

Alma Mater Studiorum - Università di Bologna

**DOTTORATO DI RICERCA IN  
SCIENZE E TECNOLOGIE AGRARIE, AMBIENTALI E ALIMENTARI**

Ciclo 34

**Settore Concorsuale:** 07/B2 - SCIENZE E TECNOLOGIE DEI SISTEMI ARBOREI E FORESTALI

**Settore Scientifico Disciplinare:** AGR/03 - ARBORICOLTURA GENERALE E COLTIVAZIONI ARBOREE

**EVALUATION OF A 2D MULTILEADER TRAINING SYSTEM FOR IMPROVING  
SUSTAINABILITY AND PRECISION ORCHARD MANAGEMENT APPLICATIONS  
IN ITALIAN APPLE ORCHARDS**

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**Esame finale anno 2022**

*This thesis is dedicated to Denise,  
thanks for believing in me even when I did not.*

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## ABSTRACT OF THE THESIS

In the last decades, climate change (CC) is affecting human habits and crop productions due to pollution and emissions of industrial activities. Agricultural sector is negatively influenced by CC and exploiting land surfaces and inputs for crop production, is responsible for the 13% of global greenhouse gasses emissions (GHGs).

Fruit growing sector is considered as a minor agricultural sector (9% and 5% of world's crops production and cultivated land surfaces respectively), but together with vegetables, it represents the main exported food worldwide. Precision Agriculture (PA) can be considered as one of the approaches of integrated farm management. In the last decades, it gave an indisputable improvement in agricultural production efficiency and sustainability. Precise crop load management (PCLM) is part of PA and it is being developed and improved to support sustainability and profitability of the fruit production industry. Fruit crop specialized management operations such as thinning, pruning and harvesting requires high skills level, and result difficult to be standardized and mechanized/automated through PA applications. Orchard systems based on planar two-dimensional canopies are known from long time. In the last decades they are raising interest because their ability to create thin and well exposed fruiting walls with a simple architecture that can support mechanization and automation. Lately, interest has been focused on multileader planar systems, because of their capacity to better follow the physiological plants bearing increasing also yields. The combination of innovative multileader planar training systems with the application of precision fruit growing (PFG) techniques dedicated to crop load management can be a valid solution to increase sustainability, yield, and profitability of fruit production.

The presented study aimed to evaluate the productive and physiological behaviour of a 2D apple training systems in the Italian environment both investigating the possibility to increase yield and PCLM resolution. Another objective was to find valuable thinning thresholds guaranteeing high yields and matching fruit market requirements for apples.

The thesis consists in three studies carried out in a Pink Lady®- Rosy Glow apple orchard trained as a planar multileader training system (double guyot). Fruiting leaders (uprights) dimension, crop load, fruit quality, flower and physiological (leaf gas exchanges and fruit growth rate) data were collected and analysed. The obtained results found that uprights present dependence among each other and as well as a mutual support during fruit development. However, individual upright fruit load and upright's fruit load distribution on the tree (~ plant crop load) seems to define both upright independence from the other, and single upright crop load effects on the final fruit quality production. Correlations between fruit load and harvest fruit size were found and thanks to that valuable thinning thresholds, based on different vegetative parameters, were obtained. Moreover, it comes out that an upright's fruit load random distribution present a widening of those thinning thresholds, keeping un-altered fruit quality. For this reason, uprights resulted a partially physiologically-dependent plant unit. Therefore, if considered and managed as independent, then no major problems on final fruit quality and production occurred. This partly confirmed the possibility to shift crop load management to single upright.

The finding of the presented studies together with the benefits coming from multileader planar training systems suggest a high potentiality of the 2D multileader training systems to increase apple production sustainability and profitability for Italian apple orchard, while easing the advent of automation in fruit production.



# CHAPTER 1

## Introduction & Aim of the Study

### Sustainability, Climate Change and Sustainable Agriculture

Sustainability is a complex topic that has been described with many definitions (Arena *et al.*, 2009; O'Brien *et al.*, 2015). In one of its original meanings, sustainability meant “to use only of those natural, renewable resources that people could continue to rely on their yields in the long term” (WOR, 2015; Dictionary, 2021) and was initially applied to silviculture (Von Carlowitz, 1713; WOR, 2015; Environment & Society Portal, 2022). Nevertheless the concept of keeping a balance between human needs and environmental limits can be tracked through the history until ancient time (Van Zon, 2002; Ponting, 2007). Probably, the most quoted and widely adopted definition of sustainability comes from the United Nations’ report titled “Our common future” (OCMR) (WCED, 1987; Keeble, 1988; Environment&SocietyPortal, 2022) where is highlighted, from the perspective of different sectors (economic, environmental, social, industrial, energetic), how the exponential development of the human society was not considering a fundamental aspect in relation to human well-being and perpetuation: the equity. This particularly from a timing point of view. In fact, coming back to the wide-assumed definition of sustainability, it is reported in the OCMR as “meeting the needs of the present without compromising the ability of future generations to meet their own needs”; this is the fundamental principle guiding sustainable development concept (the blueprint for attaining sustainability), defined as well in the OCMR (WCED, 1987).

Nowadays, it is commonly recognised that sustainability concept can be considered made up of “pillars” representing the main dimensions involved in the sustainable development process; these dimensions are intertwined and not separate, because each of them is at the same time part of the problem and of the solution in a dynamic and changing context, searching for the balance. Numbers of pillars/dimensions can vary according to the different authors’ description and the emphasis on which they point towards in their argument (Gibson *et al.*, 2001; Giddings *et al.*, 2002; Lozano, 2008; Waas *et al.*, 2011; UNESCO, 2022). Despite this, the “widest” and commonly accepted, view of sustainability is related to the triple bottom line concept (also known as 3Ps concept/model – People, Planet, Profit) in which sustainability is represented by three dimensions that are economy, environment and society. Those are connected between each other, with sustainability placed in the middle as a common goal (Figure 1.1).

As summarized by Waas *et al.* (2011)(revisiting Spangenberg, 2004) sustainability pillars can be resumed in:

- an economic growth supporting for long-term welfare creation to satisfy essential needs for jobs, income, food, energy, water, sanitation, social security, and consumption opportunities;
- an environmental protection to conserve and enhance the resource base and to keep within the Earth's environmental limits for a long term perspective;
- a social justice to achieve an equal distribution of welfare, equal access to natural resources and equal opportunities between people (gender, social groups, etc.).

To these pillars, Waas *et al.* (2011) added the important figure of the Institution, that have to achieve the task of: “changing and merging environment and economics in decision making and enforcing the common interest through greater public participation, locally and internationally”. In this way the sustainability includes the willingness, giving to the Institution a higher responsibility, to take actions to change toward a sustainable development.

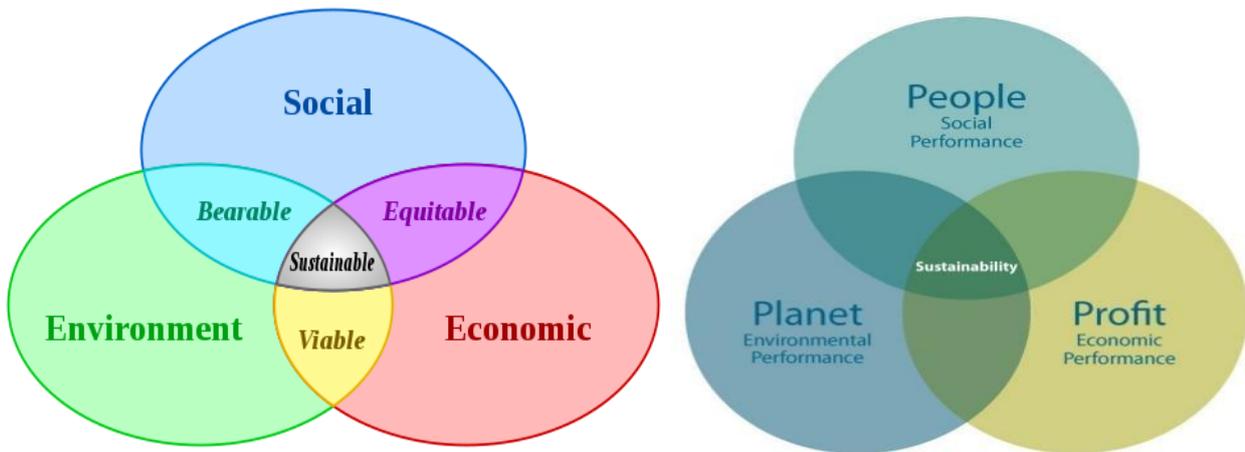


Figure 1.1 - On the left: The “triple bottom line” concept representation with sustainability placed in the middle of overlapping circle (i.e., pillars) areas - source: Adams, 2006. On the right: The same representation in relation to the so called “3Ps” model – People, Planet, Profit – source: Arslan *et al.*, 2017.

As clearly implied, environmental sustainability is a fundamental milestone to achieve sustainable development (Langhelle, 2000; Waas *et al.*, 2011). In the last decades, all world is facing climate change (CC) that is altering the normal environmental conditions in which humanity was used to live in the past centuries. Even if CC can be considered as part of the natural process occurring across different ages, the changes occurring during the last century resulted too rapid to be part of a natural Earth process, and mainly caused by a quick increase of green-house gasses (GHGs – e.g., CO<sub>2</sub>, CH<sub>4</sub> etc.) (Ripple *et al.*, 2019). This situation alters the normal atmospheric processes and energetic dynamics, inducing a mean temperature increase, those together conduce a shift of the typical climate in the different climatic regions (IPCC, 2019a, 2021). For examples, temperate climate regions tend to present more tropical climate characteristics. Others environmental problematic situation, related to CC, are ice melting, sea level increase, increase in the occurrence of extreme climatic events and temperatures (e.g., floods, storm/hurricane, droughts period etc.) (IPCC, 2014, 2019a, 2021). Scientific community stated that the rapid increase of GHGs in the last century, as well as the consequential effect on the environment (i.e., CC and related problems), is clearly related to anthropic activities (IPCC, 2014, 2019b, 2021; Ripple *et al.*, 2019).

Agricultural sector by itself, account for about 11-13% GHGs emissions (IPCC, 2014, 2019a) and results as the main water and land user, with about 70% of freshwater consumption (WWAP, 2012; United Nations, 2018) and 51% of ice-free land surface in the world (cropland-12%, pasture-37%, forestry-22% - IPCC, 2021). The fruit sector only accounts for 9% of world’s crops production and 5% of world’s cultivated land surfaces, but together with vegetables represents the main exported food type worldwide (20% of the world’s food export) (FAO, 2021, 2022). By this, it is clear how agricultural in general, as well as fruit growing sector, should increase its sustainability.

The concept of sustainable agriculture refers to a multifunctional system inclusive of all practices aiming to achieve all the goals described in the sustainable development process (Tilman *et al.*, 2002; Renting *et al.*, 2009; Robinson, 2009; Huang *et al.*, 2015). Integrated Farm Management (IFM) can be considered as the main and widest practice used to reach sustainable agricultural goals. IFM is defined as a “farming system where high-quality organic food, feed, fibre and renewable energy are produced by using resources such as soil, water, air and nature as well as regulating factors to farm sustainably and with as little polluting inputs as possible” (UNI 11233-2009 European standard). Many are the practices with which IFM can specifically act; some examples can be the integrated crop management (ICM – e.g., crop rotation, intercropping and many other agronomical approaches – Finch, 2002), the integrated pest management (IPM – e.g., minimize chemicals use, increasing biological control and agroecosystems bio-diversity – Boller *et al.*, 2004; Matthews, 2017) and the application of precision agriculture principle to improve system efficiency (Shannon *et al.*, 2018).

The concept of precision agriculture (PA) was firstly introduced around forty years ago and really matches with the aims of agricultural sustainability (Escolà *et al.*, 2021). In fact, in the last decades its applications gave an undiscussable support in reducing pollution and inputs while increasing

production efficiency in many crop species. The current official definition of PA comes from the International Society of Precision Agriculture (ISPA, 2019) and states: "Precision agriculture is a management strategy that gathers, processes and analyzes temporal, spatial and individual data and combines it with other information to support management decisions according to estimated variability for improved resource use efficiency, productivity, quality, profitability and sustainability of agricultural production" (Kerry *et al.*, 2021).

PA is based on a reiterative/cyclic process made up of 4 phases (Zude-Sasse *et al.*, 2016; Kerry *et al.*, 2021), that can be extracted from its definition:

1. Data collection and localisation: this phase is related to identify the spatial variability present in the field in order to take that into account in the successive decision-making process. This phase requires sensors to collect and locate (spatially and temporally) the data. Type, modality, and sensors utilized for data collection can follow different approaches, but they can be generally subdivided in remote and proximal sensing techniques, in relation to operative distance from the objective and which sensor(s) can operate the data collection (Zude-Sasse *et al.*, 2021). Regarding spatial and temporal location, these are generally done exploiting global navigation satellite system (GNSS) coordinates and date-time recording. Data collection should be as automated as possible, since the amount of data cannot be acquired and processed manually in reasonable time frames for the following applications (Aggelopoulou *et al.*, 2013; Zude-Sasse *et al.*, 2016). For this reason,, a lot of research has been done in data collection automation, working on sensors development and application, pursued in lasts years on wireless sensors network (WSN), internet of things (IoT) configurations, remote sensing, and computer vision solutions.
2. Data analysis and prescription definition: after the collection, data need to be analysed in relation to their spatial variability in order to obtain variability maps representing the actual status of the field in relation to one or more variable. This can be done by the use of geographic information systems (GISs), that are software able to store, manage, analyse and visualize geo-referenced data (Chang, 2016). After representing the field variability then the need shifts to its management. Usually this is achieved by modifying the prescription of a management input according to the crop spatial needs detected in field; "prescription maps" represent suitable input dose/level, modified according to the in-field spatial needs according to the analysed crop.
3. Management of the variability through differentiated input application: prescription maps are then utilized on machines or implements able to distribute inputs in the field with variable rate application (VRA); these machines are able to modify input applications in real-time actuating a precision management according to the present field variability.
4. Evaluation of the management decision: evaluating the suitability of the variable inputs in relation to the expected result. This phase is fundamental to adjust prescription maps creation in order to match precise application with desired results. In relation to this phase, it can be added that, for certain management inputs, the reiteration of phase 1 after phase 4 helps in the evaluation process, as well as in defining field temporal variability for the investigated variables.

PA process can be applied to many different agricultural management activities such as irrigation (Fernández, 2017; González *et al.*, 2018; Cohen *et al.*, 2021), pesticides application (Gil *et al.*, 2007; Campos *et al.*, 2020), fertilizers distribution or seeding operations (Reckleben *et al.*, 2017), crop load management and thinning (Godes *et al.*, 2013; Piani *et al.*, 2021; Li *et al.*, 2022) , and many others (Shannon *et al.*, 2018).

Nowadays, PA is strictly connected to automation and robotization of agricultural processes, this to guarantee a valuable, repeatable and accurate management, while facing problems such as wide field surfaces and lack of labour for agricultural sector (Zhang, 2018). Considering this fact and the cyclic processes exposed, a lot of effort has been done in the last 10-20 years, to compress phase 1 to 3 in a single one, to achieve a real-time VRA directly operated by machines and robot; for the moment, phase 4 remains tied to human expertise, but tending to future, an integration of this phase into the machines/robot is envisioned. Examples of real-time VRA can be seen in the intelligent sprayers that during a field application are able to adapt the doses of pesticides to crop/canopy and weather (i.e. wind speed) condition on the basis of detected field variability (Doruchowski *et al.*, 2009; Zande *et al.*, 2010; Doruchowski *et al.*, 2011). The advances in computer sciences, machine vision and hardware computing power, will bring commercially high precise autonomous robot with different functions as chemicals

spraying (Underwood *et al.*, 2015; Seol *et al.*, 2021; ACFR-RIPPA, 2022), weed control (Conesa-Muñoz *et al.*, 2015; Raja *et al.*, 2020a,b), automatic seeding and fertilization (Bhimanpallewar *et al.*, 2020), scouting/data collection (Ünal *et al.*, 2020) and many other activities (Grimstad *et al.*, 2017; Escolà *et al.*, 2021; Kerry *et al.*, 2021).

## Precision Fruit Growing

PA is a set of procedures fully applicable to fruit/woody crops, and PA solutions for this agricultural sector are generally defined with the broad term “precision horticulture” solutions, including also vegetables crops. As suggested by Corelli Grappadelli *et al.* (2019) “precision fruit growing” (PFG) could be a more suitable term considering the specific issue related to fruit crops (e.g. tree architecture), that differs profoundly if compared to a broad-acre vegetable crop, such as tomato. Some authors refer to PFG also as precision orchards management (POM) techniques (Qian *et al.*, 2015; Breen *et al.*, 2021; Mengoli *et al.*, 2020). Considering the intrinsic diversity between tree crops and other horticultural products, fruit production also presents some specific operations such as tree training, pruning, thinning, and harvesting, that generally require personnel with high skills (i.e., knowledge, experience, adaptability). Because of that, in most cases all these specific operations are still carried out manually, making fruit production one of the most labour-intensive crops. The difficulties that make PFG hard to be applied through mechanization/automation are mainly related to the complex 3D tree and orchard structure. The tree architecture in fact, evolve with time in a multi-year dependent management perspective that needs to take into account decision made in the past to organize the future field operations (Zhang, 2018; Zhang *et al.*, 2019).

At global level this gap is trying to be achieved through an increase of research, studies and development efforts on automation and mechanization of many productive operations in tree fruit crops; as previously reported, in the last years this automation point towards robot based platform to autonomously carrying out operations for substituting or supporting manual labour (Karkee *et al.*, 2021). Despite the difficulties present in tree crops, many PFG applications are currently utilized in field. Operations such as pruning, thinning, and selective harvest are probably the most investigated practices in automation of valuable crops such as fruit production. Together account for most of the required labour, being the most expensive management operation in fruit production (dedicated to fresh consumption); and these are probably the main motivations pushing research to investigate on these topics (Gallardo and Brady, 2015; He and Schupp, 2018). Examples of PFG solutions currently studied or utilized are:

- Precise orchard plantation (exploiting GNSS) that increases surface efficiency (Karkee *et al.*, 2021).
- Precise irrigation solution that varies widely from a “simple” irrigation system and water use efficiency (WUE) improvement through drip /sub-surface drip irrigation (Martínez *et al.*, 2014; Wang *et al.*, 2021), to an IoT automation of timing and watering volume thanks to decision support systems (DSSs) estimating the crops evapotranspiration loss based on real time orchard conditions (Manfrini *et al.*, 2018; Irriframe, 2022), up to variable rate irrigation (VRI or precision/cite-specific irrigation) application that points to single plants water demand restitution (Fernández *et al.*, 2010; Gutiérrez *et al.*, 2017; Peters, 2018; Escolà *et al.*, 2021). The main aim of these precision irrigation solutions is to improve water use efficiency and lowering inputs in fruit production.
- Precise chemical applications with inputs, waste, and pollution reduction. These solutions are represented by chemicals VRA dose adjustment (Llorens *et al.*, 2010; Karkee *et al.*, 2013; Gil *et al.*, 2014; Miranda-Fuentes *et al.*, 2016) with experimental smart sprayer solution (Underwood *et al.*, 2015; Seol *et al.*, 2021) or already on the market (FEDE, 2022; Waatic, 2022).
- Regarding pruning, mechanization is applied since long time ago, thanks to the development of mechanical pruners (i.e., generally cutting bars or hedgers) that in some cases as viticulture, brought to a meaningful increase in operation efficiency (Gatti *et al.*, 2011), also if a manual adjustment remains often a need. In vine crops, in recent years automated pruners, were presented commercially showing to able to carry out the complete pruning process (including those high-level skills cited above), with the drawbacks of operational speed (Botterill *et al.*, 2017; He *et al.*, 2018; Karkee *et al.*, 2021). In other fruit crops that require higher precision in the pruning

operations (i.e., pome and stone fruit), the mechanical support gives only assistance in increasing the pruners efficiency; automation is stalling, to this mechanized plus manual adjustment, with a lot of work done in defining objective rules for pruning (Karkee *et al.*, 2014; Karkee *et al.*, 2021), and a lot of work going on in relation to machine tree architecture understanding and robot prototype testing (Karkee *et al.*, 2014; Karkee *et al.*, 2015; Amatya *et al.*, 2016; Tabb *et al.*, 2017; He *et al.*, 2018; Zhang *et al.*, 2018; Majeed *et al.*, 2020; Karkee *et al.*, 2021).

- In fresh fruit productions, thinning is a key operation to ensure product quality; for this management operation many automation studies have been published, mainly focusing on flower thinning through chemical application (Kon *et al.*, 2018) or mechanical thinning (Glenn *et al.*, 1994; Solomakhin *et al.*, 2010; FruitTec, 2022). These techniques are widely adopted in the field. Lately, researchers are studying higher level solution matching vision systems with thinning solutions (Lyons *et al.*, 2019; Escolà *et al.*, 2021), so to move towards automation and precision application of thinning. Currently a consistent manual adjustment is still needed as for pruning operation, but some types of PFG support can be given to workers thanks to tools that precisely suggest right fruit load based on vegetative development of the fruiting element. An example is the equidisk/MAFCOT wheel (Kon *et al.*, 2012; Kon and Schupp, 2013), a tool for workers that suggest the proper crop load (fruit number) on the basis of the branch diameter on which thinning is going to be applied.
- Mechanical harvest systems investigation started around the '60s (Coppock, 1961; Lamouria *et al.*, 1961) while selective harvesting automation can be found in the literature almost 30 years later (Grand, 1987; Harrell and Levi, 1988). Interest on this topic is still on going and research activities are mainly focused on matching growers requirements in term of harvesting efficiency as well as other technical problems (Karkee, Silwal and Davidson, 2018; Karkee, Zhang and Silwal, 2021). Fruit harvesters have been present commercially since long time, but they are generally based on a “shake and catch” principle. For this reason, they are exploited only in industry transformation (i.e., cider apple, citrus for fruit juice, etc.) and dried fruit crops (e.g. nuts) because of the damages and the high percentage of waste they can produce during the fruit harvest directed to the fresh market. Lately few commercial prototype/solutions for selective automated harvesting became available (Karkee, Silwal and Davidson, 2018; Karkee, Zhang and Silwal, 2021); these exploit robotic arms/hands solutions, air-assisted sucking systems and even drones (FFRobotics, 2022; RoboticPlus, 2022; TevelAerobotics, 2022).
- An interesting application, indirectly related to the management operations, is yield mapping. For fresh consumption fruit species, precise yield mapping result useful in a-posteriori evaluation of management decision undertaken during the season; this technique coupled with field data (up to flowering stage) can be exploited to estimate production, allowing to schedule labour and hiring, and giving feedbacks on production quality and quantity to the postharvest sector (Manfrini *et al.*, 2020). It can be useful also for activities that relay on knowing the crop load (e.g., thinning operation) both to schedule labour and defining different management decision. Yield mapping in fruit crops remain an open field research with few commercial services (e.g., GreenAtlas, 2022) and lot of research published, lately focusing on computer vision solutions (Stajanko *et al.*, 2004; Linker *et al.*, 2012; Godes *et al.*, 2013; Rahnemoonfar and Sheppard, 2017; Horton *et al.*, 2017; Krikeb *et al.*, 2017; Kamilaris and Prenafeta-Boldú, 2018; Bortolotti *et al.*, 2021; Escolà *et al.*, 2021; Kerry and Escolà, 2021; Piani *et al.*, 2021; Li *et al.*, 2022).

This overview clearly explain how PFG needs a multidisciplinary approach: certain and objective rules are needed to manage orchards variability in a precise way (Breen *et al.*, 2021); these rules depend on crops biological / physiological / phenological base, and connected to environmental conditions. Horticulturalist and plant physiologist skills are needed to define the best approaches to manage the field variability in PFG, thus connecting the fruit and the automation industry and allowing them to speak the same language and interact to each other. This, in the near future, will lead to machines able to detect those bio-physiological parameters on which horticultural science will rely upon to define operational rules and provide guidance to the growers. This overview on PFG application is important to point out that a lot has been done, but clearly more can be achieved, particularly with automation and autonomous systems exploitation in order to improve fruit production sustainability. As reported by Zhang (2018) one of the main issue of PFG application and automation is the tree complexity in relation

to its 3D architecture. By this, simple, narrow, accessible and productive orchard systems had been developed in recent years, in order to ease the application and automation of PFG (Long *et al.*, 2015; Dorigoni and Micheli, 2018; Tustin *et al.*, 2018; Whiting, 2018; Karkee *et al.*, 2021; Musacchi *et al.*, 2021).

## Precise Crop Load Management in Apple Production

Focusing on a specific fruit growing requirement, crop load management can be considered one of the most important for apple (*Malus domestica* Borkh.) production. Crop load management is expansive and time consuming, but necessary to ensure a high quality yield (Schupp *et al.*, 2008). Fruit growth and quality depend on many different factors (e.g., genetic, climatic, tree age, water and nutrient supply, etc.) including crop load (Dennis, 2003; Zhang CaiXi and Chen Du, 2018). Generally, most fruit species often set more fruit than necessary if growing conditions are optimal (Westwood, 1993; Robinson, 2003; Robinson *et al.*, 2013b; Zhang, 2018). Excessive crop load levels conduce to smaller and lower-quality fruits (Wunsche and Ferguson, 2010; Kon and Schupp, 2018). Moreover, it affects also yields, physiological response as the tree bearing capacity, return bloom in addition to fruit storage diseases (Wunsche and Ferguson, 2010; Kon and Schupp, 2018). Indeed, a low crop load level can be an issue as well, because generally lead to low yields and low quality fruits, too large for the markets and with a reduced post-harvest life (Robinson *et al.*, 2013b). Regarding yields, excessive crop can conduce to the danger situation of alternate bearing, in which a season of heavy crop load is followed by a low crop load one; this situation is generally hard to be solved, resulting in a difficult management and in a high income variability during the orchard life. Thus, crop load adjustments are needed to achieve a high and remunerative production (i.e., matching market quality requirement).

Focusing more on apple production, many studies on crop load effects were published, reporting its effect on harvest fruit size (that is an inverse relation), and on many other quality proprieties such as color development, firmness, and soluble solids concentration. Light crop load generally increase these cited parameter, favouring fruit quality, while for ripening low crop load levels can conduce to an advanced maturity that is not suitable for the post-harvest life (Dennis, 2003; Wunsche and Ferguson, 2010; Charles *et al.*, 2018; Kon and Schupp, 2018). Considering that the main market requirements for apple production are fruit size, appearance and absence of defect, it is clear the importance of crop load management practice for apple cultivars.

PFG is trying to answer to this request through a crop load management as precise as possible, this to direct the production to match market requirement and maximize the profits. Thus, the objective is to set the most suitable fruit load for each plant/productive element according to its fruiting potential. Fruiting potential can be defined as the amount of fruit matter that a plant/productive structure is able to support until the harvest in relation to its available resources. Indeed that depends on its seasonal capacity of carbon fixation (more information in Ferree and Warrington, 2003; Taiz *et al.*, 2015). Fruit growers can use mainly two management operations for setting the tree fruit load: pruning and thinning.

The pruning technique aims to remove wood to adjust tree architecture, improving light distribution within the canopy and renewing productive tree structures while eliminating unproductive ones (e.g., suckers) (Forshey *et al.*, 1992; Ferree and Schupp, 2003; Kon and Schupp, 2018). Winter Pruning is the first crop load management operation during the productive season. It consists of the removal of plant structures as shoots and branches hence reducing floral buds' number and consequentially fruit potential production. Thus, important attention should be paid during pruning operations to leave the right number of floral buds to guarantee enough fruitlets for the following season but avoiding an excessive crop load. In relation to precise crop load management, the artificial spur/bud extinction (ASE) (Lauri *et al.*, 2004; Tustin *et al.*, 2012), can be considered a pruning operation. ASE consists in a manual floral buds (or spur) removal present on dormant plants, to set a maximum seasonal fruiting potential (i.e., floral buds load). ASE is a thinning method applied preventively on buds, and as manual fruit thinning, it makes possible to choose which buds to leave (bigger and right positioned) inside the canopy, exploiting particular species/cvs fruiting behaviours (e.g., prefer apical shoot floral buds in some apple cvs -Fuji,Gala).

Before thinning, the second main crop load management operation, is possible to cite also artificial pollination, that can be considered a precise crop load management operation as well because its possibilities in driving fruit set particularly for those non-self-pollinating plants (or with low pollination

rates) (Zhang CaiXi and Chen Du, 2018). This technique is based on pollen distribution (generally applied with standard sprayers or air-assisted distributors) that aim to increase the pollination and fruit set rate. This technique shows promise for managing the crop load of apple orchards (Zhang CaiXi and Chen Du, 2018), and more advanced and precise techniques, for precisely pollinate plants are under investigation and development (Ohi *et al.*, 2018; Strader *et al.*, 2019).

The second, main management operation establishing the tree crop load is thinning. This can be applied on flowers or fruits. Regarding flower thinning, also if not widely adopted because very labor-expansive, hand flower thinning is used in some regions (e.g., Washington state - USA), for some high valuable cultivars. Despite this, for apple crop flower thinning is generally adopted through chemicals applications that eliminate flowers or make them unable to be pollinated/proceed to the fruit set; a large number of chemicals products were described in literature, exploiting different active principle and methods of actions (Kon and Schupp, 2018). Chemicals fruit thinners are also exploited, even if their efficiency is significantly variable in relation to plant status and weather condition during and after the treatment (Musacchi and Greene, 2017; Kon and Schupp, 2018). Mechanical thinning is generally utilized for flower thinning operations and exploits the principle of mechanically damage /detach flowers with different methods (e.g., rotative plastic whip, pneumatic air-blade - FruitTec, 2022a ). For fruit thinning, mechanical solutions are currently present (e.g., Eclairvale, 2022) but probably less utilized than for flowers. As can be understood, both mechanical and chemical thinning method improve operations efficiency also if resulting not highly precise and selective. Chemical and/or mechanical thinning are generally followed by manual fruit thinning, that still the most used techniques, to precisely adjust the ending crop load .

Nowadays, crop load management is shifting to more objective rules and protocols on which to base pruning/ thinning operations. An example of these objective rules are guidelines for ASE application. ASE was described by Lauri *et al.* (2004), as an effective mean to increase fruit size and mitigate biennial bearing in the centrifugal training system (Lauri, 2008; Lauri *et al.*, 2011); this was later confirmed also by other authors (Tustin *et al.*, 2012; van Hooijdonk *et al.*, 2014; Breen *et al.*, 2015, 2016b; Tabing *et al.*, 2016). In these studies, the optimal crop load relies upon the carbohydrate's availability for the fruiting element (i.e., estimating it through branch cross-sectional area -BCA-). This was possible by expressing crop load levels as fruit density in relation to a vegetative parameter units (as suggested by Lombard *et al.*, 1988). This allowed to express similar crop load levels also when different tree vegetative development was present. A more precise and physiologically based approach brought to an increase in yield as well as quality in apple production (Lauri *et al.*, 2004, Tustin *et al.*, 2012). By this achievement, a device to support workers during ASE or fruit thinning was developed and it started to be utilized across the world (Kon *et al.*, 2012; Kon and Schupp, 2013). Other studies relied on the utilization of fruit density, as crop load expression, and in last decade a lot of studies has been developed in investigating suitable crop load level for different apple cultivars in different environment and training systems (Elfving and Schechter, 1993; McArtney *et al.*, 1996; Wright *et al.*, 2006; Embree *et al.*, 2007; Robinson, 2008b; Tustin *et al.*, 2012; Robinson *et al.*, 2013a; Breen *et al.*, 2015, 2016a; Castro *et al.*, 2015; Serra *et al.*, 2016; Anthony *et al.*, 2019).

