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STARCH DISTRIBUTION IN PEAR TREE ORGANS IN RELATION TO TRAINING SYSTEMS, ROOTSTOCKS AND FRUIT QUALITY

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“It is forbidden not to smile at problems,
not fight for what you want, abandon all for fear,
not to realize your dreams”

Alfredo Cuervo Barrero

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ABSTRACT

Starch is the main form in which plants store carbohydrates reserves, both in terms of amounts and distribution among different plant species. Carbohydrates are direct products of photosynthetic activity, and it is well know that yield efficiency and production are directly correlated to the amount of carbohydrates synthesized and how these are distributed among vegetative and reproductive organs. Nowadays, in pear trees, due to the modernization of orchards, through the introduction of new rootstocks and the development of new training systems, the understanding and the development of new approaches regarding the distribution and storage of carbohydrates, are required.

The objective of this research work was to study the behavior of carbohydrate reserves, mainly starch, in different pear tree organs and tissues: i.e., fruits, leaves, woody organs, roots and flower buds, at different physiological stages during the season. Starch in fruit is accumulated at early stages, and reached a maximum concentration during the middle phase of fruit development; after that, its degradation begins with a rise in soluble carbohydrates. Moreover, relationships between fruit starch degradation and different fruit traits, soluble sugars and organic acids were established. In woody organs and roots, an interconversion between starch and soluble carbohydrates was observed during the dormancy period that confirms its main function in supporting the growth and development of new tissues during the following spring. Factors as training systems, rootstocks, types of bearing wood, and their position on the canopy, influenced the concentrations of starch and soluble carbohydrates at different sampling dates. Also, environmental conditions and cultural practices must be considered to better explain these results. Thus, a deeper understanding of the dynamics of carbohydrates reserves within the plant could provide relevant information to improve several management practices to increase crop yield efficiency.

key words: carbohydrate allocation, carbohydrate storage, crop yield efficiency, gas-chromatography, non-structural, pear training systems, rootstocks, soluble carbohydrate, spectrophotometer.

INTRODUCTION

1.1 A general overview

In fruit trees, yield efficiency and productivity are directly correlated to the amount of carbohydrates synthesized and how these are distributed among vegetative and reproductive organs (Oliveira and Priestly, 1988). Basically, yield improvement is achieved by a successful management of source-sink relationships and utilization of assimilates within the plant (Ho, 1988; DeJong and Grossman, 1995). The potential of modifying plant carbohydrates allocation and their utilization within the tree has been long a topic of interest for tree fruit industry, with the objective to optimize economic yields (Wright, 1999) as well as a research topic for the plant physiologists. Seasonal carbohydrates dynamics in different tissues and organs, are particularly well defined in many species, such as apple (Stutte et al., 1994; Berüter et al., 1997; Brookfield et al., 1997; Naschitz et al., 2010), avocado (Scholefield et al., 1985; Whiley et al., 1996a, b), kiwi (Richardson et al., 1997; Miller et al., 1998; Boldingh et al., 2000; Moscatello et al., 2011), grapevine (Zapata et al., 2004), citrus (Goldschmidt and Golomb, 1982), sweet orange (Monerri et al., 2011), pistachio (Nzima et al., 1997; Elloumi et al., 2014), and sweet cherry trees (Keller and Loescher, 1989), since most cultural practices that alter carbohydrates allocation are commonly used in tree fruit production (Wright, 1999). However, scarce information is available today for the European pear cultivation. Moreover, demands of new markets, low yields and ageing of existent orchards triggered into the pear growers the need of orchard modernization. The increase of planting density, the selection of new dwarfing rootstocks (such as dwarfing clones MC and Adams), and the development of new training systems (e.g. Bi-axis) (Musacchi, 2008a; Sansavini et al., 2008; Musacchi, 2011; Musacchi et al., 2011) allowed to improve techniques and management of pear orchards to ensure higher yields, as well as, an improvement in fruit quality.

1.1.1 Pear production and principal cultivars

Pear production in the Europe Union reaches approximately to 2.2 million of tons, being, Italy, the main pear producer in EU, with 707,000 tons yearly, that are equal to 31% of EU pear production, followed by Spain, Belgium and Netherlands, with a 16%, 14.9% and 14.7%, respectively (World Apple and Pear Association, 2015). Nowadays, European pear crops is based on a very few cultivars (Musacchi et al., 2011). In fact, only four cultivars: 'Conference' (39.1%), 'Abbé Fétel' (15.7%), 'Williams BC' (11.3%) and 'Rocha' (7.7%), accounted roughly to 74% of total pear production in the Europe Union (EU-28) (World Apple and Pear Association, 2015). 'Abbé Fétel' is the most important cultivar in Italy, and represents to 35% of pear industry (Musacchi, 2008a). Moreover, in the Emilia-Romagna region, this cultivar has shown an increase of planting in the last years, in fact, the values of plant certification in the Region's nursery from 2003 showed that the production of this cultivar never fell below of 55% of nursery output (Galli et al., 2011).

1.1.2 Pear rootstocks and training systems

Recently, the pear industry has experienced some innovations shifting from low planting density orchards with medium vigor rootstocks towards high density ones (10 – 13,000 trees ha^{-1}) by the use of quince rootstocks, able to control tree size, promote earlier bearing and improve fruit quality (Musacchi, 2008b, 2009). In Italy, more than 90% of orchards are grafted on quince (*Cydonia oblonga*) rootstocks (Musacchi, 2009). The selection of new quince rootstocks, such as dwarfing clones like Adams and MH®, has allowed to improve the high density planting (HDP) management. Moreover MH®, being less vigorous than Sydo®, has obtained a positive effect on fruit size, while Adams has been of interest for growers for its high yield efficiency and good dwarfing vigor (Musacchi, 2009).

The use of dwarfing rootstocks is not just the only real alternative to increase the planting density, there is, also, the choice of the suitable training system (Musacchi, et al., 2011). Different orchard designs, according to planting density, are in fact used. Starting from lower density (palmette or hedgerow systems), through mid-to-high

density (spindle and its offshoots), up to high-density (V-shaped) and very high-density (vertical axis) (Musacchi, 2008b). New ideas concerning tree shape include plants with two or four axes, where the main objective is to split the vigor over more branches. One of this innovative tree shape, Bi-axis, directly provided from the nursery, consists in two preformed axes, planted along the row, in order to have a high and continuous fruiting wall (Dorigoni et al., 2011; Gagliardi et al., 2014). Indeed, the main advantages are: the high early yield, a good light exposure of fruit for high fruit quality, the control of tree growth and, as a consequence, the reduction in pruning time (Musacchi, 2008a; Dorigoni et al., 2011).

The success obtained in high density orchards has been achieved mainly by the correct choice of training system-rootstock combination. In this way, due to the wide availability of combinations that growers have available, appropriate guidelines focused on orchard management oriented to improving yield efficiency and fruit quality, must be determined and transferred to the industry, in order to facilitate the choice (Musacchi 2008b, 2009).

1.2 Non-structural carbohydrate reserves

Carbohydrates are the direct products of photosynthetic activity and constitute a source of energy and metabolites as well as structural basic components (Sivaci, 2006). A considerable portion of the dry matter produced through photosynthesis is stored into cell walls as cellulose, hemicellulose and lignin and therefore it is not available for further utilization by the plant (Goldschmidt and Golomb, 1982). Soluble carbohydrates, which are used to meet current plant requirements, are constituted by monosaccharides (glucose and fructose), oligosaccharides (sucrose mainly), and sugar alcohols; among those, sorbitol is quantitatively the most meaningful in the *Rosaceous* species. While, starch and hemicelluloses, used to satisfy future plant needs, are classified as insoluble carbohydrates (Oliveira and Priestly, 1988). The tree's stored carbohydrates are a reserve pool which support the trees performance during the dormancy or others periods of critical demand (Goldschmidt and Golomb, 1982; Oliveira and Priestly, 1988; Kozlowski, 1992; Whiley et al., 1996a; Flore and Layne, 1999), and later be remobilized to support metabolism and growth, particularly after the

dormancy period, in the following spring (vegetative growth and early fruit development), during the most intense respiration activity, when carbon demand may exceed carbon supply by photosynthesis (Hoch et al., 2003; Elloumi et al., 2014).

1.2.1 Starch, structure and localization in plants

Starch is the predominant non-structural insoluble carbohydrate reserve in plants (Slattery et al. 2000; Blennow, 2004; Streb and Zeeman, 2012), in terms of the amount contained, the widespread of its distribution among the different plant species, and for the carbon economy of many organs, tissues and cell types in the plant (Martin and Smith, 1995; Smith and Zeeman, 2006; Geigenberger, 2011). Starch has widely distributed in almost all green plants and in various types of plant tissues and organs, as in leaves, stems, roots, tubers, fruits and seeds, where it is found as water–insoluble granules of different sizes and specific morphologies (Blennow, 2004; Mukerjea et al., 2009; Keeling and Myers, 2010).

Normal starches consist of semicrystalline granules, usually composed of a mixture of two polysaccharides: amylase (20-30%) and amylopectin (70-80%), which represent approximately 98-99% of the dry weight (Streb and Zeeman, 2012; Keeling and Myers, 2010; Tester et al., 2004). Amylose is defined as a linear α-(1→4) glucan, while amylopectin is the highly branched component of starch, a larger α-(1→4) glucan with 5-6% α-(1→6) branch linkages (Rasmussen and Henry, 1990; 1995; Blennow, 2004; Zeeman et al., 2007; Mukerjea et al., 2009; Geigengerber, 2011).

Moreover, the starch is one of the primary products of photosynthesis (along with sucrose) in the leaves of most plants and serves to buffer the variation in photosynthates availability (Zeeman et al., 2007; Streb and Zeeman, 2012). During the day, the starch accumulated in the leaf, is degraded during the subsequent night, and it is remobilized toward sink organs, providing a continued supply of soluble sugars in absence of photosynthesis, to support respiration and growth (Zeeman et al., 2007; Sulpice et al., 2009; Keeling and Myers, 2010; Streb and Zeeman, 2012). In this way, the starch found in the chloroplasts of leaves, can be seen as a short-term carbohydrate reservoir and is often named “transitory starch”, due to his diurnal rise and fall fluctuations in these tissues (Slattery et al., 2000; Streb and Zeeman, 2012). Long-term storage of starch is

localized into the amyloplasts, specialized starch-containing plastids, conspicuously evident in the non-photosynthetic storage organs such as tubers, roots and seeds (Slattery et al., 2000). It is the so-called “storage starch”, that serves as a medium- to long-term energy source to fuel growth processes (Streb and Zeeman, 2012). Transitory and storage starch granules can be distinguished on the basis of physical characteristics: size, shape and composition (Tester et al., 2004).

Because starch is the principal constituent of the edible organs of many agronomic species, its synthesis also influences crop yield (Slattery et al., 2000). The physical properties of storage starch, which in turn are dictated by its structure, are responsible for its specific uses in the food and manufacturing industries. Advances have been made in understanding genetics and biochemistry of starch synthesis in crop plants. Furthermore, starch remains a keystone in almost all the world’s food and feed chains and has even now become one of the world’s most important source of biorenewable energy (biofuel) (Keeling and Myers, 2010; Streb and Zeeman, 2012).

1.2.2 Starch synthesis

Starch is synthesized in plastids, which in storage organs are called amyloplasts, while in transitory organs such as leaves are called chloroplasts (Keeling and Myers, 2010). The starch biosynthesis involves three enzymes: ADP-glucose pyrophosphorylase (ADPGPPase or AGPase), starch synthase (SS), and starch branching enzyme (SBE) (Martin and Smith, 1995).

In the leaves, a pathway for the conversion of Calvin-Benson cycle intermediates into ADP-Glc is widely accepted (Figure 1). A fraction of assimilated carbon (triose-phosphate) is exported to the cytosol for sucrose synthase, while the other fraction is retained in the chloroplast for starch synthesis (Zeeman et al., 2007). Chloroplastic isoforms of phosphoglucose isomerase (PGI) and phosphoglucomutase (PGM) catalyze the conversion of fructose 6-phosphate (Fru6P) into glucose 1-phosphate (Glc1P). ADPGPPase uses Glc1P and ATP (provided by photophosphorylation at the thylakoid membrane), to generate ADP-Glc and inorganic pyrophosphate (PPi) (Zeeman et al., 2007; Geigenberger, 2011; Streb and Zeeman, 2012).

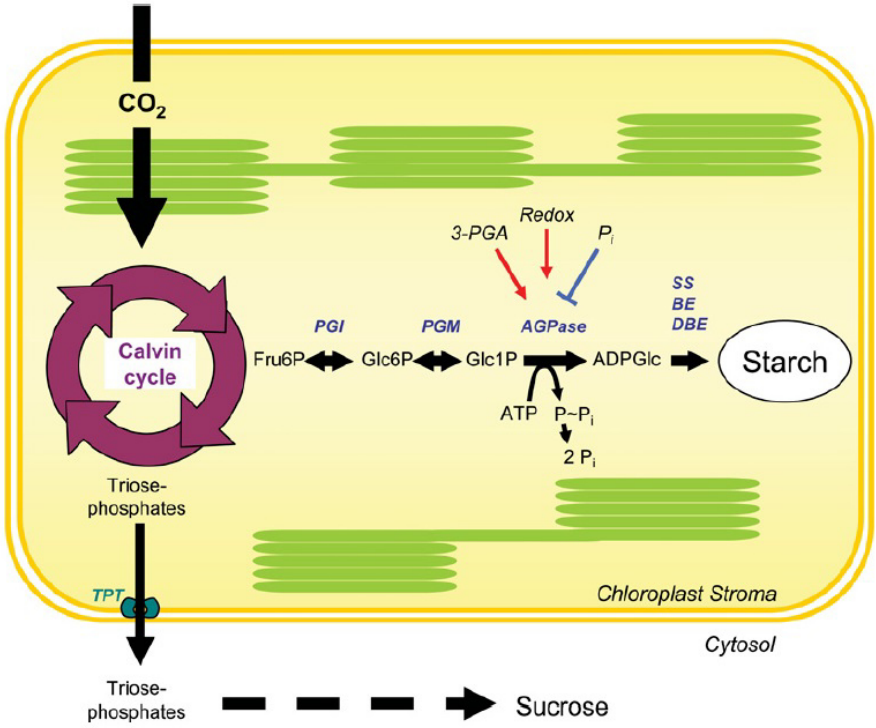

Figure 1. Schematic representation of the pathway of starch synthesis in chloroplasts (Zeeman et al., 2007).

In the case of non-photosynthetic tissues, sucrose derived from photosynthesis, are transported through the phloem sap to the storage organ (Berüter et al., 1997; Keeling and Myers, 2010) (Figure 2). In the cell cytosol, sucrose is converted to uridine diphosphate glucose (UDP-glucose) and fructose by sucrose synthase, the UDP-glucose being subsequently converted to glucose 1-phosphate (Glc1P) in the presence of pyrophosphate (PPi) by UDP-glucose pyrophosphorylase. This is then converted to glucose-6-phosphate (Glc6P) by phosphoglucomutase. The Glc6P is translocated across the amyloplasts membrane by specific translocators and is converted to Glc1P. There is some evidence that, in cereals at least, , Glc1P may be: (a) directly translocated into the amyloplasts or (b) be converted to, and translocated as, adenosine diphosphate glucose (ADP-glucose). Glc1P within the amyloplasts is also converted to ADP-glucose and provides glucose residues for amylose and amylopectin biosynthesis (Martin and Smith, 1995; Tester et al., 2004; Keeling and Myers, 2010; Geigenberger, 2011).

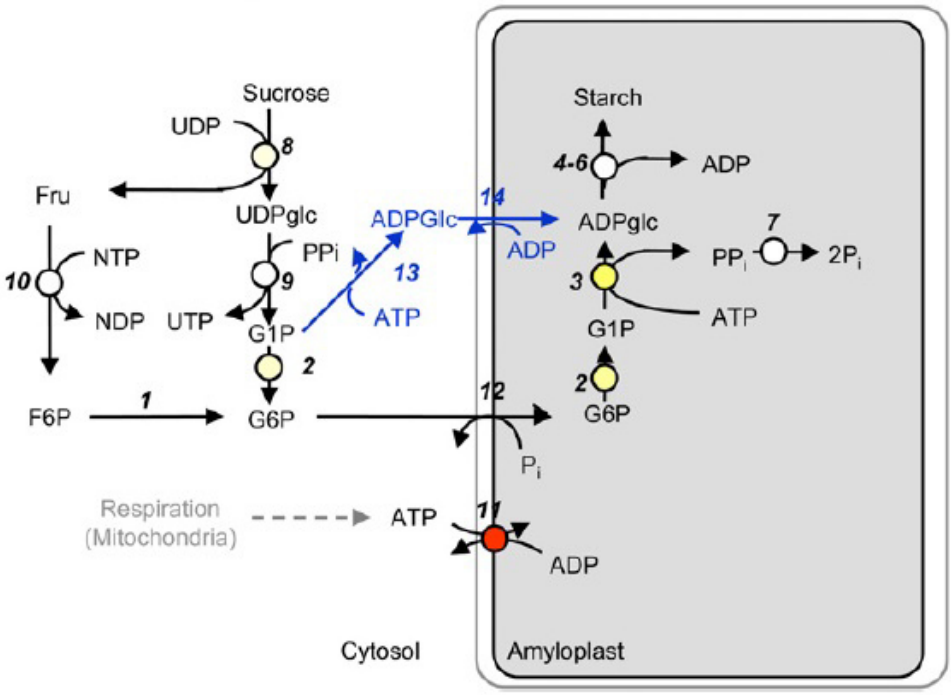

Figure 2. Schematic representation of the pathway of starch synthesis in non-photosynthetic tissues (Geigenberger, 2011). Enzymes involved in the starch synthesis: (1) PGI; (2) PGM; (3) AGPase; (4) SS; (5) SBE; (6) starch-debranching enzyme; (7) inorganic pyrophosphatase; (8) Suc synthase; (9) UDP-Glc pyrophosphorylase; (10) fructokinase; (11) ATP/ADP translocators; (12) Glc-6-P/Pi translocators; (13) cytosolic AGPase; and (14) ADP-Glc/ADP translocators.

1.3 Methods for starch determination

Starch has often used as an indicator of the tree status, being a long-term reserve in trees, so the starch quantification is a useful tool of physiological information needed to determine potential crop yields in perennial plants (Rasmussen and Henry, 1990; Bellasio et al., 2014). Carbohydrates include both soluble (soluble carbohydrates) and insoluble (starch) substances, so specific techniques are required for extraction and qualitative and quantitative assays (Loescher et al., 1990). Therefore, it is necessary to isolate starch from the other components present in the sample, prior to carrying out starch analysis. Furthermore, starch granules are composed of a mixture of two

polysaccharides, amylose (20-30%) and amylopectin (70-80%) (Tester et al., 2004; Blennow, 2004; Mukerjea et al., 2009; Keeling and Myers, 2010; Streb and Zeeman, 2012). For this reason, starch concentration cannot be determined directly because it is contained within a structurally and chemically complex matrix, which is inaccessible to the chemical reagents used to determine its concentrations. In this way, methods for routine starch analysis generally require quantitative breakdown of the polymer to the monomer, glucose, afterwards the last compound is quantitatively determined, often colorimetrically (Carter and Neubert, 1954; Rasmussen and Henry, 1990; Rose et al., 1991; Seager and Haslemore, 1993; Bellasio et al., 2014).

Generally, water-soluble sugars are extracted prior to starch analysis from the tissues samples, and starch content is determined in the solid residue (Chow and Landhäusser, 2004; Bellasio et al., 2014). Usually, two methods are used for soluble sugars extractions: hot ethanol and methanol:chloroform:water solutions (Chow and Landhäusser, 2004; Bellasio et al., 2014). Long (1916) classified the methods to determine starch under three procedures: reduction (chemical), polariscopic (physical), and colorimetric methods. Chemical methods are based on the enzymatic or acid hydrolysis of starch (Norgia et al., 2008). In woody tissues, due to the presence of non-starchy compounds, the determination of starch by chemical or polarimetric methods is difficult (Gur et al., 1969). Chow and Landhäusser (2004) found that the amount of interfering substances is closely related to the sugar content in the plant tissues. Regarding the physical methods, the values obtained refer to raw starch, because both products dextrins and hemicelluloses are dissolved, and the determination occurred on solution by a polarimetric technique (Norgia et al., 2008). These procedures, when small amounts of plant material are available, have a little value; being in this case, a colorimetric determination an alternative choice (Gur et al., 1969). Regarding the colorimetric determinations, the method based on the anthrone reagent is more adequate when it is not necessary to determine the concentrations of individual sugars (Edwards et al., 2011); on the contrary, when the determination of each single soluble sugar is necessary, in this case high-performance liquid chromatography (HPLC) or gas chromatography (GC) are the chosen methods and better results could be expected (Seager and Haslemore, 1993; Bartolozzi et al., 1997; Hoch et al., 2003).

