.9810
	lower vs upper	1.8292	0.3447
I _{AD} (index)	Bi-axis vs Slender Spindle	-0.1161	0.3129
	inner vs outer	-0.0317	0.8351
	lower vs middle	0.0162	0.9406
	lower vs upper	-0.1596	0.1024
SSC (Bx°)	Bi-axis vs Slender Spindle	0.1222	0.8351
	inner vs outer	0.4000	0.0560
	lower vs middle	0.1292	0.7812
	lower vs upper	0.7417	0.0027
Firmness (kg/cm ²)	Bi-axis vs Slender Spindle	-0.0889	0.8351
	inner vs outer	0.1039	0.7990
	lower vs middle	-0.3125	0.3370
	lower vs upper	-0.2958	0.3447

8. General Conclusions

The effect of two training systems, the Slender Spindle and the Bi-axis, and two pruning methods, Standard and the Click, on fruit quality have been the main aims of this present study. The combined use of different softwares in particular the PlantToon® and the I_{AD} allowed to precisely describe the tree canopy structure and the exact position of each fruit as well the quality traits and ripening stage.

The fruit position, one of the factors, resulted to have a strong influence on fruit quality compared to the training systems per se. The fruits on the upper part of the canopy resulted to have higher quality traits compared to the other parts of the tree in both training systems. Even though the Bi-axis spreads its vigour in two axes giving the tree a flat shape, it resulted not very different compared to the Slender Spindle, which has a compact canopy. In spite of the differences in shape, these trees resulted to have similar fruit light availability in different parts of the canopy. In fact, the inner and outer fruit positions of both training systems didn't show any fruit quality differences probably due to the fact that their narrow tree shapes provide a good light distribution through the canopy tree. Another factor that aids in this homogeneity of light availability is also the fact that the study was conducted on Gala cultivar, known to have less vigour. On one hand as a result, it allows to maintain a compact tree shape even in the Slender Spindle training system. On the other hand, a closer look shows that Bi-axis shows a limited space distance between the 2 axis (60 cm), compared to the Slender spindle distance which is 1 meter. This is a positive factor in the Bi-axis because despite the less space it still gave similar results on fruit quality compared to the Slender Spindle.

Removal of selected structural limbs during dormant pruning in winter allow to increase light transmission through the entire canopy in summer, which provides an improvement on radiation conditions allowing an enhancement of production of premium quality fruit. Continued production of premium-quality fruit on mature orchards of Gala apple primarily depends on the maintenance of the correct light environment within the tree canopy. This helps to simplify the practical operations at harvest reducing at the same time the high costs that apple crop orchard require.

The wood age and time of flowering are also important factors, which influenced fruit quality and determined fruit variability within the canopy tree. Lateral complex branches produce fruits on different kinds of wood age which bloom at different times with a result of fruit quality variability. Fruit thinning is one option to maintain the quality but more often not sufficient to reduce fruit heterogeneity. The adaptation of new pruning strategies has shown to be essential in the intensive plantation to obtain homogeneous and high quality apples within the canopy. The Click method proved to be a powerful pruning technique to increase fruit quality in the orchard. The continuous

branch renovation proposed by this method allows producing fruits on young wood with similar age improving fruit colour, size and homogeneity within the canopy.

Crop load confirmed to be another important factor that influenced fruit quality. The Bi-axis has shown to be affected in the same way as that of the Slender Spindle system. A higher crop load did penalize fruit overcolour and fruit size particularly in Gala cultivar given that this cultivar is known to produce smaller size fruit naturally.

Fruit Texture showed to be a powerful tool to describe fruit quality giving a better evaluation comparing traditional methods such as fruit flesh firmness using the traditional penetrometer. No significant results were found both comparing the two training systems, fruit position and pruning methods probably because it was very difficult to find differences in texture using this sophisticated method on the same variety even though trained in a different way.

The different maturity stages reached by the fruits were mainly affected by the height from the ground in the canopy. Fruit ripeness was not always related to fruit position and linked with fruit over colour and size and this is one of the causes of fruit quality heterogeneity at harvest and in the market. This highlights the importance and need of an ideal training system or pruning strategy with an aim to provide sufficient and more homogenous light availability within the canopy to obtain a higher percentage of fruit quality. To produce good fruit quality is a very important goal not only for the grower but also for the whole fruit chain in the market.

The market requirements for highly quality apple fruits demands high percentage of skin colour, good size, and representative flavour and textural properties. Often very colourful fruits of innovative strains could be immature at harvest or by waiting for the red colour development; fruit could be over mature by the time they are harvested.

It will be sensible to reward growers for the fruit characteristics that create consumer demand such as crispness, juiciness, soluble solid and flavour. In fact it has been observed that the consumer buys with the eyes. Therefore if the consumer is guaranteed, a high quality product along with external eye catching colours the consumer will pay a slightly higher price for the higher quality fruit. This is certain, as consumers are more concerned about internal qualities than external appearances only.

In conclusion it is recommended for further study to understand other factors involved in fruit quality variability within the canopy tree, which have been beyond the scope of this thesis, to provide information to the grower about the application of new techniques to increase fruit quality.

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