An advance in mechanical flower thinning was the adoption of vision systems able to real-time estimate flower density on the plant (Hočevár *et al.*, 2014) so to adapt the thinning intensity (i.e., operative rotating speed) according to the actual tree flower load (FruitTec, 2022b). This solution is based on a strong correlation between flower pixel density extractable from an image and the real flower density present on a plant (Piani *et al.*, 2021). This information can be exploited also in creating flower intensity variability maps for non-real time operation (such as flower/yield mapping). Currently more advanced systems for flower detection and density estimation are under development (Lyons *et al.*, 2015; Wouters *et al.*, 2015; Underwood *et al.*, 2016; Lyons and Heinemann, 2019; Li *et al.*, 2022).

Chemical thinning, both for flowers and fruit, also is increasing in precision by improving its efficiency thanks to timing application taking in to account weather forecast and/or plants status (Robinson *et al.*, 2013b) or by utilizing models able to maximize efficacy by estimating key biological process affecting the thinning results (Kon and Schupp, 2018; Allen *et al.*, 2021). Intelligent sprayers are envisioned as well for chemical thinners applications, to increase both precision and efficiency, with chemicals reduction up to 90% (Kang *et al.*, 2014).

As a more complete example, Robinson *et al.* (2014) presented preliminary results on a protocol for “precision crop load” management tested on 20 commercial apple orchards (spindle tree shapes) in

the US. The proposed method included both pruning and thinning in the process, with a lot of effort spent in increasing chemical thinning efficiency (so to reduce manual work needs). The described protocol is based on a step wise methodology where to control pre and post crop load management operations, in order to correctly adjust the fruit load in the successive steps. Information on how to calibrate the protocol are undertaken on few (e.g., 5) representative plants, then generally applied after each step on to the whole orchards. The protocol starts by defining a target yield and quality (i.e., fruit weight) by which to calculate a target number of fruit per tree so to obtain the desired yield. A defined fruit per tree number is firstly applied by a precise pruning (i.e. ASE technique) and accounting fruit set issues (i.e. late frost, scarce pollination) (Robinson *et al.*, 2013b, 2014; Francescatto *et al.*, 2020). The second step is addressing a precision chemical thinning (Robinson *et al.*, 2013b) that consist in 4-5 time chemical thinner spraying applications, in a short windows of time. That aims to bring crop load as close as possible to the target one. Spraying and timing applications rely on a carbon balance model as a predictive tool for the thinning efficacy (Lakso, Robinson and Greene, 2006; Robinson and Lakso, 2011; Greene *et al.*, 2013). After this chemical thinning phase, fruit load will be precisely manually adjusted to the target fruit number per tree. The precision crop load management protocol proposed by Robinson *et al.* (2014), is a clear example of PFG solution integrating different operations. Although not spatially precise, without considering the single tree variability within the orchard, it implements the precision in time of each management operation to maximize their effectivity and to achieve the production goal. A further goal for a possible future improvement could be to include tree variability in the protocol by evaluating single plant resource availability (i.e., vegetative development as leaf area or trunk cross-sectional area -TCA-).

Despite all the already described solutions for improving precision crop load management it is clear the main issue in adopting them is connected to the cost increases due to their implementation timing (e.g., manual fruit thinning, ASE, multiple step chemical application, control protocols, etc.). This explains also why physiologically based crop load management solutions are not widely applied yet. At the same time, Robinson *et al.*, 2013 described that this effort is justified by increases in revenue of +12-27K€ ha<sup>-1</sup>. Therefore, automation of this management operation is highly expected.

Energy, economic and environmental sustainability are crucial for the success of any agricultural production system in the future. Reduction of farming inputs (including labour), optimization of crop yield and quality, and protection of the environment will all become more important (Karkee *et al.*, 2021). The goal of performing precision tree fruit production is to implement an effective and sustainable management practice based on observing, measuring and responding to inter- and intra-orchard variability so to gain the highest possible yield and economic returns (i.e., produce quality). Automation and robotics have played and continue to play an important role in achieving these often competing goals in farming (Zhang, 2018; Karkee *et al.*, 2021).

At the present days automation results difficult for many dedicated tree crops management operations, such as crop load management ones, due to complexity of tree crops and due to the difficulties in obtaining information needed for performing it fast and precise way. For precise crop load management some of these information are bud/flower/fruit number, their 3D position on the plant as well a plant vegetative parameter like leaf area, shoot length and TCA/BCA. As cited above, to face problems related to tree and orchard complexity, new automated-adapted (sometimes called “robot-ready” - He and Schupp, 2018) orchard systems have been developed and are increasing their adoption worldwide, this to favour also PFG application and automation , so to be able to improve more, fruit production sustainability.

## **2D Orchard Systems for Precision Fruit Growing**

Fruit production system is a specific combination of orchard layout and management that is meant to improve trees production efficiency. The system include management variables such as tree spacing and layout, rootstock-scion interaction, tree-training method and mature canopy shape as well as pruning method and plant support system (Ferree and Warrington, 2003). With fruit orchard systems and tree training, growers want to apply a strategic manipulation of fruiting and vegetative habit with the goal of increasing production quantities and quality in a manner that is sustainable and profitable (Robinson, 2003; Musacchi and Greene, 2017;Whiting, 2018).

The concept of “training” fruit trees, as adapting them to grower necessities, come from the ancient, at the beginning of plant’s domestication. Grafting, for example, is a “training” technique that aim to exploit characteristics of different plants, to meet grower necessities. Proof of this technique are cited in different version of the Bible, and it seems to be tracked back to 2000 B.C. (Meng *et al.*, 2012), with certain proof of its utilization around 500 B.C. (Mudge *et al.*, 2009). Nowadays, the tree “training”, refers more to the creation of a particular tree architecture that is suitable for fruit growers’ management needs. Without any tree manipulation intervention, most of the orchard trees would grow to great height and girth, forming a globular/triangular canopy structure not suitable for an efficient orchard management. A meaningful and extreme example is the sweet cherry (*Prunus avium*) tree grown in a wild environment being able to reach up to 40m of height; other fruit species can reach lower heights (e.g., *Pyrus communis* -20m; *Malus domestica* -12m; *Prunus persicae*, *Prunus domestica*, *Prunus armeniaca* -8m) in their natural environment, but still not comparable with modern orchards maximum desirable height (i.e., 2.5-4m) (Lang and Whiting, 2021). Iteratively, throughout the domestication of tree fruit crops for large-scale production, orchardists and pomologists, in both empirical and theoretical ways, have learned and studied horticultural management strategies to improve both the quantity and quality of fruit produced, while controlling and manipulating tree architecture (Whiting, 2018). In the past, tree fruit orchards (e.g., apple, citrus, cherry, peach, pear) have been low density, based on vigorous or seedling rootstocks, and trained to open-center or globular type architectures. The unwieldy nature of these complex canopies does not lend them to precise management of growth or fruiting, and the processes of harvest and pruning were especially labor intensive (Whiting, 2018).

Traditionally, one the most utilized and effective strategy for reducing tree height and vigour has been to subdivide and diffuse tree vigour between multiple main leaders, different in number and position on the basis of the training system considered (e.g., open vase training system is the first examples with many others like candelabro, palmette, etc. – Musacchi and Greene, 2017). The commercial introduction of dwarfing, precocious rootstocks for many fruit species (e.g., apples and pears from 1960s, cherries from 1990) provided a more powerful tool for growers and facilitated the advent of high-density orchards comprised of smaller tree canopies that are managed more easily with increased precision and efficiency (Zhang, 2018; Lang and Whiting, 2021). As example, in the last years, modern apple and pear orchards tree densities range from 1000 to 6000 trees ha<sup>-1</sup>, with some systems using densities up to 10,000 trees ha<sup>-1</sup>. This increase in tree density has been made possible by the development of dwarfing rootstocks mainly (Ferree and Warrington, 2003; Robinson, 2007; Musacchi *et al.*, 2021). Grafting scions onto peculiar rootstocks is utilized not only for controlling tree size and vigour, but also for improving precocity, improving fruit quality, and improving resistance to pests and environmental conditions (Rom and Carlson, 1987; Gao *et al.*, 1992; Hirst and Ferree, 1995; Fallahi *et al.*, 2002; Amiri *et al.*, 2013; Musacchi *et al.*, 2021).

Mature orchards productivity is very closely related to total light interception despite the plating density or training system considered. This has been repeatedly demonstrated during the years, for different fruit species and with several studies focused on apple (Cain, 1972; Jackson and Palmer, 1972; Palmer and Jackson, 1977; Jackson, 1980, 1985; Palmer, 1981; Barritt, 1989, 1991; Palmer, 1989; Robinson and Lakso, 1991; Robinson and Stiles, 1991; Robinson *et al.*, 1991, 1993; Robinson, 1992a; Wunsche *et al.*, 1996; Palmer and Adams, 1997; Wunsche and Lakso, 2000; Corelli-Grappadelli and Lakso, 2007; Tustin and van Hooijdonk, 2016; Tustin *et al.*, 2001, 2018, 2022).

Early studies results on apple orchard productivity and fruit quality already concluded that large trees were unsuitable for efficient and remunerative fruit production due to poor light interception and distribution within the canopy (Heinicke, 1963; Looney, 1968). After these preliminar studies, research pointed out that in the first years of orchards establishment, light interception is poor due to the uncomplete canopies development (Jackson, 1970) and the solution found was to increase tree densities to maximise early stages and lifetime orchard light interception and, consequentially, the yields (Jackson, 1970; Palmer and Jackson, 1974; Corelli and Sansavini, 1989). This proposed solution is one of the main motivations that drove an increase in planting densities, in the modern orchard systems.

Considering light distribution within the tree, shaded areas of the canopy generally produce smaller fruit size, poorer fruit color, lower return bloom and weak fruiting spurs (Jackson, 1970; Jackson *et al.*, 1977; Robinson *et al.*, 1983; Barritt *et al.*, 1987; Lakso *et al.*, 1989; Palmer, 1989; Warrington *et al.*, 1996; Wunsche *et al.*, 1996; Robinson, 2003; Zhang, 2018; Whiting, 2018). Thick canopies do not receive enough light in the inner parts for an adequate fruit production and multiyear tree management

(Heinicke, 1963; Looney, 1968; Verheij and Verwer, 1973) leading to the problems just cited above. Voluminous tree canopies present also a lower general CO<sub>2</sub> assimilation efficiency (Forshey and McKee, 1970) due to the canopy self-shading that leads to many inefficient leaves not supporting CO<sub>2</sub> assimilation as the well illuminated ones, present in the outer part of the canopy. Whiting (2018) concluded that light interception and light distribution are two key factors in the production of quality fruits. Sometimes they compete each other according to tree architecture and leaf area development due to self-shading inside the canopy that can conduce to a not optimum CO<sub>2</sub> assimilations and tend to decline if LAI increase after some optimal levels dependant on training system and its related self-shading level.

These needs (i.e., increasing light interception while reducing plant self-shading and having reduced tree vigour and canopy dimension) bring to the concept of developing “hedgerows” shaped training systems (also called planar systems). These systems consist in a canopy architecture mainly developed in two dimensions (2D), that are generally height and width (along the orchard’s rows), so to reduce canopy thickness (perpendicular to the orchard’s rows). This particular canopy shape promotes better light interception and light distribution thanks to the lower self-shading of the tree, improving also fruit quality and yields (Jackson and Sharples, 1971; Jackson, 1989; Saure, 1990; Warrington *et al.*, 1996; Steyn *et al.*, 2002; Ferree and Warrington, 2003; Robinson, 2003; Corelli-Grappadelli and Lakso, 2007; Miller *et al.*, 2015; Dorigoni and Micheli, 2018; Tustin *et al.*, 2018). Tree shapes aimed to restrict canopy development to two dimensions are not an agronomic innovation; they were used from centuries (e.g., “espalliers” and “candelabro” shaped trees from 16<sup>th</sup> century with examples in Italy and France), mainly with gardening purpose, making fruiting walls along fences and walls delimiting gardens borders or areas (Hugard, 1981; Ferree and Warrington, 2003; Musacchi *et al.*, 2021).

Taking apple as example crop, two-dimensional orchard training systems are part of the economic evolution of fruit production occurred over the past 50-70 years (Gallardo and Brady, 2015; Dallabetta *et al.*, 2021). The first commercial apple orchards with planar canopies, called the “palmette” training system, were developed in the mid-1950s, by Italian fruit growers. The main goal of this tree shape was to improve orchard labour efficiency by the use of platforms in management operations (e.g., thinning pruning, harvest) compared with the traditional vase-shaped fruit trees typical of that time (Robinson, 2003). This system led to significant technical innovations particularly for pruning operations, also favouring the adoption of higher planting densities, consequentially increasing the yields (Sansavini, 1983). Palmette training system were quite adopted in Italy and France up to 1980-1990 when dwarfing rootstocks became more common for apple orchards favouring single leader training systems with reduced tree vigour (e.g., spindle shape trees). Many different planar training systems were developed in this 50-70 years’ time frame with higher or lower planting density. Some examples reported by Robinson (2003) are: the Penn State thin-wall trellis (Tukey, 1978), The Lincoln canopy (Dunn and Stolp, 1981), The Ebro trellis (Tustin *et al.*, 1989), The Solen system (Lespinasse, 1989), The tabletop bed (Palmer and Jackson, 1977; Preston, 1978).

Even this concept of tree architecture was adopted in the last 50-70 years, it never become a reference training system for apple production, after the palmette “decline”. In fact, the worldwide acceptance for modern apple orchard was developed around late ‘80s and is still widely based on a single leader tree with tridimensional (3D) shape (e.g., tall spindle, slender spindle, vertical central leader, etc.) ranged between 1000 to more than 5000 trees ha<sup>-1</sup>, with wide interrow spacing (>3m)(Robinson *et al.*, 1991; Tustin *et al.*, 2001; Palmer *et al.*, 2002).

After the beginning of XXI century, different authors (Lang, 2005; Robinson, 2007, 2008a; Robinson *et al.*, 2013a; Tustin, 2014) described a shift from 3D single leader/mono-axis tree to a tall thin fruiting walls (i.e., planar) with the aim to increasing light interception and decreasing self-shading, as the future in orchard systems. This vision was directly associated to an increase in yield and fruit quality and a reduction of manpower thanks to thinner and simpler canopies architecture able to match engineering needs for automation and mechanization in fruit crops. Additionally, some authors (Robinson *et al.*, 2013a ; Tustin *et al.* 2018), suggested a reduction in tree densities (<3000 plant ha<sup>-1</sup>), thus depleting planting costs while maintaining orchards profitability (Tustin *et al.*, 2018; Zhang, 2018). This is also confirmed by the number of studies on 2D training system published in the last decades and in which the authors confirmed its potential in relation to the improvement of yields, fruit quality, sustainability, mechanization, automation and application of PFG principles (Robinson, 2003; Musacchi, 2007; Dorigoni *et al.*, 2011; Dorigoni and Micheli, 2014, 2015, 2018; Dorigoni, 2016; Tustin and Van

Hooijdonk, 2016; Stanley *et al.*, 2018; Tustin *et al.*, 2018; Whiting, 2018; Zhang, 2018; Scofield *et al.*, 2020; Dallabetta *et al.*, 2021; Musacchi *et al.*, 2021; Lezzer *et al.*, 2022).

Coming to more recently developed planar systems, as suggested by Lang (2005) and Scofield *et al.* (2020), to increase productivity, new orchards systems cannot be designed without considering plants physiological principles. By this fact horticulturalist working on “innovative” training systems for a sustainable and modern fruit production, needed to match their own needs with plants physiological bases. Explanation of these bases, and motivation of the solutions utilized can be found, as example in Dorigoni, (2016), Tustin *et al.* (2016), Dorigoni *et al.*, (2018), Tustin *et al.* (2018), Lang *et al.* (2021), Musacchi *et al.* (2021). To summarize these studies, the needs remain the ones already presented above (increase in yields, sustainability, produce high-quality, automation, etc.) and physiologically based solution was mainly found in developing planar training systems created with a multileader approach that conduce to a wide distribution of plant vigour between leaders, and make tree structure, more systematic, standardized and simpler, to ease automation (Robinson, 2003; Lauri *et al.*, 2004; Lauri, 2008; Zhang, 2018; Musacchi *et al.*, 2021; Tustin *et al.*, 2022). Examples of these systems can be found in promising new planar systems such as Bibaum® (Musacchi, 2007). This system was one of the first, commercially adopted, attempt in exploiting multileader system to achieve a continues fruiting wall while reducing planting densities. This not reducing orchard yields thanks to the high number of fruiting leader maintained (2 per tree), whose hectare densities result positively related to orchards yields - Lordan *et al.*, 2018; Dallabetta *et al.*, 2021); other examples of recent planar systems are planar cordon, guyot and upright fruiting offshoot (UFO) systems, utilized on different fruit species, that are and based on the distribution of vertical semi-permanent fruiting leaders on one, or more, horizontal permanent axis (or “cordons”) (Long *et al.*, 2015; Dorigoni and Micheli, 2018; Tustin *et al.*, 2018, 2022; Lang, 2019a, 2019b; Scofield *et al.*, 2020; Lang and Whiting, 2021).

As planar systems are often also considered the so-called Y- or V-shaped systems those aim to double the canopy surface, compared to a standard vertical planar system. Y-shaped systems consist in rows of trees with a low vertical trunk and two opposing arms trained either side of the trellis and tilted towards the row alley. V-shaped systems are generally created by planting reduce vigour trees (spindle trees), alternatively tilted in opposite direction towards the row alley; in these systems can be also used palmette-like shaped trees that create thinner canopies compared to spindle trees. More recently also 2D multileader planar systems cited above are utilized to create these inclined systems; in this case the horizontal structure remain generally the same, while vertical leaders are alternatively tilted toward row alley. Y- and V-shaped systems present thin planar canopies that create two-sided, continuous and inclined fruiting walls per each planting row, increasing the “productive surface”. The development and early success and adoption of these training systems is mainly related to their higher light interception and yields (Robinson, 2003; Zhang *et al.*, 2015b) obtained for standard wide inter-row planting distances (i.e.>2.5-3m) if compared to vertical planar training systems or single leader one; this thanks to the doubled fruiting wall surface and the higher number of fruiting leaders generally present. First examples of these systems reported by Robinson (2003) are The Tatura trellis (van den Ende *et al.*, 1987), Geneva Y trellis (Robinson *et al.*, 1989; Robinson, 1992b, 1998), The MIA trellis (Hutton *et al.*, 1987), Mikado and Drilling systems (Widmer and Krebs, 1997). Coming to more recent days, the systems such as planar cordon and UFO cited above, are reported also with a Y or V shaped architecture that resulted to improve fruit production (Breen *et al.*, 2020; Lang and Whiting, 2021).

Modern fruit cultivation clearly needs to fill the gap of automation and precision VRA application that is present if compared to filed-crops; this also need to face the lack of skilled labour availability in the fruit sector (Whiting, 2018; Zhang, 2018). As reported by He and Schupp (2018), tree training systems are critical to the success of adopting automation in orchard production and that detection and accessibility to branches and fruits is a key factor for automating labour intensive operations. The same authors underline how wide canopies and complex branching hierarchies are critical issue for automation and that are being reduced in modern orchard thanks to the reduced tree dimensions.

This consideration is more true when observing planar multileader canopies in which tree architecture is more simplified and “customizable” conducing generally to lower canopies variability and complexity (Du *et al.*, 2013). Different studies confirm the suitability of 2D systems for improving technological application and mechanization as well as management efficiency. Ampatzidis and Whiting (2013) confirmed an increase (+5-15%) of hand-picking efficiency for UFO sweet cherries, thanks to its

easy-to-access architecture, compared to other canopy systems. For the same species and system, the simple architecture made able unskilled laborers to make >95% correct cuts in dormant pruning, after a brief explanation of pruning rules (Long *et al.*, 2015; Whiting, 2018). Regarding mechanization, Chen *et al.* (2012) found that UFO training systems (both Y and vertical shaped) reduce the force needed for picking fruit by automatic machines. For technological application, Zhang *et al.* (2015; 2016) developed a model with which to calculate precise planar canopy light interception pattern so to improve planting distance and physiological studies on these systems, while Amatya *et al.* (2016) underline how extremely narrow width, uniformity, and simplification of planar canopies in a upright fruiting offshoots (UFO) system is ideal for the application of recent advances in orchard machine vision and sensing technologies. Zhang *et al.* (2020) exploited simplified planar canopies (vertical and V shaped), deliberately avoiding 3D canopies, to increase key canopy parameters extraction for mechanical apple harvesting. Amatya *et al.* (2016) resulted to be able to detect branches for automated harvesting in planar canopies. As last example, Bortolotti *et al.* (2021) found that planar canopies improve computer vision by 2-11% for apple crop load estimation if compared to standard 3D tree shapes. Many other examples of 2D multileader systems easing technological application could be done, with a late interest focused on computer vision application to improve orchard automation and robotic applications (more information in Karkee *et al.*, 2021). It is clear how this planar architecture strategically facilitates the adoption of mechanization/automation for those tree crops specific tasks such as pruning, harvest and thinning improving labour efficiency by simplifying cultural practices (Whiting, 2018).

Concluding, in relation to all have been discussed, future orchard systems are highly probable to be based on multileader planar training systems for many fruit species. Apple and sweet cherry appear the readiest crops, with promising results and studies also on apricots, plums, peaches/nectarines, and pears.

Summarizing, these systems result promising because of the following characteristics:

- exploit the natural growth behaviour, and morphology, of most of temperate fruit crops to physiologically control tree vigour; with this system is possible adapt tree architecture (i.e., upright leader number) to plants vigour, so to keep reduced tree dimension in different environmental x genetic conditions.
- Increase/optimize light interception and distribution, with benefits for flower bud differentiation, CO<sub>2</sub> assimilation and fruit development and fruit quality (size, color, sweetness, texture), with considerable increase of yields and profitability.
- Reduce canopy thickness, exposing most of the fruit so to improve precise quantification and to favour observation and fruit access, easing yields and fruit quality management.
- Simplify canopy fruiting units, conducing to a lower fruiting wall variability easing management operation as well as increasing labour efficiency and precision.
- Accommodate the application of sensing technologies and algorithmic decision-making solutions, promoting the introduction of automated/robotics management solution into the orchard

## **Aim of the Study**

The fruit industry needs to increase its sustainability in crops production. This can be achieved increasing yields by improving surface production efficiency. Precision fruit growing principle and solutions can be a reliable tool for integrated farm management and to increase production efficiency.

Innovation on fruit growing sector is achieved by multileader planar tree architectures thanks to their potential ability to increase yields and precision fruit growing applications. Match fresh fruit market quality requirements is fundamental for orchard profitability. Key management operations to drive fruit quality rely in crop load management. Thanks to innovative multileader planar tree architectures tree dedicated management operations, such as thinning, are possibly mechanizable.

The combination of innovative multileader planar training systems with the application of precision fruit growing techniques dedicated to crop load management can be a valid tool to highly increase yields, profitability and, consequentially, sustainability of apple production.

The presented study aims to evaluate the ability to increase yields, of “Double Guyot” multileader planar training system for apple production in the Italian environment (Po’ valley region, Emilia-Romagna). Productive limits (i.e. maximum crop load levels), able to maintain fruit quality premium class market requirements, will be investigated for the apple variety under testing (Pink Lady® - Rosy Glow). The possibility to increase the thinning management unit resolution, from single tree to single fruiting leader within the tree, will be investigated both at physiological and productive level; this to favour a more precise, ease and possibly mechanizable, crop load management. The final aim is to develop a valuable thinning thresholds protocol (i.e., crop load levels) able to guarantee high yields and match markets requirements. The idea is to transfer the thesis results as an operational guideline for Emilia Romagna apple fruit growers who take the challenge in adopting 2D multileader training system.

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## CHAPTER 2

# Individual upright physiological traits in an apple multileader training system

### ABSTRACT

Light intercepted by the tree drives photosynthesis and seasonal light integrals have been correlated to yields. Innovative training systems have recently been proposed those share the concept of forming planar (2D) fruiting walls instead of 3D voluminous canopies. Examples of such innovative training systems are the UFO, the planar cordon, and the double-guyot. These are based on vertical semipermanent fruiting leaders (uprights), inserted onto one or two permanent horizontal cordons, with the aim to form very thin planar canopies. This shift towards ultra-thin hedgerows canopy shapes increases light interception and distribution while adapting the orchard for automation/robotization. For the precise management of such trees, it would be useful to know whether the uprights are physiologically independent of each other or not. In this study are reported results from a 4-year-old Rosy Glow orchard trained as multileader, grafted on Pajam 2 rootstock, planted at 1443 trees ha<sup>-1</sup>, growing in the Po Valley (Ferrara) - Italy. Fruit number per upright, upright dimensions, leaf gas exchanges and return bloom data were collected on uprights with contrasting, naturally occurring crop loads (high and low); Fruit quality per upright was also evaluated. Half of the uprights were girdled to investigate interconnection between them. Crop load shown to stimulate photosynthesis in both upright types, with girdled shoots presenting lower photosynthesis level if compared to intact ones. Girdled uprights shown a better fruit skin color (intensity and extension), and a higher values of SSC and flesh firmness compared to intact uprights. Yield and quality were correlated to upright length, cross sectional area and leaf area. From these correlations thinning crop load thresholds are proposed. Return bloom was altered by the treatments, but no alternate fruit bearing was induced. These results indicate that the uprights are physiologically connected and should not be considered independent from each other; crop load seems to define upright independence level. Accurate management of crop load at the upright level could be useful in improving fruit production and quality under heavy crop loads.

### ABBREVIATIONS

A = Leaf net photosynthesis  
E = Leaf transpiration  
gs = Leaf stomatal conductance  
HG = Treatment with high crop load and girdling  
HNG= Treatment with high crop load and no girdling  
LG= Treatment with low crop load and girdling  
LNG= Treatment with low crop load and no girdling  
SSC = Soluble solid content  
UCA = Upright cross-sectional area

### INTRODUCTION

Innovative training systems are being proposed to increase yields, sustainability, automation, and novel technologies while optimizing fruit quality. In many cases, the standard 3-dimensional training systems are being abandoned, to favor less voluminous and complex tree shapes, like fruiting walls/hedges. Yield is positively correlated with light interception (Jackson and Palmer, 1972; Wunsche *et al.*, 1996; Corelli-Grappadelli and Lakso, 2007) and two-dimensional canopies aim to increase light interception to increase yield and consequently their sustainability. 2-D systems, by improving light interception/distribution within the canopy, improve fruit quality as well; when considering bi-color apple varieties (such as Gala, Fuji, Pink Lady, etc.), these can benefit more than mono-color varieties from 2D systems light distribution, that improve skin coloration, favouring premium price to the growers (Jackson and Sharples, 1971; Jackson, 1989; Saure, 1990; Warrington *et al.*, 1996; Steyn *et al.*,

2002; Miller *et al.*, 2015; Musacchi and Serra, 2018 ). At the same time, these training systems can ease the automation and mechanization (Zhang, 2018) by reducing tree and orchard complexity. The reduced plant vigour attainable in 2-D(multileader) orchards (Dorigoni and Micheli, 2014) allows the tree to better tolerate mechanical topping and hedging, if compared to many central leader-based shapes, both in the dormant or summer seasons, easing the renewal of old structural wood with productive one without inducing a vigorous response (Dorigoni and Micheli, 2014, 2015).

In recognition of these potential advantages, two-dimensional canopy systems are becoming widespread, e.g., the Upright Fruiting Offshoot (UFO) (Long *et al.*, 2015) typically used for sweet cherry in U.S, and the “ 2-D Trellis” and planar cordon (Tustin *et al.*, 2016 and 2018) for apple in New Zealand, the US, Australia. A training system similar to planar cordon, used mainly on apple and called “Guyot” (Dorigoni and Micheli, 2018), is under advanced testing in some commercial farms in Italy. It is based on one (guyot) or two (double guyot) horizontal axes on which a variable number of shoots (leaders) are trained vertically as semipermanent fruiting elements called uprights (Dorigoni and Micheli, 2018).



Figure 2.1 – Examples of a double guyot training system for 4<sup>th</sup> leaf Pink Lady®-Rosy Glow apple trees, grafted on Pajam 2 rootstock and grown in Po valley (Ferrara) - Italy.

This multileader system (i.e., double guyot) differs from the planar cordon, not for the trellis structure required, basically the same with trellis wire placed every 50cm of height from the cordons plane, but mainly for the number of uprights and their spacing. Uprights number is fixed for the planar cordon (i.e., 10 uprights placed every 30cm approx. along the two cordons), and more variable for the guyot (i.e., 8-14 uprights distributed along the two cordons of the plants). Planting distances differ as well with planar cordon presenting wider planting distance along the row (generally 3m between the plants) compared to the guyot in which is more variable (2-2.8m).

While 2D multileader systems are being increasingly adopted, many physiological traits of apple trees trained with these innovative systems have not been adequately studied. In view of the increasing automation of orchard management, it is interesting to test whether the multileader systems should still be managed on a tree basis, or whether the focus could shift to the individual upright. In the latter case, a more precise management of the crop should be based on this growth unit, instead of the tree.

In a farer future, this knowledge might inform Decision Support Systems (DSSs) dedicated to crop load management and based on image analysis, such as the ones for disease (e.g., Polder *et al.*, 2021).

The aim of the study is to test from the physiological and productive point of view the possibility to shift form the whole tree to a single leader (upright) the crop load management in multileader training systems. Moreover, a quality assessment at crop load upright level have been investigated.

## MATERIALS AND METHODS

The study was carried out in 2020 (starting in June because of COVID-19 restrictions), in Voghiera, Ferrara, Italy (Lat: 44.765105, Long: 11.757721) in a 4-year-old commercial Pink Lady®-Rosy Glow apple (*Malus domestica* Borkh.) orchard trained as double guyot (Dorigoni and Micheli, 2018) and grafted on Pajam 2 rootstock. The orchard has a planting distance of 3.3m between the rows and 2.1m along the row, with 1443 trees ha<sup>-1</sup>. Each tree features from 4 to 7 uprights on each horizontal cordon. Considering tree density, since the total number of uprights per tree varied between 8 and 14, a range of 11,500 to 20,000 uprights ha<sup>-1</sup> existed. Integrated management techniques were applied to the orchard.