1.3.1 Starch Pattern Index (SPI)

Starch degradation during maturation is one of the most important indicators for predicting the optimal harvest dates for apples (Brookfield et al., 1997), usually assessed by an iodine test, in which equatorially cut fruits are dipped in an iodine solution and stain patterns are rated (Smith et al., 1979; Fan et al., 1995; Brookfield et al., 1997; Peirs et al., 2002), by a starch pattern index (SPI). The cultivars exhibits a characteristic starch pattern, during maturation, shown by iodine staining, as starch is degraded in fruits (Smith et al., 1979). This pattern proceeds in the cortical tissues either in a circular or a radial way (Peirs et al., 2002). The SPI is a subjective index measure estimated with color charts, ranging from 1 (all stained) to 10 (not stained) (Peirs et al., 2002), however, this scale is not uniform all over the world, e.g., in Canada harvest date is based on nine stage charts (Smith et al., 1979), while in the United States and in New Zeeland, is based in references chart with six stages (Fan et al., 1995; Brookfield et al., 1997). Indeed, this procedure provides only a qualitative indication of the total starch. Additionally, the rate of change of the starch index could vary according the year and locations (Smith et al., 1979). The starch-iodine reaction has also been incorporated into the routine procedures for the determination of the starch content in pear (Stow, 1988; Le Lezec and Belouín, 1994; Agar et al., 1999). However, Stow (1988) described this technique as an unreliable guide to the optimum harvesting period for pear fruits.

1.4 Source - sink relationship

Carbon partitioning implicates the transport of assimilates from source organs to various sinks, and their distribution (Fanwoua et al., 2014). Sinks for photoassimilates, nutrients, water, and phytohormones include reproductive (flowers and fruits) and vegetative (shoots or roots) tissues (Flore and Layne, 1999). The carbohydrates, produced during photosynthesis, can be used directly for growth in the same leaves (source), or, to a large extent, can be translocated to meet the current requirements of sinks, determining different patterns of growth (Oliveira and Priestley, 1988; Wolstenholme and Whiley, 1997). In leaves, starch synthesis occurs at higher rates when carbon assimilation is high, relative to the demand for carbon export, and at lower rates when assimilation is low, relative to the demand from the rest of the plant (Martin

and Smith, 1995). Variations in rates of carbohydrates transport from source to sink indicate internal competition. Among fruits, this competition is reflected in small fruits, delayed fruit maturity, less color and quality (Kozlowski, 1992), which may be amplified when assimilates supply is limited (Ho, 1988). DeJong and Grossman (1995) noted that source limitations could be explained by: insufficient amount of assimilates needed to support the sink growth, disability to translocate assimilates, or strong competition among sinks. In this way, the source-sink relationship changes during the growing season, as well as, the sink strength of individual organs and number of sinks competing for a common pool of carbohydrates (Roitsch, 1999). The concept of sink strength refers to the capacity of an organ or tissue to import carbohydrates. This ability varies mainly with species, tree vigor and age of tree (Kozlowski, 1992), as well as, also the sink demands and seasonal development pattern of the plant changes with time (Flore and Layne, 1999). This sink capacity according Ho (1988) can be measured by the product of sink size (cell number) and sink activity, which is represented by the activity of the key enzymes of carbohydrates metabolism, being both genetically determined.

However, the allocation of imported assimilates is substantially different from one sink organ to another, based on the rate of the imported assimilates. In meristematic tissues, the imported assimilates are used mainly for growth, and only a small amount would be stored temporarily, this sink organs are classified as *utilization* sinks (Ho, 1988; Kozlowski, 1992). Developing fruitlets, during the cell division period, are defined as utilization sinks, whereas in cell enlargement period act as *storage* sinks (Mehouachi et al., 1995). In *storage* organs such as fruit, stem tuber, and root, substantial amounts of imported assimilates are stored, being subsequently remobilized, according to the metabolic needs (Ho, 1988; Kozlowski, 1992).

1.5 Seasonal cycle of carbohydrate reserves

In woody organs, the quantitative and qualitative variations of starch and soluble carbohydrates during the season have been studied in several species (Cameron, 1923; Scholefield et al., 1985; Wolstenholme and Whiley, 1997; Whiley et al., 1996a, b; Miller et al., 1998; Zapata et al., 2004; Sivaci, 2006). During the natural leaves fall, in

autumn, after crop has been harvested, a maximum concentration of starch is observed. Then a decline in starch content and loss in dry weight over winter, accompanied by a mobilization of reserves from the roots and old stem to the meristematic regions (dormancy release), is observed in spring. Early stages of growth in spring depend on the reserves accumulated during the previous growth season, in this way, after bud break and flowering phase and before current season's extension growth begins, carbohydrates replenishment starts again. From then on, during the season growth, the carbohydrate concentration remained low until mid-summer. The annual patterns of accumulation of starch differ among species and genotypes, according their growth characteristic, as well as, the time of fruit ripening of the cultivars (Kozlowski, 1992) and age of the trees (Chalmers and Van den Ende, 1975). Chalmers and Van den Ende (1975) proposed that, in small-young trees, assimilates are partitioned mainly to frame and root growth, while in large-mature trees, the greatest percentage goes to the fruit.

Between deciduous and evergreen trees a great difference exists, the former practically dependent on stored carbohydrates for early spring growth, whereas the latter having leaves in winter, partly reduce this dependence on stored resources (Wolstenholme and Whiley, 1997). Also, in evergreen trees, yields have been related to the starch concentrations during the previous dormant period, in this way, a low level of starch storage, due to a heavy cropping, often ends in low yields, whereas high levels of starch concentrations result in high fruit yields (Scholefield et al., 1985; Whiley et al., 1996b).

Fruit, like apples, pears and kiwifruits, during their development, import assimilates that are used mainly in the synthesis of structural polysaccharides for growth, respiration, and carbohydrates storage (Berüter and Feusi, 1997). In apple and kiwifruit, a pattern of accumulation and degradation of starch has been described (Berüter and Feusi, 1997; Berüter et al., 1997; Brookfield et al., 1997; Boldingh et al., 2000, Berüter, 2004; Moscatello et al., 2011). Generally, at earlier stages of growth, fruits begin to accumulate starch in plastids, reaching a maximum at the onset of maturation, later on, starch is progressively degraded and give rise to an increase of soluble carbohydrates, producing, as a side effect, an increased sweetness during ripening.

1.6 Influence of cultural factors and management practices

Numerous studies pointed out the influence of the characteristics of scion (Boldingh et al., 2000; Berüter, 2004), rootstock (Gur and Samish, 1965; Gaudillère et al., 1992; Caruso et al., 1997; Olmstead et al., 2010) and training system (Stutte et al., 1994; Caruso et al., 1999) on carbohydrates partitioning and storage in several species. Although the tree vigour is modified by the rootstock, the influence on carbohydrate reserves depend on: the type of carbohydrate and on the period of the season (Gaudillère et al., 1992), the age of the tree (Olmstead et al., 2010), and the sink strength of growing fruits (Kozlowski, 1992). Regarding the training systems, considering that sources and sinks are connected to each other conducting and supporting shoot structures, the knowledge of the role of branch architecture acquire a great importance to understand the process of distribution of assimilates in fruit trees (Fanwoua et al., 2014). Other researchers have been focused on the effect of different levels of crop-load on starch accumulation in fruits and woody tissues of kiwi (Richardson et al., 1997), apple (Naschitz et al., 2010), persimmon (Park, 2011), citrus (Goldschmidt and Golomb, 1982), sweet orange (Monerri et al., 2011; Dovis et al., 2014) and pistachio trees (Nzima et al., 1997; Elloumi et al., 2014).

The accumulation of carbohydrates reserves is particularly sensitive to late-season stresses and management practices that, reducing starch accumulation, can greatly influence metabolism and growth of plant in the following year (Loescher et al., 1990). Defoliation treatment, depending on time and intensity of application, can influence the concentrations of starch and soluble sugars in fruits and woody organs during the growing season (Mehouachi et al., 1995; Hudina and Štampar, 2002; Cruz-Castillo et al., 2010), or before natural leaf fall, it could affect the root growth and flower and vegetative buds development for the following season (Oliveira and Priestley, 1988;). Otherwise, a summer pruning practice does not only remove photosynthetic sources, as defoliation does, but also removes vegetative sinks, redirecting the allocation of carbohydrates (Loescher et al., 1990). In one of the first approaches on carbohydrate reserves dynamics in pear trees, Cameron (1923) noted that trees non-headed began to store starch earlier than the headed trees, due to a major presence of young spurs. In this way, all orchard practices which maintain an optimal leaf area and delay leaf senescence could allow a greater assimilation during the postharvest period, resulting in a sufficient

or optimal accumulation of carbohydrate reserves to support initial growth and development in the following spring (Kozlowski, 1992; Tustin et al., 1997).

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AIMS OF THE THESIS

From the information collected and discussed above, the knowledge of plant carbohydrates status could give an useful indicator about tree health and its potential performance (Wolstenholme and Whiley, 1997), as well as, the balance between the supply and demand for assimilates at different stages of development or under stress conditions, and about availability of carbohydrate reserves (Boldingh et al., 2000). In this way, understanding the effects of different cultural factors and management practices on the carbohydrate storage and allocation will provide useful information for a consistent fruit production (Park, 2011).

Seasonal carbohydrates reserves in fruit trees have been studied over many years and in several species, focusing among seasonal reserve accumulation, distribution and utilization. Moreover, these studies have improved and provided more accurate methodologies of analysis, that allow a better understanding of the results, that can be evaluated under physiological aspects. However, in European pear trees, knowledge about carbohydrates reserves is still weak; which makes it necessary to investigate and update the experience so far obtained.

The carried out research has involved the study of the dynamics of carbohydrates reserves, particularly starch, in various organs of pear tree, from physiological and horticultural perspectives, throughout different phenological stages. 'Abbé Fétel' was the cultivar chosen to conduct this research, because is the most important cultivar in Italy accounting up to to 35% of the domestic market. The specific objectives were:

- To determine the behavior of starch and soluble carbohydrates in fruits and leaves during the growing season, and establish relationships between the starch degradation in fruit and different fruit quality parameters.
- To study the starch and soluble carbohydrates dynamics in wood, roots and flower buds, during the dormancy period (between post-harvest and dormancy release), in relation to training system, rootstock, and bearing wood.

To accomplish these aims, four experiments were conducted under field conditions and presented and discussed in the following chapters.

SEASONAL BEHAVIOR OF STARCH AND SOLUBLE CARBOHYDRATES IN FRUITS AND LEAVES OF 'ABBÉ FÉTEL' PEAR TREES DURING GROWING SEASON

3.1 Introduction

It is know that the initial growth and development of vegetative and reproductive organs of deciduous tree species get considerable proportions of their carbohydrates requirements from stored reserves (Hansen, 1967b; Loescher et al., 1990, Nzima et al., 1997; Caruso et al., 1997; Flore and Layne, 1999; Kühn, 2006; Lakso and Goffinet, 2013). A different point of view is perceived in fruit-bearing evergreen trees, such as citrus and avocado, where early stages of reproductive development are highly dependent on current photosynthetic carbohydrates availability (Goldschmidt and Golomb, 1982; Scholefield et al., 1985; Finazzo et al., 1994). The contribution of stored carbohydrates to flower and fruit development in deciduous trees depends, partially, on timing of this development (Loescher et al., 1990). Therefore, the knowledge of space-time of the phenological phases of vegetative and reproductive development is crucial to understand dynamics of carbohydrate reserves on fruit trees. In *Prunus*, flowering takes place before leaf emergence, consequently, in the absence of new photoassimilates, early stages of fruit development will be supported by pre-stored reserves (Kühn, 2006), and may be mainly dependent on the mobilization of root reserves (Loescher et al., 1990). Species that initiate shoot growth before anthesis, such as grapevines and kiwi, reserve mobilization is important for both shoot growth and flowering (Piller et al., 1998), until the leaves produce enough assimilates to meet sink demand (Flore and Layne, 1999).

Similar behavior was found in grapes, which has been suggested for apple trees too, because leaves are almost fully expanded before anthesis and the main part of fruit development occurs after leaves development (Loescher et al., 1990). Hansen (1971) reported that apple flowers depend on reserves only during their earliest stages of development, after this, leaves photosynthesis becomes the major source of carbohydrates for flower and subsequent fruit growth.

The tree life phases are accompanied by source-sink relationships as well as changes with respect to the sink organs competing for a common pool of carbohydrates (Roitsch, 1999). Different reproductive sinks (flowers and fruits) and vegetative sinks (shoots or roots) compete for photo assimilates nutrients, water, and phytohormones. Essentially all tree organs, at some stages of development, would act as sinks, i.e. receivers of assimilates. In terms of assimilate transport, the ability of a sink organ to import assimilates is its sink strength (Ho, 1988). Sink strength of individual organs varies with time of the year, and age of the plant, as the sink demands and seasonal development pattern of the tree change with the time (Flore and Layne, 1999). Fruits act strongly as "sinks", i.e., organs which attract metabolites from the translocation system. This can induce a strong growth reduction in the canopy of apple tree caused by a heavy crop (Hansen, 1967a). Others organs change roles between sinks and sources during the season growing progresses (Nzima et al., 1997).

The final fruit size of pears at harvest is determined by both fruit cell division and cell expansion. The former occur in the first few weeks following flower fertilization and it is thought to be influenced by the relative sink strength of the fruit and the availability and efficiency of assimilates and nutrients supply to it (Webster, 2002). Pome fruits in active growth are supplied by the C-assimilates such as sorbitol and sucrose produced by leaf photosynthesis. These C-sources enter in fruit metabolism and accumulate as fructose, sucrose, malic acid, and starch (Berüter et al., 1997). In citrus, it is known that during the cell division period developing fruit import assimilates from old leaves, due to new young leaves do not export photosynthates until their growth is completed (Mehouachi et al., 1995). In this way, developing leaves and growing fruits are competing sinks. Therefore, during the transition in the fruit from cell division to cell enlargement, young leaves move from being a sink to a source and begin to export assimilates to the fruit (Mehouachi et al., 1995).

Assimilates production in leaves is modulated by the demand for photoassimilates (Lakso et al., 1999). Thus, to some extent, the supply is adjusted to demand by the developing fruits (García-Luis et al., 2002). In deciduous fruit trees, the transport of carbohydrates depends locally on the differences in concentration between sources and sinks (Bruchou and Génard, 1999). Finazzo et al., (1994) investigated in avocado trees cv. 'Petersen' the translocation pattern of photoassimilates between developing fruitlets

and leaves, stated that there was not a competition between these sinks for current photoassimilates at earlier stages of growing season, due to the presence of enough availability of carbohydrates.

In the present work, were determined the dynamics of accumulation and degradation of starch and soluble carbohydrates concentrations, in fruits and leaves of 'Abbé Fétel' pear trees, trained at Spindle system and grafted on two rootstocks, Sydo® and Quince C, during the growing season, expressed as days after full bloom (DAFB). An additional goal was to evaluate possible differences in carbohydrates concentrations in fruits from different types of bearing wood.

3.2 Materials and Methods

3.2.1 Plant material and cultivation conditions

Trial was carried out throughout three growing seasons (2012-2013-2014) on seventeen-year-old pear trees (*Pyrus communis* L.) cv. 'Abbé Fétel', trained as a Spindle and grafted on two rootstocks: Sydo® (medium vigor) and Quince C (dwarfing). Planting distance was 3.6 x 1.40 m for Sydo® and 3.6 x 0.7 m for Quince C.

The orchard (North-South oriented) was located at the Experimental Station of the Bologna Agriculture Faculty, in Cadriano, Italy (44°54'88.53''S; 11°38'59.30''W). The orchard was managed following standard cultural practices (i.e. fertigation, disease and pest control). Homogeneous trees in size and vigor were selected within the orchard for the experimental trail on the basis of the TSCA.

3.2.2 Fruit and leaf sampling

Timing of samples collection during the growing season was defined as days after full bloom (DAFB); consequently the date of full bloom was registered each year. The full bloom, corresponding to the 100% of open flowers, took place on March 30, 2012, April 17, 2013, and March 27, 2014. At each sampling date, 12 fruits in the middle zone of the tree (between 1.0 to 1.5 m high) and from the external part of the canopy were

collected randomly per treatment (2 rootstocks) to determine later the soluble carbohydrates and starch concentration. During the first two seasons of the experiment (2012 - 2013) fruits were collected only from 3-years-old branches; whilst in the third year (2014) fruits were harvested from different types of bearing wood: brindle-type shoots, 2-years-old branches, 3-and-over-years-old branches, and short-old spurs. Simultaneously, in the first two years of study, for each sampling date, the leaves more nearby to the bearing wood where sampled fruits were held, were collected.

Each sampling was done early in the morning and the vegetal material was directly transferred in a cooler to the laboratory for analyses. Samples were collected in each year of evaluation, at different DAFB, as shown in Table 1. The commercial harvest took place at 152, 142, and 157 DAFB, respectively.

Table 1. Sampling dates, expressed as days after full bloom (DAFB) for each evaluation year.

	Days after full bloom						
Year	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
2012	97	115	131	<u>152</u>	171	195	
2013	51	63	79	93	107	127	<u>142</u>
2014	90	121	133	<u>157</u>			

DAFB underlined corresponded to commercial harvest.

3.2.3 Fruit and leaf growth

Fresh fruit weight was determined on 12 fruits for treatment at each sampling date. While average leaves fresh weight was calculated from all leaves collected. Fruit and leaf dry weights were determined on subsamples placed in a freeze dryer for 7 and 3 days, respectively (HETO drywinner, DW3, Denmark). From these values, the daily rate of dry matter (mg day^{-1}) in the developing organs was calculated.

3.2.4 Productive parameters

At harvest time, twelve trees for each treatment, Spindle/Sydo® and Spindle/MH® were evaluated. All fruits were picked and then, number and weight of total fruits were recorded.

3.2.5 Starch and soluble carbohydrates determinations

3.2.5.1 Preparation of material

One 2-mm-thick equatorial slice from each of the 12 fruits (3 biological replications with 4 pooled fruit each one) was collected. For leaves, three biological replications of 50 units each one, were determined. Fruit flesh and leaf samples were immediately frozen in liquid nitrogen and stored at -80 °C to be later used for the determination of the soluble carbohydrates and starch concentrations. Successively, the samples were dried in a freeze dryer, reweighed and ground till a fine powder with a mill then stored in airtight containers at room temperature. For the analysis, three technical replications of 50 mg for fruit and, 100 mg for leaves of dry weight each were used.

Carbohydrates were analyzed using a modification of the method described by Roe (1955). A heat-stable amyloglucosidase, needed to hydrolyze the starch contained in the finely ground plant material to glucose, was used. The glucose produced was measured colorimetrically with the anthrone method (Marangoni et al., 1980) by a spectrophotometer (VIS-UV Varian mod. Cary 1E).

3.2.5.2 Determination of soluble carbohydrates concentrations

Soluble carbohydrates, including sorbitol, sucrose, glucose, fructose, raffinose, and inositol (Loescher et al., 1990) were extracted by 5 ml of methanol:chloroform:water solvent (MCW, 12:5:3, v/v/v) added to the tissues, 50 and 100 mg, for fruits and leaves, respectively, and let it act for 30 min shaking by hand 2 - 3 times. Then, samples were centrifuged 10 min x 5000 *g* and supernatants were collected, and pellets washed in 5

ml of MCW and re-centrifuged. This step was repeated two more times and the three pooled supernatants, so resulting, were evaporated in a rotary evaporator (Heidolph, Hei-VAP Value/G3) at 60 °C, 100 rpm until organic molecules were totally evaporated. The aqueous residue was brought to a volume of 10 ml with distilled water. To remove the interfering phenolic compounds, 0.2 g polyvinylpolypyrrolidone (PVPP, Sigma Aldrich) were added in each sample, shaken and then centrifuged. Afterwards, the resulting clear supernatant was ready to be measured by the spectrophotometer.