The experiment was carried out on 36 uprights chosen according to their fruit number (*FN*) to upright length ratio (*Len\_FD*, Factor 1). This is computed with Eq.1 where *FN* is the fruit number of the upright and *Length(cm)* is the length of the upright, in cm, taken from the point of insertion on the horizontal cordon. *FN* and *Length(cm)* measurements occurred on 03/06.

$$Len\_FD(\text{fruit}/\text{cm}) = \frac{FN}{Length(\text{cm})} \quad (\text{Eq.1})$$

These 36 uprights were chosen on a total of 6 trees (6 uprights per tree) where no artificial crop load adjustment was applied. 18 uprights were selected on 3 trees, whose value for the ratio was greater than 0.1 (High, H crop load treatment). Likewise, on 3 other trees, 18 shoots were selected with a ratio lower than 0.05 (Low crop load, L). The threshold values had been determined based on a preliminary assay of upright size and crop load (n=250). Natural crop load condition was used to characterize contrast in fruit load for different uprights. Half of the uprights in each group (H and L) were randomly girdled at the base (Factor 2), around 5cm above the insertion on horizontal cordon. A strip of bark 0.5 cm wide was removed, on 03/06, exposing the underlying wood, so to isolate the fruiting unit in respect to phloem flux. This yielded the second factor of girdled (G) and not girdled (NG). The two factors were arranged in a split-plot design, resulting in 4 treatments (each with 9 reps) as follows: HG and HNG, high crop load, girdled and non-girdled, respectively; LG and LNG, low crop load girdled, and non-girdled, respectively.

On each upright, leaf gas exchanges were measured 3 times during the season (on 20/07, 13/08 and 11/09) by a LI-COR 6400 (LI-COR, Lincoln, NE, USA) before solar noon, on randomly selected well expanded shoot leaves. Length and diameter of each upright were recorded at harvest (25/10) respectively by a digital caliper (Mitutoyo, Kanagawa, Japan) and a standard measuring tape. Measurements were taken as presented previously for length, while upright diameter was measured 10 cm above the insertion onto the cordon (above the girdle). From measurements took on 25/10 upright cross-sectional area (UCA -cm<sup>2</sup>) and upright volume (Vol. - cm<sup>3</sup>) were calculated. Upright leaf area (LA - m<sup>2</sup>) per upright was calculated by estimating the total number of leaves, on lateral shoots and spurs, and multiplying that number by the average leaf area for the two categories of shoot. The number of leaves on each upright (*Leaf Number* in Eq.2) was estimated by Eq. 2 where *a* = 0.3098, *b* = 5.6751 and *total shoot length(cm)* is the sum of all upright shoots' length (collected manually by measuring tape). *a* and *b* were obtained by developing an empirical relation between shoot length and shoot leaf number ( *r* = 0.82; *n* = 310 shoots analyzed). All spurs on uprights were counted, and the average number of leaves per spur was determined by counting leaves on 300 randomly chosen spurs. The average area of shoot and spur leaves was determined on 300 leaves, per shoot type, that were collected randomly and measured by a LI-COR 3100C leaf area meter (LI-COR, Lincoln, NE, USA).

$$LeafNumber = a * total\ shoot\ length(\text{cm}) + b \quad (\text{Eq.2})$$

At harvest, fruit were strip-picked, manually counted and their quality parameters were evaluated within each upright. Fruit maximum equatorial diameter (Diam, mm) and weight (g) were measured by digital caliper (Mitutoyo, Kanagawa, Japan) attached to an external memory ([www.hkconsulting.it](http://www.hkconsulting.it)) and a digital scale (KB1200, KERN & SHON, Germany) respectively. Flesh firmness (FF, Kgf) was determined by a penetrometer (GÜSS Fruit Texture Analyser, GÜSS, South Africa) with an 11 mm diameter tip after removing the fruit peel. Soluble solid content (SSC, °Brix) was determined by measuring the refractive index of the juice for each fruit portion with a digital refractometer (ATAGO PAL-1, ATAGO, Japan). Color parameters were measured by a Chroma meter (CR-400, Konica Minolta, Japan) on blush (reddest area) and background ("greenest" area) sides of each fruit (most and least exposed to the sun) and expressed

as CIE L\*C\*h\* color-space coordinates directly from the instrument; one-flash measurement was used to collect the color data. Individual fruit color extension was estimated by assessing the percentage fruit area covered by blush color (red-colored surface) from visual observation.

Return bloom were evaluated in the spring of 2021 during full bloom (occurred on 02/04). Flower clusters were manually counted on each upright.

Several flower densities (FLD - using spring 2021 data) and fruit densities (FD - using 2020 harvest data) variables were computed, with Eq.3 and Eq.4, respectively, where, “*P*” refers to the vegetative parameter considered and “*Pu*” to the respective measure unit (e.g., *P* = UCA, *Pu* = cm<sup>2</sup>). The calculated FLDs were expressed as number of flower clusters (FLN) per upright cross-sectional area unit (UCA\_FLD – FLN /cm<sup>3</sup>), leaf area unit (LA\_FLD – FLN /m<sup>2</sup>), length unit (Len\_FLD – FLN /cm) and volume unit (Vol\_FLD – FLN /cm<sup>3</sup>) parameters of each upright. FDs were expressed as number of fruit per upright cross-sectional area unit (UCA\_FD – FN /cm<sup>2</sup>), leaf area unit (LA\_FD – FN /m<sup>2</sup>), length unit (Len\_FD – FN /cm) and volume unit (Vol\_FD – FN /cm<sup>3</sup>) parameters of each upright.

$$P\_FLD(FLN/Pu) = \frac{FLN}{P(Pu)} \quad (\text{Eq. 3})$$

$$P\_FD(FN/Pu) = \frac{FN}{P(Pu)} \quad (\text{Eq. 4})$$

These parameters were computed because describe flower and fruit load (respectively for FLD and FD) not only as a number, but considering the vegetative development of the upright considered.

Data were analyzed according to a split-plot design by R and R-Studio software using “agricolae” and “stats” packages. Because the distribution of the dataset was not normal, due to the particular set up of the trial searching for “extreme” condition in fruit-to-upright-length ratio, the non-parametric Kruskal-Wallis test and pairwise Wilcoxon Post-HOC were used to test statistical differences among treatments.

For leaf gas exchanges, data coming from different date of measurement was found non-significantly different between each other, therefore, these parameters were analyzed together with the statistical tests cited above.

Regarding upright length, fruit number and derived parameters coming from different date of measurement (03/06 and 25/10), these resulted stat. non different between each other; therefore, data from 25/10 only were analyzed, with the same statistical test above. Harvest data only were analyzed because they better represent the productive result of the entire season.

Pearson’s r correlation coefficient was used to investigate correlations between fruit quality and crop load.

## RESULTS

### *Leaf Gas Exchanges*

Leaf gas exchanges were affected both by crop load and girdling (Table 2.1). Photosynthesis seemed to be more affected by girdling than crop load, with lower rates in HG than in both non girdled treatments, even though a 75% reduction was found in LG compared to HG units. Stomatal conductance did not respond to crop load in NG units, whereas it greatly decreased (-66%) in the G uprights, where, again, crop load strongly affected the response. Substomatal CO<sub>2</sub> was highest in LNG, mirroring the lower A compared to HNG, and it was lower in both girdled treatments, reflecting their much reduced, and different between themselves, conductance. Transpiration followed the same pattern of photosynthesis.

Table 2.1. Leaf gas exchanges expressed as photosynthesis (A) ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), stomatal conductance ( $g_s$ ) ( $\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ), substomatal  $\text{CO}_2$  ( $\mu\text{mol CO}_2 \text{ mol air}^{-1}$ ), leaf transpiration (E) ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ) for 'Rosy Glow' apple grown in the Po Valley -Italy. Mean separation in columns by Wilcoxon Post-HOC test.  $P < 0.05$ . (N=27).

	<b>A</b>	<b><math>g_s</math></b>	<b>Subst.<math>\text{CO}_2</math></b>	<b>E</b>
<b>HNG</b>	19.7 a	0.180 a	191.8 b	5.29 a
<b>LNG</b>	17.2 b	0.167 a	203.7 a	4.58 b
<b>HG</b>	12.9 c	0.110 b	178.9 c	3.22 c
<b>LG</b>	4.8 d	0.035 c	111.6 d	1.00 d
<b>Sign.<sup>1</sup></b>	***	***	***	***

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

### Fruit Quality

Within the same crop load, G improved firmness up to 7%, SSC up to 16% and color extension up to 12% without causing an advancement in maturity as expressed by flesh firmness, which was not reduced by girdling, but to the contrary increased (Table 2.2). Fruit size in H treatments was not affected by girdling, as diameter and weight were not different, whereas they were higher in LG than in LNG. Moreover, a trend can be overseen between fruit mean diameter and weight; indeed, they were higher for G with an increase of up to 3% and 11% respectively, compared to the NG (for L situation) (Table 2.2).

Crop load clearly affected fruit quality in both G and NG (Table 2.2), decreasing fruit size up to 9% and 37% for diameter and weight respectively; the same result can be observed for the other quality parameters.

Table 2.2. Fruit quality parameters expressed as fruit number (FN), diameter (Diam) (mm), Weight (g), flesh firmness (FF) (kgf), SSC ( $^{\circ}\text{Brix}$ ), Color extension (Col. Ext) (% of covered area) for 'Rosy Glow' apple grown in the Po Valley -Italy. Mean separation in columns by Wilcoxon Post-HOC test.  $P < 0.05$ . (N=9 for FN; N=46-305, for other variables).

	<b>FN</b>	<b>Diam</b>	<b>Weight</b>	<b>FF</b>	<b>SSC</b>	<b>Col. Ext</b>
<b>HNG</b>	34 a	69.9 c	145 c	8.72 d	14.4 d	67.5 d
<b>LNG</b>	5 d	75.0 b	185 b	9.16 c	15.2 c	79.4 b
<b>HG</b>	26 b	70.4 c	152 c	9.38 b	16.7 b	75.7 c
<b>LG</b>	7 c	77.4 a	208 a	9.72 a	17.1 a	84.4 a
<b>Sign.<sup>1</sup></b>	***	***	***	***	***	***

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

Table 2.3 presents the fruit color parameters achieved by each treatment (CIE  $L^*$ ,  $C^*$  and  $h^*$  color-space coordinates) while Figure 2.2 shows these parameters in their relative color representation. The parameter  $L^*$  appears higher in NG than G, for the same crop load level, and in H compared to L, for the same girdling condition; this both for blush and background color although not always significant.  $C^*$  values decrease in NG and H, while red color, expressed by  $h^*$  in the blush side of the fruit, decreases from H to L and from NG to G ( $h^*$  lower values correspond to a redder color); the opposite occurs for background color. Figure 2.2 visually summarizes the results of the color analysis, ranking the treatments for blush and background colors from the greenest to the reddest, that are respectively HNG, LNG, HG, LG.

Table 2.3. Fruit color intensity expressed as CIE L\*, C\*, h\* color space coordinates for both blush (BL) and background color (bg) for 'Rosy Glow' apple grown in the Po Valley -Italy. Mean separation within columns by Wilcoxon Post-HOC test. P < 0.05. (N=46-305).

	<i>L*<sub>BL</sub></i>	<i>C*<sub>BL</sub></i>	<i>h*<sub>BL</sub></i>	<i>L*<sub>bg</sub></i>	<i>C*<sub>bg</sub></i>	<i>h*<sub>bg</sub></i>
<b>HNG</b>	54.0 a	39.5 c	38.2 a	70.3 a	33.8 d	21.7 b
<b>LNG</b>	50.2 b	44.2 b	34.8 b	68.2 b	37.0 c	37.9 ab
<b>HG</b>	49.6 b	44.8 b	33.6 b	66.2 c	38.8 b	46.3 a
<b>LG</b>	46.2 c	47.6 a	30.4 c	65.1 c	46.6 a	52.8 a
<b>Sign.<sup>1</sup></b>	***	***	***	***	***	***

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at p ≤ 0.05, p ≤ 0.01 or p ≤ 0.001, respectively.

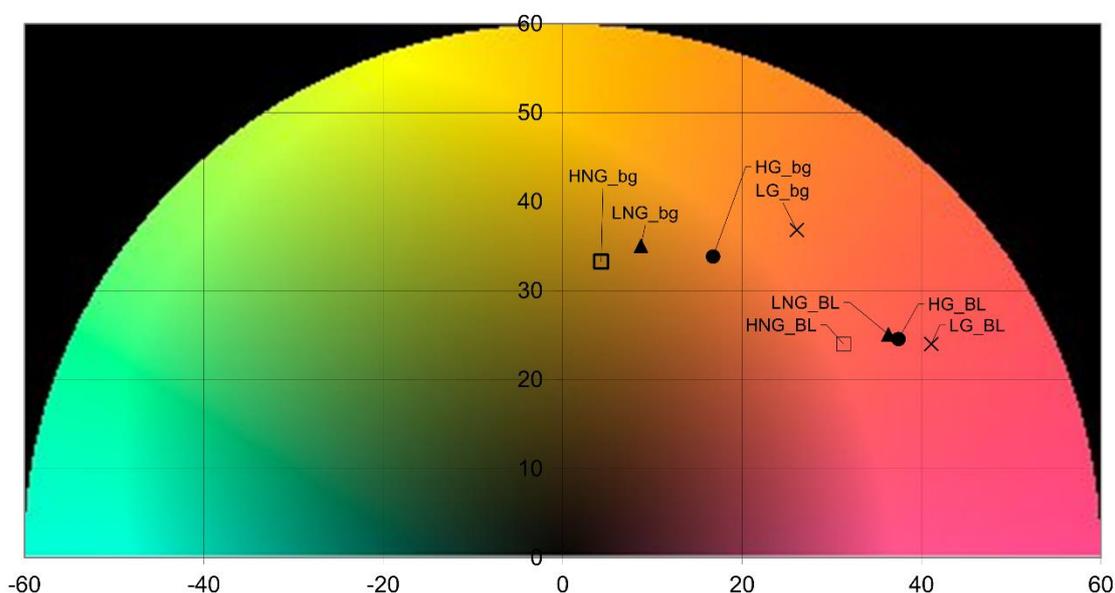


Figure 2.2 - Fruit color representation, per each treatment, in CIE L\*C\*h\* color-space for 'Rosy Glow' apple grown in the Po Valley -Italy. “\_bg” means “background”; “\_bl” means “blush”. (N=46-305).

### Allometric-Quality Correlations

Correlations between allometric data and fruit quality and productive parameters were analyzed by Pearson's r values and their respective p-values are presented in Table 2.4. Fruit diameter and weight present a good correlation with UCA and Vol. and LA only in the case of HG. Flesh firmness shows no relevant correlations (only a single value in LG against LA) while SSC shows notable, always negative, correlation values with all the allometric parameters in LNG. LG presents a good correlation only for LA parameter. Col.Ext. presents always negative correlation with UCA, Vol and LA only in HG. Col.h\* parameter (h\*<sub>BL</sub> in Table 2.3) was considered as the most representative for red blush color. This parameter shows correlation with UCA, Vol. and LA both in HG and LNG, but with opposite signs: correlations are negative for HG and positive for LNG.

Table 2.4. Linear correlations between quality and vegetative parameters<sup>2</sup> for 'Rosy Glow' apple grown in the Po Valley -Italy. Pearson's "r" coefficients and relative p-values for linear correlation between parameters are presented. Bold-underlined values ( $r > 0.60$ ) were considered as notable correlations. (N=9).

	<b>HG</b>		<b>HNG</b>		<b>LG</b>		<b>LNG</b>	
	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>
<sup>3</sup> Diam vs UCA_FD <sup>2</sup>	<b><u>-0.75</u></b>	***	-0.29	*	-0.48	***	-0.32	*
<sup>3</sup> Diam vs Vol_FD <sup>2</sup>	<b><u>-0.71</u></b>	***	-0.46	***	-0.46	***	-0.30	*
<sup>3</sup> Diam vs LA_FD <sup>2</sup>	<b><u>-0.61</u></b>	***	-0.21	ns	-0.48	***	-0.36	**
<i>Weight vs UCA_FD<sup>2</sup></i>	<b><u>-0.80</u></b>	***	-0.34	**	-0.46	***	-0.09	ns
<i>Weight vs Vol_FD<sup>2</sup></i>	<b><u>-0.76</u></b>	***	-0.44	***	-0.41	***	-0.09	ns
<i>Weight vs LA_FD<sup>2</sup></i>	<b><u>-0.64</u></b>	***	-0.15	ns	-0.34	**	-0.05	ns
<sup>3</sup> FF vs LA_FD <sup>2</sup>	-0.15	ns	-0.31	*	<b><u>-0.69</u></b>	***	0.49	***
<sup>3</sup> SSC vs UCA_FD <sup>2</sup>	-0.53	***	-0.18	ns	-0.54	***	<b><u>-0.78</u></b>	***
<sup>3</sup> SSC vs Len_FD <sup>2</sup>	-0.14	ns	-0.03	ns	-0.48	***	<b><u>-0.65</u></b>	***
<sup>3</sup> SSC vs Vol_FD <sup>2</sup>	-0.60	***	-0.28	*	-0.53	***	<b><u>-0.73</u></b>	***
<sup>3</sup> SSC vs LA_FD <sup>2</sup>	-0.59	***	-0.46	***	<b><u>-0.77</u></b>	***	<b><u>-0.73</u></b>	***
<sup>3</sup> Col.Ext. vs UCA_FD <sup>2</sup>	<b><u>-0.96</u></b>	***	0.11	ns	-0.36	*	0.13	ns
<sup>3</sup> Col.Ext. vs Vol_FD <sup>2</sup>	<b><u>-0.92</u></b>	***	-0.20	ns	-0.43	***	0.22	ns
<sup>3</sup> Col.Ext. vs LA_FD <sup>2</sup>	<b><u>-0.73</u></b>	***	-0.14	ns	-0.32	*	-0.51	***
<sup>3</sup> Color (h* <sub>BL</sub> ) vs UCA_FD <sup>2</sup>	<b><u>-0.91</u></b>	***	-0.21	ns	0.14	ns	<b><u>0.90</u></b>	***
<sup>3</sup> Color (h* <sub>BL</sub> ) vs Vol_FD <sup>2</sup>	<b><u>-0.93</u></b>	***	0.09	ns	0.20	ns	<b><u>0.88</u></b>	***
<sup>3</sup> Color (h* <sub>BL</sub> ) vs LA_FD <sup>2</sup>	<b><u>-0.81</u></b>	***	0.16	ns	0.10	ns	<b><u>0.81</u></b>	***

<sup>1</sup> ns. \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively

<sup>2</sup> UCA\_FD, Len\_FD, Vol\_FD and LA\_FD are Fruit density (FD) expressed as the ratio of fruit number by upright cross-sectional area, length, volume and leaf area respectively

<sup>3</sup> Diam, FF, SSC, Col.Ext, Color (h\*BL) are fruit diameter, flesh firmness, soluble solid content, color extension and color hue angle for blush respectively

The correlations between allometric data and mean fruit diameter are shown in figures 2.3-2.6. For Pink Lady-Rosy Glow in Italy, premium quality specifications can vary from year to year, but generally are a good fruit appearance (i.e. no fruit defect and blush area >50% with an intense red color) and a minimum fruit size of 70 mm. Considering these specifications, the following fruit densities were found, that can be considered as minimum thresholds limiting fruit of size to less than 70 mm (figures 2.3-2.6):

- 12-15 fruit/cm<sup>2</sup> of upright cross-sectional area
- 0.10-0.15 fruit/cm of upright length
- 0.20-0.30 fruit/cm<sup>3</sup> of upright volume
- 25-35 fruit/m<sup>2</sup> of upright leaf area

For LG and LNG mean fruit diameter is always above 70mm while for HG and HNG this only happens for half of the uprights. Figure 2.3 shows the general negative relationship between mean fruit diameter and fruit number per upright cross-sectional area; the strongest relations are present in girdled uprights (-0.75 and -0.48 for H and L respectively) while weaker ones are shown by non-girdled treatments. Crop load threshold appears to be defined at 12-15 fruit/cm<sup>2</sup> of UCA and overall relationship between these parameters appears quite interesting ( $r = -0.79$ ).

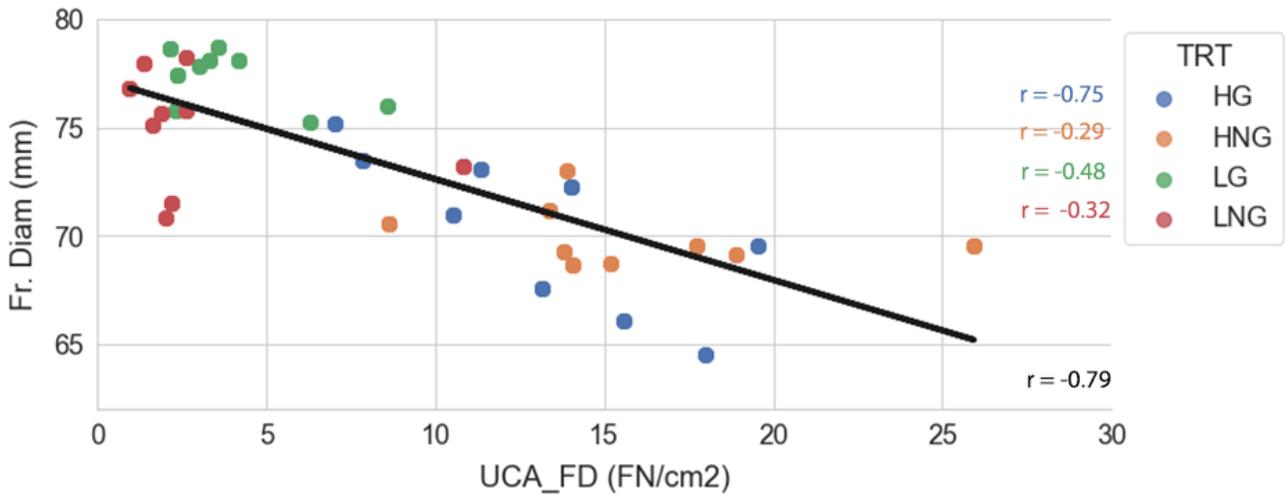


Figure 2.3 – Scatterplot showing mean upright fruit diameter (Fr.Diam) vs mean upright UCA fruit density (UCA\_FD) for ‘Rosy Glow’ apple grown in the Po Valley. Correlation coefficient values are presented for each treatment (N=9). The black line represents the general trend among all the treatments (N=36).

Fruit density per upright length (Figure 2.4) shows a wider and more variable range for the crop load threshold above which 70mm fruits number is reduced (0.10-0.15 fruit/cm of upright length). Here treatments show generally low correlation values with opposite behavior: negative and positive correlation respectively for isolated(G) and connected (NG) uprights. The general correlation again appears quite clearly (r = -0.74) and negative.

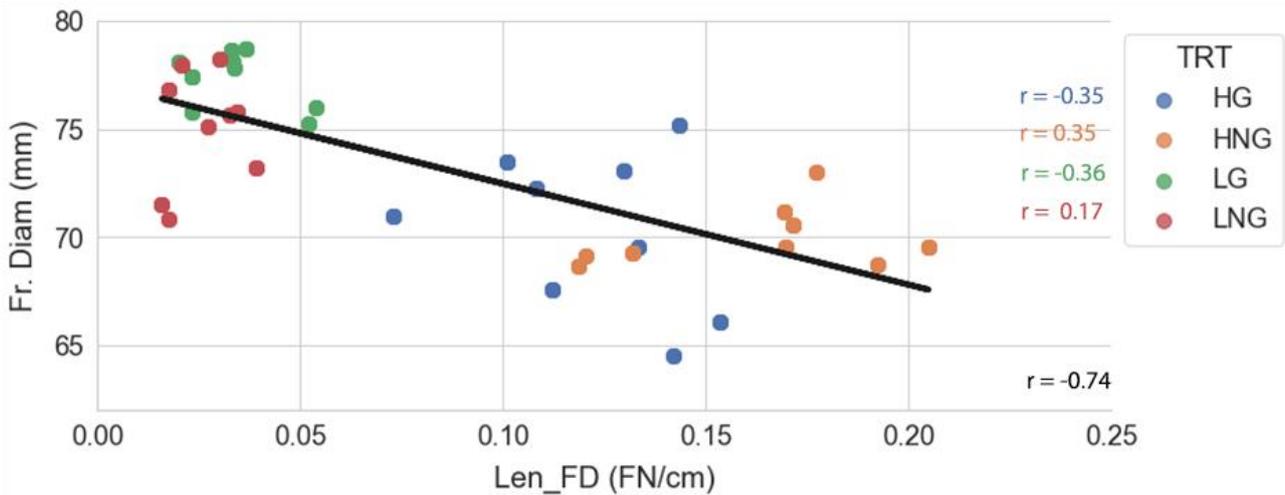


Figure 2.4– Scatterplot showing mean upright fruit diameter (Fr.Diam) vs mean upright lenght fruit density (Len\_FD) for ‘Rosy Glow’ apple grown in the Po Valley. Correlation coefficient values are presented for each treatment (N=9). The black line represents the general trend among all the treatments (N=36).

Volume fruit density seems to have a less variable crop load threshold, compared to length fruit density, with large fruit mainly present for values lower than 0.2-0.3 fruit/cm<sup>3</sup> of upright volume. HG presents the highest r correlation values and LNG the lowest with the remaining treatments scoring the same; overall r correlations value is -0.72 (Figure 2.5).

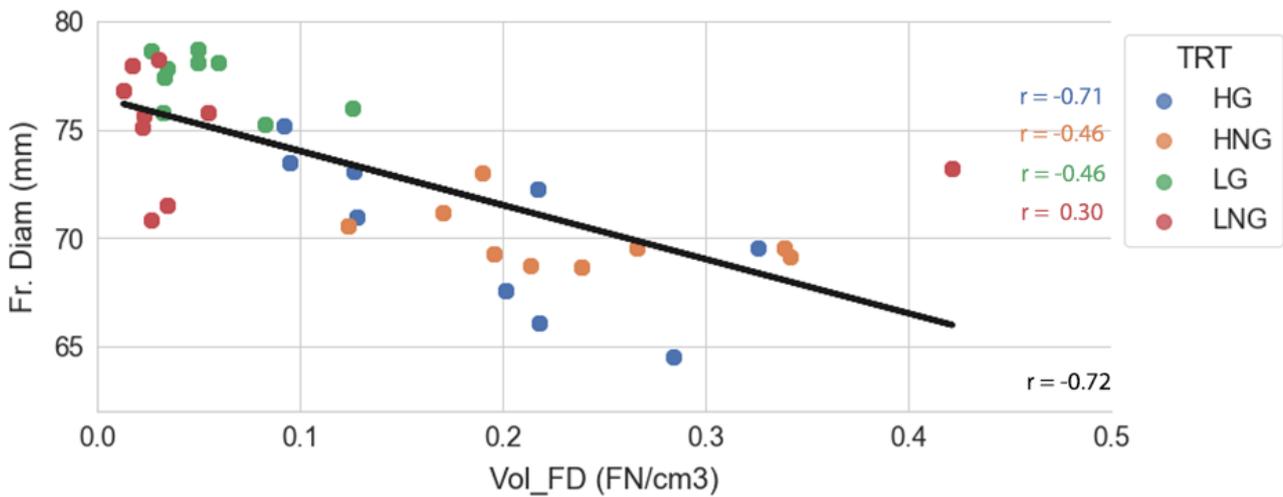


Figure 2.5– Scatterplot showing mean upright fruit diameter (Fr.Diam) vs mean upright volume fruit density (Vol\_FD) for ‘Rosy Glow’ apple grown in the Po Valley. Correlation coefficient values are presented for each treatment (N=9). The black line represents the general trend among all the treatments (N=36).

When fruit density is expressed in terms of leaf area (Figure 2.6), a behaviour similar to fruit density per upright length (Figure 2.4) appears, with a wide and variable crop load threshold range (25-35 fruit/m<sup>2</sup> of upright leaf area). Higher r values are present for G than for NG. The general relation is similar to the one found for upright cross-sectional area (Figure 2.3).

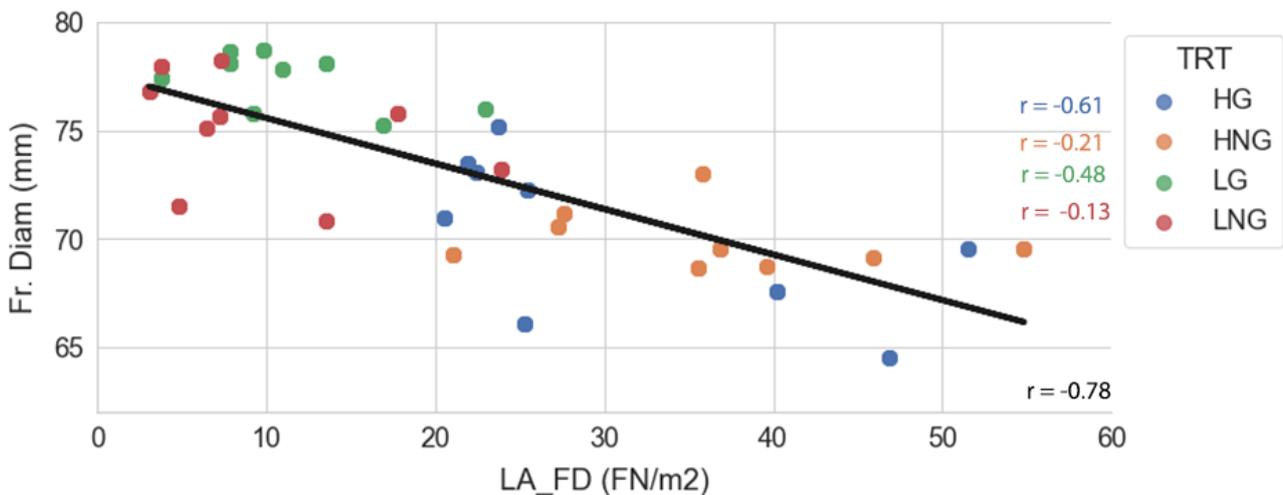


Figure 2.6– Scatterplot showing mean upright fruit diameter (Fr.Diam) vs mean upright leaf area fruit density (LA\_FD) for ‘Rosy Glow’ apple grown in the Po Valley. Correlation coefficient values are presented for each treatment (N=9). The black line represents the general trend among all the treatments (N=36).

### **Return Bloom**

Girdling seems to promote flower number and volume density in L while not in H when compared to NG. For H crop load, G and NG result different only for length fruit density, while not in the other parameter, also if a tendency of HNG to higher values is present, but for leaf area. For G condition, H and L crop load result always different if not for flower number, while for NG, H and L result similar in any parameter if not for leaf area fruit density. This last parameter is the only one clearly driven by crop load level, that present same result for both girdling situation. (Table 2.5).

Table 2.5. Flower cluster number (FLN), flower density in relation to upright UCA (FLN/cm<sup>2</sup>), length (FLN/cm), volume (FLN/cm<sup>3</sup>), and leaf area (FLN/m<sup>2</sup>) for 'Rosy Glow' apple grown in the Po Valley -Italy -, for 2021 spring data. Mean separation within columns by Wilcoxon Post-HOC test. P< 0.05. (N=9).