3.2.5.3 Determination of starch concentration

The insoluble residue (pellet), resulting after the soluble carbohydrates extraction, was saved and used to determine the starch concentration. The pellet was re-suspended in 2 ml of distilled water and autoclaved for 20 min at 120 °C (to facilitate the enzymatic digestion). Successively, starch was enzymatically digested (breakdown of the polymer to the monomer, glucose) by incubation at 45 - 50 °C in a water bath for 1 h with a solution made with 1 ml 2M Na-acetate buffer plus 1 ml of 200 mM Na-acetate buffer, both a pH 4.5, and 1 mg amyloglucosidase from *Aspergillus niger* (Sigma, E.C. 3.2.1.3), thereafter allowed to stand at room temperature for 12-24 hours. After this digestion period, samples were centrifuged, and supernatants were placed in a 10 ml flask. The residue was centrifuged one time more with a solution of 1 ml of Na-acetate buffer and 1 ml of distilled water. So, a third centrifugation was applied on the residue with a solution of 2 ml of distilled water. The three supernatants were combined, and were brought to a final volume of 10 ml with distilled water. Afterwards, each sample was ready to be measured by spectrophotometer.

3.2.5.4 Spectrophotometer analysis

One ml aliquot from each of the 10 ml sample was taken, and 10 ml of anthrone reagent added in a glass tube with glass cap. Subsequently, samples were shaken and maintained in boiling water for 15 minutes in the dark, then cooled to room temperature for 30 minutes (keeping the dark). Finally, samples were quantified by reading the absorbance by spectrophotometer (VIS-UV Varian model Cary 1E) at 620 nm of

wavelength (λ); concentrations were obtained by comparison with a standard curve of glucose (0 – 1000 μg ml^{-1}).

Total soluble carbohydrates and starch concentrations are reported as glucose equivalents (milligrams per gram of dry weight tissue) and corrected for the appropriate dilutions. Starch concentration was also multiplied by 0.9. This factor accounted for the mass of glucose theoretically hydrolyzed from a unit mass of starch (McCready et al., 1950; Seager and Haslemore, 1993; Smith and Zeeman, 2006; Bellasio et al., 2014).

3.2.6 Statistical analysis

Analysis of variance (ANOVA) and means separation followed by SNK Test with a significance level of $p<0.05$ were performed on all collected data and were elaborated with a SAS® software package (SAS Institute, Cary, NC, USA).

3.3 Results

3.3.1 Fruit growth

The fruit's growth curve of 'Abbé Fétel' pear, measured through three seasons, fitted well a single sigmoid curve, as shown in Figure 1. Fruits harvested from the same bearing wood (3-and-over-years-old branches) during the season registered significant differences between rootstocks only at 97 DAFB in the first year of study (data not shown), where Sydo® rootstock presented the highest value, being a 21.5% higher than Quince C, respectively. Moreover, among fruits collected from different types of bearing wood (2014), no differences were found within each combination (training systems/rootstock) (Table 2).

The average fresh leaf weight for both years (2012-2013), exhibited a similar trend during the period under evaluation, between 264.6 and 316.1 mg (average 289.0 ± 53.3 S.E.) and, as shown in Figure 2, did not showed significant differences ($p = 0.35$). This is mainly due to the fact that the weight was measured after the active growth period, when the leaves were already fully expanded. Whereas, for the leaf dry weight, an

increase of 24.9% was observed between 50 and 100 DAFB, after this phase only a slight dry weight percentage increase was observed, then remained constant until the end of the evaluation (average 43.4% ± 0.2). Furthermore, among all sampling date significant differences were found ($p \leq 0.0001$) (Figure 2).

3.3.2 Starch and soluble carbohydrates in fruits

In Figure 3A - B is shown the behavior of starch and soluble carbohydrates concentration in fruits of 'Abbé Fétel' pear trees, according the rootstocks and year of evaluation. Sydo® and Quince C rootstocks did not present significant differences for starch concentration at each sampling date, in the same year of assessment (Figure 3A). However, for the soluble carbohydrates concentration were found differences at 97 and 127 DAFB ($p<0.01$), for the first and second evaluation seasons, respectively (Figure 3B), where Sydo® rootstock presented the highest value, being a 51.6 and 18.7% higher than Quince C, respectively.

The starch level in fruit reached a maximum value during the middle phase of fruit development, and later in the season it has been almost fully degraded with a simultaneous increase in soluble carbohydrates, corresponding to the period of fruit maturation. The highest starch amounts were registered at 115 and 107 DAFB, for the first and second year, regardless of the rootstock (Figure 3A). The maximum concentrations for Sydo® rootstock corresponded to 167.8 and 128.7 mg starch g^{-1} DW, whereas for Quince C corresponded to 175.9 and 128.7 mg starch g^{-1} DW, for the first and second year of evaluation, respectively. The average values between rootstocks for year (as no differences were found) corresponded to 3.5% and 1.9% of fruit fresh weight (17.2% – 12.3% of dry weight). In addition, the maximum levels were registered approximately 5 weeks before commercial harvest, which corresponded to 152 and 142 DAFB, respectively. Also, from these results, it is noteworthy that season 2013 recorded lower values than season 2012.

On the other hand, fruits collected from different types of bearing wood at different dates during the last season of experiment (2014), did not show differences in terms of starch and soluble carbohydrates concentration in both combinations of training system/rootstock (Figure 4A - B, Table 3). In this way, fruits showed a similar behavior

during growth and maturation on the tree. Nevertheless, the statistical analysis reported significant differences between the two rootstocks (Figure 5). Unlike the results of the first two years, Sydo® and Quince C rootstocks presented differences at 90 and 121 DAFB for starch concentration, where Quince C showed the highest value, being a 20.9% and 11.0% higher than Sydo®, respectively. Whilst for soluble carbohydrates, differences were found at 121 and 157 DAFB, with a high significance ($p \leq 0.001$), showing the same behavior of previous years, where Sydo® rootstock obtained the highest value.

Another parameter analyzed in fruits harvested from different types of bearing wood, was the daily rate of increase in soluble carbohydrates and decrease in starch cocentrations (mg day^{-1}). No differences were found within each combination of training system/rootstock, either for starch and soluble carbohydrates (Table 4, vertical comparison). However, between rootstocks were presented differences (horizontal comparison). Sydo® rootstock obtained a daily rate of increase in soluble carbohydrates of 28.2% and 47.6% higher than Quince C, in branches of 2 and 3-years-old, respectively. Whereas, the daily rate of starch decrease, was only significant for fruits held by short-old spurs, being in this case the rootstock Quince C that obtained the highest value (Table 4).

3.3.3 Starch and soluble carbohydrates in leaves

Starch in leaves showed a different behavior to that observed in fruits (Figure 6). The two-year experiment (2012 - 2013) allowed a better understanding of this performance. The starch pattern could be divided mainly in three different stages. Initially, from 50 to 100 DAFB, a decrease in the leaf starch concentration can be observed, which corresponded to the period when fruit actively stored starch. Subsequently, a phase without major changes until 150 DAFB, and finally, from harvest until the last sampling of the first year, an increase in the value was observed (Figure 6). In the second year of the experiment, a windstorm, some days before to the harvest sampling, occurred, so, unfortunately leaves were compromised for the following subsequent samplings. In fact, an abrupt decrease was observed at 142 DAFB (Figure 6), which was accompanied of a significant increase of soluble carbohydrates (Table 5). On the other hand, the statistical

analysis showed significant differences between rootstocks only in the first date of sampling in both seasons 2012 and 2013 (51 and 97 DAFB, respectively), showing Quince C as the rootstock with the highest leaf starch concentration (Figure 6).

In the case of soluble carbohydrates, the rootstocks presented significant differences only in the second year at 63, 79 and 107 DAFB (Table 5); nevertheless, it was not possible to establish a trend for the differences between the rootstocks. The general pattern, throughout the two seasons assessed, showed a stable performance from the beginning of the experiment until 150 DAFB (average 53.9 and 52.6 mg Glc g^{-1} DW, respectively), from this point, similarly to what was observed for the starch concentrations, an increase in the concentration of soluble carbohydrates occurred.

3.4. Discussion

Seasonal starch pattern of Abbé Fétel' pear fruits (Figure 3A) observed in this trial followed patterns of accumulation and degradation that have been described for other species (Brookfield et al., 1997; Berüter et al., 1997; Berüter, 2004; Gawęda and Ben, 2010 for apple; Wegrzyn and MacRae, 1995; Miller et al., 1998; Richardson et al., 1997, 2004; Boldingh et al., 2000; Moscatello et al., 2011 for kiwifruits).

During the first stage of fruit growth, fruit are acting as 'utilization sink' rather than 'storage sink', because a great demand to support their high metabolic activity and rate of cell division is needed (Mehouachi et al., 1995). Moscatello et al. (2011) stated that in this period, in which strong cell division occurs, most of the carbon found in the fruit is allocated to structural components. Wegrzyn and MacRae (1995) confirmed that during the first 60 DAFB of kiwifruit development, the rate of starch accumulation was minimal. Moreover, Brookfield et al. (1997) noted that, in the first four weeks of fruit growth, the starch concentrations decreased in apple cultivars 'Royal Gala' and 'Fuji' and, after this, a period of fruit abscission normally occurs, while the remaining fruits on the tree accumulate starch again. From these studies a consistent pattern emerged, the starch begins to accumulate in fruit after the phase of cell division which, depending on the cultivar and the fruit development duration, occurs approximately between 30 and 50 DAFB for apple (Brookfield et al., 1997; Berüter et al., 1997; Berüter, 2004; Gawęda and Ben, 2010), whereas in kiwifruit, fruit starch accumulation has been

observed between 40 and 80 DAFB (Wegrzyn and MacRae, 1995; Miller et al., 1998; Richardson et al., 1997, 2004; Boldingh et al., 2000; Moscatello et al., 2011). In this experiment, 'Abbé Fétel' pear fruit registered the minimum value at 51 DAFB, which corresponded to 24.7 mg g^{-1} DW, being the starting point for starch storage in fruits.

Several authors have pointed out the beginning of starch breakdown, which coincides with the peak of its concentration, for different apple cultivars, indicating dates between 105 and 112 DAFB, and for kiwifruits between 130 and 160 DAFB. According to the experimental values obtained in this study, where the starch hydrolysis began at 115 and 107 DAFB, for the years 2012 and 2013 respectively, our data agree with the previous ones reported in the literature concerning other species.

The result that starch and soluble carbohydrates concentration in fruits, collected from different types of bearing wood, presented the same trend (Figure 4, Table 3), can be explained in agreement with Chalmers and Van den Ende (1975)'s findings in peaches. They stated that the growth of the fruit, roots and tree frame (old structures) is competitively inter-related throughout the life of the tree. Moreover, that sink strength of the individual organs varies with time of the year, and age of the plant, as the sink demands and seasonal development pattern of the plant change with time (Ho, 1988; Flore and Layne, 1999; García-Luis et al., 2002). Thereby, in small-young trees, assimilates are partitioned mainly to frame and root growth, to promote a rapid fill their allotted space, while in large-mature trees, the greatest percentage goes to the fruit (Chalmers and Van den Ende, 1975). The latter represents the case of this experiment, because the trees were in their nineteenth year at the time of this work.

DeJong and Grossman (1995) stated that in peach source limitations may result from different reasons, such as: insufficient assimilate availability, inability of the translocation systems, or competition from other sinks. Regarding the latter hypothesis, this is supported by the fact that dry weight of the leaves (Figure 2) showed no major increases along the season, therefore leaves never behaved as a strong sink competing for assimilates, conversely, leaves are usually a source of assimilates. In respect of the former, supply limitation, Finazzo et al. (1994) in the 'Petersen' avocado trees reported that the leaves began to export photoassimilates when they reached about 35% of final mean midvein length. As described by Lakso and Goffinet (2013), the pattern of support for apple growth show a transition as the season progress, among the different types of

development leaves. Initially, are the spur leaves to bear fruit growth (approximately until 20 DAFB); afterward, are the leaves on the lateral shoots in the spurs to support them. Finally, over-12-leaves-long extension shoots can start to support the fruit (from 40 to 60 DAFB). Hansen (1967a) found that the majority of the ^{14}C exported from the leaves was translocated to the fruit linked at the same spur shoot; this is in accordance with previous studies indicating that the proximity of a sink to assimilate sources is a determining factor for its growth rate (DeJong and Grossman, 1995). Consequently, as soon as the vegetative growth stops, assimilates from leaves can be dedicated to the fruit growth (Lakso and Goffinet, 2013). In the case of evergreen trees, the current photosynthesis becomes relevant at earlier stages of the season, to support the growth of both developing fruitlets and leaves (Finazzo et al., 1994).

For the years evaluated, data showed clearly that in 2012 higher values for fruit starch and soluble carbohydrates were obtained respect to season 2013 (Figure 3A). Gawęda and Ben (2010) and Brookfield et al. (1997) stated that dynamics of changes in the starch concentration, revealed considerable similarities between the analyzed cultivars, but great differences between the years of the experiment. In the present trial, two different quince rootstocks seemed not to have influence on the behavior of starch, because no significant differences between them were presented, either in 2012 and 2013 seasons. Richardson et al. (1997) in kiwifruit noted that the crop-load altered the concentration of starch in the fruit during growth, with a higher percentage of starch allocation in fruits belonging to lightly-cropped vines compared to those held by heavier crop vines. This result also agree with those obtained by Klages et al. (2001) in 'Braeburn' apple, where fruits from trees with a high crop-load (≈ 340 fruits tree^{-1}) showed lower starch concentrations compared to fruits from low-cropping trees (≈ 140 fruits tree^{-1}). This could support our results, since 2012 season presented a low crop-load (average 27 fruits tree^{-1}), while 2013 corresponded to high crop-load (average 65 fruits tree^{-1}). However, this disagrees with the results obtained by Park (2011) in 'Fuyu' persimmon trees, where with a lower leaf/fruit (L/F) ratio (high crop-load) the starch was partitioned more to the fruits and less to the roots. However, it is noticeable to remark that, in this experiment author used four-year-old tree (young trees), exhibiting an active competition between reproductive and vegetative organs, as mentioned above (Chalmers and Van den Ende, 1975), this because, under higher L/F ratio (low crop-load) more carbohydrate were available on the accumulation in perennial parts. Hansen

(1967a) hypothesized that the presence of "sinks" in plant affects the rate of translocation of assimilates from leaves, consistent with previous studies and present results. Moreover, García-Luis et al. (2002) stated that the differences in fruit growth rate occurred only when there were no limitations in carbohydrate supply in the tree. Nowadays, there are few studies that have considered this factor on the dynamics of fruit starch, making it necessary to be taken into account in future research.

In respect of soluble carbohydrates, the greatest differences found between rootstocks (Figure 5, Table 4) could be mainly attributed to the different vigor of them. Sydo®, among the quince rootstocks is characterized by inducing a medium vigor, while Quince C is a dwarfing rootstock (Musacchi, 2011). This aspect can influence the canopy development, obtaining a smaller leaf area on Quince C than Sydo®, and therefore, lower yield efficiency. Significant differences on yield between these rootstocks have been reported (Musacchi et al., 2011).

Regarding the behavior of starch and soluble carbohydrates in leaves (Figure 6, and Table 5), they showed a similar pattern to that previously studied in other species (Finazzo et al., 1994; Miller et al., 1998; Boldingh et al., 2000). Finazzo et al. (1994) stated that in avocado the peak in assimilate import and the onset of assimilate export occurred during the same stage of leaf maturation, i.e. at the end of cell division, when the average mean midvein length was between 30 and 50 mm. In kiwifruit, Boldingh et al. (2000) reported that mature leaf is reached approximately at 50 DAFB, which coincide with the onset of starch accumulation in fruits. Miller et al. (1998) observed also in kiwifruit, a gradual decline in the concentration of both starch and total soluble sugars over the growing season until harvest. That trend was not observed in this experiment. A decrease of leaf starch was only observed during the phase of starch accumulation in fruit (from 50 until 100 DAFB), after that, the values were stable until harvest, in accordance to Boldingh et al. (2000). In the case of soluble carbohydrates, these were stable without major changes until harvest. The decrease of starch concentration could be attributed to the high demand for assimilates in fruits. Hansen (1967a) pointed out that the apple fruit, providing an efficient "sink", accelerate photosynthates translocation from proximate leaves compared to leaves on spur without fruits. Indeed, Park (2011) in persimmon and Elloumi et al. (2014) in pistachio, obtained in de-fruited trees a content of starch approximately six fold higher than trees

presenting a high crop-load. The same argument statement could explain why we obtained subsequent increase after harvest, either for starch and soluble carbohydrates, this because picked fruit did not act any more like an effective "sink". During the maturation phase of the fruit, the values were stable, as also observed by Miller et al. (1998), mainly because the sink strength of fruit is progressively reduced. It is important to highlight that leaves are considered as a 'transitory storage' of starch, owing mainly to the fact that they serve as carbon fixing organs during the day and that starch is degraded during the subsequent night to provide a continued supply of sugars to sustain respiration and growth.

3.5. Conclusions

The present work confirmed that 'Abbé Fétel' pear fruits follow a typical pattern of accumulation and degradation of starch that has already been described for other species. This pattern through the years presented differences related to the concentration and timing of the maximum level of accumulation and onset of starch degradation, which could be related to factors of management practices and environmental conditions. The differences of vigor between the two rootstocks evaluated, Sydo® and Quince C did not influence the accumulation trend of the starch, however, a slight effect was observed for the soluble carbohydrates in fruits and leaves.

On the other hand, the protocol developed for the determination of starch and soluble carbohydrates in this experiment, allowed to obtain reliable and homogenous values among the samples, throughout the season.

3.6 Literature cited

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3.7 Figures and tables

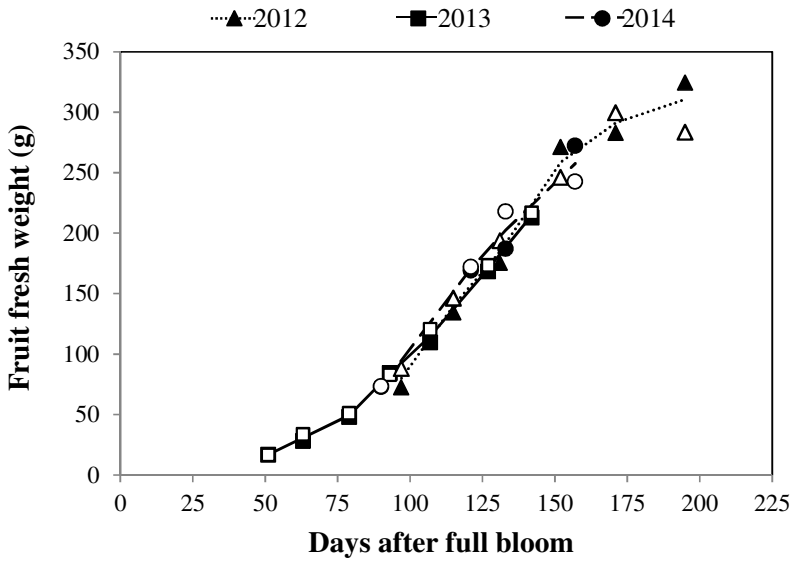

Figure 1. Fruit fresh weight of 'Abbé Fétel' pear fruits during three seasons: 2012 (Δ, ▲), 2013 (■, □), and 2014(○, ●), as days after full bloom (DAFB), from both rootstocks: Sydo® (open symbols) and Quince C (close symbols). Values are means of 12 fruit of average size per treatment.

Table 2. Fruit fresh weight (g) according the types of bearing woods at different days after full bloom, per each combination of training system/rootstock, in year 2014.