	<i>FLN</i>	<i>UCA</i>	<i>Length</i>	<i>Volume</i>	<i>Leaf Area</i>
<b>HNG</b>	29.2 ab	12.9 ab	0.138 a	0.184 b	29.0 b
<b>LNG</b>	25.4 b	13.1 ab	0.125 ab	0.176 b	42.4 a
<b>HG</b>	27.6 ab	12.8 b	0.122 b	0.174 b	29.4 b
<b>LG</b>	30.4 a	15.3 a	0.137 a	0.211 a	42.9 a
<b>Sign.<sup>1</sup></b>	*	***	**	**	***

<sup>1</sup> ns. \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively

## DISCUSSION

### Leaf Gas Exchanges

Girdling had a visible effect on all gas exchange parameters. This indicates that uprights are not independent of each other, even in this quite “unusual” training system for apple trees. Indeed, the phloem interruption reduced the carbohydrate translocation between the uprights with a dramatic reduction of all gas exchanges when the sink, in this case fruit load, was weak. This confirms literature reporting decline in net photosynthesis rate after weakening of the sink demand (Paul and Foyer, 2001; Fan *et al.*, 2010; Taiz *et al.*, 2015). A reduction in the gas exchanges was also present in the NG but at lower instance. This probably indicates that a sink-demand coming from other organs out of the single upright stimulated leaf gas exchanges. This seems supported, in L, by a higher leaf gas exchange reduction for the isolated uprights (LG) compared to the connected ones (LNG). The degree of reduction/stimulation of gas exchanges might also depend on the distance that carbohydrates must travel among the intact uprights. It seems likely that, the greater the distance between uprights that can export and those that need support, the lower the effect on gas exchanges. This will need to be investigated more in future studies, but higher carbon partitioning priority for short sink-source transport distances are proposed in literature, supporting this hypothesis (Cieslak *et al.*, 2011; Tustin *et al.*, 2022). An adding remark is that other uprights, not analyzed, were present on the tree and being involved in the sink-demand gradient of the tree, they surely affected leaf gas exchanges results for NG treatments.

### Fruit Quality

Girdling seemed to improve fruit quality due to the isolation from the rest of the plant. In this situation, for moderate crop loads, source limitation did not occur and all the fruit in the upright could attain satisfactory growth. At the same time G did not cause any advance in maturity, as shown by flesh firmness, which was probably increased by the greater availability of dry matter resources during the late cytokinesis stages. The greater availability of resources, during the cell proliferation stage, could have induced a higher cell number in G, and this consequentially increased flesh firmness for this treatment, considering that all fruit were exposed to the same environmental condition. Charles *et al.* (2018) found that higher fruit cell number, resulted in higher fruit flesh firmness, this without applying girdling condition, while Fallahi *et al.* (2018) found higher firmness for fruit on girdled trees compared to the ones on non-girdled trees; in this last case, authors suggested that higher availability of Ca, due to the phloem interruption, for the fruit on girdled trees can be the motivation of the higher firmness of the fruit instead of an higher fruit cells number. If G induced higher cell number, then bigger fruit size would be expected for G treatments, considered the known positive relation between cell number and final fruit size, (Harada *et al.*, 2005; Charles *et al.*, 2018). Despite this, G did not show a clear effect on fruit size (diameter and weight), which was not different in H while it was in L.

These different fruit sizes and weights highlight crop load as the strongest driver for fruit development. Indeed, HG was not able to overcome the source limitation to growth, but on the other hand, LG presented larger fruit than LNG, indicating that LNG uprights are likely exporting resources to the rest of the tree. Probably HNG uprights benefited from external inputs which allowed them to achieve the same fruit size as HG. On the other hand, HG might well have been too heavy, making crop load a limitation. Considering HG fruit number (24% fewer fruit than HNG) and leaf area dedicated to each fruit (0.037 vs 0.031 m<sup>2</sup> fruit<sup>-1</sup> for HG and HNG respectively), HG should be favoured in fruit development compared to HNG. The lack of size differentiation between HG and HNG could be partially dependent on girdling application timing. Being applied in the late cytokinesis stage, G effect on fruit size and weight, could have been limited if compared to earlier timing of application. If not, the presented situation suggests again that resource exchange happened in favour of HNG increasing its fruit size up to HG ones.

A confirm to that can be seen also from fruit weight : diameter (w:t) ratios that can be utilized to inferring about fruit cortical density (~ fruit cell number). For these ratios, treatments ranking is LG (2.68, a) > LNG (2.46, b) > HG (2.16, c) > HNG (2.07, c), with same statistical results as for fruit size. By this it seems that higher fruit cortical density was actually present for LG compared to LNG, while not for HG compared to HNG. These results seem to support what exposed above, since HNG reached HG fruit size, probably thanks to a similar fruit cells number and not thanks to a higher cells volume. Considering the lower leaf area dedicated to each fruit, the similar fruit size and fruit cortical density (~ fruit cell number) of HNG, compared to HG, an external support to HNG fruit development seemed occurred. Again, out-of-trial uprights present on the trees were involved in the definition of inter-uprights sink-demand gradient and so they affected the obtained results.

Regarding FF results, the higher w:t ratios of LNG compared to HG is not reflected in FF results. LNG bigger fruits, with higher w:t ratio compared to HG, should present higher FF than HG, if cortex density (~ fruit cell number) was responsible of this parameter. From the results, It seems more probable that FF was driven, in this case, by other factor than cell number contrasting the findings exposed above from Charles *et al.* (2018), supporting instead the hypothesis from Fallahi *et al.* (2018).

The results for G clearly showed how crop load strongly drove other fruit quality parameters: the higher the crop load, the lower the fruit quality. This is supported by literature as reported by Jakopič *et al.*,(2013), for example, who showed how apple SSC, size and color intensity are highly affected by crop load, decreasing their levels when crop load level increases. The same was found by Meland (2009), Robinson and Lopez (2012) and Serra *et al.*(2016). Also the blush color of the fruit, mainly represented by the parameter h\*\_BL (together with C\*\_BL), seems to be promoted by girdling first, and then by the lower crop load (L); this could be again related to the higher resource (i.e., carbohydrate) availability in G (compared to the respective NG crop load level) and L (compared to H). This situation could have led to a higher synthesis of uridine phosphate sugars, that are precursors of anthocyanin compound responsible for skin red color (Ban *et al.*, 2009) , and mainly represented by cyanidin-3-galactoside in red apple fruit skin (Lancaster and Dougall, 1992; Jakopič *et al.*, 2013).

In HNG, background h\* values suggest a redder color for the background (Table 2.3): this is due to a math artifact caused by the average of several negative values (i.e. color in the “green” area of CIE L\*C\*h\* color space) with values higher than those presented for “h\*\_BL” (pointing to yellowish background color).

### **Allometric-Quality Correlations**

The figures from 2.3 to 2.6 consider different fruit density expressions for NG and G treatments. Within these, L generally presents a high fruit diameter while H a lower size. Confirming what already exposed above regarding heavy crop load effect on fruit size (Meland, 2009; Robinson and Lopez, 2012; Jakopič *et al.*, 2013; Serra *et al.*, 2016). The correlation within G showed a stronger suggesting that fruit density is the overriding driver of fruit size, more important than the phloem connection.

HG presents almost always the highest r coefficients, and this implies that girdling matched with high crop load tends to emphasize the correlation; LG seems to produce generally clearer correlations

compared to LNG. The less clear NG situation supports the theory of non-independence between uprights: when resources can be exchanged, the correlations become weaker, likely due to the trafficking of carbohydrates among uprights. On the other hand, both NG treatments show correlations supporting the hypothesis that crop load is the main driver for fruit development despite girdling.

For H, girdling caused higher values above 70mm for fruit diameter, and also if size was not different (Table 2.2) a possible trend can be seen in the scatterplots (Figures 2.3-2.6) where HG shows more points above the 70mm fruit diameter if compared to HNG.

The fruit density levels presented in the results could be investigated as possible preliminary thinning threshold for single upright management to guarantee fruit size above 70 mm.

Fruit number per UCA (Figure 2.3) will probably be the most useful indicator to guide thinning intensity, as reported by the utilization of the “MAFCOT wheel” (or Equifruit Disk) in the field (Lespinasse and Lauri, 1999; MAFCOT, 2000; Kon *et al.*, 2012). This is supported by the fact that fruit density expressed on UCA was clearer cut than the other expressions tested.

### **Return Bloom**

Crop load seems to clearly drive return bloom in isolated uprights, while in NG the situation is less clear, probably reflecting the considerations about carbohydrate export outlined above. Only for leaf area flower density clear effect of crop load is present also in NG condition confirming the effect of H crop load in reducing return bloom also for connected upright. This support literature findings highlighting the inverse relation between crop load and return bloom (Serra *et al.*, 2016; Stefanelli *et al.*, 2018). Considering that leaf area is the main source supporting flower and fruit development, it is interesting notice the clear effect of crop load on this flower density, also for NG treatments, if compared to the other results. It might be that this more sink-source linked fruit density parameter, is able to better represent relation between flower inhibition and crop load levels.

Considering the aforementioned possible (and preliminary) crop load threshold, all the treatments present a flower density near to those thresholds. Pink Lady® -Rosy Glow variety can set up to 5-6 fruit per cluster, generally reduced to 1-3 (max) fruits per cluster during the thinning. Considering that only 1 fruit per flower cluster counted will be retained (a negative scenario), then the crop load treatments analyzed in this trial seem to guarantee an adequate flower differentiation to keep the same apple production in the following season. This suggest that treatments did not induce alternate bearing. Said that, is recognized how alternate bearing is strongly dependent on genetics characteristics (Byers, 2003), so results obtained can be considered valid only for the studied variety and need to be verified for biennial cultivars such as Fuji or Honeycrisp.

## **CONCLUSIONS**

In this work, it was investigated whether, in a modern 2-D training system, management techniques such as crop load regulation and girdling, could be adopted at the scale of the whole tree, as opposed to the individual fruit bearing upright. To do this, it was set up a set of contrasting crop load by girdling conditions and assessed them by physiological as well as horticultural parameters. This set up indicates that crop load is the overriding factor determining whether the individual upright can be considered as the appropriate management unit. For low crop conditions, the supply of carbohydrates to the fruit appears to be quite sufficient in both girdled and intact conditions. However, even though high crop load conditions appear able to stimulate the upright physiology (i.e., by sustained gas exchanges) they will prevent attaining large fruit unless they are able to attract carbohydrates from other, assimilate exporting, uprights. In fact, under high crop loads, girdling was not able to improve fruit size and quality attributes compared to the intact shoots. This is a clear indication that uprights are not to be considered as independent growth units in all cases. More work is needed to define the most useful and easy to determine allometric parameters capable of assessing this dependency.

Correlations between fruit density and quality parameters, were found, mainly for the two opposite situations of isolated high crop load level (HG) (where negative correlations were found for size and color) and connected low crop load level (LNG) (where negative correlations were found for SSC and positive for color).

For this 2D-training system, some fruit density thresholds were presented, which should improve fruit size independent of girdling. In the orchard, fruit number/cm<sup>2</sup> of UCA can be the most useful and applicable by the growers; in case of not uniform upright length development, fruit number per cm of length or per cm<sup>3</sup> of volume can be used as well.

Regarding return bloom, crop load seems the main driver and girdling enhances flower induction, but only for low (L) crop load. Connected (NG) uprights seem to help balancing flower production between high and low loaded uprights; for all the treatments in this study, return to bloom appeared more than enough in order to guarantee acceptable production in the following season.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mazzoni Farms and Michele Gerin for granting access to the orchard making this study possible.

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## CHAPTER 3

# Upright as fruiting unit for precise crop load management in a multileader training system: relationships between fruit load and fruit quality for apple

### ABSTRACT

Two-dimensional (2D) orchard training systems are innovation occurred over the past 50-70 years. In the last decades 2D training system are becoming increasingly used and, particularly multileader planar systems, are raising their interest in apple (*Malus domestica* Borkh.) production. Currently there is a lack of physiological, agronomical, and managerial information for these tree training systems; this needs to be overcome to obtain clear and objective instruction to address fruit sector management operations. A shift from the concept of entire plant management to a single leader (or upright), as fruiting management unit, is envisioned to ease orchard operation such as pruning and thinning. This study investigated upright crop load effect on its final fruit production, for 2020 season, according to three crop load level (H~14, M~10, L~3 fruit/cm<sup>2</sup> of upright-cross-sectional area-UCA) in a 4yrs old, multileader Pink Lady®-Rosy Glow apple system, located in Ferrara, Italy. The girdling technique was applied, on part of the trees in trial, to investigate interdependency between the different plant leaders. Allometric, flowering, fruit growth, production, and quality parameters were analysed so to find relationships allowing solid guidelines for the crop load management at upright level. A comparison between results for uprights placed on trees with, and without, girdled leaders is presented. Results confirmed the presence of an inverse relationship between upright crop load and final fruit quality; this was underlined by absolute growth rate and fruit size only for leaders placed on trees with girdled uprights, but not for the remaining trees. Unequivocal relationship between treatments and fruit quality was not found, but possible maximum thinning thresholds for high quality production were proposed (~10 fruit/cm<sup>2</sup> UCA). Treatments did not affect return bloom and vegetative growth. All treatments, but not H-girdled, led to a good quality apple production (fruit size >70mm) witnessing the absence of a limiting condition for leaders on intact trees (with no-girdled uprights) at the tested crop load levels. Uprights interconnection was confirmed, but the level of independence of each upright seems defined by individual uprights fruit load distribution and whole plant fruit load. Ultimately, crop load management based on a single upright unit seems feasible, but to obtain robust guidelines more studies are needed.

### ABBREVIATIONS

AGR = Absolute growth rate	IntactDS = Dataset with no girdling condition
BCA = Branch cross-sectional area	L = Low crop load condition
Col.Ext = Fruit Color extension color	LA = Leaf area
DAFB = Day After full bloom	LG= Low crop load girdled treatment
DS/DSS = Dataset / datasets	LI = Low crop load treatment for IntactDS
FD/FDs = Fruit density/ fruit densities	LNG= Low crop load not girdled treatment
FF = Flesh firmness	M = Medium crop load condition
FLD/FLDs = Flower density/flower densities	MG = Medium crop load girdled treatment
FLN = Flower cluster number	MI= Medium crop load treatment for IntactDS
FN = Fruit number	MNG = Low crop load not girdled treatment
G = Girdled condition	NG = Not girdled condition
GirdledDS = Dataset with girdled upright	SPI = Starch pattern index
H = High crop load condition	SSC = Soluble solid content
HG = High crop load girdled treatment	TCA = Trunk cross-sectional area
HI = High crop load treatment for IntactDS	UCA = Upright cross-sectional area
HNG= High crop load not girdled treatment	

## INTRODUCTION

Extremely narrow, two-dimensional (2D) orchard training systems are relatively new systems, that represent the most recent evolution of apple orchards that has taken place over the past 50-70 years (Palmer and Jackson, 1977; Tukey, 1978; Preston, 1978; Dunn and Stolp, 1981; Tustin *et al.*, 1989, 2018; Lespinasse, 1989; Sansavini, 1993; Corelli-Grappadelli, 1998; Robinson, 2003; Musacchi, 2007; Dorigoni *et al.*, 2011; Dorigoni and Micheli, 2018; Dallabetta *et al.*, 2021; Musacchi *et al.*, 2021). Even this concept of tree architecture was adopted along this timeframe, current 2D canopies thickness is intended for less 50 cm circa, from side to side of the row, and so far cry from even the thinnest 2D canopies of yesteryear. For current modern orchard, this greatly compresses the thickness of the single leader tree (e.g., tall spindle, slender spindle, vertical central leader, etc.), whose planting density range between fewer than 1000 to more than 5000 trees ha<sup>-1</sup>, with wide inter-row spacings (>3m) (Robinson *et al.*, 1991; Tustin *et al.*, 2001; Palmer *et al.*, 2002). The wide diffusion of central leader tree is mainly attributable to the utilization of dwarfing rootstocks that fostered the increase in orchard tree density (Ferree and Knee, 1997; Palmer and Adams, 1997; Tustin *et al.*, 2001; Ferree and Warrington, 2003; Musacchi *et al.*, 2021) (up to 6666 trees ha<sup>-1</sup>) leading to a substantial improvement in yields (Lordan *et al.*, 2018; Dallabetta *et al.*, 2021). After the beginning of the XXI century, 2D training systems have been adopted more frequently, in the quest to enhance yield, sustainability as well as mechanization and technological applications. Different authors (Robinson, 2007; Robinson *et al.*, 2013; Tustin, 2014) envisioned the future in orchard systems as a shift from mono-axis trees to tall, thin fruiting walls aiming to increase light interception and decrease shelf-shading. This vision was directly connected to an increase in yield and fruit quality (Tustin *et al.*, 2022) and a reduction of manpower thanks to thin and simple canopies able to match engineering needs for automation and mechanization problems (Zhang, 2018). A reduction in tree densities was also suggested (<3000 plant ha<sup>-1</sup>), to control planting and managements costs while maintaining orchard profitability (Tustin *et al.*, 2018; Zhang, 2018). Therefore, in the last two decades, ultra-thin 2D training system have been proposed for different fruit species. The apple industry interest, in different regions of the world (e.g., USA, Australia, Italy, New Zealand), is lately focused on multileader training systems that feature a high number of fruiting uprights (i.e., from 2 to more than 10) grafted to a single root system. This shape allows to better control tree vigour, reducing tree density and increasing orchard yields, sustainability and homogeneity in relation to management operations (e.g. pruning- spraying) (Dorigoni and Micheli, 2014, 2018; Dorigoni, 2016; Tustin *et al.*, 2018; Zhang, 2018).

2D training systems shift the focus from the single tree to homogeneous fruiting walls/rows but the level of independence of each of the fruiting wall elements (uprights) relative to the whole plant is largely unexplored, yet. In their approach, Tustin *et al.*, 2022 considered that the narrow/non-branched structure of their planar cordon concentrates canopy leaf area quite close to developing fruit, increasing carbon partitioning priority for short sink-source transport distances (Cieslak *et al.*, 2011). If confirmed, this would imply that planar training systems can shift the granularity of management operations to the single upright level, thus improving the overall precision in orchard management. However, this is still an open question due to a lack of information of the relationships between upright and tree; in fact, no grower guidelines for the management of this training system are available yet.

Breen *et al.*, (2021) asserted that in orchard, more specifically using innovative 2D training systems, there is a need for precise instructions for the complex management operations (e.g., pruning and thinning), that require a thorough understanding of the plant behaviour, so to be able to match it with unskilled labor and/or future automation/robotization.

To shift to single-leader management, a better understanding of upright independence is needed. In a recent study, on the double-guyot training system (Dorigoni and Micheli, 2018), Bortolotti *et al.* (in press) found that independence among uprights, seems to depend on the individual upright fruit load.

Crop load management is fundamental for high quality and profitable fruit production, but adequate crop load levels for training systems with a high number of leaders (i.e., >5) are currently unknown, or under early investigation. Multileader trees seem able to support crop load levels twice those for single leader trees (i.e., 8-12 vs 4-6 fruit/cm<sup>2</sup> of branch cross-sectional area -BCA- for planar cordon and spindle trees respectively)(Breen *et al.*, 2021). Despite this preliminary information, these crop load levels need to be better investigated and tested considering the interaction and the effect of the tree genetics and the growing environments (Breen *et al.*, 2021).

Shifting the management focus to the single upright could facilitate carrying out operations such as pruning and thinning, because of the simplified structural unit on which to work on. This promises to help the training, and improving efficiency, of unskilled field operators, as well as favouring automation, for such operations, in the near future. If clear and objective guidelines would be available, unskilled personnel and possibly machines/robots could replace (or support) humans in carrying out field operations (Robinson *et al.*, 2013; Tustin *et al.*, 2018; Zhang, 2018; Breen *et al.*, 2021).

The presented study aims to better investigate the effects of the individual upright crop load on final fruit size and quality, both from an agronomic and physiological point of view. The experiment examines the differences between girdled and intact upright, placed on different tress, so to define the specific resources allocations thus to obtain a possible crop load management guidelines at single-leader resolution, on 2D multileader training systems.

## MATERIALS AND METHODS

The study was carried out in 2020 in a commercial Pink Lady®-Rosy Glow apple (*Malus domestica*, Borkh.) orchard located in Ferrara, Italy (Lat: 44.765105, Long: 11.757721). The 4-year-old orchard was trained as a multileader system named double guyot (Dorigoni and Micheli, 2018) and grafted on Pajam 2 rootstock. This training system (Dorigoni and Micheli, 2018) is similar to a planar cordon (Tustin *et al.*, 2018) and based on two main branches that are bent down to an almost horizontal position (10-15° inclination) during the first year; from these horizontal permanent cordons, a variable number (4-7 per cordon) of vertical shoots are trained as semi-permanent fruiting leader (upright). Thus, each tree features between 8 and 14 uprights. The orchard spacing is 3.3m x 2.1 m, resulting in 1443 trees ha<sup>-1</sup>, and was managed following standard integrated management techniques.

Eighteen trees were selected in the orchard, yielding a total of 177 uprights. The uprights were rated according to their fruit number : upright length ratio (Len\_FD) and divided in three classes. The crop load of all the uprights was not imposed chemically or manually but reflected the upright natural fruit bearing capacity.

The Len\_FD ratio was computed with the following formula, Eq.1, where *FN* is the number of fruits on an upright and *Length(cm)*, the upright length in centimeter. *FN* and *Length(cm)* were collected on 03/06.

$$Len\_FD(fruit/cm) = \frac{FN}{Length(cm)} \quad (\text{Eq.1})$$

The threshold class values were determined in a preliminary assay on a sample of different upright sizes and crop loads (n=250). The mean Len\_FD of the sample was calculated and then, by adding and subtracting from it the sample standard deviation, crop load treatment thresholds were defined. Low (L), medium (M) and High (H) crop load corresponded to Len\_FD <0.05, 0.05<Len\_FD<0.1 and Len\_FD >0.1 respectively. 24 uprights, per crop load selected, belonged to 3 trees (8 per tree, for a total of 9 trees). Half of the uprights in each tree were randomly girdled (on 03/06) by removing a 0.5 cm wide strip of bark at their base, to interrupt the phloem connection and isolate the fruiting unit, while the rest were left interconnected to the tree. Thus, the treatments in the girdled dataset (GirdledDS), each with 12 replications, were: high crop load girdled (HG), high crop load non-girdled (HNG), medium crop load girdled (MG) and medium crop load non-girdled (MNG), low crop load girdled (LG) and low crop load non-girdled (LNG).

The remaining 9 trees constituted the intact dataset (IntactDS), in which all the uprights were kept intact and analyzed. The crop loads in this dataset were defined as for the GirdledDS. To avoid mistakes, and for ease of discussion, treatments for IntactDS are called HI, MI and LI respectively for high, medium, and low crop load levels. Due to the natural crop load random distribution of uprights, the single intact treatments were randomly distributed between the 9 selected trees; consequently, the numerosity of uprights in the treatments were different, being higher for MI (n=42) and lower for HI (n=36) and LI (n=27).

To clarify, part of the uprights in HG, HNG, LG, LNG treatments (GirdledDS) were the same analyzed in the previous chapter (Chapter 2), while all uprights belonging to MG, MNG (GirdledDS) and to HI, MI, LI (IntactDS) are not.

In the GirdledDS, fruit growth rates were monitored along the season. Absolute growth rate (AGR – g/day) was obtained dividing the weight difference between two subsequent measurement dates of the same fruit, by the number of days elapsed between the measurements (Boini *et al.*, 2019). Diameters were converted to weights utilizing an empirical relation (Eq.2,  $r > 0.99$ ;  $n > 300$ ) obtained by regressing fruit max diameter and fruit weight (respectively *Diam(mm)* and *Weight(g)* in Eq. 2) for more than 300 fruits, as done by Boini *et al.* (2019). AGR was monitored on 36 fruit per treatment.

$$Weight(g) = 0.0006 * Diam(mm)^{2.924} \quad (Eq.2)$$

On all the uprights of both datasets, length and diameter were recorded at harvest (25/10) by a digital caliper (Mitutoyo, Kanagawa, Japan) and a standard measuring tape. Length (cm) was measured from the top to the base of the upright; upright diameter was measured 10 cm above its base (and above the girdling). From these measurements upright cross-sectional area (UCA – cm<sup>2</sup>) and volume (Vol – cm<sup>3</sup>) were calculated. Leaf area (LA – m<sup>2</sup>) per upright was also calculated as follows. An empirical relation was developed between total lateral shoot length per upright and their leaf number (Eq.3,  $r = 0.82$ ;  $n = 310$ ). Then, total lateral shoot length (*total shoot length(cm)* in Eq.3) was measured on each upright (by a measuring tape), and total shoot leaf number per upright (*LeafNumber* in Eq.3) was computed applying the formula of Eq.3. Spur shoots were counted on each upright, and the average number of leaves, per spur, was determined by counting leaves on 300 randomly chosen spur shoots. The average leaf area of shoot and spur leaves was determined on a sample of 300 leaves per each shoot category and measured by a LI-COR 3100C leaf area meter (LI-COR, Lincoln, NE, USA).

$$LeafNumber = 0.3098 * total\ shoot\ length(cm) + 5.6751 \quad (Eq.3)$$

Total upright leaf area was computed by adding the total shoot and spur leaf areas obtained by multiplying the respective average leaf areas by shoot leaf number and by spur leaf number.

At harvest time, fruit were strip-picked, counted and their quality parameters were evaluated within each upright. Fruit maximum equatorial diameter (Diam – in mm) and weight (g) were measured by digital caliper (Mitutoyo, Kanagawa, Japan) attached to an external memory ([www.hkconsulting.it](http://www.hkconsulting.it)) and a digital scale (KB1200, KERN & SHON, Germany). Flesh firmness (FF – Kgf) was determined by a penetrometer (GÜSS Fruit Texture Analyser, GÜSS, South Africa) with an 11mm diameter tip after removing the fruit peel. Soluble solid content (SSC - °Brix) was determined by measuring the refractive index of the juice for each fruit with a digital refractometer (ATAGO PAL-1, ATAGO, Japan). Starch pattern index (SPI) was evaluated by a skilled operator that classified the Lugol solution iodine reaction with the starch present on a equatorial sliced fruit; SPI classification was done following the ten degradation classes (1-least ripen; 10- most ripen) suggested by Ctifl in “Le code amidon pomme” (CTIFL, 2002). Color parameters were measured by a Chroma meter (CR-400, Konica Minolta, Japan) on the blush and background sides of the fruit (most exposed and least exposed to the sun) and expressed as CIE L\*C\*h\* color-space coordinates (directly obtained from the instrument) where L\* represent lightness, C\* represent “chroma” (higher values means more intense color tone) and h\* “hue angle” (lower values means redder color tone – pure red = 0); C\* and h\* coordinates were considered as the most representative for the study analysis; one-flash measurement was used to collect the color data. Individual fruit over-color extension was also estimated by assessing the fruit area percentage covered by blush color (red-colored surface) from visual observation.

The fruit quality parameters were measured on all the fruit of GirdledDS; in the IntactDS only fruit diameter and weight were measured.

Flower density (FLDs - using spring 2021 data) and fruit density (FDs - using 2020 harvest data) variables were computed (with Eq. 4 and 5 respectively). Flower and fruit densities were expressed as number of flower clusters and fruits (*FLN* and *FN* in equations 4 and 5, respectively) divided by the chosen vegetative parameters (“*P*” in equations 4 and 5) that are UCA, length, volume and leaf area of each upright. “*Pu*” in the equations below means parameter measure unit (respectively cm<sup>2</sup>, cm, cm<sup>3</sup>, m<sup>2</sup> for the above cited parameters “*P*”).

$$P\_FLD(FLN/Pu) = \frac{FLN}{P(Pu)} \quad (Eq. 4)$$

$$P\_FD(FN/Pu) = \frac{FN}{P(Pu)} \quad (Eq. 5)$$

Computed FLDs ( $P\_FLD$  in Eq.4) were UCA\_FLD (FLN /cm<sup>2</sup>), Len\_FLD (FLN /cm), Vol\_FLD (FLN /cm<sup>3</sup>) and LA\_FLD (FLN /m<sup>2</sup>). Computed FDs ( $P\_FD$  in Eq.5) were UCA\_FD (FN /cm<sup>2</sup>), Len\_ FD (FN /cm), Vol\_ FD (FN /cm<sup>3</sup>) and LA\_ FD (FN /m<sup>2</sup>).

These parameters describe flower and fruit load considering the vegetative development of the uprights, and so better representing uprights sink/source condition. FD were chosen to express fruit load (instead of fruit number per upright) for that reason, and utilized in correlation analysis with fruit quality parameters, after harvest 2020.

The experimental designs for the two datasets were a balanced split plot design and an unbalanced randomized block design for GirdledDS and IntactDS respectively.

Data were analyzed with R and R-Studio using the “agricolae” and “stats” packages. ANOVA procedures and SNK POST-HOC method, were used to test statistical differences among treatments in both the datasets.

Data regarding upright length, fruit number and extracted parameters (e.g., fruit densities) resulted not stat. different between the two collection dates (i.e., 03/06 and 25/10); therefore, only harvest-time data (25/10) were chosen for the analysis, and presented in the results, because they better represent the tree productive result of the season.

For evaluating statistical differences among class size distributions in the different treatments, a correspondence multivariate analysis was performed, followed by a cluster analysis, with a chi-square ( $\chi^2$ ) test (Greenacre, 2007).

Pearson’s r correlation coefficient was used to investigate correlations between fruit quality and crop load. Only the correlations with  $r > 0.6$  are presented, as they are considered valuable by the authors.

## RESULTS

### *Allometries*

No statistical differences are present between the treatments neither for GirdledDS (in none of the possible crop load and girdling combination, Table 3.1,) neither for IntactDS (Table 3.2), for allometries. This shows a comparable vegetative growth situation in both the datasets. Despite this, for GirdledDS, some tendencies can be exposed: MG generally presents the lowest values in terms of vegetative parameters (UCA, Vol and LA) if not for length; on the other hand, LG presents the highest values for UCA, length and Vol. H has an intermediate behaviour showing highest values for LA. Generally, L and H seem to present similar magnitude for UCA, length and LA, but not for Vol. G shows lower values of UCA compared to NG, in all crop load levels while for Length, Vol and LA it shows lower values for H and higher for L. It is shown a tendency where G condition seems to increase vegetation (but not UCA) in L crop load level, while reducing it in H.

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Table 3.1. Allometries for “GirdledDS”: Upright Cross-sectional Area (UCA)(cm<sup>2</sup>), Length (cm), Volume (Vol)(cm<sup>3</sup>) and Leaf Area (LA)(m<sup>2</sup>) for ‘Rosy Glow’ apple grown in Ferrara (Italy) and trained as multileader. Mean separation in columns by SNK test.  $P < 0.05$ . (N=12)

	<b>UCA</b>	<b>Length</b>	<b>Vol</b>	<b>LA</b>
<b>HG</b>	1.99	199	144	0.837
<b>MG</b>	1.58	197	111	0.626
<b>LG</b>	2.38	226	183	0.832
<b>HNG</b>	2.02	204	144	0.899
<b>MNG</b>	1.78	189	122	0.642
<b>LNG</b>	2.47	202	176	0.733
<b>Sign.<sup>1</sup></b>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

Table 3.2 presents IntactDS trends. In this case also, no statistical differences are present between the crop load treatments. However, LI presents generally the highest values for all the parameter (Length, Vol, LA), but not for UCA that reach the maximum in MI and minimum in HI.