		Days after full bloom (DAFB)			
Combination	**Type of bearing wood**	90	121	133	157
		g fruit^{-1}			
Splindle/Sydo®	Brindle-type shoot	78.3[z]	183.5	197.0	274.1
	2-years-old	75.0	167.2	232.1	240.3
	3-years-old	72.3	176.9	246.3	218.1
	Short-old spurs	67.8	161.0	196.5	232.8
	Significance	**n.s.[y]**	**n.s.**	**n.s.**	**n.s.**
Splindle/Quince C	Brindle-type shoot	64.6	169.7	199.5	307.3
	2-years-old	77.0	165.8	170.4	274.1
	3-years-old	81.3	166.4	211.8	260.9
	Short-old spurs	68.5	175.1	166.4	246.8
	Significance	**n.s.**	**n.s.**	**n.s.**	**n.s.**

[z]Values are means of 12 fruit of average size. [y] Significance level: n.s. = not significant.

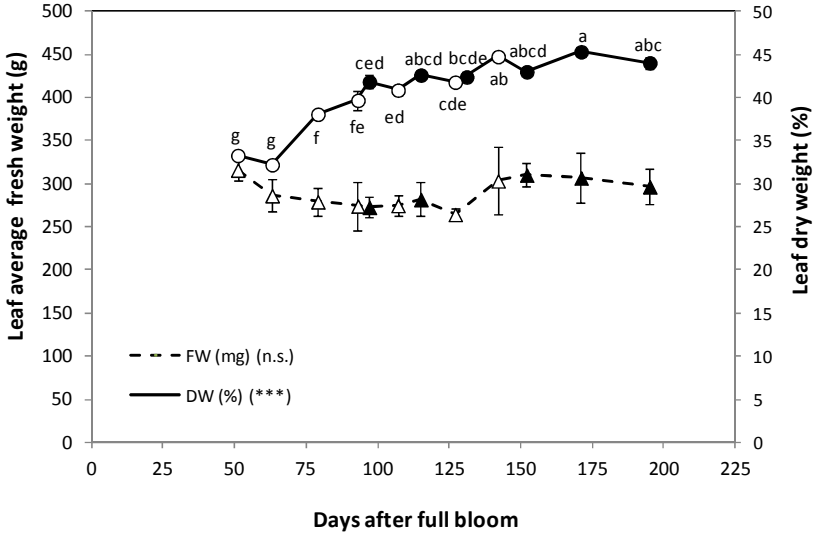

Figure 2. Fresh weight (mg, dashed line with triangles) and dry weight (%, full line with circles) in leaf of 'Abbé Fétel' pear fruit, harvested from branches over 3 years-old, during the growth and maturation on the tree, as days after full bloom. Data refers to the 2012 (closed symbols) and 2013 (open symbols). Values mean ± S.E. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

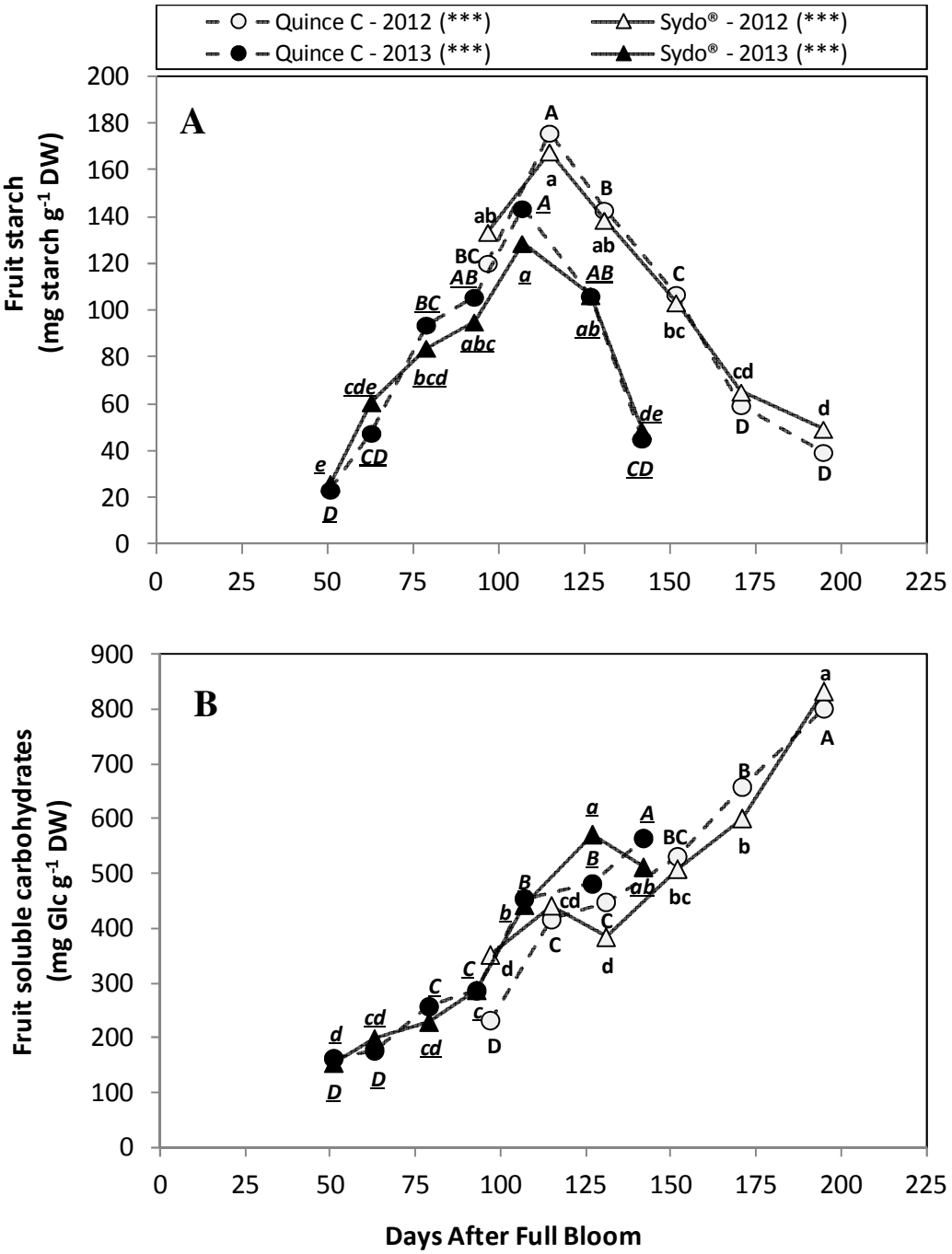

Figure 3. Starch (A) and soluble carbohydrates (B) concentration (mg g^{-1} DW) trends in fruits of 'Abbé Fétel' pear trees, from both rootstocks: Sydo® (Δ, ▲) and Quince C (○, ●), during growth and maturation on the tree, as days after full bloom, for two evaluation years: 2012 (open symbols), and 2013 (close symbols). Capital letters indicate significance for Quince C rootstock, while small letters for Sydo®. Letters in italics and underlined corresponded to 2013 evaluation. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

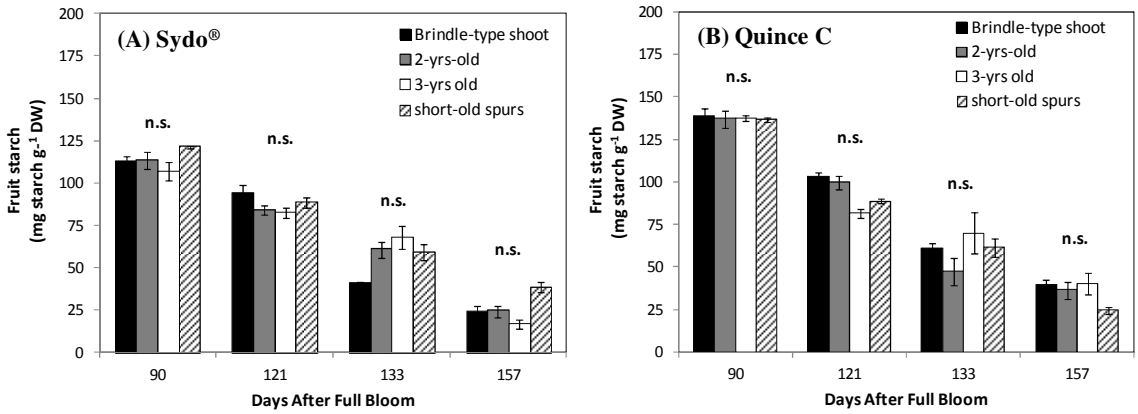

Figure 4. Fruit starch concentration (mg starch g^{-1} DW) among different types of bearing wood per each combination: (A) Spindle/Sydo®; (B) Spindle/Quince C, during the growth and maturation on the tree, as days after full bloom, in year 2014. Error bars are means ± S.E. (n=3). n.s. = not significant.

Table 3. Fruit soluble carbohydrates concentrations (mg Glc g^{-1} DW) for each training system/rootstock combination, among different types of bearing wood, at different days after full bloom during growth and maturation on the tree, in year 2014.

		Days after full bloom			
Combination	**Type of bearing wood**	90	121	133	157
		mg Glc g^{-1} DW			
Spindle/Sydo®	Brindle-type shoot	276.2	512.3	690.6	682.7
	2-years-old	249.6	531.6	700.2	725.0
	3-years-old	223.4	534.8	737.1	730.7
	Short-old spur	286.0	543.3	646.2	742.8
	Signif.	**n.s.**	**n.s.**	**n.s.**	**n.s.**
Spindle/Quince C	Brindle-type shoot	250.5	440.5	554.2	611.9
	2-years-old	275.3	401.6	501.8	646.9
	3-years-old	289.5	443.5	519.7	632.9
	Short-old spur	255.2	415.9	520.6	662.3
	Signif.	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Values are means of three technical replicates. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

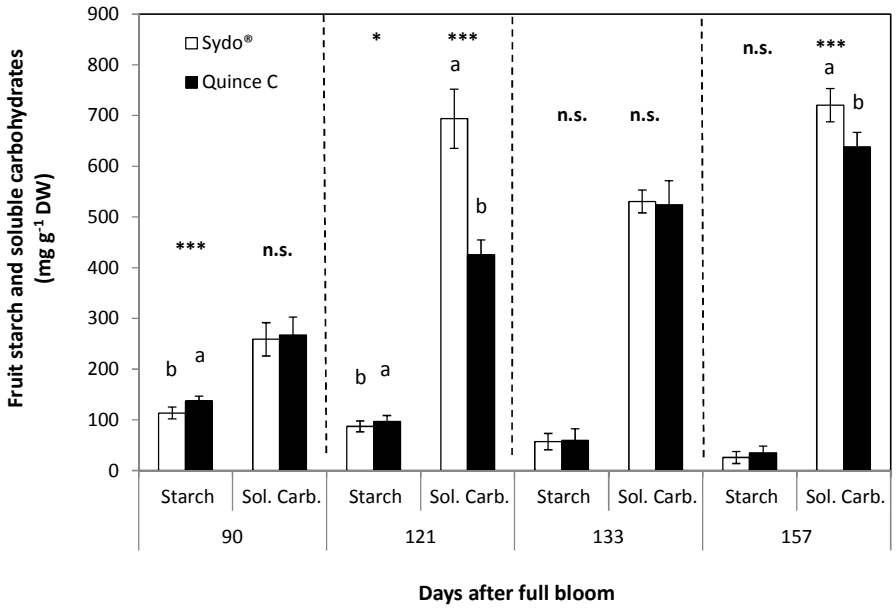

Figure 5. Fruit starch and soluble carbohydrates concentration at different days after full bloom, for both rootstocks: Sydo® (white bars) and Quince C (black bars), in the 2014 year. Values are means of three technical replicates ± S.E. Small letters indicate significant differences between rootstocks. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

Table 4. Daily rate of increase in soluble carbohydrates and decrease in starch concentrations (mg day^{-1}) in fruits harvested from different types of bearing wood: BR, brindle-type shoot; BR 2, 2-years-old; BR 3, 3-years-old; and S-spurs, short-old spurs, for each rootstock, Quince C and Sydo®, from 90 DAFB until harvest (157 DAFB).

	Soluble carbohydrates					**Starch**				
Bearing wood	Quince C		Sydo®			Quince C		Sydo®		
	mg day^{-1}				***Signif.***	mg day^{-1}				***Signif.***
BR	5.39 ± 0.14		6.07 ± 0.36		**n.s.**	1.49 ± 0.15		1.33 ± 0.12		**n.s.**
BR 2	5.54 ± 0.30	**B**	7.10 ± 0.36	**A**	*	1.50 ± 0.28		1.33 ± 0.28		**n.s.**
BR 3	5.13 ± 0.10	**B**	7.57 ± 0.34	**A**	**	1.45 ± 0.18		1.34 ± 0.18		**n.s.**
S-spurs	6.08 ± 0.24		6.82 ± 0.22		**n.s.**	1.67 ± 0.11	**A**	1.24 ± 0.11	**B**	*
Signif.	***n.s.***		***n.s.***			***n.s.***		***n.s.***		

Values are means of three technical replicates. Capital letters discriminate in horizontal way (between rootstocks), while small letters in vertical way (among bearing wood). Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

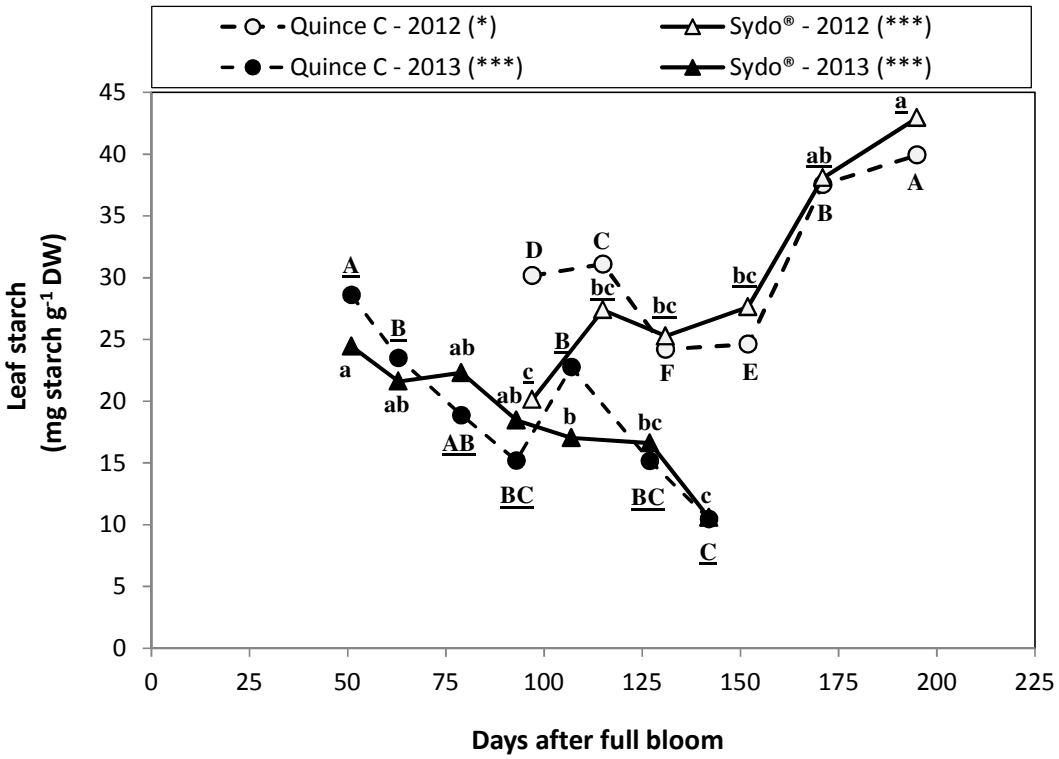

Figure 6. Leaf starch concentration (mg starch g^{-1} DW), during two evaluation seasons: 2012 (open symbols) and 2013 (close symbols), as days after full bloom, from both rootstocks: Sydo® (Δ, ▲) and Quince C (○, ●). Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

Table 5. Leaf soluble carbohydrates concentrations (mg Glc g^{-1} DW) for the seasons 2012 and 2013, at different days after full bloom, for each rootstock: Quince C and Sydo®.

Year	DAFB	Quince C	Sydo®	
		mg Glc g^{-1} DW		***Signif.***
2012	97	54.2 ± 2.3 bc	52.9 ± 0.6 ab	**n.s.**
	115	54.9 ± 4.3 bc	57.7 ± 1.6 ab	**n.s.**
	131	49.7 ± 1.3 c	50.9 ± 1.3 b	**n.s.**
	152	57.3 ± 2.0 bc	53.8 ± 3.6 ab	**n.s.**
	171	62.8 ± 1.0 b	67.6 ± 2.9 a	**n.s.**
	195	72.9 ± 3.1 a	67.9 ± 6.8 a	**n.s.**
	Signif.	***	*	
2013	51	58.2 ± 3.7 b	55.3 ± 1.0 ab	**n.s.**
	63	45.1 ± 0.8 c B	52.0 ± 0.3 b A	***
	79	60.2 ± 1.2 ab A	46.4 ± 2.9 b B	**
	93	51.9 ± 1.4 bc	52.8 ± 2.5 ab	**n.s.**
	107	52.6 ± 0.9 bc A	48.5 ± 0.4 b B	**
	127	54.2 ± 2.2 bc	53.9 ± 2.0 ab	**n.s.**
	142	67.5 ± 1.4 a	60.7 ± 2.6 a	**n.s.**
	Signif.	***	**	

Values are means of three technical replicates (average ± S.E.). Capital letters in the horizontal way indicate significant differences between rootstocks, while small letters in vertical way among DAFB. Significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

STARCH DEGRADATION IN 'ABBÉ FÉTEL' FRUIT IN RELATION TO FRUIT QUALITY PARAMETERS, SOLUBLE SUGARS AND ORGANIC ACIDS

4.1 Introduction

Starch has been synthesized in fruit since several weeks before harvest, afterward its degradation begins. This process of hydrolysis is contemporary to a rise in soluble sugars content, being this an indicator of the incipient fruit maturity stage (Wegrzyn and MacRae, 1995; Berüter, 2004). During fruit development, the main translocate soluble sugars, from the leaves (sources), are in form of sorbitol and sucrose, which enter into fruit metabolism to be converted into glucose, fructose, sucrose, malic acid and starch (Berüter et al., 1997; Hudina and Štampar, 1999; Berüter, 2004; Dugalic et al., 2014). In many of the *Rosaceae* species sorbitol is the main translocated carbohydrate (Tanase et al., 2002), and accounts for 60 to 90% of the carbon exported from the leaf (Loescher, 1987). The increase of soluble sugars has been related with an increase in dry matter (Ackermann et al., 1992). Factors as assimilates supplied to fruit, fruit metabolism and increase of fruit volume, determinate the sugars concentrations during all the fruit development (Génard et al., 2003).

The organic malic and citric acids are mentioned as the major products of the metabolism during fruit growth in apples and pears, showing subsequently a decrease at maturity stage (Hudina and Štampar, 1999; Berüter, 2004). As the growing season progresses, changes in metabolites in both, source and sink, often take place (Hudina et al., 2007). In pear fruit, the composition of soluble sugars and organic acids determines characteristics as sweetness and sourness (Eccher Zerbini, 2002; Hudina et al., 2012). In this way, levels of soluble sugars and organic acids are important factors in determining the sensory quality of ripe fruit (Ackermann et al., 1992; Berüter, 1998; Hudina and Štampar, 1999; Tanase et al., 2002; Hudina et al., 2007; Dugalic et al., 2014). In addition, it is noteworthy that the contents of sugars and organic acids are cultivar, environmental and cultural dependent (Lobit et al., 2006; Colaric et al., 2006; Hudina et al., 2007; Sha et al., 2011; Hudina et al., 2012; Dugalic et al., 2014).

Fruit quality is determined by external (shape, size, and appearance) and internal (taste and texture) characteristics (Dewulf et al., 1999). Fruit maturity is normally assessed by the determination of parameters known as maturity indices (Brookfield, 1997). Several indices of maturity as: soluble solids concentration (SSC), titratable acidity (TA), ratio of soluble solids to titratable acidity (SSC/TA), skin color and, flesh firmness (FF), are utilized to decide the optimal harvest time, which is crucial for storage management and final fruit quality (Kader, 1999). Recently, a new fruit index has been developed; the DA-meter is a portable, easy to use Vis/NIR instrument capable to measure the Index of Absorbance Difference (I_{AD}) (Ziosi et al., 2008; Costa et al., 2009). This index is calculated to difference in absorbance between two precise wavelengths: 670 nm (near the Chlorophyll-a absorption peak) and 720 nm (background of the spectrum) (Nyasordzi et al., 2013).

In apples, the starch degradation has been related to the stage of maturity, using for this the Starch Pattern Index (SPI). Starch conversion into sugars is one of the most important indicators for predicting the optimal harvest dates for apples (Brookfield et al., 1997; Peirs et al., 2002), which is usually assessed using an iodine test that provides a visual pattern of the total starch content. This technique has the disadvantage that starch concentration is not quantitatively measured (Peirs et al., 2002), but needs to be estimated following a scale. On the other hand, this scale is not uniform all over the world, e.g., in Canada harvest date is based on nine stage charts (Smith et al., 1979), while in the United States and in New Zeeland, is based in references chart with six stages (Fan et al., 1995; Brookfield et al., 1997). Conversely, in pear fruits the starch index is less utilized even if some studies reported the use of this procedure (Stow, 1988; Le Lezec and Belouín, 1994; Agar et al., 1999). However, Stow (1988) described this technique as an unreliable guide to determine the optimum harvesting period of pear fruits. On the other hand, some studies of starch degradation in pear fruits have been conducted during postharvest storage (Murayama et al., 2002). They proposed that starch degradation and respiratory climacteric occurs independently, and are unrelated, as also been found in kiwifruits (MacRae et al., 1992).

There are many fruit features that change during the last phase of fruit development, so the challenge is to define which of these might best reflect the approach that will be useful for our purposes and objectives. Previous researches have shown the variations of

fruit quality parameters, soluble sugars and organic acids in different cultivars of pear trees, nevertheless, there is no available information about the relationship between the evolution of these parameters and starch degradation in developing pear fruit. Therefore, the objectives of this experiment were: 1) to determine the starch degradation kinetics on fruit, in order to evaluate if it could be correlated to the main fruit quality parameters, soluble sugars and organic acids, 2) to contribute to the optimal harvest time assessment.

4.2 Materials and Methods

4.2.1 Plant material

Trial was carried out for two consecutive seasons (2012-2013) on seventeen-year-old pear trees (*Pyrus communis* L.) cv. 'Abbé Fétel', trained at Spindle and grafted on two rootstocks: Sydo® (medium vigor) and Quince C (dwarfing). Characteristics of the experimental site are described in paragraph 3.2.1.

4.2.2 Experimental design and fruit sampling

This trial was conducted at the same time of the first experiment (Chapter III). In this way, simultaneously, when fruits were sampled to determine starch and soluble carbohydrates concentrations, other 12 fruits from 3-years-old branches were harvested for each rootstock (treatment) and transported to the laboratory, in plastic trays, to assess fruit quality parameters. These fruit were used for fruit quality evaluation on the same day of their harvest: non-destructive indices (fruit weight, I_{AD}, skin color coordinates, ethylene production, fruit ratio height/width); and destructive indices (firmness, soluble sugar content, pH and acidity), were performed.

The amounts of alcohol soluble sugars (fructose, glucose, sucrose, maltose, trehalose, raffinose and xylose), organic acids (malic, succinic, citric, tartaric, quinic and ascorbic acid) and, sugar alcohols (sorbitol, mannitol and inositol) were specifically determined

by gas chromatography technique (GC) using the method previously described by Bartolozzi et al. (1997) for apricot fruits, with some modifications (Kuznetsova, 2013).

The timing of evaluation corresponded to those of the first experiment, at 97, 115, 131, 152, 171 and 195 days after full bloom (DAFB) in 2012 and at 51, 63, 79, 93, 107, 127 and 142 DAFB in 2013. The commercial harvests took place at 152 and 142 DAFB, respectively.

4.2.3 Starch determination

4.2.3.1 Preparation of material

The protocol used to prepare the material for the analysis was the same previously described in paragraph 3.2.5.1, chapter III.

4.2.3.2 Determination of starch concentration

The protocol used to determine starch concentration was the same previously described in paragraph 3.2.5.3, chapter III.

4.2.3.3 Spectrophotometer analysis

The protocol used to measure the starch concentrations in the prepared samples ready for the spectrophotometer analysis, was the same previously described in paragraph 3.2.5.4, chapter III.

4.2.4 Soluble sugars and organic acid determinations

For these analyses, the same fruit samples prepared to determine starch and soluble carbohydrate concentrations were used in order to have a more reliable and accurate result regarding the behavior of fruits at different sampling dates.

4.2.4.1 Preparation of samples for gas chromatography

Fifty mg of dry weight of each replicate (3 replicates) for treatment and sampling date were dissolved in 5 ml imidazole extraction buffer (imidazole buffer 0.1 *M* in 50% ethanol, pH=7.0) for 25 min, vortexing now and then, in order to avoid the acid hydrolysis of sucrose into fructose and glucose by maintaining a neutral pH (Bartolozzi et al., 1997). Subsequently, samples were centrifuged at 5,000 x g for 5 min (Eppendorf 5804 R, Hamburg). Then, supernatants were withdrawn and collected in a plastic tube. After that, 1 ml aliquot from each sample was transferred into chromatography vials (Supelco analytical, Sigma-Aldrich S.), and 500 µl of internal standard (B-phenyl-glucopyranoside, 1 g/500 ml 50% ethanol) was added, then, the samples were dried by an air stream at 34 °C for 48 h, in this way all the liquid phase was completely evaporated (anhydrous samples).

4.2.4.2 Derivatisation

Alcohol-soluble sugars, carboxylic acids, amines, and others, can be silylated. The process involves the replacement of a proton with a trialkylsilyl group, usually trimethylsilyl (-SiMe3). Generally, the substrate is deprotonated with a suitable strong base followed by treatment with a silyl chloride (e.g. trimethylsilyl chloride). Silyl derivatives are generally less polar and more thermally stable than their precursor organic compounds. The introduction of a silyl group gives derivatives enhanced volatility, making the derivatives suitable for analysis by gas chromatography.

According to Bartolozzi et al. (1997), this procedure was accomplished in three steps. Firstly, after 48 h, the anhydrous samples were treated with 400 µl of pyridine and vortexed to completely dissolve the pellets. Secondly, 200 µl of hexamethyldisilazane (HMDS) were added and, finally, 100 µl of trimethylchlorosilane (TMCS). Then, the

samples were vortexed one more time and heated at 60 °C in a water bath for 2 h for the final step of derivatisation. Afterward, the samples were cooled and stored at 4 °C, until the moment of injection into the gas chromatograph.

4.2.4.3 Gas chromatograph analysis

One µl of each sample was analyzed on a 3900 gas chromatograph (Varian, Inc, USA), equipped with a flame ionization detector (FID) and a CP-8401 auto-injector. Helium (He) was used as a carrier gas, while hydrogen (H_2), nitrogen (N_2) and, oxygen (O_2) were used as FID gases. Equipment specifications, as described by Kuznetsova (2013) are: a column Varian CP-Sil 5CB (30 m of length), injector and detector temperatures, 125 °C and 300 °C, respectively, and, 17.75 min duration of each sample run.

Particularly, for this experiment, the alcohol soluble sugars: fructose, glucose and, sucrose, the organic acids: malic and quinic; and, the sugar alcohols: sorbitol and inositol were measured. The results were expresses as mg g^{-1} of dry weight.

4.2.5 Fruit quality parameters

4.2.5.1 Non-destructive measurements

Fruit maturity was assessed at harvest by measuring the I_{AD} index with the DA-meter device (TR Turoni, Forli, Italy). The surface color of each fruit was assessed with a Minolta Chroma Meter model CR-400 (Minolta, Tokyo, Japan). The CIELAB coordinates (L*, a*, b*) were measured on both equatorial zone of each fruit with the average used to calculate Hue angle ($h\circ=\tan^{-1}(b^*/a^*)$) and Chroma ($C^*=((a^*)^2+(b^*)^2)^{1/2}$) to characterize changes in skin color from green to yellow during ripening. Both systems function on the principle that the combined coordinates of the three values define a color. In fact the two color spaces are interconnected as a* and b* are the ground values for calculating hue angle and Chroma. Hue angle is the most commonly used parameter when measuring fruit color (McGuire, 1992; Núñez-Delicado et al., 2005).

Fruit height (H) and width (W) were measured with a digital caliper and then, ratio = H/W was calculated.

Ethylene production was measured on six fruits for each treatment and sampling date as described by Gutierrez (2014). Each fruit was placed into a jar (1.0 L), tightly sealed and thereafter left at room temperature for 1 h. The measurements were done by a gas chromatograph instrument (Dani HT 86.01, Milan, Italy). Ethylene concentration was calculated, and expressed as, nanoliter per gram of fresh weigh per 1 h (nL h^{-1} g^{-1} FW).

4.2.5.2 Destructive measurements of fruit quality parameters

Fruit flesh firmness (FF) was measured (previous skin removal with a peeler) with a FTA GS-14 texture analyzer (Guss, Strand, South Africa) equipped with the plunger traditionally used for stone fruits (8 mm ø). FF value corresponds to the average of two measurements performed on both fruit cheeks and were expressed in kg cm^{-2}.

The twelve fruit were divided in three replicates (4 fruit each) and slices from four fruit were pooled and juiced together to give a composite sample analyzed for soluble sugar content (SSC), using a thermo-balanced PAL-1 refractometer (Atago, Tokyo, Japan), and titratable acidity (TA) using a semi-automatic titrator (Compact-S Titrator, Crison, Modena, Italy). TA was measured, and percentage of malic acid equivalents were determined (g l^{-1} malic acid).

4.2.6 Statistical analysis

All the collected data at the different sampling dates were statistically analyzed by SAS statistical software (SAS Institute, Cary, NC, USA) and differences between means evaluated using Student-Newman-Keuls (SNK) test, with a significance level of $P<0.05$. Evolutions of the fruit quality parameters, soluble sugars and organic acids were expressed as function of days after full bloom, by descriptively figures. Relations between starch hydrolysis in fruits and fruit quality parameters were described by linear correlation (coefficient of determination) and Pearson Product-Moment Correlation (r) were defined. Additionally, the collected data were submitted to a principal component

analysis (PCA), used statistical software Statgraphics Plus 5.1 (Statpoint Technologies, Warrenton, WA, USA).

4.3 Results

4.3.1 Fruit quality parameters during the growing season

Changes in quality parameters during the growing seasons (2012 – 2013) in fruits of 'Abbé Fétel' pear trees grafted on Quince C and Sydo® rootstocks are reported respectively in Table 1 and 2. In general, as the season came along, fruit weight (FW) and soluble sugars content (SSC) increased, while flesh firmness (FF), I_{AD} index, acidity and hue angle decreased. At both harvests (152 and 142 DAFB), regardless of rootstock, fruits picked in the 2012 season showed numerical differences for FW and FF compared with those of 2013 season (Table 1 - 2). The FW measured at harvest in the 2012 was a 27.0% and 13.4% higher for Quince C and Sydo® rootstocks than values registered at harvest in the 2013, respectively; whereas for the FF, this accounted a 14.8% and 28.0%, respectively. On the other hand, within the same year, no differences were found for FF (Figure 1C – G) and FW (Figure 1A – E) between rootstocks, except for the weights at 97 DAFB in the year 2012 (p-value<0.01). Regarding the I_{AD} index, fruits registered values above 2.00 from the first measurements until the middle phase of fruit development (Figure 1B – F), when the decrease occurred simultaneously with the onset of starch hydrolysis in fruits (115 and 107 DAFB, in the 2012 and 2013, respectively) (Figure 3). Moreover, in the second year (2013), the "I_{AD} index stable period" ($I_{AD} \approx 2.0$) lasted longer, mainly because the sampling began at earlier stages of fruit development. Between rootstocks, significant differences emerged in both years, at 115 and 131 DAFB for the 2012, and at 63 and 93 DAFB for the 2013 (Figure 1B – F), where fruit from Sydo® rootstock retained higher I_{AD} index values than those from Quince C. Regarding SSC, significant increases until harvest were observed, following this date, no meaningful variations were registered (Table 1 - 2). In addition, no significant differences between rootstocks were found in 2012 (Figure 1D), whereas the following year, at 93 DAFB, fruit from Sydo® had higher SSC than those from Quince C (Figure 1H).

Ethylene concentrations showed no major changes during the season. In fact, only after harvest a noticeable increase was observed in the first year of evaluation, for both rootstocks (Table 1 - 2). About color measurements, Hue angle presented significant differences only for Sydo® rootstock in the first season, it decreased along with the fruit development, i.e., a change in skin color from green (hue = 115.0°) to greenish-yellow (hue = 100.6°). Instead, differences were found for Chroma (saturation), both for Quince C and Sydo®, mainly, a general increase of saturation, i.e. from dullness to vividness value, was observed toward the end of the season (Table 1 - 2).

4.3.2 Alcohol soluble sugars, organic acids and sugar alcohols

Glucose and fructose concentrations (mg g^{-1} DW), during the season, showed similar behaviors, regardless of the treatment, although with different ranges (Table 3 - 4, Figure 2C - D). In both rootstocks, these metabolites increased in fruit along with its development. At harvest time, fructose concentration registered amounts three-fold higher than the glucose concentrations. In general, the concentrations in the 2012 year were lower compared to those in the 2013 year. In the case of sucrose, unlike fructose and glucose, during the first sampling dates, constant values around zero mg g^{-1} DW were registered until 131 and 107 DAFB, for 2012 and 2013 year, respectively (Table 3 - 4, Figure 2F). After this, a sharp increase of this metabolite was measured and it continued to rise still after harvest (2012 year). Quince C and Sydo® rootstocks, showed the same behavior for fructose and sucrose concentrations, within the same year of evaluation (Figure 2C – F), whereas for glucose a difference between rootstocks at 127 DAFB in the second year emerged (Figure 2D).

Regarding the alcohol sugar, inositol, only for Spindle/Sydo® during the second growing season (2013) differences (p-value<0.05) were observed (Table 4). Overall there were no large variations and values remained close to 0.0 mg inositol g^{-1} DW. The averages corresponded to 0.24 ± 0.04 and 0.23 ± 0.08, for Spindle/Quince C, in 2012 and 2013, respectively (Table 3), while for Spindle/Sydo® were 0.22 ± 0.06 and 0.18 ± 0.06, respectively (Table 4). On the other hand, for sorbitol significant differences among sampling dates were registered in the 2012 for both rootstocks (Table 3 - 4).

Finally, the organic acids, malic and quinic acid had different trends. Quinic acid clearly showed a decrease in fruits as long as growing season progressed (Table 3 - 4). Rootstocks showed the same behavior, without differences between them (Figure 2B). Between the first sampling dates of 2013, the amount of quinic acid abruptly decreased, approximately until 100 DAFB, thereafter, this value was asymptotic to a minimum, (harvest time). Malic acid during the evaluation season did not present a stable trend. In Spindle/Quince C combination no differences were found for this metabolite in both years (Table 3), although, in 2013 a numerical variability was observed. For Spindle/Sydo®, differences were observed in the 2013, but they were due to a low amount registered at the beginning of evaluations (Table 4, Figure 2A).

4.3.3 Relationship among starch degradation and fruit quality parameters, soluble sugars and organic acids

The onset of fruit starch degradation marked the beginning of correlations between this process with the several fruit quality parameters, soluble sugars and organic acids. Regardless of the rootstock, the highest starch concentrations were registered at 115 and 107 DAFB, for the first and second year, respectively (Figure 3), and from these points the quality analyses started. Moreover, because the rootstocks did not show great differences between them, either for fruit quality parameters and metabolites, the collected data of the two rootstocks were analyzed as a whole for to correlate with starch degradation.

In the first season (2012), FW and SSC showed an inverse relationship with starch degradation in fruit, with Pearson correlation coefficients (r) of -0.97 and -0.89, respectively (Table 5). Indeed, FW and SSC increased when starch in fruit decreased, throughout fruit maturation stages. While for FF, I_{AD} index, acidity and hue angle positive relationships were obtained. The correlation coefficients (r) corresponded to 0.96, 0.74, 0.90 and 0.59, respectively (Table 5), therefore, as the starch was degraded in fruit, these variables also decreased, throughout fruit maturation. Regarding the pH and Chroma, these variables did not show any correlation, with Pearson values close to 0.0 (Table 5). However, for all these variables mentioned above positively or negatively correlated to starch degradation, only for FW, SSC and FF, the coefficient of

determination (R^2) was highly significant ($p<0.001$), indicating that a linear equation explained the response of these variables regarding of fruit starch degradation. Whereas for acidity and I_{AD} index, the coefficients of determination were lower in magnitude but equally significant (R^2 = 0.79 and 0.47, respectively). In the second year (2013), FW, SSC, FF, I_{AD} index, pH and hue got the same behavior of the previous year, i.e., those who obtained a positive or negative correlation, maintained the trend (Table 5). Whilst, the significant result obtained in the first year for acidity, was not confirmed in the second year. On the other hand, both pH and Chroma reported an opposite behavior in 2013. In the 2013, these parameters obtained negative correlation coefficients (-0.85 and -0.91, respectively) and significant coefficients of determination, R^2 = 0.72 and 0.83, respectively ($p <0.05$).

As a result of analysis of both years, the parameters that showed a consistent and stable pattern separately, i.e. for each season, were those significant together, i.e., fruit weight, soluble sugars content, flesh firmness and I_{AD} index (Table 5, Figure 4). The linear equations of these relationships are reported in Table 6. As showed, the coefficient of determination was 0.80 for soluble sugars content and 0.84 for flesh firmness, which obtained a $p<0.001$. This result indicated that more than 80% of the variability, either for the SSC and FF, was explained by the degradation of starch in the fruit. For the fruit weight, R^2 = 0.60 was lower compared to FF and SSC, although the p-value was rather significant ($p<0.001$). Instead, for I_{AD} index was obtained a R^2 = 0.52, being the least significant variable.

Regarding the metabolites, that were previously described, sorbitol and malic acid did not show large variations during the growing seasons (2012 – 2013) (Figure 5), in fact, these were not significantly related to the fruit starch degradation (Table 7). The average amounts recorded were 32.0 and 40.9 mg g^{-1} DW for sorbitol in 2012 and 2013 (Figure 5A – C), respectively, while for malic acid corresponded to 2.9 and 3.0 mg g^{-1} DW (Figure 5B – D), respectively. On the other hand, glucose, fructose, sucrose, inositol and quinic acid obtained a significant correlation in the first year (2012) (Table 7). Glucose, fructose and sucrose were negatively related with fruit starch degradation, therefore, when the starch concentration decreased in the fruit, these metabolites linearly increased (Figure 5A – B). These increases corresponded to 73.7% and 105.2% for fructose and glucose, respectively. Instead, for sucrose, an abrupt increase was observed (115

DAFB), roughly sixty-fold than initial values. In the case of inositol and quinic acid, their behaviors were positively related to the fruit starch degradation (r = 0.45 and 0.83, respectively) (Table 7). In the second year, only glucose and sucrose showed a significant relationship, maintaining the same trend of the previous year. On the whole, when all the data collected during the two experimental seasons (2012 – 2013) were plotted by a PCA analysis (Figure 6). PCA showed that the first two principal components explained 73.0% of the total variance among fruit soluble sugars, organic acids and starch degradation during fruit maturity stage. PC1, obtained the higher variability (52.3%), was defined positively for starch and quinic acid, in a lesser extent for inositol, whilst fructose, glucose and sucrose were negatively related with the starch degradation in fruits, this result was also corroborated by correlation and regression analyses in Table 7. In the PC2 the variables that explained the major variability were malic acid and sorbitol. Additionally, from this analysis it was observed that the PC1 grouped the rootstock according the DAFB. Regardless of rootstocks, glucose, fructose, sucrose, inositol and quinic acid showed a significant correlation (Table 7). However, only for glucose, a coefficient of determination above 0.50 (R^2 = 0.57) was observed, whereas inositol obtained a R^2 = 0.10, the lowest one among all the metabolites analyzed. Therefore, although if these correlations were significant (mainly due to a significant result in the first year), they were rather weak to explain the lineal association between them (R^2<0.50), in such a way, there could be other models that better explain these relationships.