Table 3.2. Allometries for “IntactDS”: Upright Cross-sectional Area (UCA)(cm<sup>2</sup>), Length (cm), Volume (Vol)(cm<sup>3</sup>), Leaf Area (LA)(m<sup>2</sup>) for ‘Rosy Glow’ apple grown in Ferrara (Italy) and trained as multileader. Mean separation in columns by SNK test.  $P < 0.05$ . (N=27-36)

	<b>UCA</b>	<b>Length</b>	<b>Vol</b>	<b>LA</b>
<b>HI</b>	1.63	188	109	0.738
<b>MI</b>	1.80	182	127	0.656
<b>LI</b>	1.74	193	131	0.759
<b>Sign.<sup>1</sup></b>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

### **Fruit Density**

Table 3.3 shows the fruit number (FN) at harvest per each treatment and relative fruit densities (FDs) expressed in terms of UCA, length, volume and leaf area. Minimum and maximum FDs values are present in LG and HNG treatment respectively, while intermediate values are present for M. For all the parameters considered, the magnitude differences between the girdling treatments are around 2 and 3-to-5 fold respectively for L to M and L to H condition. M presents generally values that are 30 to 50% smaller of H. All the FDs are statistically different among crop loads (H, M, L) but not for volume fruit density where H and M are not different (Table 3.3). Girdling (G, NG) causes no differences within the same crop load level for FDs, while only in H, presents statistical difference between G and NG for fruit number.

Table 3.3. “GirdledDS” Fruit number (FN) and fruit density (FD) in relation to UCA (UCA\_FD) (FN/cm<sup>2</sup>), Length (Len\_FD) (FN/cm), Volume (Vol\_FD) (FN/cm<sup>3</sup>), Leaf Area (LA\_FD) (FN/m<sup>2</sup>) for ‘Rosy Glow’ apple grown in Ferrara (Italy) and trained as multileader. Mean separation in columns by SNK test.  $P < 0.05$ . (N=12)

	<i>FN</i>	<i>UCA_FD</i>	<i>Len_FD</i>	<i>Vol_FD</i>	<i>LA_FD</i>
<b>HG</b>	24.2 b	13.7 a	0.122 a	0.240 a	34.0 a
<b>MG</b>	14.6 c	9.9 b	0.074 b	0.163 a	24.4 b
<b>LG</b>	6.7 d	2.9 c	0.029 c	0.039 b	8.6 c
<b>HNG</b>	29.5 a	15.6 a	0.143 a	0.237 a	36.7 a
<b>MNG</b>	14.0 c	9.3 b	0.074 b	0.157 a	23.6 b
<b>LNG</b>	6.5 d	3.1 c	0.032 c	0.049 b	11.0 c
<b>Sign.<sup>1</sup></b>	***	***	***	***	***

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

Table 3.4 shows how within each variable, for Intact DS, all crop level treatments result significantly different from each other. Values for presented variables are generally similar (FN, Len\_FD) or higher (UCA\_FD, Vol\_FD, LA\_FD) than the one presented in Table 3.3, for same crop load levels.

Table 3.4. "IntactDS" Fruit number (FN) and fruit density (FD) in relation to UCA (UCA\_FD) (FN/cm<sup>2</sup>), Length (Len\_FD) (FN/cm), Volume (Vol\_FD) (FN/cm<sup>3</sup>), Leaf Area (LA\_FD) (FN/m<sup>2</sup>) for 'Rosy Glow' apple grown in Ferrara (Italy) and trained as multileader. Mean separation in columns by SNK test.  $P < 0.05$ . (N=27-36)

	<i>FN</i>	<i>UCA_FD</i>	<i>Len_FD</i>	<i>Vol_FD</i>	<i>LA_FD</i>
<b>HI</b>	24.2 a	16.1 a	0.129 a	0.283 a	38.2 a
<b>MI</b>	13.9 b	10.5 b	0.076 b	0.197 b	26.3 b
<b>LI</b>	6.3 c	4.0 c	0.031 c	0.081 c	8.8 c
<b>Sign.<sup>1</sup></b>	***	***	***	***	***

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

### Fruit Quality

Fruit quality parameters for GirdledDS are presented in Table 3.5. Fruit diameter and weight follow a similar pattern where G seems to statistically improve dimension for L (+2.6mm and +24g), but not for H and M which have closer values.

Flesh firmness (FF) behave differently being improved by girdling in every crop load level; M and L follow the same trend grouping both for G and NG, while HG presents an intermediate behaviour; HNG scores the lowest value of all.

SSC presents values that clearly show the effect of G with values up to +2.2°Brix compared to NG. No statistical differences are present among the crop load treatments but a possible trend can be evaluated in the treatments in which the H seems to have lower values than L.

SPI presents differences only between MG and LNG, while all the other treatments show similar, intermediate values. Also here, for SPI, an increasing trend from H to L can be seen.

Table 3.5. Fruit quality parameters of "GirdledDS" expressed as diameter (Diam.) (mm), Weight (g), flesh firmness (FF) (kgf), SSC (°Brix), Starch Pattern Index (SPI) (SPI class mean) for 'Rosy Glow' apple grown in Ferrara (Italy) and trained as multileader. Mean separation in columns by SNK test.  $P < 0.05$ . (N=60-324).

	<i>Diam.</i>	<i>Weight</i>	<i>FF</i>	<i>SSC</i>	<i>SPI</i>
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<b>HG</b>	69.9	c	150	c	9.53	ab	16.5	a	8.96	ab
<b>MG</b>	71.7	c	164	c	9.68	a	16.9	a	8.78	b
<b>LG</b>	77.4	a	208	a	9.82	a	17.1	a	9.04	ab
<b>HNG</b>	70.2	c	150	c	8.73	c	14.3	b	9.00	ab
<b>MNG</b>	71.1	c	158	c	9.18	b	14.8	b	8.98	ab
<b>LNG</b>	74.9	b	184	b	9.14	b	15.1	b	9.16	a
<b>Sign.<sup>1</sup></b>	***		***		***		***		*	

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

In Table 3.6 fruit quality parameters are shown for IntactDS, where only fruit diameter and weight are present. For these two parameters treatments do not show differences and the presented values result very similar between all the treatments with a range of less than 1 mm and 4 g for diameter and weight respectively.

Table 3.6. Fruit quality parameters of “IntactDS” expressed as diameter (Diam) (mm), Weight (g) for ‘Rosy Glow’ apple grown in Ferrara (Italy) and trained as multileader. Mean separation in columns by SNK test.  $P < 0.05$ . (N=169-870).

	<b>Diam</b>	<b>Weight</b>	<b>FF</b>	<b>SSC</b>	<b>SPI</b>
<b>HI</b>	72.3	170	NA	NA	NA
<b>MI</b>	72.5	171	NA	NA	NA
<b>LI</b>	72.9	174	NA	NA	NA
<b>Sign.<sup>1</sup></b>	ns	ns			

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

NA: not available

Color development in GirdledDS is described in Table 3.7, with color extension (Col. Ext.) and color intensity parameters. Col.Ext. shows higher values in G compared to NG. A clear trend among crop loads shows H with lower values while L tends to have higher colored surfaces in both G and NG.

The color intensity parameters  $h^*$  and  $C^*$  follow a similar pattern to color extension improving in G compared to NG; an inverse relation to crop loads is present for  $C^*_{BL}$  and  $C^*_{bg}$ , while  $h^*_{BL}$  and  $h^*_{bg}$  present an opposite behaviour. Ranking from greenest to reddest shows HNG first, followed by MNG, LNG, HG, MG, LG respectively, for blush color. The same ranking is valid for background colors, also if  $h^*_{bg}$  values suggest differently: this is due to math artifact caused by the average of several negative values (i.e. color in the “green” area of CIE  $L^*C^*h^*$  color space) with values higher than those presented for “ $h^*_{BL}$ ” (pointing to yellowish background color).

Table 3.7. “GirdledDS” Fruit Color extension (Col. Ext) (% of blush area) and intensity parameter expressed as  $C^*$  and  $h^*$  coordinates, in  $L^*C^*h^*$  color space, for both blush (BL) and background (bg) color, for ‘Rosy Glow’ apple grown in Ferrara (Italy) and trained as multileader. Mean separation in columns by SNK test.  $P < 0.05$ . (N=60-324).

	<b>Col. Ext</b>	<b><math>h^*_{BL}</math></b>	<b><math>C^*_{BL}</math></b>	<b><math>h^*_{bg}</math></b>	<b><math>C^*_{bg}</math></b>					
<b>HG</b>	76.0	b	34.4	bc	44.0	b	36.7	ab	38.4	bc

<b>MG</b>	81.6	ab	32.2	cd	45.5	b	54.0	a	39.5	b
<b>LG</b>	86.3	a	30.4	d	47.5	a	52.8	a	46.9	a
<b>HNG</b>	67.1	c	38.1	a	39.5	d	27.1	b	33.8	d
<b>MNG</b>	75.0	b	36.1	ab	41.3	c	44.0	ab	34.3	cd
<b>LNG</b>	77.9	b	34.4	bc	44.5	b	48.7	ab	37.0	bcd
<b>Sign.<sup>1</sup></b>		***		***		***		*		***

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

### Fruit Size Class Distribution

In Figure 3.1 are presented the mean upright fruit size class distribution per treatment for 6 marketable diameter classes (<65mm, 65-70mm, 70-75mm, 75-80mm, 80-85mm, >85mm). LG and LNG behave similarly presenting the highest frequency (47% and 43% respectively) for the 75-80mm class; LNG presents then more fruit below the 75-80mm class (37%) while LG is more balanced in frequency of fruit above (27%) and below (26%) this class.

HNG and MG follow a similar pattern where the highest frequency is in 70-75mm class (with 40% and 43% respectively); MG seems balanced for fruit above (23%) and below its median, with a slightly higher frequency below that (37%), while HNG presents a class distribution clearly shifted towards lower diameter classes, presenting 65-70mm as its second highest frequency class (37%), comparable to MG (26%).

The MNG and HG treatments present a flatter distribution, shifted towards the smallest diameter class compared to the treatments previously discussed; most of the fruit belong to classes <75mm. The most represented class is 70-75mm for both the treatments with frequency values of 32% and 28% (respectively for MNG and HG), that is close to the frequency recorded for 65-70mm (29-27% for MNG-HG); HG presents the highest frequency value for unmarketable fruit (<65mm) with 23%. Cluster analysis and a  $\chi^2$  test shows statistical differences between L (G and NG cluster) and all the other treatments; the other treatments are not different among each other in their distribution.

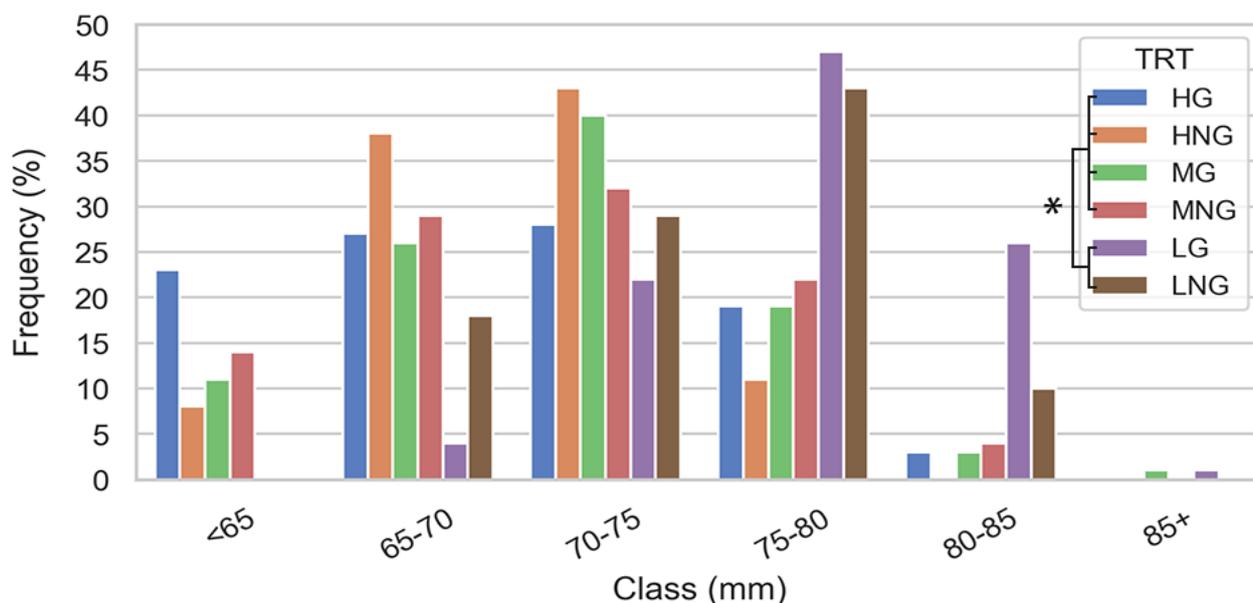


Figure 3.1 - Fruit size class distribution of "GirdledDS" for classes: <65mm, 65-70mm, 70-75mm, 75-80mm, 80-85mm, 85+. Frequencies are expressed as percentage in respect to the total number of fruits (N=60-324). Data for 'Rosy Glow' apple grown in Ferrara -Italy- and trained as multileader. \* in the legend means statistical difference in the distributions between the cluster [LG;LNG] and the cluster [HG;HNG;MG;MNG].

Figure 3.2 shows a normal distribution pattern for the IntactDS. In general, the fruit size class distribution is shifted towards higher fruit diameters in LI, even though this same treatment shows the highest percentage of unmarketable fruit (<65mm - 9 %). The most represented fruit size class is 70-75mm for all the treatments with values of 41%, 45% and 45% respectively for LI, MI, and HI; the second most represented class is 65-70mm for HI (26%) while 75-80mm for LI (27%); MI presents both 65-70mm and 75-80mm class as second most represented fruit size class with a value of 23%. However, the cluster analysis and  $\chi^2$  test do not show statistical differences between the treatments.

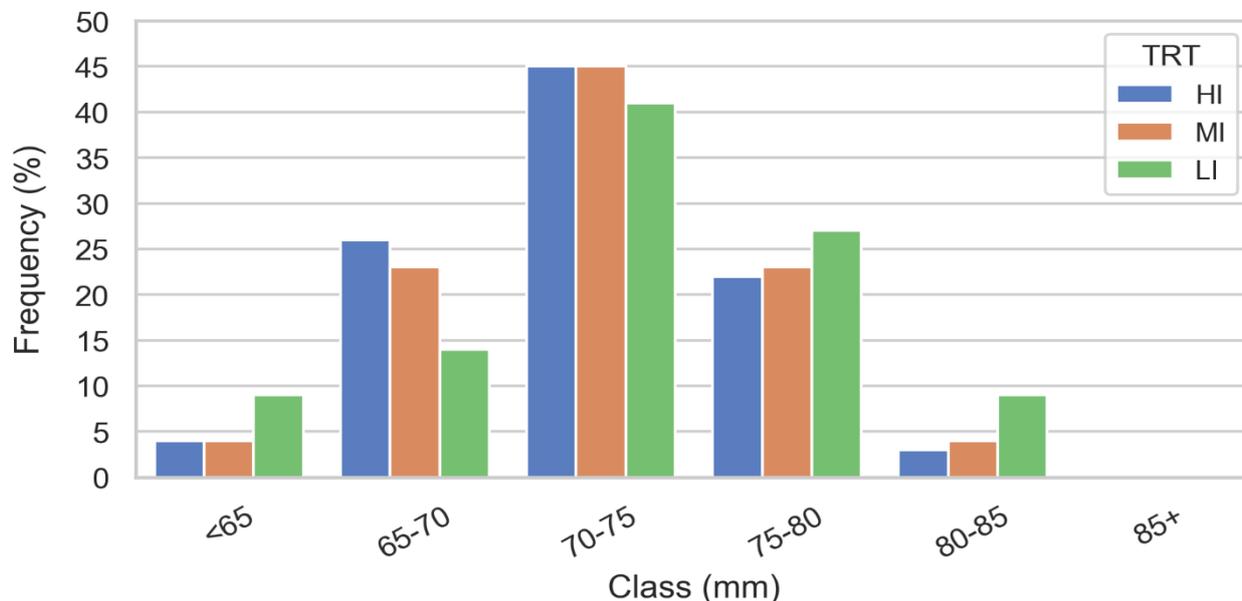


Figure 3.2 - Fruit size class distribution for “IntactDS” for classes: <65mm, 65-70mm, 70-75mm, 75-80mm, 80- line plot sns85mm, 85+. Frequencies are expressed as percentage in respect to the total number of fruits (N=169-870). Data for ‘Rosy Glow’ apple grown in Ferrara -Italy- and trained as multileader.

### **Fruit Growth**

Fruit growth data were collected only in the GirdledDS, as it was wanted to test differences in growth between G and NG. Full bloom occurred on 04/04/2020. In the first period of measurement, from 60 (girdling date) to 75 days after full bloom (DAFB), girdling seems to speed up fruit absolute growth rate (AGR) for each crop load level (Figure 3.3), while afterwards differences are observable between G and NG only in L, and until 118 DAFB.

At 131 DAFB, LG and LNG present significantly higher values than HG, while the other treatments present intermediate values. At 146 DAFB, only AGR values for LNG and MNG are different (higher) from all the other treatments. Generally highest AGR are present for L treatment in both girdling conditions, while H shows the lowest with intermediate values for M.

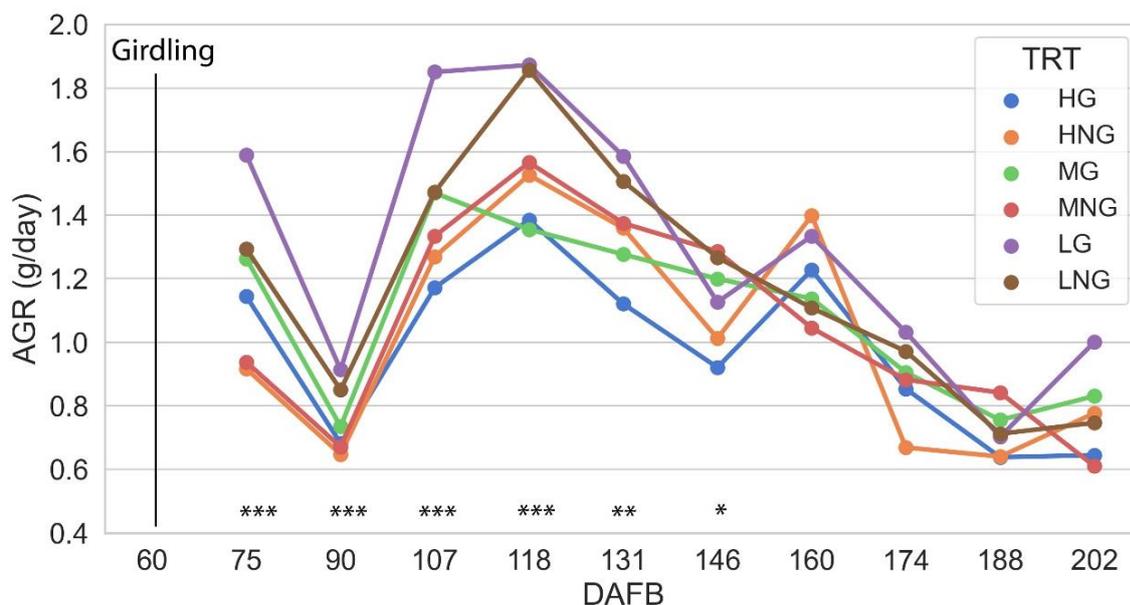


Figure 3.3 – “GirdledDS” Fruit Absolute Growth Rate (AGR) for different measurements along 2020 season for ‘Rosy Glow’ apple grown in Ferrara (Italy) and trained as multileader. Measurements’ timings are expressed as days after full bloom (DAFB). Girdling was applied at 60 DAFB. \*, \*\* and \*\*\* means significant effect  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively. (N=36).

Mean seasonal AGRs, presented in Figure 3.4 (X axis), shows the exact same trend and statistical differentiation as fruit size and weight classification (Table 3.5). Mean seasonal AGR values per treatments are: 1.09 (HG), 1.21 (MG), 1.46 (LG) and 1.16 (HNG), 1.1 (MNG), 1.33 (LNG) grams per day<sup>-1</sup>. Statistical differences are present for LG (1<sup>st</sup> statistical group “a”), LNG (2<sup>nd</sup> statistical group “b”) and the other treatments (belonging to the 3<sup>rd</sup> stat group “c”); statistical significance presents a  $P < 0.001$  for ANOVA analysis.

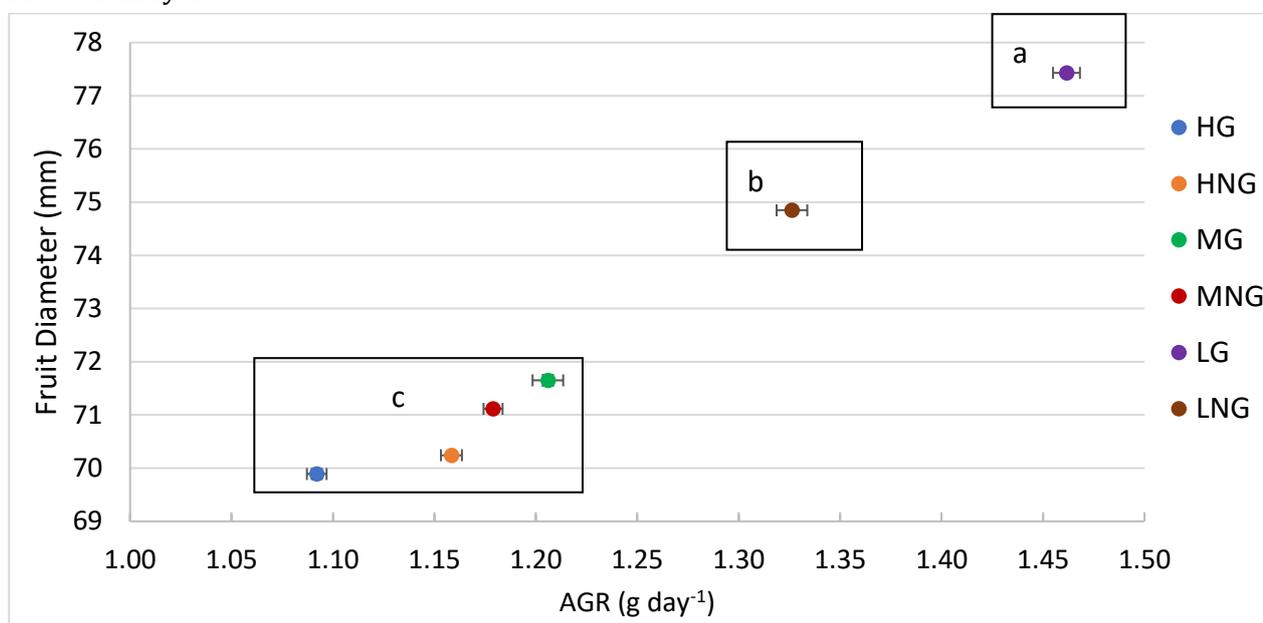


Figure 3.4-Scatterplot presenting mean fruit diameter (Y axis) and mean seasonal AGR (X axis) per treatment of “GirdledDS”, for ‘Rosy Glow’ apple grown in Ferrara (Italy) and trained as multileader. Standard error bars for axis Y are present but not visible due to the factor scale. (N=12)

### Allometric-Quality Correlations

Correlations between fruit density expressions and fruit quality were investigated (Table 3.8). Only correlations with  $r > 0.60$  were considered valuable. G condition presents many correlations: fruit

size presents a strong, negative correlation with UCA fruit density in H while M shows negative correlations both with UCA\_FD and Vol\_FD. In G condition, UCA\_FD shows negative correlations to H and M and a positive one for L, for fruit weight, which also appears correlated to volume fruit density in M and L. SSC always shows negative correlations with fruit density: with UCA\_FD and Len\_FD for LNG and with LA\_FD both for MG and HG; in HG, SSC shows correlation also with Vol\_FD. The color extension appears correlated only in G and always negatively with UCA, volume (in H, M and L), and LA fruit density (in H and M). Color ( $h^*_BL$  – blush color tone), presents similar correlation of color extension for HG, but with positive and higher rates; Vol\_FD presents relationship also in MG, NG and LNG, while UCA\_FD is correlated only with MG.

HNG did not show any correlations between fruit quality and fruit density data.

IntactDS data are not presented because no correlations with values of  $r > 0.60$  were found.

Table 3.8. Linear correlations between fruit quality and vegetative parameters per each treatment of “GirdledDS”, for 4 yrs-old ‘Rosy Glow’ apple trees grown in Ferrara (Italy) and trained as multileader. Pearson’s “r” coefficients and relative p-values for linear correlation between parameters are presented. Values in bold characters are considered in the text ( $r > 0.60$ ). (N=12)

	<i>HG</i>		<i>MG</i>		<i>LG</i>		<i>HNG</i>		<i>MNG</i>		<i>LNG</i>	
	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>
<sup>3</sup> <i>Diam vs UCA_FD</i> <sup>2</sup>	<b>-0.71</b>	**	<b>-0.62</b>	*	0.29	<i>ns</i>	-0.34	<i>ns</i>	-0.47	<i>ns</i>	-0.15	<i>ns</i>
<sup>3</sup> <i>Diam vs Vol_FD</i> <sup>2</sup>	-0.58	*	<b>-0.61</b>	*	0.29	<i>ns</i>	-0.42	<i>ns</i>	<b>-0.63</b>	*	-0.30	<i>ns</i>
<sup>3</sup> <i>Weight vs UCA_FD</i> <sup>2</sup>	<b>-0.71</b>	**	<b>-0.70</b>	*	<b>0.67</b>	*	-0.32	<i>ns</i>	<b>-0.44</b>	<i>ns</i>	0.13	<i>ns</i>
<sup>3</sup> <i>Weight vs Vol_FD</i> <sup>2</sup>	-0.53	<i>ns</i>	<b>-0.75</b>	**	<b>0.72</b>	*	-0.31	<i>ns</i>	<b>-0.61</b>	*	-0.02	<i>ns</i>
<sup>3</sup> <i>Weight vs LA_FD</i> <sup>2</sup>	-0.54	<i>ns</i>	-0.38	<i>ns</i>	<b>0.63</b>	<i>ns</i>	-0.08	<i>ns</i>	-0.45	<i>ns</i>	<b>0.61</b>	<i>ns</i>
<sup>3</sup> <i>FF vs LA_FD</i> <sup>2</sup>	-0.18	<i>ns</i>	0.12	<i>ns</i>	<b>-0.65</b>	<i>ns</i>	-0.28	<i>ns</i>	0.00	<i>ns</i>	0.08	<i>ns</i>
<sup>3</sup> <i>SSC vs UCA_FD</i> <sup>2</sup>	-0.46	<i>ns</i>	-0.13	<i>ns</i>	-0.29	<i>ns</i>	-0.16	<i>ns</i>	-0.19	<i>ns</i>	<b>-0.69</b>	*
<sup>3</sup> <i>SSC vs Len_FD</i> <sup>2</sup>	-0.21	<i>ns</i>	0.07	<i>ns</i>	-0.36	<i>ns</i>	0.20	<i>ns</i>	-0.18	<i>ns</i>	<b>-0.74</b>	*
<sup>3</sup> <i>SSC vs Vol_FD</i> <sup>2</sup>	<b>-0.68</b>	*	-0.29	<i>ns</i>	-0.13	<i>ns</i>	-0.35	<i>ns</i>	-0.38	<i>ns</i>	-0.53	<i>ns</i>
<sup>3</sup> <i>SSC vs LA_FD</i> <sup>2</sup>	<b>-0.72</b>	**	-0.39	<i>ns</i>	<b>-0.76</b>	*	-0.54	<i>ns</i>	-0.48	<i>ns</i>	-0.46	<i>ns</i>
<sup>3</sup> <i>Col.Ext. vs UCA_FD</i> <sup>2</sup>	<b>-0.70</b>	**	<b>-0.61</b>	*	<b>-0.70</b>	<i>ns</i>	0.12	<i>ns</i>	-0.39	<i>ns</i>	0.00	<i>ns</i>
<sup>3</sup> <i>Col.Ext. vs Len_FD</i> <sup>2</sup>	-0.55	<i>ns</i> *	-0.10	<i>ns</i>	0.44	<i>ns</i>	0.04	<i>ns</i>	-0.40	<i>ns</i>	-0.58	<i>ns</i>
<sup>3</sup> <i>Col.Ext. vs Vol_FD</i> <sup>2</sup>	<b>-0.72</b>	**	-0.59	*	<b>-0.76</b>	<i>ns</i>	-0.11	<i>ns</i>	-0.48	<i>ns</i>	0.31	<i>ns</i>
<i>Col.Ext. vs LA_FD</i> <sup>2</sup>	<b>-0.78</b>	**	<b>-0.68</b>	*	-0.38	<i>ns</i>	-0.23	<i>ns</i>	-0.45	<i>ns</i>	-0.53	<i>ns</i>
<sup>3</sup> <i>Color (h*_BL) vs UCA_FD</i> <sup>2</sup>	<b>0.77</b>	**	<b>0.61</b>	*	0.59	<i>ns</i>	0.06	<i>ns</i>	0.24	<i>ns</i>	0.47	<i>ns</i>
<sup>3</sup> <i>Color (h*_BL) vs Vol_FD</i> <sup>2</sup>	<b>0.85</b>	***	<b>0.72</b>	**	<b>0.66</b>	<i>ns</i>	0.36	<i>ns</i>	0.26	<i>ns</i>	<b>0.72</b>	*
<sup>3</sup> <i>Color (h*_BL) vs LA_FD</i> <sup>2</sup>	<b>0.88</b>	***	0.47	<i>ns</i>	0.34	<i>ns</i>	0.43	<i>ns</i>	0.27	<i>ns</i>	0.18	<i>ns</i>

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

<sup>2</sup> UCA\_FD, Len\_FD, Vol\_FD and LA\_FD are Fruit density (FD) expressed as the ratio of fruit number by upright cross-sectional area, length, volume and leaf area respectively

<sup>3</sup> Diam, FF, SSC, Col.Ext, Color(h\*BL) are fruit diameter, flesh firmness, soluble solid content, color extension and color hue angle for blush, respectively

Apples to be sold in the Pink Lady®-Rosy Glow marketable category must achieve specified minimum quality requirements for size (60-65mm), appearance (no/minimum defect) and color (minimum surface with bright “pink” color, i.e. 20-60% based on the premium class considered) (UNECE, 2020; Pink Lady® quality manual, 2021). Relationships between fruit diameter at harvest and upright fruit density were investigated with the aim to create a list of FD thresholds below which most of the fruit would achieve a premium size for Italian Pink Lady®-Rosy Glow apple market (i.e.,  $\geq 70$  mm). The Figures 3.5 to 3.8 present the scatterplots of these relations.

From the data available in this study, most of the fruit with diameter  $\geq 70$  mm seem to occur below the following densities:

- 8-12 fruit/cm<sup>2</sup> of upright cross-sectional area
- 0.07-0.10 fruit/cm of upright length
- 0.15-0.20 fruit/cm<sup>3</sup> of upright volume
- 20-30 fruit/m<sup>2</sup> of upright leaf area

Fruit quality results underline that most of the fruit  $>70$ mm size belong to both G and NG in the L (which have no fruit below 72mm diameter on average) and M treatments. In H, fewer than 50% of fruit achieve this size. The G coefficients seem higher than those for NG (Table 3.8). The strength and the type (e.g., positive or negative) of correlation depend on the treatment, as represented in Table 3.8. The scatterplots from figure 3.5 to 3.8 show also general correlations among all the treatments; UCA\_FD and Vol\_FD show the highest general correlations with fruit diameter (both with  $r = -0.72$ ), while Len\_FD the lowest ( $r = -0.58$ ), and LA\_FD presenting an intermediate value ( $r = -0.65$ ).

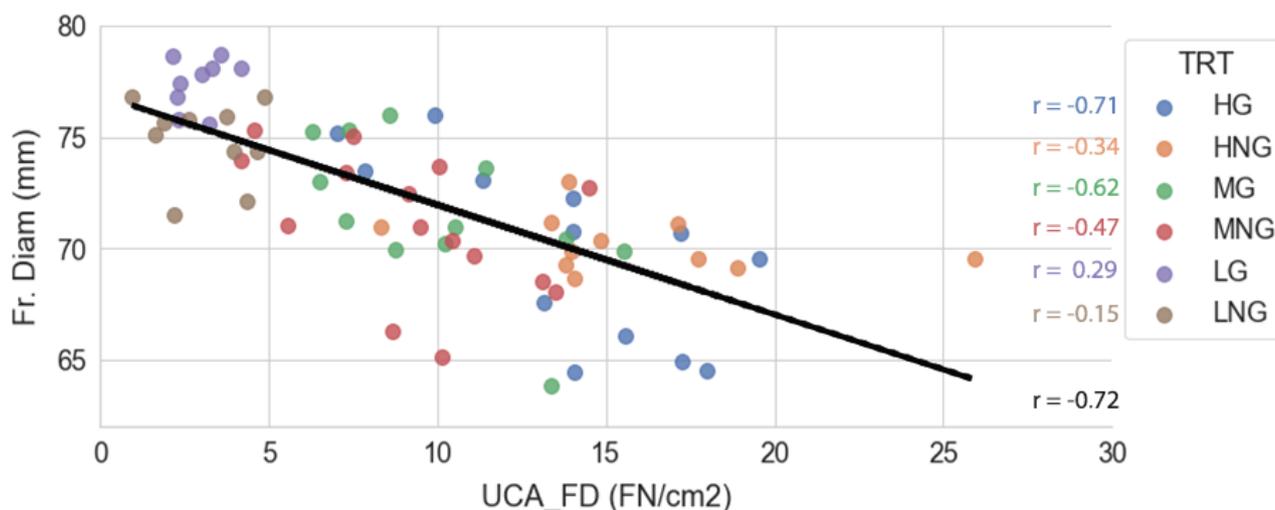


Figure 3.5 – Scatterplot showing mean upright fruit diameter (Fr.Diam) vs mean upright UCA fruit density (UCA\_FD) for ‘Rosy Glow’ apple grown in Ferrara (Italy) and trained as multileader. Correlation coefficient values are presented for each treatment (N=12). The black line represents the general trend among all the treatments (N=72).

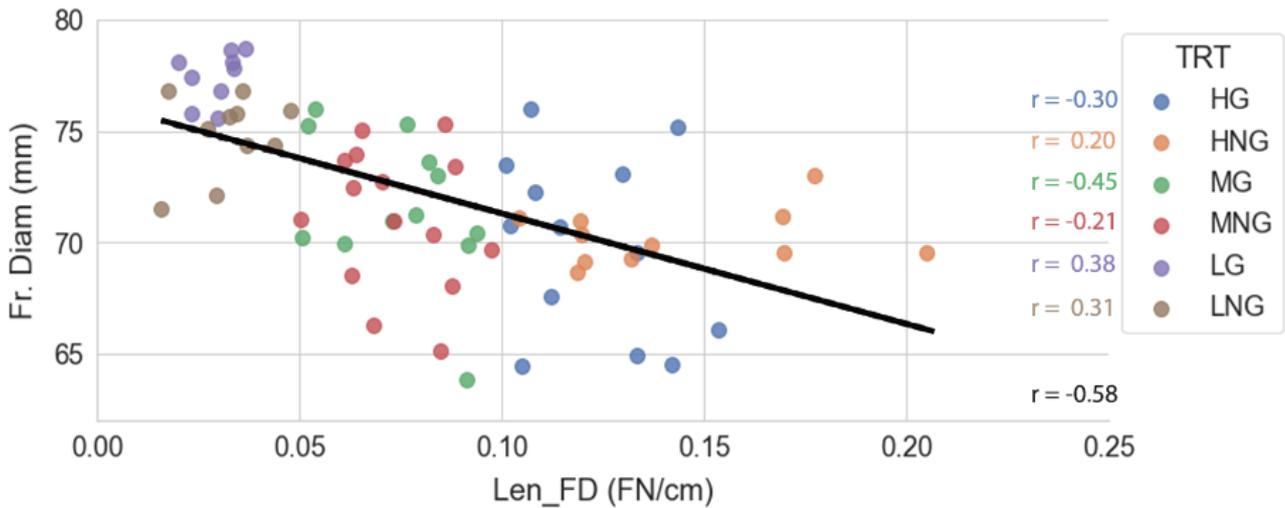


Figure 3.6- Scatterplot showing mean upright fruit diameter (Fr.Diam) vs mean upright length fruit density (Len\_FD) for 'Rosy Glow' apple grown in Ferrara (Italy) and trained as multileader. Correlation coefficient values are presented for each treatment (N=12). The black line represents the general trend among all the treatments (N=72).

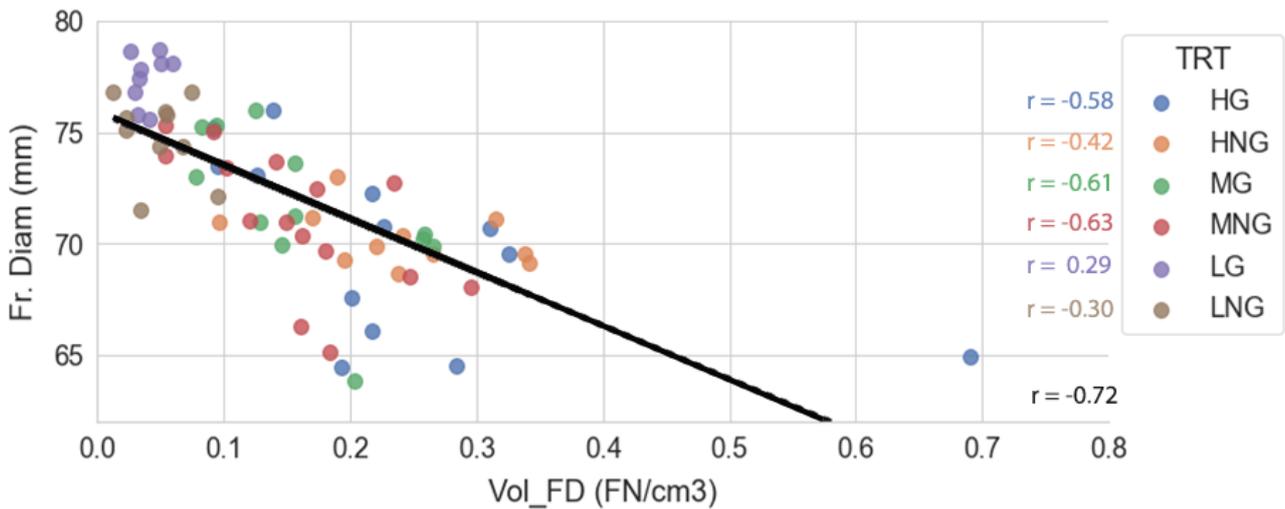


Figure 3.7- Scatterplot showing mean upright fruit diameter (Fr.Diam) vs mean upright volume fruit density (Vol\_FD) for 'Rosy Glow' apple grown in Ferrara (Italy) and trained as multileader. Correlation coefficient values are presented for each treatment (N=12). The black line represents the general trend among all the treatments (N=72).

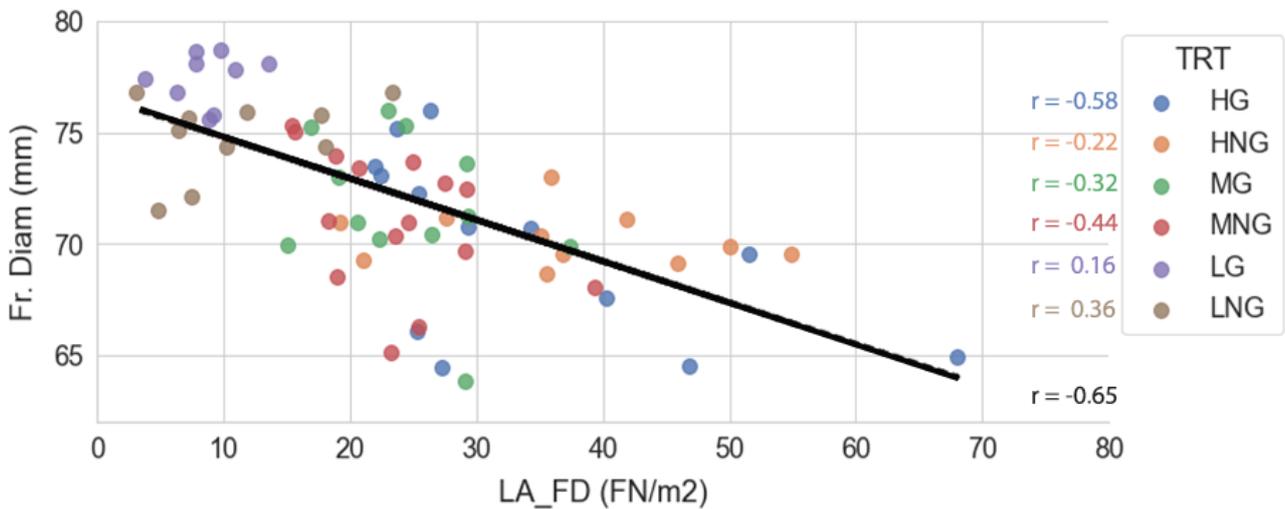


Figure 3.8 - Scatterplot showing mean upright fruit diameter (Fr.Diam) vs mean upright leaf area fruit density (LA\_FD) for 'Rosy Glow' apple grown in Ferrara (Italy) and trained as multileader. Correlation coefficient values are presented for each treatment (N=12). The black line represents the general trend among all the treatments (N=72).

### Return Bloom

The analysis of the distribution between the different datasets of flower cluster number in spring 2021 is presented in Table 3.9, for GirdledDS. No significant differences are present between the treatments for any of the flower densities (FLDs) evaluated. Flower clusters number (FLN) and densities appear sufficient to reach the crop load and FDs recorded during the previous season and exposed in Table 3.3. Despite no difference, highest values for flower densities are present for LG (in UCA, Len and LA FLDs) and MNG (in UCA and Vol FLDs).

Table 3.9. GirdledDS flower clusters number (FLN) and flower density (FLD) in relation to UCA (UCA\_FLD) (FLN/cm<sup>2</sup>), length (Len\_FLD) (FLN/cm), volume (Vol\_FLD) (FLN/cm<sup>3</sup>), Leaf Area (LA\_FLD) (FLN/m<sup>2</sup>) for 'Rosy Glow' apple grown in Ferrara (Italy) and trained as multileader - 2021 spring data. Mean separation in columns by SNK test. P< 0.05. (N=12)

	<i>FLN</i>	<i>UCA_FLD</i>	<i>Len_FLD</i>	<i>Vol_FLD</i>	<i>LA_FLD</i>
<b>HG</b>	24.6	12.6	0.116	0.189	29.0
<b>MG</b>	22.8	12.7	0.105	0.182	35.1
<b>LG</b>	31.8	14.8	0.141	0.201	41.8
<b>HNG</b>	25.6	12.9	0.122	0.193	30.5
<b>MNG</b>	21.6	14.8	0.116	0.251	38.2
<b>LNG</b>	25.6	12.4	0.126	0.193	40.8
<b>Sign.<sup>1</sup></b>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively.

Similar results are present for the IntactDS (Table 3.10) where all flower densities in the various treatments show no differences, but for leaf area flower density where treatment MI scores the highest value, LI the lowest and HI being intermediate.

Table 3.10. IntactDS Flower clusters number (FLN) and flower density (FLD) in relation to UCA (UCA\_FLD) (FLN/cm<sup>2</sup>), length (Len\_FLD) (FLN/cm), volume (Vol\_FLD) (FLN/cm<sup>3</sup>), leaf area (LA\_FLD) (FLN/m<sup>2</sup>) for 'Rosy Glow' apple grown in Ferrara (Italy) and trained as multileader - 2021 spring data. Mean separation in columns by SNK test. P< 0.05. (N=27-36)

	<i>FLN</i>	<i>UCA_FLD</i>	<i>Len_FLD</i>	<i>Vol_FLD</i>	<i>LA_FLD</i>
<b>HI</b>	26.3	16.7	0.136	0.273	38.2 ab
<b>MI</b>	24.2	17.4	0.131	0.305	43.1 a
<b>LI</b>	25.8	16.6	0.123	0.277	33.9 b
<b>Sign.<sup>1</sup></b>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>	*

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively.

## DISCUSSION

### *Allometries*

No statistical differences were found between the crop load treatments (Table 3.2- IntactDS), and their combination with girdling condition (Table 3.1-GirdledDS), and the vegetative parameters considered. It appears as less developed uprights grew on intact plants (Table 3.2), where only MI presented values similar to MG and MNG; a comparison between the high and low crop loads in both DSs show larger differences in vegetative parameters. The absence of statistical differences in both DSs induced by similar crop load levels is noteworthy, as many papers have shown a negative relation between vegetative growth and crop load (Giuliani *et al.*, 1997; Wunsche and Ferguson, 2010; Forshey and Elfving, 2011). Therefore, a differentiation induced by treatments was expected in this trial. On the other hand, many authors found no major effects of fruiting on vegetative growth and plant/canopy development (Palmer, 1992; Wunsche and Ferguson, 2010; Yuri *et al.*, 2011), showing that these relationships may depend on other factors, beyond the ones considered here, such as environmental condition, plant genetics, rootstock and training system utilized. Thus, crop loads, in both DSs, registered in this trial did not alter vegetative responses, but a trend due to crop load effect seems present; an additional consideration could be that the crop loads attained (matched or not with girdling) were not sufficiently extreme to statistically alter the vegetative response in this training system, considering its intrinsic vigour control (Dorigoni and Micheli, 2014; Dorigoni, 2016). In add the dwarfing rootstock utilized in this trial (Pajam 2) surely reduced the tree vigour and most probably also the vegetative response to crop loads; for the same crop load levels, more vigorous rootstocks could present different results regarding vegetative response.

### *Fruit Density*

The sectional area of different tree organs (e.g. TCA, BCA etc.) have been used very often to express thinning thresholds as “crop density” (Lombard *et al.*, 1988), and subsequently to assist growers in setting crop loads according to it, particularly when manual thinning or artificial spur/bud extinction (ASE) approach is applied (Lauri *et al.*, 1995, 1997, 2004; Tustin *et al.*, 2011, 2012; van Hooijdonk *et al.*, 2014). In slender-spindle trees, suggested thinning thresholds for obtaining constant and high-quality apple production, vary from 6 to 12 fruit per cm<sup>2</sup> of TCA. This variability is due to environmental parameters, management or genetic traits, that can impact fruit quality and yield at harvest for the same crop load levels (Elfving and Schechter, 1993; McArtney *et al.*, 1996; Wright *et al.*, 2006; Embree *et al.*, 2007; Robinson, 2008; Robinson *et al.*, 2013; Castro *et al.*, 2015; Serra *et al.*, 2016; Anthony *et al.*, 2019). Breen *et al.*, (2016) presented a crop load threshold based on branch cross-sectional (BCA) for artificial spur extinction, in tall spindle trees. These authors show how the increase from 3 to 5 fruit/BCA cm<sup>2</sup> induced a reduction in fruit production for “Gala” apples, keeping fruit dimensions acceptable (e.g. >156 g). For similar values of ASE (e.g. 5 fruit/BCA cm<sup>2</sup>) Tustin *et al.*, (2012) found, for “Scifresh” apples trees trained as tall spindle vertical axe, that alternate bearing was reduced in the following year and fruit set was improved (+22% of fruit set) compared to the unmodified natural crop load (18 fruit/ BCA cm<sup>2</sup>). By these results, values of 4-6 fruit/BCA cm<sup>2</sup> could be assumed as a branch based thinning threshold for marketable apple production in spindle trees (Tustin *et al.*, 2012; Breen *et al.*, 2015, 2016; Tabing *et al.*, 2016).

For GirdledDS, M treatment presents a UCA\_FD in range with the above-mentioned TCA based threshold (9.3 and 9.9 fruit/cm<sup>2</sup> for NG and G respectively); this treatment was used to test the suitability of generally recognized plant-based density thresholds (i.e., expressed as TCA), for 2D systems, where it is interesting to consider the upright, and not the whole tree, as the individual fruiting element. On the other hand, L (UCA\_FD = 3.1 and 2.9 fruit/cm<sup>2</sup> for NG and G respectively) and H (UCA\_FD = 15.6 and 13.8 fruit/cm<sup>2</sup> for NG and G respectively) crop loads were used to check the range of acceptable variation of the threshold that would still maintain high quality production in multileader training systems. For IntactDS, slightly higher values (i.e., UCA fruit density: 16.1, 10.5, 4.0 fruit/cm<sup>2</sup> for HI, MI and LI respectively) are present if compared to the same crop load level exposed above for GirdledDS, but also IntactDS treatments are in line for testing TCA based thinning thresholds, on UCA level, in a girdling free condition.

Comparing treatment values with BCA based thresholds (i.e., 4-6 fruit/cm<sup>2</sup> BCA reported above - Tustin *et al.*, 2012; Breen *et al.*, 2015, 2016; Tabing *et al.*, 2016), treatment's FDs present values slightly lower, in low treatments, almost doubled, in medium treatments, and up to three folds, in high treatments, for both the DSs.

The relationship between parameters as upright length, volume, leaf area to FDs were also investigated to understand if other fruit density indexes could be exploited for guiding crop load management in multileader training system or the existence of valuable relationship between them.

Considering, the different crop loads investigated in the trial, and the fruit quality results presented (Tables 3.5, 3.6), it seems possible to apply and increase TCA-based fruit density at UCA level. Taking into account the values presented by Tustin *et al.*, (2012) and Breen *et al.*, (2016), BCA based fruit density level might be doubled (i.e., 9-10 fruit/cm<sup>2</sup> of BCA, similar to M treatments values), in multileader training systems, maintaining satisfactory fruit quality parameter, as suggested by Breen *et al.*, (2021) and recently confirmed by Tustin *et al.*, (2022).

### **Fruit Quality**

Results in Table 3.5 clearly show how FF and SSC were improved by G, but not by crop load. Crop loads seem not to be the strongest driver for these quality parameters, at upright level, when girdling is applied; in HNG only FF resulted with lower values compared to the others suggesting that for NG, high crop load tend to induce lower FF, probably due to carbohydrate export. Considering the restricted range of values between all the treatments for FF (min-max: 8.72-9.81 kgf) and SPI (min-max: 8.96 – 9.15), it can be said that maturity among the different treatments was quite similar, despite statistical differences were found, concluding that these treatments did not significantly alter maturation.

On the other hand, G induced an increase in FF and SSC confirming that uprights may not be independent, and that resource exchanges among them may occur. Fruit had similar ripening level, as indicated by the restricted SPI range. Thus, the differences found in FF and SSC should depend on the treatment imposed (G) and not on different levels of fruit ripening. This is in accord with studies investigating trunk girdling application, in which the girdling technique resulted to increase SSC (Pretorius *et al.*, 2004) and FF (Fallahi *et al.*, 2018).

In regard to the fruit appearance, G presents better color extension and color tone-intensity parameters than NG confirming that color development is affected by uprights interconnection. Considering that the process of apple color development is based on an increase of anthocyanin matched with a degradation of chlorophyll compounds (Lancaster and Dougall, 1992), then the avoided export (i.e. higher availability) of photosynthate in the girdled treatments, could have induced a higher synthesis of sugar precursors of anthocyanin compounds, responsible for skin red color (Lancaster and Dougall, 1992; Ban *et al.*, 2009; Jakopič *et al.*, 2013).

For the same reason (i.e., the higher availability of photosynthate), girdling was expected to increase fruit size, as generally reported in literature (Goren *et al.*, 2003; Pretorius *et al.*, 2004, Fallahi *et al.*, 2018), but high and medium load treatments were not improved neither in fruit size neither in weight. On the other hand, it is clear how LG benefitted by the isolated situation (G) increasing its fruit size compared to LNG. This situation suggests an excessive crop load for HG and MG, that cannot be supported, as happened for LG, in isolated condition. The lower size of LNG (compared to LG) can be so attributed to a resource export, to the rest of the plant, considering its intact phloem interconnection. This hypothesis is in accord with findings of Bortolotti *et al.*, (in press) and appear also supported by the result of IntactDS in which no statistical differences were found. Probably the absence of girdled uprights on the plant, matched with a random distribution of single upright fruit load, improved the mutual resource exchange that led all crop load levels to reach the same final fruit size (Table 3.6). On the contrary it was not the case for GirdledDSs, where a more equal distribution of single upright fruit load was present, and a reduced exchange possibility (due to girdling presence) led to a differentiation between the treatments. This hypothesis seems possible considering the carbohydrate unloading mechanism for apple, that involve phloem only (Zhang *et al.*, 2004), and how sink demand/ strength (i.e., fruits - crop load in this case) drives resource allocation and movement (Blanke, 2009; Taiz *et al.*, 2015).

## **Fruit Growth**

Fruit growth appears to be improved by G for all treatments in the first part of the season suggesting that girdling helps to increase growth; therefore, in the second part of the season, AGR seems to be more influenced by crop load than by girdling. Generally, AGR values tend to be statistically similar for both G and NG in each of the crop load levels, in this last phase. Since the mean seasonal AGR matched final fruit size/ weight (Figure 3.4 -Table 3.5) it can be said that the modification in AGR induced by the treatments are reflected in the harvest fruit dimension per each treatment. Only L (both for G and NG condition) results different from all the other treatments, in terms of harvest fruit size and mean seasonal AGR, those were probably source limited in their development.

AGR values confirm findings by Bortolotti *et al.*, (in press) regarding the interconnection and interexchange between different uprights and its effect on final fruit dimensions. This is shown also in GirdledDS mean seasonal AGRs (Figure 3.4) where LNG presents lower values compared to LG, but remaining NG treatments are not reduced if compared to their respective girdled fruit load treatments.

The multileader training system led to a “standardization” in mean AGRs (and so in final fruit size) for NG uprights, improving fruit dimension for those uprights with heavy crop load (H, M) while reducing it in upright with low crop load condition (L); this due to the occurrence of an inter-uprights resource exchange. This seem then confirmed in the results of IntactDS (Table 3.6) where upright crop load seems to be completely unrelated to fruit size when phloem is maintained intact. The magnitude and relation of this effect is connected to the independence level of each upright, which seems driven first by its own crop load and secondly by the one related to the whole plant and/or to the single-upright crop load distribution on the plant.

This hypothesis is in accord with literature investigating crop load/fruit quality relation for 3D training system (Wunsche *et al.*, 2000; Serra *et al.*, 2016; Anthony *et al.*, 2019) and also with literature studying the relative contribution to fruit development of different vegetative formation and their distance from the fruit itself (Wunsche *et al.*, 1996; Dennis, 2003; Ferree and Warrington, 2003; Bairam *et al.*, 2019).

## **Allometric-Quality Correlations**

Correlations between different fruit density expressions and fruit quality were investigated. Most of the strongest correlations were found in HG supporting the hypothesis (coming from the previous chapter) that heavy crop load matched with girdling condition tend to emphasize correlations presents thanks to its restrictive condition (i.e., high carbon demand, lack of carbon import / export).

The general trend for all the treatments is a negative correlation between fruit size and fruit densities (FDs), but in LG all correlations are positive. This fact is probably due to the “unidirectional” resource availability for fruit of this treatment: being the only treatment in this condition and not responding to external sink demands (as happen for LNG) correlations are altered and even with increasing FDs, fruit size increases thanks to the available resources due to the overabundant situation. The same behaviour appears again only in LNG for Len\_FD and LA\_FD and being this treatment the second less limited in term of resources (and sink-source competition), this fact seems to support what stated above. On the other hand, for the other treatments a resource limitation is always present and, depending on its magnitude, correlations appear strong.

Analysing the scatterplots presented (Figures 3.5 to 3.8), it can be seen how, despite the variability in single-treatments correlation values, the general trend among all the treatments presents generally stronger r values, being also very informative in a “overall” viewpoint.

A clear set of guidelines suggesting how to manage crop load to obtain abundant, high-quality fruit production cannot be extracted from the data presented. However, considering the correlations (Table 3.8) and the scatterplot clouds (Figures 3.5 to 3.8), it might be possible to test those natural crop loads as thinning thresholds. Table 3.11 shows the thinning thresholds that can be derived from the results of this work.

Table 3.11 possible thinning threshold for premium quality Pink Lady®-Rosy Glow apple production, for trees grown with a multileader training system in Ferrara – Italy.

	<i>Present study</i>
<b><i>UCA_FD</i></b> <sup>2</sup>	8-12 fruit/cm <sup>2</sup>
<b><i>Len_FD</i></b> <sup>2</sup>	0.07-0.10 fruit/cm
<b><i>Vol_FD</i></b> <sup>2</sup>	0.15-0.20 fruit/cm <sup>3</sup>
<b><i>LA_FD</i></b> <sup>2</sup>	20-30 fruit/m <sup>2</sup>

<sup>2</sup> UCA\_FD, Len\_FD, Vol\_FD and LA\_FD are Fruit density (FD) expressed as the ratio of fruit number by upright cross-sectional area, length, volume and leaf area respectively

Considering the above thresholds of fruit densities (Table 3.11), values seem to match with the guidelines presented in the literature (Breen *et al.*, 2021; Tustin *et al.*, 2022) confirming a value of fruit per BCA cm<sup>2</sup> of approx 10, in multileader training system; by the same studies, depending on the apple cultivar, higher or lower values could be adapted, which should be investigated.

The data from IntactDS show no correlations and from the scatterplot (data not shown) it is not possible to estimate a thinning threshold. This fact in addition with the mean fruit dimension for high, medium and low crop load treatments, for IntactDS, (Table 3.6) seem to support the conclusion regarding the strong interconnection between uprights. Here no correlation can be found because of the extremely high mutual support that strongly reduced fruit diameter differences that treatments could have induced for isolated situation.

### **Return Bloom**

The absence of statistical differences shows that the applied treatments are not inducing an alteration in flower differentiation for the following season for girdled and intact datasets. Considering IntactDS the free resource exchange could have reduced (up to cancel) treatments differences regarding return bloom analysis. It is well known that return bloom and alternate fruit bearing is strongly dependent on genetics (Byers, 2003), and it is known how Pink Lady® apples are considered not susceptible to this phenomenon. So, the presented results and considerations would not be valid for varieties susceptible to alternate bearing (e.g., Honeycrisp, Fuji, and Golden Delicious), which would need specific studies for the determination of their optimum thresholds.

## **CONCLUSION**

The present study aimed to investigate objective crop load levels that could become in the future guidelines for growers interested in growing multileader planar training systems. This experiment relied on a combination of girdled and intact fruiting units (uprights), placed on the same and on different trees, that were assessed for a number of vegetative and fruiting parameters.

This study pinpoints the interconnection (i.e., lack of independence) among uprights, that appear capable, in the intact situations tested, to exchange resources that are reflected in fruit size and quality at harvest. Upright response to crop load, and its relevance to production, needs to be investigated more to gain a better knowledge on the uprights export onset determinants, for those uprights not having a strong enough sink demand within themselves. If this will be clarified, apple production could shift to the individual upright as management element, facilitating tree management in novel orchards, that are being developed for reduced human intervention (i.e., robotization) and requiring less skilled labour to be managed.

More specifically, results regarding fruit densities, correlation analyses and thinning thresholds show the following: i) none of the crop loads (and girdling condition) altered the vegetative growth creating an unbalanced situation; ii) Only the low crop load level altered mean seasonal fruit AGR and consequentially fruit dimensions, both in girdled and non-girdled condition; iii) realistic upright-based thinning thresholds were proposed for high quality apple production; iv) correlations between fruit densities and final fruit size were not particularly strong for single treatment, but general trends

highlight a valid negative correlation; iv) return bloom was not affected by none of crop load levels and girdling condition.

Finally, an important consideration needs to be done: in both the datasets, all the treatments reached the market mandated minimum diameter of 70mm for premium fruit class. Only one treatment combination (HG) missed the 70mm target, but only for 0.11mm, pointing out that, in a more productive market-oriented point of view, all crop loads tested seem able to support high quality production. Moreover, although the study is preliminary, the results confirm that, for apple multileader training system such as double-guyot, it is possible to apply generally accepted indices, such as the TCA-based crop loads for high quality fruit production at the single upright level and, by this, future management on this fruiting element seem possible.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mazzoni Farms and Michele Gerin for granting access to the orchard making this study possible.

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## CHAPTER 4

# Multileader training system: productive and vegetative relationships between single leader and whole plant in apple

### ABSTRACT

The present study investigated the relationship between crop load and harvest fruit quality considering two different fruiting units as the whole plant and the single vertical leader (or “upright”). The trial was carried out in a commercial orchard, trained as multileader, of a 4-year-old Pink Lady®-Rosy Glow apple (*Malus Domestica* Borkh.) located in Ferrara (Po’ valley - Italy). Crop load treatments (i.e., high - limiting, medium - commercial, and low- not-limiting) were defined both at plant and upright level. For each tree in trial (n=9), allometric, productive, quality, and flowering parameters were recorded. Three parallel analyses were conducted comparing results at whole plant and at single upright scale. Correlations between crop load, fruit densities and fruit size were investigated. The aim of the study is to state the opportunity to shift the tree management to the single upright and define possible thinning thresholds for multileader training system in the Emilia-Romagna region. Results report that upright cannot be considered as a completely independent unit. Its independence seems to increase according to balanced distribution of fruit load between the plant uprights together with crop load. For all the crop load treatments no effect on the return bloom and vegetative development was observed neither a clear relation between single upright crop load and final fruit size and quality. No upright thinning thresholds were found, but negative correlations between fruit size and whole plant crop load was obtained. Among all the treatments only the highest crop load reduced fruit size; despite this all treatments reached a premium quality production (fruit size  $\geq 70$ mm), suggesting that up to 16 fruits per cm<sup>2</sup> upright sectional area can be a possible target for high quality Pink Lady®-Rosy Glow production, on multileader training systems. These results underline the chance to apply precise crop load management at upright level, despite the upright interconnection.