4.4 Discussion

Traits defining fruit quality, such as sweetness, sourness and texture, are influenced by the contents of sugars and organic acids, which depend on the cultivars, climatic conditions and cultural practices such as: crop-load, nutrition and irrigation (Ackermann et al., 1992; Hudina and Štampar, 1999; Klages et al., 2001; Tanase et al., 2002; Hudina et al., 2007; Sha et al., 2011; Hudina et al., 2012; Dugalic et al., 2014). Our study was referred to a specific cultivar, ‘Abbé Fétel’, grafted on two rootstocks, Quince C and Sydo®. The results of fruit quality parameters, soluble sugars and organic acids evaluations, did not show significant differences between rootstocks, however

significance emerged between years, and this could be attributed to different climatic conditions and management practices.

Firmness decrease at the onset of maturity is a common characteristic in most fruit species. Flesh firmness, as well as soluble sugars content, showed to be highly correlated to starch degradation (Table 6), whereas for the fresh weight and I_{AD} index, when both years were analyzed together, a weak but significant linear equation was observed (Table 6). However, it is noticeable that the fresh weight, referred to each single growing season, showed to be the most significant trait related to starch degradation (Table 5). This result could be attributed to differences in crop-load, which also explained the differences found at harvest on the average fresh weight, regardless of the considered rootstock (Table 1 – 2). Klages et al. (2001), found a positive correlation between starch concentration and fruit weight (r = 0.98), during the phase of accumulation of starch; however, the concentrations of starch varied with the crop-load level, which determined different levels of maturity of the fruits at the time of sampling. According to Lurie et al. (2013), the skin degreening (loss of chlorophyll) and flesh softening are two processes that are synchronized during peach ripening. Correlations between the I_{AD} index and ethylene concentrations, flesh softening, and chlorophyll loss have been established in peach fruit ripening (Ziosi et al., 2008). Nyasordzi et al. (2013) at harvest, and Gutierrez (2014) starting from one month before harvest, obtained in apple fruits, a good correlation between I_{AD} index and starch degradation, measured by starch pattern index (SPI), that this a qualitative rather than a quantitative index. In pear fruits, one study was carried out to define SPI regressions in the cvs. 'Williams', 'Conference' and 'Doyenné du Comice' (Le Lezec and Belouin, 1994), however, there are few studies available that have related this index with others fruit quality parameters in pear fruits (Agar et al., 1999), in contrast to what happens in apple (Brookfield et al., 1997; Peirs et al., 2002; Nyasordzi et al., 2013). The fact the I_{AD} has not gotten an optimal coefficient of determination for the linear correlation (R^2 = 0.52) could be also attributed (as for fresh weight) to differences between years, mainly related to different beginning of starch degradation (Brookfield et al., 1997). Regarding the ethylene measurements, since this experiment was carried out following physiological maturation (on-tree) (Kader, 1999), ethylene production was undetectable (Table 1 – 2), in this way, this trait cannot represent a good predictor of harvest time; neither a

parameter to be related to the starch concentration in fruit maturity stage. Moreover, studies conducted on pear postharvest (Murayama et al., 2002) and kiwifruits (MacRae et al., 1992) noted that the starch degradation occurred before the ethylene production rate showed an increase. Nevertheless, it is noteworthy to considerer the type of tissue under analysis, because starch degradation begins in the fruit core and progresses outwards (Le Lezec and Belouin, 1994); disregarding this aspect can lead to different outcomes.

In 'Abbé Fétel' pear fruits, malic and quinic acid were the most representative organic acids. At earlier stages of fruit development, roughly until 70 DAFB, quinic acid was the most abundant one (Figure 2B); after this moment, its concentration progressively declined, which could be explained by an increase of dry weight during the cell growth phase (Ackermann et al., 1992). Zhang et al. (2010) in 'Honeycrisp' apple fruit reported that from 42 DAFB, only the quinic acid decreased with fruit development. Instead, malic acid throughout the season showed a more stable pattern, being the most abundant organic acid during fruit maturity stage (Figure 2A). Generally, the composition of organic acids in pear is more variable that of the sugar (Eccher Zerbini, 2002). Sha et al. (2011), in different cultivars of pear, pointed out that malic and citric acid were the major organic acids contained in fruit, in agreement with Hudina and Štampar (1999), Colaric et al. (2006) and Hudina et al. (2007); whereas quinic acid was present in a lower amount. They also noted that malic acid was the major compound among all organic acids, in agreement with the results obtained for apple fruit (Ackermann et al., 1992; Berüter, 1998). However, malic acid was not related to starch degradation in fruit (Table 7, Figure 6), whereas, quinic acid showed a positive correlation (Figure 6), even if the coefficient of determination was not fairly significant (Table 7). A research conducted on apple fruits postharvest, showed a decrease of malic acid, attributed to its role, together with sugars, in metabolic process (respiration) (Ackermann et al., 1992).

Our results showed that sorbitol was the main translocated soluble sugar in pear fruits; in agreement with other studies (Hudina and Štampar, 1999; Colaric et al., 2006; Hudina et al., 2007), including some on apple fruits (Berüter, 1985; Berüter, 2004; Zhang et al., 2010). During the season, sorbitol was present in constant and higher amounts compared to sucrose, which has been found as the main form used for sugar transport in plum (Dugalic et al., 2014) and peach fruits (Génard et al., 2003). The

constant concentrations of sorbitol registered, during apple fruit growth, were explained by the fact that, this metabolite was continuously converted to glucose, fructose and sucrose (Ackermann et al., 1992). These two sugars, sorbitol and sucrose, varies in concentration and function, according to the organs in which they are found and their developmental stage (Dugalic et al., 2014). Berüter (1985) noted in apple cv. 'Golden Delicious', that at early stage of fruit development (cell division) sucrose was the major source, whereas during the sugar storage period (cell enlargement) sorbitol was the predominant sugar. According to Berüter (1998) and Génard et al. (2003), the concentrations of glucose and fructose were related to metabolic transformation of sorbitol and, to a lesser degree, to the hydrolysis of sucrose in the early stages of fruit development; in agreement with our results (Figure 2). These hexoses (glucose and fructose) can be stored or, become precursors for starch synthesis. In our experiment, glucose was the soluble sugar that showed the best correlation with starch degradation (Figure 6). Regarding this fact, Berüter (1985), Hudina and Štampar (1999) and Zhang et al. (2010) stated that starch synthesis is dependent on glucose level, this because during the cell expansion period the increase of glucose concentration preceded that of starch accumulation, and throughout this period the glucose remained stable, so later on, at the onset of starch degradation (several weeks before harvest) increased again. Regarding fructose concentrations, Berüter et al. (1997) described a steady increase, as it was also observed in our experiment (Figure 5A), and was the most abundant soluble sugar among all determined (Colaric et al., 2006); this is mainly due to the fact that this metabolite was not used for starch synthesis, making it more available for its accumulation. For this reason the fructose concentrations were three-fold the amounts of glucose, in agreement with Berüter (2004) for the high acid genotype of apple fruit 'Usterapfel'. On the other hand, for sucrose was evidenced a slight increase when starch was degraded, being more evident at the end of the evaluations (Figure 2F). This result could be explained, in part, by starch degradation in fruit (Berüter el al., 1997; Berüter, 2004), which was corroborated by a negative significant correlation (Table 7, Figure 6), as well as, synthesized from the existent sorbitol (Hudina and Štampar, 1999).

4.5 Conclusion

This work studied the relationship between different parameters to define fruit quality and fruit starch degradation. From the results, it was possible to determine, during fruit growth and maturation, changes and trends of all variables. The rootstocks evaluated, Quince C and Sydo®, did not show great differences throughout the growing season, for all variables measured. The starch degradation in fruits, which started several weeks before harvest, was positively correlated with fruit weight, soluble sugar content and quinic acid concentration, whereas was negatively correlated with flesh firmness, I_{AD} index, glucose and fructose concentrations, in line with works on other species. The correlations obtained, presented different degrees of linear association (coefficient of determination, R^2), although for all, the p-value was highly meaningful ($p< 0.001$), except for I_{AD} index, which gets a $p<0.01$. In this way, a linear association for those with a R^2 ca. 0.50, might not be the best equation to explain this association.

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4.7 Figures and table

Table 1. Fruit qualitative parameters for Spindle/Quince C combination, both year of evaluations, 2012 and 2013, regarding the days after full bloom (DAFB).

Year	DAFB	Fruit weight (g)	Flesh firmness (kg cm^{-2})	SSC (°Brix)	I_{AD}	ratio H/W (mm)	pH	Acidity (malic acid g l^{-1})	Chroma	Hue	Ethylene (nL h^{-1} g^{-1} FW)
2012	97	72.3 e	14.8 a	11.8 c	2.23 a	1.98	3.90	4.57 a	38.9	112.8	0.00 b
	115	134.5 d	9.9 b	12.4 c	2.18 a	1.84	3.79	3.89 b	40.1	112.5	0.00 b
	131	175.6 c	8.5 c	13.6 b	1.95 ab	1.92	3.86	3.63 b	40.4	108.7	0.08 b
Harvest	152	271.3 b	6.2 d	14.7 a	1.97 ab	1.98	3.96	2.82 c	39.8	108.6	0.01 b
	171	282.9 b	5.5 d	14.7 a	1.88 b	1.88	3.98	2.72 c	37.9	109.2	0.01 b
	195	324.3 a	2.0 e	15.5 a	1.11 d	2.04	3.86	2.61 c	40.1	110.6	12.71 a
Significance		***	***	***	***	**n.s.**	**n.s.**	***	**n.s.**	**n.s.**	***
2013	51	16.6 f			2.25 a	2.07 a					
	63	27.9 f			2.25 a	1.98 ab					
	79	48.0 e			2.29 a	1.92 abc					
	93	84.2 d	11.1 a	10.1 d	2.18 a	1.88 bcd	3.44 c	4.75 a	38.8 b	126.0	0.00
	107	109.4 c	9.2 b	12.4 c	2.19 a	1.71 d	3.65 b	3.02 b	38.9 b	125.1	0.01
	127	168.2 b	5.6 c	13.7 b	1.90 b	1.74 cd	3.61 b	5.45 a	39.6 b	116.4	0.01
Harvest	142	212.9 a	5.4 c	16.3 a	1.71 c	1.75 cd	3.91 a	2.62 b	42.2 a	112.2	0.02
Significance		***	***	***	***	***	***	***	***	**n.s.**	**n.s.**

Mean values followed by same small letters do not differ significantly according to SNK test (p=0.05). Significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

Table 2. Fruit qualitative parameters for Spindle/Sydo® combination, both year of evaluations, 2012 and 2013, regarding the days after full bloom (DAFB).

Year	DAFB	Fruit weight (g)	Flesh firmness (kg cm^{-2})	SSC (°Brix)	I_{AD}	ratio H/W (mm)	pH	Acidity (malic acid g l^{-1})	Chroma	Hue	Ethylene (nl/gFWh)
2012	97	87.9 e	14.5 a	11.5 c	2.28 a	1.94	4.04 a	4.03 a	37.4 b	115.0 a	0.01 b
	115	146.0 d	10.8 b	12.8 b	2.30 a	1.82	4.03 a	3.34 b	39.4 ab	114.1 a	0.03 b
	131	193.7 c	7.8 c	12.8 b	2.14 ab	1.90	3.93 a	3.57 b	41.2 a	111.3 b	0.02 b
Harvest	152	246.0 b	6.4 d	15.1 a	2.02 b	1.97	3.99 a	2.71 c	40.5 a	110.2 b	0.03 b
	171	299.5 a	5.3 e	16.0 a	1.74 c	1.85	3.98 a	2.68 c	39.8 ab	109.7 b	0.01 b
	195	283.3 a	2.5 f	15.0 a	1.33 d	1.91	3.81 b	2.65 c	40.6 a	100.6 c	13.53 a
	Significance	***	***	***	***	**n.s.**	***	***	***	***	***
2013	51	16.7 g			2.35 ab	2.14 a					
	63	33.6 f			2.41 a	1.96 b					
	79	51.1 e			2.29 b	1.85 bc					
	93	82.8 d	12.2 a	11.0 d	2.29 b	1.76 bc	3.68 b	4.94 a	37.4 b	113.3	0.00
	107	120.5 c	8.7 b	12.8 c	2.25 b	1.85 bc	3.71 b	2.90 b	37.7 b	117.9	0.01
	127	173.2 b	6.1 c	14.2 b	1.95 c	1.75 bc	3.64 b	5.95 a	38.3 b	133.4	0.01
Harvest	142	216.8 a	5.0 c	15.3 a	1.81 d	1.66 c	4.07 a	2.18 b	42.2 a	120.6	0.02
	Significance	***	***	***	***	***	***	***	***	**n.s.**	**n.s.**

Mean values followed by same small letters do not differ significantly according to SNK test (p=0.05). Significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

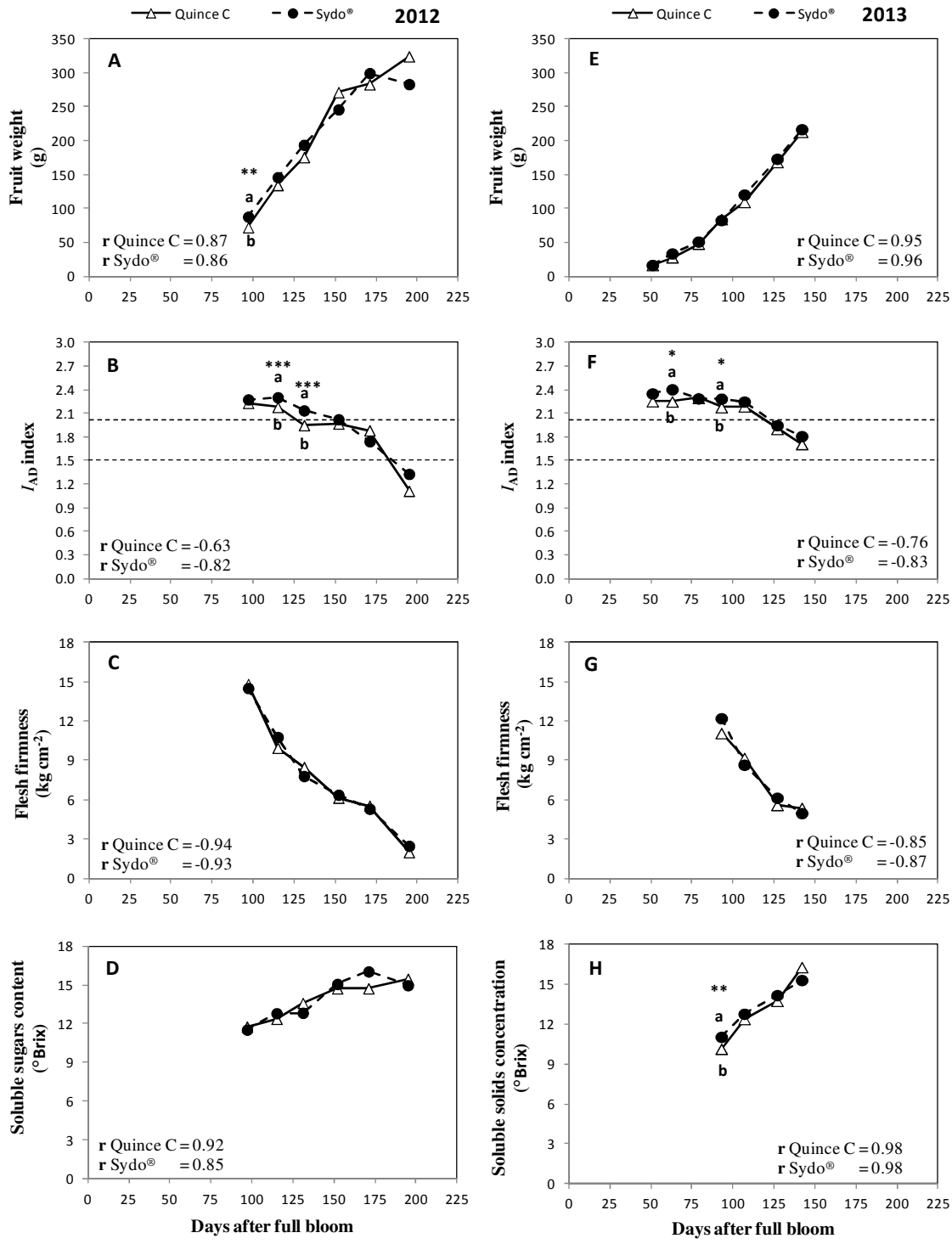

Figure 1. Behavior of fruit weight (A, E), I_{AD} index (B, F), flesh firmness (C, G) and, soluble sugars content (D, H) during the growing season, as days after full bloom, for both combination: Spindle/Quince C (Δ) and Spindle/Sydo® (●) and evaluation years: 2012 (A, B, C and D) and 2013 (E, F, G and H). Statistical parameter evaluated: coefficient of Pearson (r). Significance level, *=p<0.05, **=p<0.01, ***=p<0.001.

Table 3. Fruit soluble sugars and organic acids for Spindle/Quince C combination, for both evaluation years, 2012 and 2013, regarding the days after full bloom (DAFB).

Year	DAFB	Glucose	Fructose	Sucrose	Sorbitol	Inositol	Malic acid	Quinic acid
					mg g^{-1} DW			
2012	97	24.65 c	86.99 e	0.35 c	32.54 a	0.29	2.85	2.57 a
	115	26.97 bc	120.28 d	0.30 c	31.60 a	0.27	2.72	1.65 b
	131	32.48 b	141.79 cd	0.65 c	33.37 a	0.29	2.79	1.03 c
Harvest	152	49.73 a	166.58 bc	1.54 c	33.16 a	0.22	2.66	0.72 c
	171	52.90 a	178.61 ab	6.16 b	31.55 a	0.23	2.75	0.70 c
	195	56.74 a	201.97 a	10.12 a	24.40 b	0.15	2.75	0.69 c
	Significance	***	***	***	*	**n.s.**	**n.s.**	***
2013	51	15.08 b	42.43 b	0.00	32.47	0.07	1.56	6.64 a
	63	20.54 b	58.27 b	0.00	41.90	0.15	3.10	5.98 a
	79	20.16 b	77.47 b	0.00	38.41	0.25	3.28	4.06 b
	93	31.79 b	97.83 b	0.00	33.66	0.31	2.37	1.68 c
	107	36.49 b	247.81 a	0.83	51.66	0.33	2.86	0.98 c
	127	35.26 b	187.10 a	1.94	44.02	0.16	3.63	0.92 c
Harvest	142	68.09 a	226.73 a	6.19	39.72	0.30	1.92	0.94 c
	Significance	*	***	**n.s.**	**n.s.**	**n.s.**	**n.s.**	***

Mean values (n = 3) followed by same small letters do not differ significantly according to SNK test (p=0.05). Significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

Table 4. Fruit soluble sugars and organic acids for Spindle/Sydo® combination, for both evaluation years, 2012 and 2013, regarding the days after full bloom (DAFB).