### ABBREVIATIONS

FF = Flesh firmness  
FLN = Flower cluster  
FN = Fruit number  
Hp = High plant crop load treatment  
Hu= High upright crop load treatment  
LA = Leaf area  
LAI = Leaf area index  
LI = Daily representative light interception  
Lp= Low plant crop load treatment  
Lu= Low upright crop load treatment  
Mp= Medium plant crop load treatment  
Mu= Medium upright crop load treatment  
PlantDS = Dataset based on plant crop load treatments  
Sh.LA= Shoot leaf area  
Sp.LA = Spur leaf area  
SPI = Starch pattern index  
SSC = Soluble solid content  
TCA = Trunk cross-sectional area  
UCA = Upright cross-sectional area  
UprightDS = Dataset based on upright crop load treatments

## INTRODUCTION

Following from the previous chapters, the objective of the present study was to investigate the relationship between crop load and fruit size and quality comparing two different fruiting levels (i.e., possible thinning management unit): whole plant or single upright (i.e., vertical leader). Differently from the previously presented studies, here the focus was on completely intact trees (with none of the uprights girdled) on which all the present uprights were analysed, and then compared to results at whole tree level. At the single upright level, the analysis was carried out in the two conditions: considering it as a separate and independent fruiting unit, for crop load management, or as dependent from its connection to the plant. The investigation wanted to better clarify the possibility to shift the crop load management strategy of a multileader training system to the single upright, as well as to define possible thinning thresholds, to suggest to growers using 2D multileader systems, for high quality, profitable apple production, for Emilia Romagna (E.R.) region - Italy.

## MATERIALS AND METHODS

Data were collected in 2020 season, on trees belonging to a 4-year-old commercial Pink Lady®-Rosy Glow apple (*Malus domestica*, Borkh) orchard, grafted on Pajam 2 rootstock and located in Voghiera, Ferrara, Italy (Lat: 44.765105, Long: 11.757721). The orchard was trained as a double guyot system (Dorigoni and Micheli, 2018), with a planting distance of 3.3m x 2.1m and a tree density of 1443 plants ha<sup>-1</sup>. In this training system each tree has from 4 to 7 uprights on each horizontal axis: the total number of uprights per tree ranges between 8 and 14 with an upright density of 11,500-20,000 ha<sup>-1</sup>.

Data analyzed in this study come from 9 trees chosen randomly, based on their crop load. This was the naturally occurring crop load, as these plants were not subjected to chemical or manual thinning. Productive and quality parameters were analyzed on a whole plant or single upright basis and taking into account their crop load levels. For the analysis presented here, the data were organized according to two dimensions, generating the plant-based dataset (PlantDS) and the upright-based dataset (UprightDS). The 9 trees analyzed in this trial are the same utilized in the previous chapter for IntactDS, so not presenting girdling on it.

Plant crop load treatments were defined using the mean value of ~100 fruit per tree (a representative crop load value for Pink Lady®-Rosy Glow apple spindle trees in E.R. region) multiplied by 2 considering that double-guyot approximatively halved tree density for a standard orchard (~ 3000 trees ha<sup>-1</sup>). This crop load level (i.e., ~100 fruit per tree) represent the target crop load for growers pointing to a production of 50t ha<sup>-1</sup> (standard for E.R region) consisting of fruits falling in the first premium class fruit size (i.e., 70mm ~ 150g). Based on fruit counts, three crop load levels were defined as follows: high (Hp, N=34), presenting more than 210 fruit tree<sup>-1</sup> (resulting in 13.1 fruit per cm<sup>2</sup> of trunk cross-sectional area - TCA); low (Lp, N=36), having less than 130 fruit tree<sup>-1</sup> (resulting in 5.1 fruit/TCA cm<sup>2</sup>), and medium (Mp, N=35) presenting an intermediate crop load (i.e., 130 < Mp < 210 fruit tree<sup>-1</sup> - resulting in 10.1 fruit/ TCA cm<sup>2</sup>). Three trees for each crop load level served as replicates. Data grouped with this approach will be referred as PlantDS.

At upright level, crop load treatments were defined using thresholds based on fruit number to upright length ratio (i.e., length fruit density – Len\_FD) obtained in a preliminary assay where the length of 250 randomly chosen uprights was measured and their fruit number counted. The mean length fruit density was calculated and by adding and subtracting the standard deviation, crop load thresholds for the three treatments were obtained. Values <0.05, between 0.05-0.1 and >0.1 were chosen for low (Lu), medium (Mu) and high (Hu) crop load levels, respectively. Length fruit density was chosen for treatment differentiation because manual thinning applied by the grower on the rest of the orchard was based on upright length. Eq.1 was used to compute length fruit density (*Len\_FD* in Eq.1) where *FN* is upright fruit number and *Length(cm)* is upright length.

$$Len\_FD(\text{fruit}/\text{cm}) = \frac{FN}{Length(\text{cm})} \quad (\text{Eq.1})$$

All the uprights (N=105) present on the 9 plants for PlantDS were assigned to one of the crop load treatments based on their Len\_FD. The uprights belonging to the different treatments were randomly

distributed among the 9 trees due to the normal distribution of fruit densities, and their numbers were, 42 (Mu), 36 (Hu) and 27 (Lu). Data grouped with this approach will be referred as UprightDS.

Measurements of length (cm) and diameter (Diam - cm) of all the uprights were collected by a digital calliper (Mitutoyo, Kanagawa, Japan) and a standard measuring tape, at harvest time (25/10). Upright length was measured from their insertion onto the horizontal cordon to the top, while diameter was measured 10 cm above the insertion. From these data upright cross-sectional area (UCA, cm<sup>2</sup>) and volume (Vol, cm<sup>3</sup>) were calculated. Leaf area (LA, m<sup>2</sup>) on each upright was calculated by estimating the total number of leaves on lateral shoots and spurs multiplied by their average leaf area. The number of shoot leaves on each upright was estimated by developing an empirical relation (Eq.2), regressing shoot length and shoot leaf number, which was applied to the total shoot length per upright. This relation is shown in Eq.2 ( $r = 0.82$ ;  $n = 310$ ) where *LeafNumber* is the total shoot leaf number per upright and *total shoot length(cm)* is the sum of all upright shoots' length. Spurs were counted, and the average number of leaves per spur was determined by counting leaves on 300 randomly chosen spurs. The average area of shoot and spur leaves was determined on 300 leaves per shoot type that were collected and measured by a LI-COR 3100C leaf area meter (LI-COR, Lincoln, NE, USA); total lateral shoots leaf area (Sh.LA, m<sup>2</sup>) and spur leaf area (Sp.LA, m<sup>2</sup>) were then summed together to obtain the whole upright LA.

$$\text{LeafNumber} = 0.3098 * \text{total shoot length(cm)} + 5.6751 \quad (\text{Eq.2})$$

The PlantDS was obtained from the sum (for plant level) and the mean (for upright level) of the values of those upright structuring the plants falling in the whole-plant based crop load treatment (i.e., Hp, Mp, Lp). Moreover, on PlantDS also trunk cross-sectional area (TCA, cm<sup>2</sup>), leaf area index (LAI), and daily representative light interception (LI, %) were collected. LAI was calculated dividing plant LA by the orchard surface allotted to each tree. LI was estimated, using methodologies from Zhang *et al.* (2015, 2016), applying image analysis of tree shades. Images were taken, in a window of -3 and -2 hours before solar noon, perpendicularly to the ground where a white background was lied; then a binary classification was applied on the images to discriminate tree-shade-pixel, from background-pixels, to compute a porosity index. Equations from Zhang *et al.*, (2016) were then applied to estimate the correct LI.

Harvest occurred on 25/10; on this date, plants were strip-picked and all fruit per upright were counted (FN), sized (Diam, mm) and weighed (g). A digital calliper (Mitutoyo, Kanagawa, Japan) attached to an external memory ([www.hkconsulting.it](http://www.hkconsulting.it)) and a digital scale (KB1200, KERN & SHON, Germany) were used for these determinations. A sample of 25 fruit per tree were collected randomly choosing 2 healthy fruit per upright in each tree; when not possible due to uprights numerosity at least 1 fruit per upright was chosen, then remaining fruits were randomly collected from as many different upright as possible until reaching 25 fruits collected. On the collected fruit samples, the following fruit quality parameters were recorded: i) flesh firmness (FF, Kgf), determined by a penetrometer (GÜSS Fruit Texture Analyser, GÜSS, South Africa) with an 11 mm diameter tip after removing a slice of fruit peel; ii) soluble solid content (SSC, °Brix), determined by measuring the refractive index of fruit juice with a digital refractometer (ATAGO PAL-1, ATAGO, Japan); iii) starch pattern index (SPI), evaluated by a skilled operator, based on the ten degradation classes suggested by Ctifl (CTIFL, 2002); iv) color parameters by a Chroma meter (CR-400, Konica Minolta, Japan) on the blush and background sides of the fruit and expressed as CIE L\*C\*h\* color-space coordinates (directly computed by the instrument) where L\* represents lightness, C\* represents "chroma" (higher values mean more saturated color tone) and h\* "hue angle" (lower values mean redder color tone, pure red = 0); C\* and h\* were considered as the most representative color coordinates and were the only one analyzed; one-flash measurement was used to collect the color data; v) individual fruit red overcolor was estimated by assessing the percentage fruit area covered by blush color (red-colored surface) from visual observation (Col.Ext. - %).

Return bloom was investigated collecting flower cluster number (FLN), per each upright, during 2021 full bloom, occurred on 02/04. Flower and fruit density variables were computed dividing the flower cluster number (data from 2021 season) and fruit number (data from harvest 2020) respectively for the following vegetative parameters: TCA (cm<sup>2</sup>), UCA (cm<sup>2</sup>), length (cm), volume (cm<sup>3</sup>), LA (m<sup>2</sup>), Sh.LA (cm<sup>2</sup>), Sp.LA (cm<sup>2</sup>) and LAI; this was done looking for a more sensible way of expressing crop load

aiming to establish optimum thinning thresholds via correlation analyses. Equations 4 and 5 below were utilized for computing flower and fruit densities; in the equations, “*P*” refers to the vegetative parameter considered, from the ones cited above, and “*Pu*” to the respective measure unit of those parameters.

$$P\_FLD(FLN/Pu) = \frac{FLN}{P(Pu)} \quad (\text{Eq. 4})$$

$$P\_FD(FN/Pu) = \frac{FN}{P(Pu)} \quad (\text{Eq. 5})$$

The experimental design for both the datasets was a complete randomized block, balanced for PlantDS and unbalanced for UprightDS. Data were analyzed with R and R-Studio using “agricolae” and “stats” packages. ANOVA procedure was used to test for statistical differences among treatments and the SNK post-hoc test for mean separation. For fruit size classes distribution, a correspondence multivariate analysis was performed, followed by a cluster analysis, with a chi-square ( $\chi^2$ ) test (Greenacre, 2007); the same analysis was utilized to test for differences among upright distribution both in PlantDS and UprightDS. Pearson’s *r* correlation coefficient was used to investigate correlations between fruit quality and crop load; only the correlations equal to or exceeding  $r > 0.6$  were considered.

## RESULTS

### Allometries

Figure 4.1A represents the plant structure composition of PlantDS (Hp, Mp, Lp) in terms of UprightDS treatments (Hu, Mu, Lu). Plants presenting high crop load (Hp), have the highest frequency of high load uprights, followed by Mu, while only a few occurred in Lu. A mirroring situation occurs for Lp plants; for Mp the distribution is shifted towards higher frequencies of high vs low loaded uprights (Figure 4.1A). Cluster analysis and  $\chi^2$  testing, revealed a difference between Hp and Lp, while Mp was intermediate and did not differ from either of the other classes.

Figure 4.1B represents the distribution of uprights (UprightDS) on PlantDS plants. High load uprights (Hu) are more frequent on Hp plants and less on Lp and Mp. Low load uprights (Lu) are mostly occurring on Lp plants, with only few on Hp and Mp. Medium loaded uprights (Mu) present a more balanced distribution, with most of the uprights in Mp, and the rest almost equally distributed on Lp and Hp. Cluster analysis and  $\chi^2$  test show the same results presented for data in Figure 4.1A.

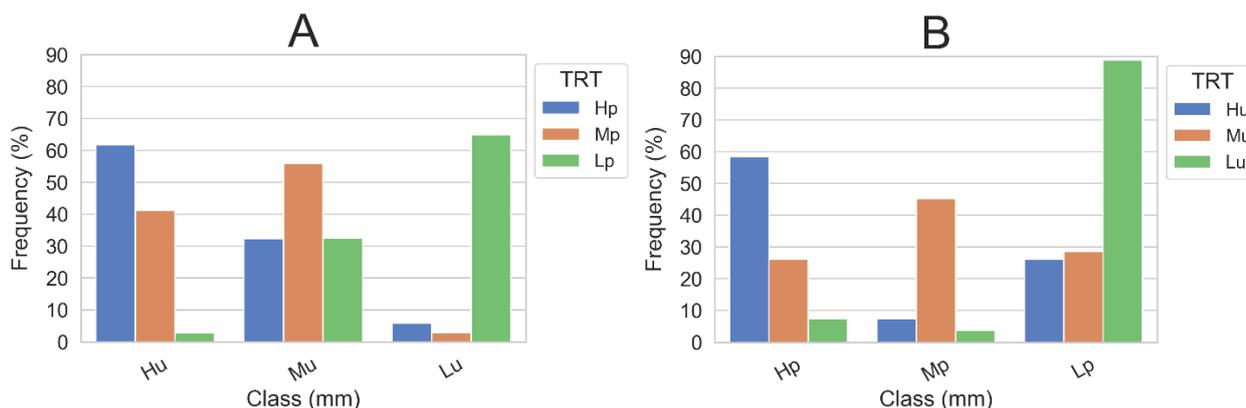


Figure 4.1- A. PlantDS plants upright composition in base of UprightDS treatments; B. UprightDS uprights distribution on plantDS treatments. Data are presented for ‘Rosy Glow’ apple grown in E.R. region - Italy-, trained as multileader. (N=34-36 for PlantDS; N=27-42 for UprightDS).

Considering general canopy development, Table 4.1 presents vegetative parameters both at plant level (i.e., sum of all the uprights of each tree), and at upright level (i.e., the mean of the uprights of each tree) for PlantDS. No differences appeared except for upright length both at plant and upright level

where light loaded plants (Lp) present the highest value followed by Hp and Mp. The same trend, although not statistically significant, is present for UCA, volume and leaf area (LA).

Table 4.1. Allometries for “PlantDS”: Trunk Cross-sectional Area (TCA)(cm<sup>2</sup>), Upright Cross-sectional Area (UCA)(cm<sup>2</sup>), Length (cm), Volume (cm<sup>3</sup>), Leaf area (LA)(m<sup>2</sup>) for ‘Rosy Glow’ apple grown in E.R. region- Italy-, trained as multileader. Mean separation in columns by SNK test. P<0.05. (N=9 for TCA, N=34-36 for other variables)

	<b>TCA</b>	<b>UCA</b>	<b>Length</b>	<b>Volume</b>	<b>LA</b>
<b><i>Plant Level</i></b>					
<b><i>Hp</i></b>	18.4	18.8	2096 ab	1305	7.89
<b><i>Mp</i></b>	18.4	18.0	1911 b	1219	7.10
<b><i>Lp</i></b>	23.6	23.7	2521 a	1741	9.88
<b><i>Sign.<sup>1</sup></i></b>	<i>ns</i>	<i>ns</i>	*	<i>ns</i>	<i>ns</i>
<b><i>Upright Level</i></b>					
<b><i>Hp</i></b>	NA	1.66	185 ab	115	0.696
<b><i>Mp</i></b>	NA	1.59	169 b	108	0.627
<b><i>Lp</i></b>	NA	1.92	204 a	141	0.801
<b><i>Sign.<sup>1</sup></i></b>		<i>ns</i>	*	<i>ns</i>	<i>ns</i>

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively.

NA: not available

Table 4.2 shows the vegetative parameters of the UprightDS, which also do not exhibit differences. A trend, although non-significant, shows light uprights (Lu) always scoring the highest values but not for UCA where Mu scores the highest; Mu presents the lowest values for leaf area and length, and Hu for volume and UCA.

Table 4.2. Allometries for “UprightDS”: Upright Cross-sectional Area (UCA)(cm<sup>2</sup>), Length (cm), Volume (cm<sup>3</sup>), Leaf area (LA)(m<sup>2</sup>) for ‘Rosy Glow’ apple grown in E.R. region- Italy-, trained as multileader. Mean separation in columns by SNK test. P< 0.05. (N=27-42)

	<b>UCA</b>	<b>Length</b>	<b>Volume</b>	<b>LA</b>
<b><i>Hu</i></b>	1.63	188	109	0.738
<b><i>Mu</i></b>	1.80	182	127	0.656
<b><i>Lu</i></b>	1.74	193	131	0.759
<b><i>Sign.<sup>1</sup></i></b>	<i>ns</i>	<i>ns</i>	<i>ns</i>	<i>ns</i>

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively.

### **Fruit Density**

Table 4.3 shows fruit number (FN) and fruit densities (FDs) of PlantDS, expressed in terms of different vegetative parameters. Table 4.4 presents the same results for UprightDS.

At plant level (PlantDS), differences were found between all treatments for crop loads (FN), TCA, length and volume FDs; for UCA\_FD and LA\_FD, Mp and Hp present similar, higher values than Lp. At the upright level, FN and LenFD scores differences for all three crop loads, whereas Hp and Mp are similar, and higher than Lp, for UCA\_FD, VolFD and LA\_FD.

Table 4.38. “PlantDS” Fruit number (FN) and fruit density in relation to TCA (TCA\_FD) (FN/cm<sup>2</sup>) UCA (UCA\_FD) (FN/cm<sup>2</sup>), and upright Length (Len\_FD) (FN/cm), Volume (Vol\_FD) (FN/cm<sup>3</sup>), Leaf Area

(LA\_FD) (FN/m<sup>2</sup>) for 'Rosy Glow' apple grown in E.R. region- Italy-, trained as multileader. Mean separation in columns by SNK test for P< 0.05. (N=3 for TCA\_FD; N=34-36 for other variables).

	<i>FN</i>	<i>TCA_FD</i>	<i>UCA_FD</i>	<i>Len_FD</i>	<i>Vol_FD</i>	<i>LA_FD</i>
<b><i>Plant Level</i></b>						
<b><i>Hp</i></b>	240 a	13.1 a	12.9 a	0.114 a	0.186 a	30.6 a
<b><i>Mp</i></b>	184 b	10.1 b	10.7 a	0.096 b	0.174 b	27.3 a
<b><i>Lp</i></b>	117 c	5.1 c	5.1 b	0.048 c	0.073 c	12.8 b
<b><i>Sign.<sup>1</sup></i></b>	***		**	***	*	*
<b><i>Upright Level</i></b>						
<b><i>Hp</i></b>	21.2 a	NA	14.2 a	0.110 a	0.245 a	32.7 a
<b><i>Mp</i></b>	16.2 b	NA	12.9 a	0.094 b	0.264 a	33.0 a
<b><i>Lp</i></b>	9.5 c	NA	5.6 b	0.047 c	0.090 b	13.0 b
<b><i>Sign.<sup>1</sup></i></b>	***		***	*	***	***

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively.

NA: not applicable

The UprightDS shows differences between all the treatments (Table 4.4): low, medium, and high upright crop load treatments (Lu, Mu and Hu) score as the lowest, intermediate and the highest value in each parameter, respectively.

Table 4.4. "UprightDS" Fruit number (FN) and fruit density in relation to UCA (UCA\_FD) (FN/cm<sup>2</sup>), and upright Length (Len\_FD) (FN/cm), Volume (Vol\_FD) (FN/cm<sup>3</sup>), Leaf Area (LA\_FD) (FN/m<sup>2</sup>) for 'Rosy Glow' apple grown in E.R. region- Italy-, trained as multileader. Mean separation in columns by SNK test for P< 0.05. (N=27-42).

	<i>FN</i>	<i>UCA_FD</i>	<i>Len_FD</i>	<i>Vol_FD</i>	<i>LA_FD</i>
<b><i>Hu</i></b>	24.2 a	16.1 a	0.129 a	0.283 a	38.2 a
<b><i>Mu</i></b>	14.0 b	10.5 b	0.076 b	0.197 b	26.3 b
<b><i>Lu</i></b>	6.3 c	4.0 c	0.031 c	0.081 c	8.8 c
<b><i>Sign.<sup>1</sup></i></b>	***	***	***	***	***

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively.

### Fruit Quality

Table 4.5 shows fruit quality parameters for PlantDS. At plant level no differences are shown for fruit diameter (Diam) and weight. Hp trees show a tendency towards smaller fruit compared to the other treatments. The other fruit quality parameters are always higher in Lp. Mp and Hp mostly show similar values, except for SPI, which is higher – although marginally – in Hp.

Color extension (Hp: 65%, Mp: 61%, Lp: 71%) results not different among treatments. Regarding color development, the sequence from greenest to reddest is Mp (h\*: 38.1), Hp (h\*: 34.4), Lp (h\*: 33.3) and Hp (h\*: 74.3), Mp (h\*: 73.5), Lp (h\*: 61.5) for blush and background sides of the fruit, respectively. Lp fruit is the reddest for background and blush color, while Mp and Hp are the greenest respectively for blush and background color.

At upright level, for PlantDS (Table 4.5), only fruit diameter and weight were recorded for each fruit. Hp scores the lowest for both parameters, whereas the other two treatments present similar values.

Table 4.5. Fruit quality parameters for “PlantDS” expressed as diameter (Diam) (mm), Weight (g), flesh firmness (FF) (kgf), soluble solid content (SSC) (°Brix), Starch Pattern Index (SPI) (SPI class mean) for ‘Rosy Glow’ apple grown in E.R. region- Italy-, trained as multileader. Mean separation in columns by SNK test. P< 0.05. (N=352-720 for Diam and Weight; N=25 for other variables)

	<b>Diam</b>	<b>Weight</b>	<b>FF</b>	<b>SSC</b>	<b>SPI</b>
<b><i>Plant Level</i></b>					
<b>Hp</b>	71.5	164	8.94 b	15.2 ab	8.95 b
<b>Mp</b>	73.2	176	8.85 b	14.9 b	8.83 c
<b>Lp</b>	73.5	178	9.43 a	15.9 a	9.00 a
<b>Sign.<sup>1</sup></b>	<i>ns</i>	<i>ns</i>	**	*	***
<b><i>Upright Level</i></b>					
<b>Hp</b>	70.9 b	162 b	NA	NA	NA
<b>Mp</b>	73.3 a	176 a	NA	NA	NA
<b>Lp</b>	73.3 a	176 a	NA	NA	NA
<b>Sign.<sup>1</sup></b>	***	***			

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively.  
NA: not available

No statistical differences are present in the UprightDS (Table 4.6) as well, for the fruit quality parameter recorded. Differences of fruit diameter and weight between PlantDS and UprightDS datasets are reduced to less than 2mm and 10g respectively.

Table 4.6. Fruit quality parameters for “UprightDS” expressed as diameter (Diam) (mm) and Weight (g) for ‘Rosy Glow’ apple grown in E.R. region- Italy-, trained as multileader. Mean separation in columns by SNK test. P< 0.05. (N=169-870).

	<b>Diam</b>	<b>Weight</b>	<b>FF</b>	<b>SSC</b>	<b>SPI</b>
<b>Hu</b>	72.3	170	NA	NA	NA
<b>Mu</b>	72.5	171	NA	NA	NA
<b>Lu</b>	72.9	174	NA	NA	NA
<b>Sign.<sup>1</sup></b>	<i>ns</i>	<i>ns</i>			

<sup>1</sup> *ns*, \*, \*\* and \*\*\*: effect not significant or significant at p ≤0.05, p ≤0.01 or p ≤0.001, respectively.  
NA: not available

### **Fruit Size Class Distribution**

Figures 4.2A and 4.2B show the fruit size class distribution for PlantDS and UprightDS respectively. Figure 4.2A points out how smaller fruit are more present in Hp, while reducing plant crop load, fruit size distribution shifts towards a higher frequency of bigger fruit. Despite that, for all treatments the most represented size class is 70-75mm with values of 41%, 41% and 49% respectively for Hp, Mp, Lp. The second most represented class size for Hp is 65-70mm (33%), while 75-80mm for both Mp (29%) and Lp (26%). Despite these trends, clustering analysis and  $\chi^2$  test shows no differences between the treatments for fruit size class distribution.

Figure 4.2B shows a similar pattern. UprightDS general distribution is shifted to higher fruit diameter in Lu. In this case, however, Lu, and not Hu, presents the highest fraction of unmarketable fruit (i.e., <65mm – 9%), as would be expected looking at Figure 4.2A where, at the plant level, Hp presents

the highest value (8%). In both datasets, the 70-75mm class is the most represented for all treatments (Hu-45%, Mu-45%, Lu-41%), but the second most represented class is 65-70mm for Hu (26%), vs. 75-80mm for Lu (27%), while Mu has equal values for both 65-70 and 75-80mm (23%). Once again, though, clustering analysis and  $\chi^2$  test shows no differences between the treatments.

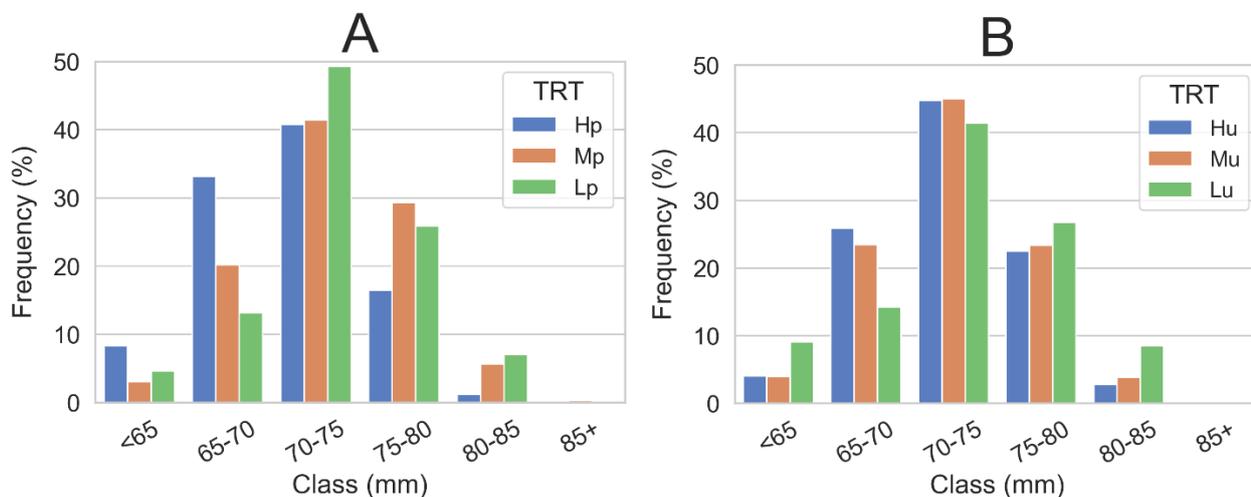


Figure 4.2 – Size class distribution for fruit harvested on ‘Rosy Glow’ apple grown in E.R. region- Italy-, trained as multileader. A. represents results for PlantDS (N=352-720); B. represents results for UprightDS (N=169-870)

### Allometric-Quality Correlations

Table 4.7 presents the correlations between fruit diameter and weight vs. different fruit densities in PlantDS; only data at plant level are shown because none of the correlations at upright level was sufficiently strong (i.e., none of them presented  $r > 0.6$ ).

Mp shows strong correlations with almost all FD expressions, except for spur leaf area fruit density (Sp.LA\_FD); the daily light interception (LI) presents the only positive correlation. Fewer correlations are shown in Hp with Len\_FD and sp.LA\_FD presenting values of  $r$  over 0.95. Perhaps not surprisingly, Lp not shows any correlation with  $r$  over 0.6. Pooling all data (“All”), interesting values appear for TCA, UCA, Volume, Sh.LA, LA and LAI fruit densities; the  $r$  values range for these fruit densities is similar (i.e.,  $0.71 \pm 0.05$ ) and the best values observed are with UCA\_FD and Sh.LA\_FD both for fruit diameter ( $r = -0.74$  for both) and weight ( $r = -0.76$  and  $-0.75$  respectively).

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Table 9.7 – “PlantDS” correlation table; Pearson “ $r$ ” coefficient and its relative p-value are presented for fruit diameter (Fr.Diam) and weight (Fr.Weight) against different fruit density parameters for ‘Rosy Glow’ apple grown in E.R. region- Italy-, trained as multileader. “All” treatment shows

correlation between the factors considered for all the three treatments grouped together. Bolded-underlined values were considered interesting ( $r > 0.60$ ). (N=34-36; for "All" N=105)

	<i>All</i>		<i>Hp</i>		<i>Mp</i>		<i>Lp</i>	
	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>	<i>r</i>	<i>p</i> <sup>1</sup>
<i>Fr.Diam vs TCA_FD</i> <sup>2</sup>	<b><u>-0.72</u></b>	***	-0.46	**	<b><u>-0.99</u></b>	***	-0.13	<i>ns</i>
<i>Fr.Diam vs LI</i> <sup>2</sup>	0.53	***	0.00	<i>ns</i>	<b><u>0.69</u></b>	***	-0.05	<i>ns</i>
<i>Fr.Diam vs UCA_FD</i> <sup>2</sup>	<b><u>-0.74</u></b>	***	0.00	<i>ns</i>	<b><u>-0.96</u></b>	***	-0.19	<i>ns</i>
<i>Fr.Diam vs Len_FD</i> <sup>2</sup>	<b><u>-0.62</u></b>	***	<b><u>-0.99</u></b>	***	<b><u>-0.94</u></b>	***	-0.39	*
<i>Fr.Diam vs Vol_FD</i> <sup>2</sup>	<b><u>-0.67</u></b>	***	0.06	<i>ns</i>	<b><u>-0.82</u></b>	***	-0.25	<i>ns</i>
<i>Fr.Diam vs Sh.LA_FD</i> <sup>2</sup>	<b><u>-0.74</u></b>	***	0.10	<i>ns</i>	<b><u>-0.98</u></b>	***	0.39	*
<i>Fr.Diam vs Sp.LA_FD</i> <sup>2</sup>	-0.44	***	<b><u>-0.96</u></b>	***	-0.10	<i>ns</i>	-0.49	**
<i>Fr.Diam vs LA_FD</i> <sup>2</sup>	<b><u>-0.72</u></b>	***	-0.18	<i>ns</i>	<b><u>-0.99</u></b>	***	0.07	<i>ns</i>
<i>Fr.Diam vs LAI_FD</i> <sup>2</sup>	<b><u>-0.72</u></b>	***	-0.18	<i>ns</i>	<b><u>-0.99</u></b>	***	0.07	<i>ns</i>
<i>Fr.Weight vs TCA_FD</i> <sup>2</sup>	<b><u>-0.74</u></b>	***	-0.29	<i>ns</i>	<b><u>-0.99</u></b>	***	-0.26	<i>ns</i>
<i>Fr.Weight vs LI</i> <sup>2</sup>	0.56	***	0.20	<i>ns</i>	<b><u>0.71</u></b>	***	0.08	<i>ns</i>
<i>Fr.Weight vs UCA_FD</i> <sup>2</sup>	<b><u>-0.76</u></b>	***	0.19	<i>ns</i>	<b><u>-0.97</u></b>	***	-0.32	<i>ns</i> *
<i>Fr.Weight vs Len_FD</i> <sup>2</sup>	<b><u>-0.65</u></b>	***	<b><u>-1.00</u></b>	***	<b><u>-0.93</u></b>	***	-0.50	**
<i>Fr.Weight vs Vol_FD</i> <sup>2</sup>	<b><u>-0.69</u></b>	***	0.25	<i>ns</i>	<b><u>-0.85</u></b>	***	-0.37	*
<i>Fr.Weight vs Sh.LA_FD</i> <sup>2</sup>	<b><u>-0.75</u></b>	***	0.29	<i>ns</i>	<b><u>-0.98</u></b>	***	0.26	<i>ns</i>
<i>Fr.Weight vs Sp.LA_FD</i> <sup>2</sup>	-0.46	***	<b><u>-0.99</u></b>	***	-0.06	<i>ns</i>	-0.60	***
<i>Fr.Weight vs LA_FD</i> <sup>2</sup>	<b><u>-0.74</u></b>	***	0.01	<i>ns</i>	<b><u>-0.99</u></b>	***	-0.06	<i>ns</i>
<i>Fr.Weight vs LAI_FD</i> <sup>2</sup>	<b><u>-0.74</u></b>	***	0.01	<i>ns</i>	<b><u>-0.99</u></b>	***	-0.06	<i>ns</i>

<sup>1</sup> ns, \*, \*\* and \*\*\*: effect not significant or significant at  $p \leq 0.05$ ,  $p \leq 0.01$  or  $p \leq 0.001$ , respectively.