Year	DAFB	Glucose	Fructose	Sucrose	Sorbitol	Inositol	Malic acid	Quinic acid
					mg g^{-1} DW			
2012	97	23.83 c	87.69 c	0.13 b	32.72 ab	0.30	3.59	2.60 a
	115	30.76 bc	106.27 c	0.00 b	29.68 ab	0.26	2.77	1.69 b
	131	40.60 b	136.22 bc	0.12 b	36.74 ab	0.30	2.99	1.24 c
Harvest	152	53.33 a	160.63 ab	0.61 b	36.15 ab	0.15	3.02	0.76 d
	171	59.30 a	204.97 a	4.82 b	38.99 a	0.09	3.73	0.72 d
	195	61.70 a	191.63 a	8.90 a	24.54 b	0.19	2.75	0.56 d
Significance		***	***	***	*	**n.s.**	**n.s.**	***
2013	51	14.03 c	39.63 c	0.00 b	29.55	0.06 b	1.52 b	6.28 a
	63	17.21 c	49.07 c	0.00 b	35.82	0.25 ab	2.79 a	5.40 b
	79	22.13 bc	73.30 bc	0.00 b	30.65	0.22 ab	3.18 a	3.49 c
	93	34.75 ab	102.74 b	0.00 b	34.60	0.28 a	3.81 a	2.01 d
	107	39.88 a	174.53 a	0.59 b	39.65	0.25 ab	3.48 a	1.38 de
	127	49.15 a	192.06 a	1.22 b	35.75	0.06 b	3.03 a	0.91 e
Harvest	142	50.08 a	206.18 a	5.39 a	34.62	0.13 ab	2.94 a	0.79 e
Significance		***	***	***	**n.s.**	*	**	***

Mean values (n = 3) followed by same small letters do not differ significantly according to SNK test (p=0.05). Significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

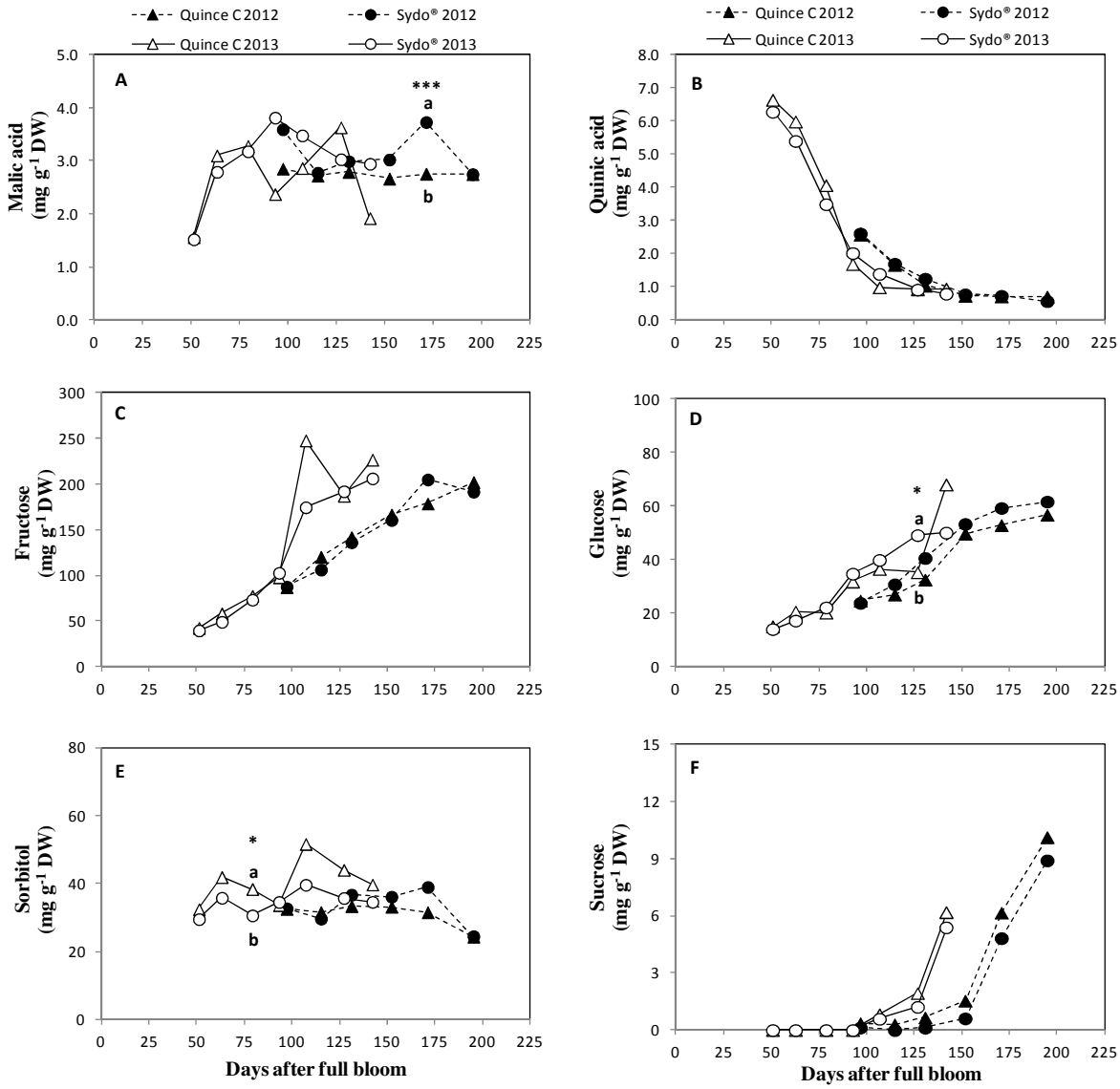

Figure 2. Fruit soluble sugars and organic acids measurements during the growing season, as days after full bloom, for both combinations: Spindle/Quince C (▲, Δ) and Spindle/Sydo® (●, ○) and years of evaluation: 2012 (black symbols, dashed line) and 2013 (white symbols, full line). Organic acids: malic acid (A) and quinic acid (B), alcohol soluble sugars: fructose (C), glucose (D) and sucrose (F), and sugar alcohol: sorbitol (E). Data are means of 3 replicates. Significance level, *=p<0.05, **=p<0.01, ***=p<0.001.

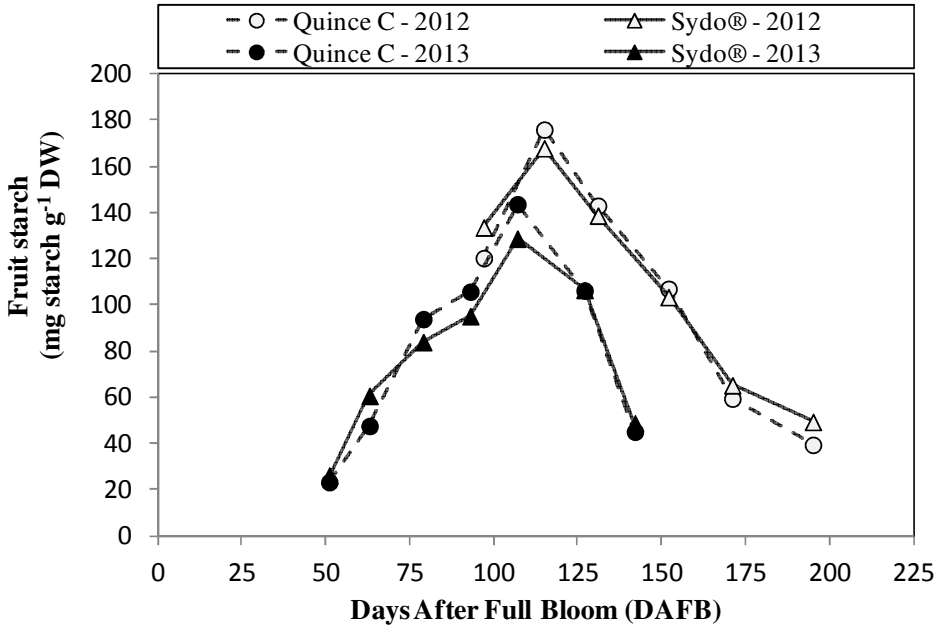

Figure 3. Starch concentration (mg g^{-1} DW) trends in 'Abbé Fétel' pear trees, from both rootstocks: Sydo® (Δ, ▲) and Quince C (○, ●), during growth and maturation on the tree, as days after full bloom, for two years of evaluation: 2012 (open symbols), and 2013 (close symbols).

Table 5. Correlations between starch degradation in fruits and different fruit quality parameters: fresh weight (FW), soluble sugars content (SSC), flesh firmness (FF), I_{AD} index, pH, acidity, Chroma and Hue angle. Statistical parameters evaluated were correlation coefficient of Pearson (r) and coefficient of determination (R^2).

Variables	2012			2013			2012-2013		
	Pearson	R^2	***Signif.***	Pearson	R^2	***Signif.***	Pearson	R^2	***Signif.***
FW	-0.97	0.95	***	-0.96	0.93	**	-0.78	0.60	***
SSC	-0.89	0.81	***	-0.97	0.94	***	-0.90	0.80	***
FF	0.96	0.90	***	0.85	0.72	*	0.92	0.84	***
I_{AD}	0.74	0.47	*	0.91	0.82	*	0.73	0.52	**
pH	0.01	0.04	**n.s.**	-0.85	0.72	*	-0.27	0.07	**n.s.**
acidity	0.90	0.79	**	0.34	0.11	**n.s.**	0.40	0.16	**n.s.**
Chroma	0.21	0.11	**n.s.**	-0.91	0.83	*	-0.31	0.09	**n.s.**
Hue	0.59	0.31	**n.s.**	0.42	0.17	**n.s.**	0.23	0.05	**n.s.**

Mean values of both treatments (Sydo® and Quince C rootstocks). Significance level, *=p<0.05, **=p<0.01, ***=p<0.001.

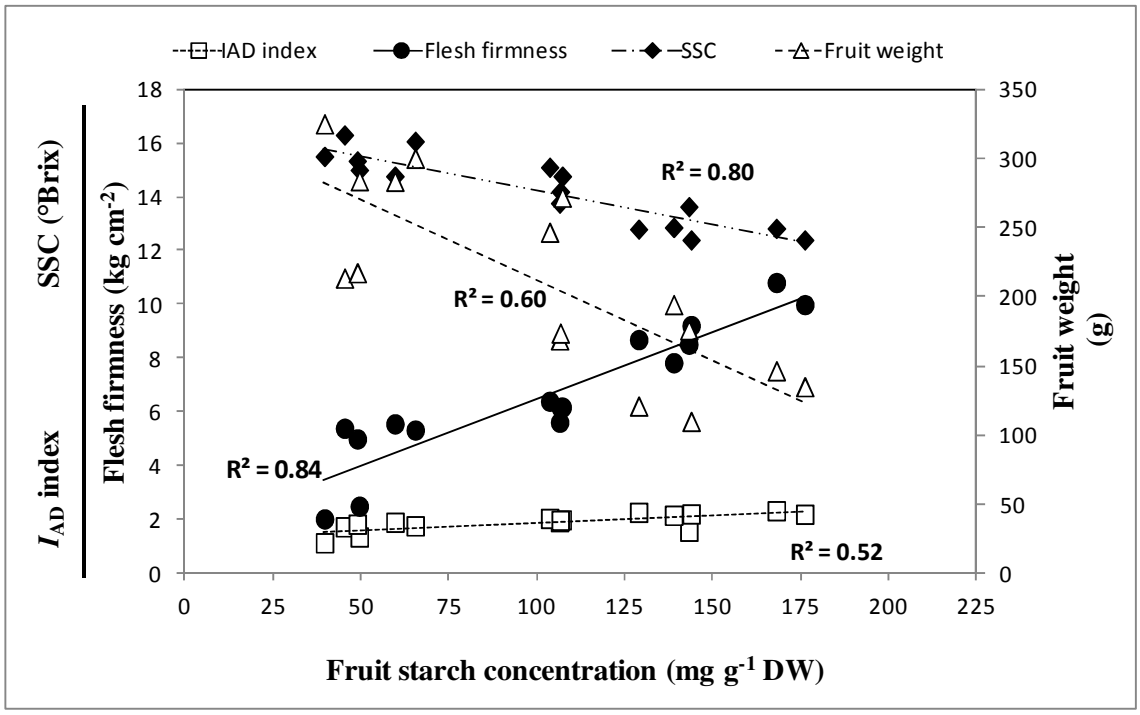

Figure 4. Correlations between fruit starch concentration and I_{AD} index (□), flesh firmness (●), SSC (♦) and fruit weight (Δ), during the two years (2012-2013). Values are means of both rootstocks, Sydo® and Quince C. R^2, coefficient of determination of linear function.

Table 6. Linear equations for starch degradation in fruit and different fruit quality parameters: fruit weight, flesh firmness, soluble sugars content and I_{AD} index. Statistical parameters reports: (a) intercepts, (b) slopes, coefficients of determination (R^2) and p-values.

Fruit starch degradation				
Variable	**a**	**b**	**R^2**	**p-value**
Fruit weight	328.10 ± 27.67	-1.16 ± 0.25	0.60	<0.001
Flesh firmness	1.49 ± 0.63	0.05 ± 0.01	0.84	<0.001
SSC	16.81 ± 0.38	-0.03 ± 0.00	0.80	<0.001
I_{AD} index	1.34 ± 0.15	0.01 ± 0.00	0.52	<0.01

Mean values for intercepts and slopes are reported ± S.E.

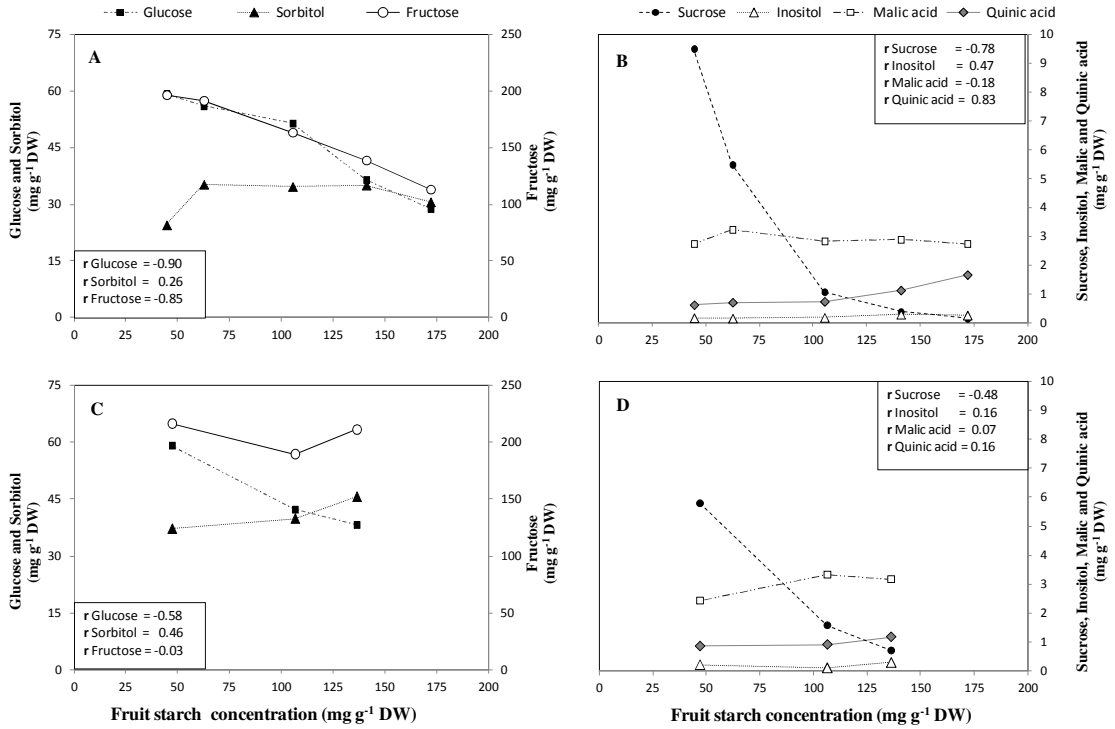

Figure 5. Correlations coefficients (r, Pearson) between fruit starch concentration and alcohol soluble sugars (fructose, glucose and sucrose), organic acids (malic and quinic acids) and sugars alcohol (sorbitol and inositol), for both years: 2012 (A - B) and 2013 (C – D). Values (n = 6) are means of both rootstocks, Sydo® and Quince C.

Table 7. Correlations between starch degradation in fruits and alcohol soluble sugars: fructose, glucose, and sucrose, organic acids: malic and quinic acid and, sugar alcohols: sorbitol and inositol. Statistical parameters evaluated were correlation coefficient of Pearson (r) and coefficient of determination (R^2).

Metabolites	**2012**			**2013**			**2012-2013**		
	Pearson	R^2	***Signif.***	Pearson	R^2	***Signif.***	Pearson	R^2	***Signif.***
Glucose	-0.90	0.81	***	-0.58	0.33	*	-0.75	0.56	***
Sorbitol	0.26	0.06	**n.s.**	0.46	0.20	**n.s.**	0.20	0.05	**n.s.**
Fructose	-0.85	0.72	***	-0.03	0.00	**n.s.**	-0.48	0.28	***
Sucrose	-0.78	0.61	***	-0.48	0.22	*	-0.67	0.46	***
Inositol	0.47	0.21	**	0.16	0.02	**n.s.**	0.32	0.11	*
Malic acid	-0.18	0.03	**n.s.**	0.07	0.00	**n.s.**	0.08	0.00	**n.s.**
Quinic acid	0.83	0.69	***	0.16	0.02	**n.s.**	0.64	0.41	***

Mean values of both treatments (Sydo® and Quince C rootstock). Significance level, *=p<0.05, **=p<0.01, ***=p<0.001.

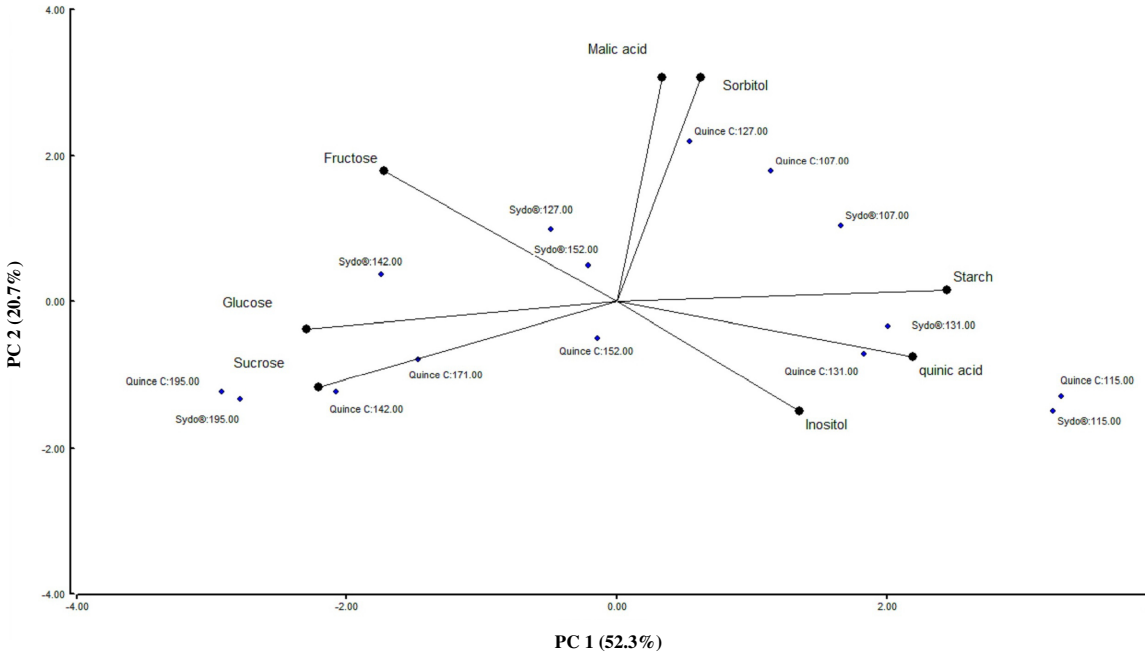

Figure 6. Bi-plot principal component analysis (PCA) of soluble sugars, organic acid and starch degradation, during fruit maturity stage, for both rootstocks (Quince C and Sydo®) and DAFB.

STORAGE DYNAMICS OF NONSTRUCTURAL CARBOHYDRATE RESERVES AT THE DORMANCY PERIOD IN WOOD, ROOTS AND FLOWER BUDS OF 'ABBÉ FÉTEL' PEAR TREES

5.1 Introduction

Annual accumulation and mobilization of carbohydrates in fruit trees, among individual organs, either reproductive and vegetative, is greatly affected by availability of carbon reserves, assimilates from photosynthesis, crop load, number and position within the branch of the different source and sink organs, as well as ability of the translocation system to deliver these resources to sinks (Wright, 1999; Samach and Smith, 2013; Fanwoua et al., 2014). Storage carbohydrates are obliged to sustain growth under periods of stress, and during bud flush and leaf growth in the following spring, until the leaves have developed a sufficient photosynthetic ability to independently support net carbon assimilation (Whiley et al., 1996a; Flore and Layne, 1999; Regier et al., 2010). Also, these reserves support any growth and metabolism of developing buds and cambium during the dormant season, as trees have no photosynthetic capacity (Keller and Loescher, 1989; Loescher et al., 1990). In many tree species, these functions are mainly supported by starch, which is degraded to soluble sugars during the dormant season, for respiration maintenance, and in spring, during bud flush (Regier et al., 2010). Besides the reserve functions of carbohydrates, soluble sugars can also serve as freezing protectants. The concentration and localization of carbohydrates within tissues are affected by several factors, such as: shoot vigor, leaf to fruit ratio, pruning technique, temperature, moisture, and light (Whiley et al., 1996a; Ikinci, 2014).