<sup>2</sup> TCA\_FD, UCA\_FD, Len\_FD, Vol\_FD, Sh.LA\_FD, Sp.LA\_FD, LA\_FD and LAI\_FD are Fruit density (FD) expressed as the ratio of fruit number by Trunk cross-sectional area, upright cross-sectional area, length, volume, shoot leaf area, spur leaf area, leaf area and leaf area index respectively

Correlations for UprightDS are not presented because none of the investigated correlations showed interesting correlation (i.e., none of them presented  $r > 0.6$ ).

Scatterplots of the contrast presented in Table 4.7 were also investigated, for both the DSs, with the aim to find a crop load threshold (i.e., thinning thresholds) above which fruit size could have been reduced; results are not shown because no thinning thresholds could be extrapolated from the scatterplots analysis.

### Return Bloom

Return bloom parameters as flower clusters number (FLN) were counted per each upright and flower cluster densities (FLD) calculated utilizing Eq. 4.

Results are not shown in table because for all the treatments, in both the datasets and levels, no statistical difference and trends were found. For completeness the following information can be given.

For PlantDS, FLN ranges from 235 (Mp) to 333 (Lp) at plant level, while from 22.7 (Lp) to 28.6 (Mp) at upright level. UCA\_FLD values, ranges are respectively 14-14.4 (Mp-Hp) for plant level, while slightly increasing to 16.8-17.1(Hp-Lp) at upright level. Len\_FLD presents very similar values between plant and upright level, that are respectively 0.12-0.13 (Mp-Lp) and 0.13-0.14 (Mp-Lp). Vol\_FLD and

LA\_FLD show lower values for plant level, respectively 0.20-0.23 (Lp-Mp) and 34.3-35.7 (Hp-Mp), compared to upright level, respectively 0.27-0.31 (Lp-Mp) and 37.9-40.8 (Lp-Mp).

For UprightDS, FLN ranges from 24.2 (Mu) to 26.3 (Hu) while UCA\_FLD, Len\_FLD, Vol\_FLD, LA\_FLD present value ranges of 16.6-17.4 (Lu-Mu), 0.12-0.13 (Lu-Mu), 0.27-0.31 (Hu-Mu), 33.9-43.1 (Lu-Mu), respectively.

## DISCUSSION

### *Allometries*

Vegetative parameters of uprights show comparable values between PlantDS and UprightDS (Tables 4.1 and 4.2). These results, coupled with the upright distribution at plant level (Figure 4.1A), suggest that for the crop load imposed the vegetative growth is not altered both at plant and upright level in either dataset, although in Mp upright length is lower than Lp, but not Hp (Table 4.1). This apparent reduced development of the Mp plants derives from previous season growth; however, despite its lower development, Mp total shoot length and upright extension growth was not different to the one of other treatments (data not shown).

Responses of vegetative growth to crop load can vary, as seen in many papers. Literature generally reports negative correlation between crop load and vegetative growth (Giuliani *et al.*, 1997; Palmer *et al.*, 1997; Wunsche and Ferguson, 2010). In the present study, neither differences among treatments neither trends nor alterations of allometric measurements, due to crop load, were found, also for the highest crop load levels occurring in Hp. This fact is not in accordance with literature reported above, but also studies reporting no major effect of crop load on vegetative response are present in literature (Palmer, 1992; Wunsche and Ferguson, 2010; Yuri *et al.*, 2011). One aspect specific to this trial, is represented by the tree structure, which aims to control vigour by spreading it in the many growing points of multileader systems (Lauri *et al.*, 2004; Lauri, 2008; Dorigoni and Micheli, 2014; Dorigoni, 2016; Tustin *et al.*, 2018, 2022). It is possible that treatments crop load levels were not sufficiently high to induce an alteration in vegetative growth or that the reduced vigour, induced by this training system, led to a situation where crop load do not result as strong factor affecting vegetative growth as the training system. In add, also the effect of rootstock should be considered, the dwarfing Pajam-2 utilized surely reduced the tree vigour and affected the vegetative response to crop loads; different results could occur for different rootstock-scion combination.

### *Fruit Density*

Crop load values, defined as fruit number per TCA, in the range 6-12 fruit/cm<sup>2</sup> have been used to set a limit for high quality apple production and yields for a wide range of genetic, environmental and management conditions in 3D training systems (Elfving and Schechter, 1993; McArtney *et al.*, 1996; Racskó, 2006; Embree *et al.*, 2007; Robinson, 2008; Robinson *et al.*, 2014; Castro *et al.*, 2015; Serra *et al.*, 2016; Anthony *et al.*, 2019). In the presented study, PlantDS values are in line with this range, with Mp (10.06), Lp (5.11), and Hp (13.11), allowing a comparison between 2D and 3D training systems. Similarly, for PlantDS and UprightDS, UCA\_FDs can be compared with known crop load thresholds based on branch cross-sectional area (BCA). In 3D tree shapes, several authors reported values of 4-6 fruit/BCA cm<sup>2</sup> as a limit for adequate production and quality (Lauri *et al.*, 2004; Tustin *et al.*, 2012; Kon and Schupp, 2013, 2018; Breen *et al.*, 2015; Tabing *et al.*, 2016; Atay *et al.*, 2021). In comparison, UCA\_FDs present similar, doubled and up to three-fold higher values for Lp (5.6) and Lu (4.0), Mp (12.9) and Mu (10.5), Hp (14.2) and Hu (16.1) respectively.

Upright crop load treatments were selected according to Len\_FD, and not TCA/BCA\_FDs, to mimic the technique adopted by the grower in whose orchard the trial was conducted.

Differences between the grouping of FDs in Tables 4.3 and 4.4 can only be attributed to the single upright crop load distribution on the plant (Figure 4.1A), considering that uprights and fruit in the trial were the same in both datasets. This uneven distribution caused medium and high crop load treatments (Mp, Hp, Table 4.3) be similar for UCA\_FD and LA\_FD, at plant level, and for UCA, LA and volume FDs, at

upright level. On the contrary, for UprightDS (Table 4.4), treatments are statistically different in all the fruit densities considered. UCA\_FD and LA\_FD can be considered as the most representative expressions of fruit density, from a physiological point of view. In fact, they can be considered as the parameters accounting for resource availability in terms of water, delivered by xylem, i.e., directly related to UCA (Lang, 1990; Ferree and Warrington, 2003; Taiz *et al.*, 2015), and photosynthates produced in the leaves (Ferree and Warrington, 2003; Taiz *et al.*, 2015). In literature TCA (Robinson *et al.*, 2013; Castro *et al.*, 2015; Serra *et al.*, 2016; Anthony *et al.*, 2019) and BCA (Tustin *et al.*, 2012; Breen *et al.*, 2015, 2016; Tabing *et al.*, 2016), ascribable to UCA in this study, are generally used to represent both tree vegetative development / vigour and resource availability in crop load studies, while LA (and fruit per LA unit or LA per fruit) is generally used in source-sink physiological investigations (Palmer, 1992; Wünsche *et al.*, 2000; Tozzi *et al.*, 2018; Bairam *et al.*, 2019). Since neither of these FDs (UCA\_FD and LA\_FD) are different for Mp and Hp, fruits of those treatments should have had the same level of water and photosynthetic resources and should have reached the same final dimension and quality level. Thus, in PlantDS for high (Hp) and medium (Mp) treatments, no statistical difference in fruit size and quality was expected due to the same sink-source relation provided to each of the fruit, since the same values of UCA\_FD and LA\_FD were present. On the contrary, a differentiation should be present for UprightDS where treatments are statistically different for all the fruit densities considered.

### **Fruit Quality**

Fruit size and quality results highlight an interesting controversy for medium load treatment in PlantDS (Mp, Table 4.5). Fruit size and weight are similar, in Mp, to low crop load treatments at both plant and upright level, suggesting a non-limiting crop load; this is also supported at upright level, where only Hp appears different from the other treatments and presents smaller fruit size. On the other hand, Hp and Mp are not different in terms of UCA and LA FDs (Table 4.3), and this should be reflected in similar fruit sizes. The medium crop load (Mp) however, behaves opposite to expectations, probably due to its upright composition at plant level and generally lower crop load compared to Hp. The higher number of medium uprights (56% vs 32% in Hp), the lower number of heavy uprights (41% vs 62% in Hp) and the lower overall plant crop load (-24% compared to Hp) may have induced higher availability of resources that were exchanged among the uprights, allowing Mp to increase its fruit size up to that of Lp. This hypothesis seems supported by Bortolotti *et al.* (In Press) and by the findings of the previously presented chapters. Probably a more balanced distribution of fruit on each upright and a non-limiting crop load, as in Mp, could promote a better resource exchange. Indeed, a similar source-sink level, in the whole tree, could avoid unidirectional resource exchange between the uprights affecting harvest fruit size in the single uprights. From this consideration, a more “rigorous” training system as the planar cordon (Tustin *et al.*, 2018), in which is defined a fixed upright number (10 per tree) and spacing (~30cm) could have helped in evaluating the occurrence and the magnitude of inter-upright resources exchange. In fact, in such a “regular” system, inter-upright sink-source relationships (i.e., sink strength) would have been less affected by variable phloem transportation distance and unbalanced upright number between the horizontal cordons, as for double guyot system, better highlighting the effect of individual upright crop load on harvest fruit size.

Considering the other fruit quality parameters (Table 4.5), Mp always shows values suggesting a condition of less mature fruit, while Lp shows more mature fruits condition. Hp was similar to Mp for FF and SSC parameters. Also SPI and color attributes (red overcolor and intensity) show Mp as the least mature of all. Literature reports how excessive crop loads tend to induce a reduction of quality parameters such as SSC and color attributes, while delaying fruit ripening (Wünsche *et al.*, 1996, 2000; Dennis, 2003; Ferree and Warrington, 2003; Serra *et al.*, 2016; Anthony *et al.*, 2019; Bairam *et al.*, 2019). SSC, color and SPI results for Mp, seems to suggest an excessive crop load level, which would contradict the results of fruit size. It is worth pointing out that the range of quality parameters presented is quite narrow (8.85-9.43 for FF, 14.9-15.9 for SSC, 8.83 -9.00 for SPI), thus the negative response of Mp should not be considered as excessively severe, for fruit quality, as these differences are too narrow to impact at commercial level. A similar consideration may be extended to fruit size, where all treatments fell in the 70-75mm class (Table 4.3).

Results on UprightDS (Table 4.6) do not show differences between the treatments, if compared to the plantDS at upright level (Table 4.5), so no clear trends appear in this case. The probable reason is

connected to the upright distribution at plant level. Figure 4.1A reports the upright make-up of heavy load plants: these are made up of 62%, 32% and 6% of high (Hu), medium (Mu) and low (Lu) uprights, respectively. By this, it was probable to find a reduced fruit size in Hu, in UprightDS, because the majority of Hu upright are placed together on heavy loaded plants (Hp) and generally in a heavier crop load condition, that should have reduced fruit size (Wünsche and Ferguson, 2010; Robinson and Lopez, 2012; Jakopič *et al.*, 2013; Serra *et al.*, 2016). The lack of this effect suggests once again that the interaction between single upright and whole plant crop load can alter the final fruit size. Data of Table 4.6 depicted a situation where heavy loaded uprights (Hu) on heavy loaded plants (Hp) presented a lower mean fruit diameter compared to the same upright type (Hu), on trees with lower crop load levels (i.e., Mp and Lp). The mean value per treatment, presented in Table 4.6, flattened these differences resulting in similar values between the upright treatments. Moreover, the unequal distribution of uprights (Hu, Mu, Lu) within plants (Hp, Mp, Lp) (Figure 4.1A), induced no statistical differences between the treatments. This was confirmed by the trend for mean fruit dimension in heavy loaded uprights (Hu), that followed the plant crop load levels on which they were placed, which increased from high to low crop loads: 71.4mm, 73.4mm and 74.0mm respectively for Hu placed on Hp, Mp, Lp trees. A different result was found for Mu and Lu, whose fruit were the largest in Mp (73.1mm and 75.4 mm, respectively), the smallest in Hp (71.0mm and 64.7mm) and intermediate in Lp (72.8mm and 73.5mm). The larger diameter for Mu and Lu, on Mp trees, confirms how a more even distribution of single upright crop load can improve fruit size under non-limited conditions (i.e., Mp). Thus, even though differences in fruit size were present in UprightDS, because of their unequal distribution, no significant differences emerged.

The interaction and the gradients between leaders may explain the fruit density, size and quality differences: a higher resource exchange activity driven by crop load condition combined with the unequal distribution of upright crop load on the plants probably occurred. This situation seems possible, considering studies regarding relative contribution to fruit development of different vegetative formation and their distance from the fruit (Wünsche *et al.*, 1996; Dennis, F., 2003; Ferree and Warrington, 2003; Bairam *et al.*, 2019). Considering the occurrence of this high resource exchange, probably the most reliable indicators for fruit quality and dimension are those from the PlantDS at plant and upright levels, in which all upright crop load level and interaction are integrated together.

### ***Fruit Size Class Distribution***

In Figure 4.2A and 4.2B fruit size class distribution presents a similar pattern for the different treatments and datasets: a general shift towards larger fruit at low crop loads, in accordance with the literature (Ferree and Warrington, 2003; Wunsche and Ferguson, 2010; Serra *et al.*, 2016).

These results seem to confirm fruit quality results: a difference in the behaviour of medium crop load between PlantDS and UprightDS, with a distribution more similar to Lp at plant level with fruit falling mostly in the 70-75mm and 75-80mm classes, compared to a normal distribution centered on the 70-75 mm class for Mu.

It is important to emphasize that fruit size and quality parameter presented both for PlantDS and UprightDS are in line with Pink Lady®-Rosy Glow defined requirements for premium quality fruit (i.e. red blush on >50% of fruit surface and size >70mm) in the Italian market. Across all treatments only 9% of fruit fell below the minimum fruit size of 65mm and at least 70% of fruit were above 70mm in Mp and Lp, while 58% for Hp. Once again, it is apparent that upright crop load levels could be adjusted – when not all were heavily cropped – in order to preserve high production without excessive losses in quality traits, even in Hu.

### ***Allometric-Quality Correlations***

Many strong correlations ( $r > 0.9$ ) for heavy and medium loaded trees were found, although data were derived from only 3 plants per treatment. For this reason, “overall” data from Table 4.7 can be considered more reliable, although many of these showed slightly lower correlation coefficient values. Most fruit densities are negatively correlated to final fruit size and weight. The best correlations found are with UCA and Sh.LA fruit densities for fruit diameter and weight, closely followed by all the others.

The lack of correlations in  $L_p$  shows that, when no source limitations are present, crop load and fruit dimensions are almost unrelated, i.e., if no correlation between crop load and fruit size can be found, crop load was set too low and did not allow to fulfil the tree potential.

It is noteworthy that when uprights were considered as fruiting elements independently from the tree (UprightDS), no correlations were found between fruit dimension and various ways of expressing crop load (i.e., fruit densities). This discrepancy can probably be attributed to the unequal crop load distribution of the uprights on each plant, probably inducing different degrees of carbohydrate partitioning within the uprights. This observation suggests that individual uprights cannot be considered as completely independent fruiting elements. This would lead to the suggestion that they should not be used as management units in multileader training systems. However, further consideration may lead to opposite conclusions, if the whole plant crop load is considered (see below).

It is however noteworthy to underscore the fact that all the treatments provided apples that can be considered as premium quality. Fruit densities, up to the heaviest crop load treatment (e.g., 13.1 fruit/cm<sup>2</sup> TCA and 14.1 - 16.1 fruit/cm<sup>2</sup> UCA), seem adequate to guarantee high quality fruit production, in this kind of training system, for Emilia-Romagna environmental conditions. These values are slightly higher than the maximum suggested for TCA based 3D plants shapes (range 6-12 fruit/cm<sup>2</sup> TCA), and around 3-times those suggested for BCA (4-6 fruit/cm<sup>2</sup> UCA). Recently Breen *et al.*, (2021) suggested, and Tustin *et al.*, (2022) confirmed, for planar cordon multileader system (Tustin, van Hooijdonk and Breen, 2018), desirable crop loads range around 8-12 fruit/cm<sup>2</sup> BCA. These values have been confirmed in this work, as also shown in previous chapters of this dissertation.

### **Return Bloom**

Data on return bloom showed no differences for the two datasets analyzed. This implies that the tested crop loads, even the highest ones, were not sufficient to induce negative effects on flower bud differentiation. Return bloom and alternate fruit bearing are strongly dependent on genetics (Byers, 2003) and Pink Lady®-Rosy Glow is considered less prone to alternate bearing than other cultivars, such as “Gala”, “Golden” or “Fuji”. Results can be considered so valid, only for the investigated Pink Lady®-Rosy Glow apple cultivars, while further studies are needed to confirm or deny obtained results for other apple cultivars trained as multileader.

## **CONCLUSION**

This study analyzed a novel 2-D training system with the goal of understanding whether or not the individual upright can be taken as the management unit in lieu of the whole tree, in a novel orchard design, intended to reach higher productivity and be more suitable for precision approaches, including robotization.

The results obtained clearly show that the single upright cannot be considered as a completely independent fruiting unit, because resources can be mobilized among leaders within the plant. The level of this mobilization depends on both upright fruit load condition, and the tree upright population in terms of crop load (i.e., the individual uprights fruit load distribution on the plant).

When the population of uprights within the tree is more homogeneous, upright independence seems related with upright fruit load (increasing when fruit load increase). When, on the other hand, different upright fruit loads are present in the tree, this independence is less evident because resources will likely flow from lower to higher crop load uprights. This resource exchange can also overcome potential losses in fruit quality for those high cropping uprights in trees with unbalanced populations.

For applying precise crop load management at upright level, each tree should have most of the fruit with the same resources available and not having a pool of resource distributed “unequally” between fruit. In other words, the best strategy would be to try and set the same fruit density for all the uprights, to guarantee similar growing conditions to each fruit. Under similar conditions, single upright precise crop load management would be applicable.

For situations where unequal fruit load occurs, single upright management approach could be applied, but production results will be more related to whole plant crop load rather than to the single upright.

Moreover, crop load levels up to H (e.g., 13 fr/cm<sup>2</sup> of TCA, 13-16 fr/cm<sup>2</sup> of UCA) appear suitable for high quality fruit production, in multileader training system considering upright interdependency. In commercial orchards it will be difficult to reach an equal crop load on all the uprights, therefore the unequal upright crop load distribution could facilitate growers in the management of such an orchard.

Further studies are needed to improve understanding of the relation between upright and whole plant crop load, to better define thresholds that can lead to switch from canopy- to upright-based management. Studies are also needed to confirm the supposed higher suitability of the double-guyot training system in the Emilia-Romagna region compared to standard spindle training system, adopting the crop load levels proposed here. Concluding, confirms are needed also for the possibility to transfer the obtained results from Pink Lady®-Rosy Glow to other apple cultivars grown in the same region.

## ACKNOWLEDGEMENTS

The authors gratefully acknowledge Mazzoni Farms and Michele Gerin for granting access to the orchard making this study possible.

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## CHAPTER 5

### General Discussion and Conclusion

In this thesis, the possibility to shift orchard management resolution to the single upright of a multi-leader training system was investigated. This was done to improve precise crop load management (i.e., thinning) for multileader planar (2D) training systems in the Italian Po Valley environment. By assessing tree responses, the hope was to define a practical guideline pinpointing achievable crop load levels at which Pink Lady® apple (*Malus domestica* Borkh.) can produce premium quality fruit for this tree shape. Through the analysis of leader physiological independence and fruit load effects on production, the upright as single fruiting element, and then the relationships between uprights and the whole plant status itself were studied.

In chapter 2, physiological results related to leaf gas exchanges pointed out a dependence among uprights and the possibility of mutual support among different uprights during fruit development; this result was supported by fruit production and quality data. Moreover, a correlation between single upright fruit load and harvest fruit size was found, indicating that accurate thinning thresholds could effectively allow to manage crop load at single upright level.

In chapter 3, part of the unanswered hypotheses raised from chapter 2 were confirmed and objective metrics (i.e., thinning thresholds) on which to base precise crop load management were searched in a larger dataset. Results confirmed the thinning thresholds values found in the previous study, despite a small reduction in fruit number. Chapter 3 highlighted that for plants with an unbalanced random distribution of single upright fruit load (i.e., unthinned tree), the mutual resources interexchange between uprights increases (up to erasing crop load effects under our conditions of study) suggesting the ability of the plant to support higher fruit load, homogeneously spread over all the uprights.

In chapter 4, a similar analysis to chapter 3 was carried out. Contrarily to the previous trial, where uprights were always considered independent fruiting elements, in this case all uprights were investigated both as parts of trees and as independently manageable units. No relationships between crop load expressions and harvest fruit size were found for individual uprights, whereas they appeared at whole plant level. On the other hand, results confirmed the preliminary hypothesis supporting the relevance of single upright fruit load distribution on the plants (~ plant crop load) in defining both upright independence and single upright crop load effects on final fruit quality production.

Results from these studies pointed out an unexpected situation: uprights are not completely physiologically independent from each other, but if considered and managed as independent management units, then no major problems on final fruit quality and production is to occur, at least at the proposed crop load levels tested here. As discussed, the level of autonomy is related to the balance in upright crop load within a tree. In fact, the level of independence of single vertical axes is related to their own fruit load, and to the homogeneity of such load within the individual plant. The more similarity among upright crop loads, the greater the independence of each single upright; this is particularly true at high fruit loads. On the other hand, the level of mutual support to fruit development can result minimal or even absent, based on the actual fruit load.

Table 5.1 shows a summary of the thinning thresholds emerged from each of the studies and the level at which single upright crop load management seems to be applicable. Yields presented were calculated on a model upright defined from the average of all the uprights of the trials (~ 200) and multiplied by the actual mean orchard upright density (i.e., 16000 uprights ha<sup>-1</sup> with a planting distance of 2.1m x 3.3m) for a fruit production consisting of 70mm/150g fruit size average.

Table 5.1- Possible thinning thresholds, and the relative yields achievable, emerged from the studies reported in this thesis. Study 1, 2 and 3 refer respectively to chapter 2, 3 and 4 of the thesis.

	<i>Study 1</i>	<i>Study 2</i>	<i>Study 3</i>
<b><u>Thinning thresh.</u></b>			
<b><i>UCA_FD</i></b>	12-15 fruit/cm <sup>2</sup>	8-12 fruit/cm <sup>2</sup>	Up to 16 fruit/cm <sup>2</sup>
<b><i>Len_FD</i></b>	0.10-0.15 fruit/cm	0.07-0.10 fruit/cm	Up to 0.13 fruit/cm
<b><i>Vol_FD</i></b>	0.20-0.30 fruit/cm <sup>3</sup>	0.15-0.20 fruit/cm <sup>3</sup>	Up to 0.28 fruit/cm <sup>3</sup>
<b><i>LA_FD</i></b>	25-35 fruit/m <sup>2</sup>	20-30 fruit/m <sup>2</sup>	Up to 38 fruit/m <sup>2</sup>
<b><u>Yields</u></b>			
<b><i>UCA Yield</i><sup>1</sup></b>	54-67 t ha <sup>-1</sup>	36-54 t ha <sup>-1</sup>	72 t ha <sup>-1</sup>
<b><i>Len Yield</i><sup>1</sup></b>	45-68 t ha <sup>-1</sup>	32-45 t ha <sup>-1</sup>	59 t ha <sup>-1</sup>
<b><i>Vol Yield</i><sup>1</sup></b>	63-95 t ha <sup>-1</sup>	47-63 t ha <sup>-1</sup>	88 t ha <sup>-1</sup>
<b><i>LA Yield</i><sup>1</sup></b>	43-61 t ha <sup>-1</sup>	35-52 t ha <sup>-1</sup>	66 t ha <sup>-1</sup>

<sup>1</sup>UCA\_FD, Len\_FD, Vol\_FD, LA\_FD are fruit density (FD) expressed as the ratio of fruit number by upright's cross-sectional area (UCA, cm<sup>2</sup>), length (Len, cm), volume (Vol, cm<sup>3</sup>) and leaf area (LA, m<sup>2</sup>) respectively.

<sup>2</sup>Yields calculated on mean values, for all the uprights analyzed in the different studies, of upright's cross-sectional area (UCA) = 1.864 cm<sup>2</sup>; length (Len) = 189.5 cm; volume (Vol) = 131.4 cm<sup>3</sup>; leaf area (LA) = 0.720 m<sup>2</sup>. Mean fruit size was considered = 70mm/150g.

Table 5.1 shows a reduction in all fruit thinning thresholds passing from the first to the second study. Considering that thinning thresholds in both studies are obtained from similar trials set up (i.e., from girdled uprights), the reduction in thresholds values can be probably due to a different, higher variability, among the uprights of the second study. On the other hand, when girdling was not applied, i.e., allowing resource exchange among uprights, in a condition of randomly distributed upright fruit loads (study 3), crop load effect was reduced, giving thinning thresholds values similar, or higher, to those in Study 1. From the point of view of ease of use in the field, thinning thresholds based on UCA and upright length are probably the most suitable for rapid, if not automatic, determination in the field, while volume and leaf area are more difficult to be applicable because less immediate and with somewhat heavy acquisition/computation needs.

Moreover, yields presented in Table 5.1 are not always acceptable for a commercial Pink Lady®-Rosy Glow apple orchard placed in the Po valley, where yields are generally 50-70 t ha<sup>-1</sup> for 3D training systems (spindle), with occasionally high yields up to >90t ha<sup>-1</sup>. The "low" yield estimates at the proposed thresholds are connected to the wide inter-row distance utilized in the study orchard (3.3m), dictated by tractor size, not tree production potential. These wide spacings reduce upright density and reduce light interception. The planar cordon orchard tested in New Zealand by Tustin *et al.* (2022), features an inter-row distance of <2.5m. This increases light interception (always <40%, in our case) and planting density, resulting in higher yields per hectare. As an example, decreasing interrow spacing in the trial orchard to 2.5, 2.25 or 2m, estimated yields should – all other aspects being equal – increase by 31%, 45% and 63%, respectively. This would return more acceptable yields and, in the case of the higher densities, much greater than the spindle's (e.g., >90t ha<sup>-1</sup> vs 60t ha<sup>-1</sup>).

The yield of the tested orchard (a 1.2 ha, 4-year-old, double guyot multileader system, Pink Lady®-Rosy Glow grafted on Pajam2, with 1443 plants ha<sup>-1</sup>) was 79 t ha<sup>-1</sup> with high quality, despite the adverse conditions of the 2020 season (late frost damage and pollinator problems) that may have reduced the total outcome. This figure is higher than the standard production for current commercial 3D orchards. The thinning approach applied by the grower was to have unskilled personnel leave 5 to 6 fruits between each of the 6 horizontal trellis wire (placed every 50-60cm in height). This resulted in a thinning level of 0.10-0.12 fruit per cm of upright length, providing a considerably higher yield than the estimates of Table 5.1 (79 vs. 45 t ha<sup>-1</sup>). This difference between obtained and estimated yields was

probably due to the small tree sample on which yields were estimated. Despite this, the suitability of these thinning thresholds is confirmed: even in a difficult season such as 2020, a production of almost 80 t ha<sup>-1</sup> was reached using this thinning procedure, confirming this crop load can be considered suitable for fruit production with yields comparable to standard 3D tree shapes. This consideration further supports the results presented in this dissertation, whereby higher production with same quality level seems possible for 2-D trees.

Moreover, if the less-than-optimal interrow planting distance lowered yields in this 2-D orchard, it can be inferred that this orchard would have yielded, if spacings were reduced and tree number increased, 31, 45, 63% more than 79 t ha<sup>-1</sup> (i.e., 103, 115, 129 t ha<sup>-1</sup>). These figures might even be higher, considering that the 4-yr-old orchard under study cannot be considered mature due to the incomplete development of the canopy where uprights had not yet attained full height (i.e., 300 cm). It appears as though consistent yields above 100 t ha<sup>-1</sup> might be possible. Inferring data for a fully developed canopy (3m height) at 2 m interrow planting distance, yields up to 164 t ha<sup>-1</sup> seem obtainable from the studied orchard.

This expectation of unprecedented yields (i.e., + >50% of best figures for 3D systems in this environment - >90t ha<sup>-1</sup>) is all but proven and needs thorough testing. However, yields reported by Tustin *et al.* (2022), for the planar cordon training system, range between 131 and 175 t ha<sup>-1</sup> for inter-row spacing of 2 m, in a 95% fully developed canopy. They also pointed out that above a certain light interception level (i.e., 60-70%) the response of yield to light interception becomes exponential, supporting the higher-than-expected yields. The same authors hypothesised a productive potential for planar multileader apple orchard of 250-320t ha<sup>-1</sup>, for the New Zealand environment.

In conclusion, the goal of this thesis was to evaluate a multileader planar apple training system, on different physiological and agronomical aspects, for its potential to improve sustainability and precision orchard management (POM) in Italian apple orchards. Despite the clear interconnection between each fruiting leader, driven concurrently by its own and the whole plant's fruit load, it appears to be possible to exploit single leader-based thinning thresholds for crop load management, favouring POM application. At the proposed thinning thresholds, fruit production can be expected, with higher fruit quality and yields similar to (or higher than) those of commercial orchards based on 3D tree shapes, if suitable interrow distances for planar systems will be utilized. Considering the actual results obtained from the test orchard in the Po Valley, the investigated training system seems to be able to increase up to 30% (i.e., to 130 t ha<sup>-1</sup>) current "best yields" for a standard, single leader tree. Theoretically, upon reaching maturity, this orchard might achieve 164 t ha<sup>-1</sup>.

Hence, perhaps the single factor that could slow the development of a similar training system lies with the lack of mechanical solutions suitable for inter-rows narrower than 2.5 m.

The finding of the presented studies, together with the benefits coming with multileader planar training systems (exposed in the introduction) suggest a high potentiality of this training systems to increase apple production sustainability also for Italian apple orchard.

Further studies are needed to confirm the results of this thesis and to investigate the possibility to transfer them to other apple varieties, in different environment.

## Literature Cited

Tustin, D.S., Breen, K.C. & Hooijdonk, B.M. van. (2022). Light utilisation, leaf canopy properties and fruiting responses of narrow-row, planar cordon apple orchard planting systems—A study of the productivity of apple. *Scientia Horticulturae*, **294**, 110778.