The whole tree may be considered as a storage organism, and reserve carbohydrates are commonly found in all the perennial parts of the tree, but the higher carbohydrate reserves concentrations are usually found in root tissues (Loescher et al., 1990; Kozlowski, 1992). Carbon partitioning process involves transport of assimilates to different organs and their distribution to different sinks. Sources and sinks are connected one to each other by shoot structures constituting the architecture of the branch (Fanwoua et al., 2014). During the autumn, carbohydrates produced in the leaves

get stored as starch, mainly in twigs, main branches, trunk and root system (Mendel and Cohen, 1967). The amount of carbohydrates stored in the roots changes during the season, with the highest reserves late in the season or during dormancy and the lowest levels in spring, after bud flush (Regier et al., 2010). It is generally thought that root and shoot compete for the assimilate resources, and that the aboveground organs have priority over the roots (Flore and Layne, 1999). Hansen (1967) stated that, after application of ^{14}C (from May to September), reserves distribution among shoots, trunk and roots, depends in particular upon the growth intensity of the various organs. Moreover, Marcelis (1996) noted that the dry matter partitioning among the sinks is mainly regulated by the sinks themselves. To describe correctly the partitioning dynamics, a parameter like "sink strength" is needed. Sink strength describes the sink's drawing power to import assimilates (Ho, 1988; Kozlowski, 1992).

Alternate bearing in avocado (Scholefield et al., 1985; Whiley et al., 1996a, b), pistachio (Nzima et al., 1997), and citrus (Goldschmidt and Golomb, 1982) appears to be closely related to the carbohydrate levels in the tree, stored after harvest until the release of dormancy period. It has been hypothesized that crop yield decreases can be a consequence of a root reserve reduction in response to severe water stress (Loescher et al. 1990; Lopez et al. 2007) or an increase in the fruit-load in the previous season (Park, 2011), which reduces carbohydrates availability to support flowering and fruit set the following season. In general, the accumulation of high concentrations of starch in woody tissues during the quiescent period before spring-blooming, results in high fruit yields, while the failure to store sufficient starch reserves (inevitably due to a previous heavy crop load), most often ends up with crop failure (Whiley et al., 1996b).

The retention for a longer period of sufficient and healthy active leaf canopy until the natural leaf fall, which can be achieved by training systems that optimize the light interception (Kozlowski, 1992), could to allow greater accumulation of carbohydrates after crop harvest, which may be associated with high yields potential in apple trees (Tustin et al., 1997) and kiwifruit vines (Kwack et al., 2014). Cruz-Castillo et al. (2010) demonstrated that a poor bloom return in kiwifruit is related to the depletion of nonstructural carbohydrates in summer caused by defoliation or leaf damage. Loescher et al. (1990) noted that, for the storage carbohydrates are the roots the most affected organ when some treatments, as defoliation or pruning, are conducted. This pattern

pointed out that photosynthesis, even when occurring late in the season, is important for normal starch accumulation.

The transition from active growth to the dormant phase is a prerequisite step for the winter survival of the plants (Ito et al., 2012). In temperate areas, typical location for deciduous trees, the most important factor for overtaking dormancy is the accumulation of a certain amount of chill hours, variable according to species and cultivar (Ito et al., 2012; Marafon et al., 2010). This exposure to low temperatures apparently induces changes in the metabolism which are necessary for the beginning of growth. Bud break requires carbon supply for metabolic reactivation and leaf primordia growth. Carbohydrates could be this source of energy for growth resumption (Bonhomme et al., 2009). The starch mobilization from reserve tissues (stems and/or roots) to the growth areas (meristems) of the woody plants, aims to increase the amount of soluble sugars potentially useful for cellular metabolism. The starch stored in the xylem parenchymatous cells is rapidly converted to sugars, particularly sucrose, and this metabolite is transported along the xylem pathway together with water to the buds, where it is directly absorbed or further hydrolyzed to glucose and fructose to supply energy and carbon precursors (Marafon et al., 2011).

The main objective of the present work was to establish and quantify the seasonal dynamics of starch and soluble carbohydrates in wood, roots and flower buds of 'Abbé Fétel' pear trees throughout two growing seasons, during the period starting after harvest and before bud break. Moreover, to evaluate the effect of two factors: level of crop-load and date of sampling, on carbohydrates stored reserves during the first season.

5.2 Materials and Methods

5.2.1 Plant material

Trial was carried out throughout two growing seasons (from October 2012 to January 2014) on seventeen-year-old pear trees (*Pyrus communis* L.) cv. 'Abbé Fétel', trained at Spindle and grafted on Quince C rootstock. The planting density was 3.6 m x 0.7 m. The orchard was located in Cadriano, Italy (44°54'88.53''S; 11°38'59.30''W), with an approximate North-South orientation.

Trunk diameter at 10 cm above grafting point was measured and trunk cross-sectional area (TCSA) was calculated. In this way, trees homogeneous in size and vigor were selected within the orchard for the experimental trails.

5.2.2 Experimental design and sampling

Twenty four trees were selected according to trunk cross-sectional area (TCSA; 60.2 ± 1.5 cm^2). Six whole trees were destructively sampled at four phenological stages. Sampling dates corresponded to:

1. Date 1 (D1): one month after commercial harvest (9 Oct. 2012);
2. Date 2 (D2): during dormancy - after natural fall of leaves (14 Jan. 2013) - before any growth activity was detectable;
3. Date 3 (D3): one month after second year fruit harvest (10 Oct. 2013);
4. Date 4 (D4): full dormancy in the second year (20 Jan. 2014).

Trees were carefully dug out by a tractor equipped with a trencher which was inserted beneath the main root system (100 cm depth). Above-ground tree organs were fractioned into the following individual components: leaves (when present), brindle-type shoots (current-season's shoot), 2-years-old branches, 3-years-old branches, 4-and-over-years-old branches, short-old spurs, trunk, and flower buds. Below-ground organs were divided in: root stump, coarse roots (thickness > 2 mm), and fine root (thickness < 2 mm). The number of flower buds was also determined. Ages of the tissues refer to their physiological ages at the beginning of each season. A randomly selected aliquot of 50 g for each type of organ was sampled for further analysis.

Additionally, in the two first dates of sampling (D1 and D2) according to crop load evaluated at harvest, trees were post-hoc subdivided in two groups: high crop-load (HCL, 3 trees) and low crop-load (LCL, 3 trees).

5.2.3 Dry matter partitioning

Immediately after all organs discrimination mentioned above, fresh weight (FW) was measured for each of them. Roots were rinsed with water to remove the soil debris and left in the open air for 40 minutes before fresh weight determinations. Subsamples were weighed and then dried in a forced draft oven at 60 °C until constant mass to determine dry weight (DW). Dry matter (%) is calculated as (DW/FW) x 100 (Palmer, 1988, 1992).

5.2.4 Fruit yield

Fruit number and yield were recorded for all trees evaluated at the commercial harvest. At the first harvest (2012), two levels of crop-load were distinguished: a high crop-load (HCL, av. 25 ± 2 S.E) and a low crop-load (LCL, av. 10 ± 1 S.E.).

5.2.5 Leaf measurements

Leaf measurements were carried out on two occasions, at the first sampling D1 and, at the natural fall of leaves (late November). The total number of leaves was counted for each assessed tree. At D1, the total was obtained by counting all the canopy leaves since the leaf senescence had not started yet. In the meantime, chosen trees for the late November sampling were surrounded by a plastic net (similar to hail net protection). This operation was done in order to collect all the leaves that otherwise would have fallen on the ground. After that, the leaves were weekly harvested from the net, between D1 and natural leaves fall. The remaining leaves still attached to the trees (average of 5%) were manually harvested. At each partial sampling date of D1-natural leaves fall interval, the number and total weight of leaves were determined.

Leaf area (LA in cm^2) was determined with a leaf area meter (LI-3100; LI-COR, Lincoln, Nebraska, USA) on a subsample from each tree, corresponding to 20% of total leaf weight/tree. Then, the total leaf area was estimated by multiplying the number of

leaves by the average leaf area obtained measuring the 20% subsample and Leaf Area Index (LAI) calculated as total leaf area on land area (Wünsche and Lakso, 2000).

5.2.6 Starch and soluble carbohydrates determinations

5.2.6.1 Preparation of material

A subsample of 50 g FW of each organ was utilized for starch and soluble carbohydrates analysis. For the flower buds, two thirds of the total weight was sampled. Subsamples were immediately frozen in liquid nitrogen and stored at -80 °C until analysis. Successively, samples were kept for 7 days in a freeze dryer (HETO drywinner, DW3, Denmark), then reweighed and, once weights were stable, ground till a fine powder with a mill and stored in airtight containers at room temperature. All organs (except flower buds), were ground by a mill (Restch SM 200, Germany) firstly with a 0.25 mm Ø sieve, and subsequently with a 0.20 mm Ø one (Fritsch pulverisette 14, Germany), in order to standardize the grinding step for all samples. Flower buds were directly ground in a 0.20 mm Ø sieve mill.

For the following analysis, three technical replicates of 100 mg DW of each organ were used.

5.2.6.2 Determination of soluble carbohydrates concentrations

The protocol used to determine soluble carbohydrates concentrations was the same previously described in paragraph 3.2.5.2, chapter III.

5.2.6.3 Determination of starch concentration

The protocol used to determine starch concentration was the same previously described in paragraph 3.2.5.3, chapter III.

5.2.6.4 Spectrophotometer analysis

The protocol used to measure the total soluble carbohydrates and starch concentrations in the prepared samples ready for the spectrophotometer analysis, was the same previously described in paragraph 3.2.5.4, chapter III.

5.2.7 Statistical analysis

The experiment was arranged as a completely randomized design. All data collected were elaborated with SAS® software for the statistical analysis of the variance and means separation was performed by Student-Newman-Keuls (SNK) test, differences were considered significant at $p \leq 0.05$. The study was conducted in a repeated measures design with six replicates (trees) for each tissue (9 tissues) per each sampling date (4 dates). A factorial experimental design was used with level of cropping and date of sampling as sources of variation, in the first assessed season.

5.3 Results

5.3.1 Fruit yield

The two fruit-load levels established in the harvest of 2012 showed significant differences for the parameters of fruit number (n. tree^{-1}) and yield (kg tree^{-1}), but this factor was not significant for the fruit weight (g) (Table 1). High Crop Load (HCL) trees showed values approximately three times higher than those in Low Crop Load (LCL) trees. Between years, differences in productive parameters were also significant. There was a marked alternate bearing, 2013 corresponded to a higher crop season compared to the previous one (2012 season). In contrast, 2013 average fruit weight (g) at harvest was a 33.2% lower than that in the 2012 (Table 1).

5.3.2 Leaf measurements

The crop-load factor, evaluated in the 2012, showed significant differences for leaves number, total leaf weight, leaf area and LAI (Table 2). For all these, the HCL presented the highest values. Regarding comparison between dates of sampling (D1-harvest versus natural fall leaves), only the total leaf weight was significantly different, with the value measured one month after harvest higher (1.67 kg $tree^{-1}$) than the one on late November (1.12 kg $tree^{-1}$). Regarding average leaf weight, the interaction between crop load and sampling date was meaningful, as shown in the Table 2. Briefly, leaves collected after harvest from trees with a LCL obtained the highest value (356.3 ± 15.2 mg), whereas leaves during natural leaves fall, regardless the level of crop-load, exhibited the lowest values (Figure 1). Other parameters evaluated were the percent of content water (% WC) and dry matter (% DM). The results showed significant differences, either for the level of crop-load or sampling date, but the interaction between them was not significant (Table 2). In this way, leaves sampled from trees with a HCL obtained a % of DM higher than in 4.6% compared with trees with a LCL. Whereas leaves sampled at natural leaves fall showed a 70.5% of DM, higher than in 48.7% respect of leaves sampled after harvest.

Soluble carbohydrates in leaves did not present a significant interaction between crop-level and date of sampling (Figure 2), unlike to what observed for the starch concentration (Figure 3). The highest values of soluble carbohydrates concentration in leaves, within each factor, were obtained in LCL trees (49.2 mg Glc g^{-1} DW), and in the sampling after harvest (76.5 mg Glc g^{-1} DW), respectively (Figure 2). Regarding the starch concentrations, differences were found between trees with different crop-load levels sampled after harvest (Figure 3), where leaves from trees with a LCL obtained the lowest concentration (12.6 mg starch g^{-1} DW), being a 53.6% lower than leaves from trees with a HCL. At natural leaves fall, the starch concentrations corresponded to 28.3 ± 0.6 and 29.2 ± 1.2 for leaves with a LCL and HCL, respectively.

5.3.3 Starch, soluble carbohydrates, and fresh weight of wood, roots, and flower buds

The two levels of crop-load established at harvest (2012) did not present significant differences in terms of dry weight, starch and soluble carbohydrates concentration for the different organs evaluated at the dates D1 and D2 (data not shown).

Fresh weight measurements for the different structures, at the four sampling, showed that 2-year-old branches, flower buds, and coarse roots (thickness> 2 mm) presented significant differences among the dates (Table 3). For these three organs, the first two dates (D1 and D2), obtained the highest values. For the flower buds, a tendency of increase of fresh weight was also observed (not statistically significant) within a single growing season between after harvest and dormancy (D1 and D2; D3 and D4), unlike to what happened in other organs (Table 3). These increases in flower buds (FB) fresh weight corresponded to a 25% and 49%, respectively for the first and second transition (October – January). However, for the average fresh weight and number of FB, significant differences were obtained among the sampling dates (Figure 4). D1 registered the highest number of flower buds (619 FB), differing statistically to the other dates. The lowest value corresponded to 149 FB, which was obtained at D3 (after harvest, 2013).

Regardless of type of organs, either flower buds (Figure 5), woody organs (Figure 6A) or roots (Figure 7A – B), a consistent and stable pattern through the growing season was observed for soluble carbohydrates. Thus, between the intervals (after harvest and dormancy) the concentrations of soluble carbohydrates increased, showing highest values during the dormancy period, in January (D2 and D4). In the FB, for both assessed years, this increase was twofold the value recorded after harvest (Figure 5). On the other hand, short-old spurs, among the woody organs aboveground, obtained the largest increases, which corresponded to a 93.6% and 74.2%, for D1 – D2 and D3 – D4 intervals, respectively (Table 4). However, this was different in the roots, both coarse and fine roots, mainly in the first interval (D1 - D2), did not differ statistically (Figure 7A - B), even if the increases were 5.5% and 10.9 %, respectively (Table 4).

Respect to starch concentration, only the woody organs showed a clear trend throughout the growing seasons; a decrease occurred between the intervals of each sampling date

(Figure 6B). Unlike of the performance for soluble carbohydrates, starch concentration in short-old spurs exhibited the smallest decline (6.5%) in the first interval (D1 – D2) (Table 4), whereas the other aboveground structures showed an average of 34.5% (± 2.0 S.E.) for the same period. In the case of flower buds, coarse and fine roots, the starch behavior previously described for woody organs was reported only in the second evaluation season, between dates D3 and D4 (Figure 5, 7A - B), showing a significant difference. On the other hand, between harvest of 2012 and the dormancy evaluated on January 2013 (D1 and D2), were registered increases of 21.8% and 18.0% in flower buds and fine roots, respectively (Figure 5, Table 5).

In the year of LCL (2012), at D1, almost all the different aboveground structures accumulated starch and soluble carbohydrates concentrations higher than those obtained in 2013 season (a heavy crop load year) at the same corresponding stage (D3) (Table 5). Exception to that regarded starch and soluble carbohydrates concentrations in short-old spur, where the highest values were recorded at D3, corresponded to 81.78 and 36.91 mg g^{-1} DW, respectively; although, only starch showed significant differences (Table 5). Nevertheless, although there were numerical differences for other structures not all were statistically significant. The greatest variation among the woody tissues existed for starch concentration within the same year, ranging from 50.2 to 102.0 mg g^{-1} DW respectively for old short spurs and 2-year-old branches. One-year-shoot and 2-year-old branches presented the highest starch concentrations values. Whilst for the soluble carbohydrates concentrations the short-old spurs presented the highest values within each season, 35.57 and 36.91 mg g^{-1} DW, respectively.

5.4 Discussion

Carbohydrates dynamics in temperate-zone fruit tree tissues reflect the interconversion between starch and soluble carbohydrates. Starch hydrolyzed during dormancy period (amylase is activated by cold temperature (Marafon et al., 2011)) is used to maintain the metabolism, and also is translocated towards organs, to support the new growth (at dormancy release) during the following spring (Keller and Loescher, 1989; Lacointe et al., 1993; Nzima et al., 1997; Bonhomme et al., 2009; Marafon et al., 2011; Ito et al., 2012). The annual cycle of nonstructural carbohydrates in woody plant organs has been

reported for several species (Scholefield et al., 1985; Keller and Loescher, 1989; Lacointe et al., 1993; Whiley et al., 1996a, b; Zapata et al., 2004; Regier et al., 2010; Park, 2011; Ito et al., 2012), but in European pear the research has been largely neglected. The studies, which have been conducted so far on other species, are consistent with our findings. Principally, the starch stored in woody tissues reaches its maximum level after harvest during fall (D1 and D3), after this, during endodormancy period (winter) and early spring is hydrolyzed and the minimum values were obtained (D2 and D4) (Figure 6B, and 7A - B). After flowering and fruit set, starch started to accumulate again in the woody tissues. Regarding soluble carbohydrates, a reverse pattern was reported; indeed, at the end of dormancy phase and in early spring, an increase in their concentration was observed, confirming a process of interconversion between them (Figure 5, 6A, and 7A – B).

When fruit enter into the maturation period, they do not act more as a sink for assimilates, and other organs, such as shoots and roots, (depending of their sink strength (growth cycle)) require assimilates in order to accumulate reserves during fall before tree enters in a dormancy period. This experiment showed the importance of leaves retention after harvest, in fact leaves sampled at October presented a significant higher amount of soluble carbohydrates respect to the natural leaves fall (Figure 2). Even if leaves were entering in a senescence period, confirmed by their weight loss (Figure 1) and %WC decrease, at late November (Table 2), they still presented a significant concentration of soluble carbohydrates (15.6 mg Glc g^{-1} DW). Thus, any kind of leaf damage occurring, from insects, diseases, viruses, or climatic events (hail) may reduce leaf area, decrease the source-sink ratio, and reduce the overall photosynthetic potential (Flore and Layne, 1999). McCamant (1988) cited by Loescher et al. (1990) noted that sweet cherry trees defoliated in August had the lowest amount of starch in all tissues, while trees defoliated at later dates had higher levels; starch in the other organs was increasing with the delay of defoliation. Whiley et al. (1996a) indicated that if the summer-grown leaves in avocado trees are maintained until spring shoot was fully accomplished, the continuity of assimilates supply during the flowering and fruit set period will be ensured. Moreover, Hudina and Štampar (2002) studied the effect of reduction of leaf area on quality of pear fruits cv. ‘Williams’ during their development, and concluded that a 30% reduction in leaf area induced a lower assimilates production. Another work on ‘Hayward’ kiwifruit reported that a 75% reduction of foliage five

DAFB decreased the rate of starch accumulation in summer on current shoots and trunk bark, but did not affect the starch concentrations in roots (Cruz-Castillo et al., 2010). Similar results were found by Kwack et al. (2014), also in kiwifruit cv. 'Goldrush' in perennial organs, with a reduction of 75 and 100% leaf area between August and September, highlighted that in October an increase of starch concentrations in roots was observed. Moreover, the significant loss of leaf area on summer-pruned trees may lead to a reduction in the carbohydrate and nutrient element concentrations in the remaining tissues limiting in this way the tree growth (Ikinci, 2014). Therefore, a defoliation treatment conducted between harvest and natural leaves fall, could affect more intensely some growth stages, which influence the crop potential, causing changes in crop phenology and fruit development (Tustin et al., 1997).

The differences found between starch concentrations accumulated at October of each year, could be attributed at the differences in the crop load level (Table 5). During the growing season there is an overlapping period between vegetative and reproductive growth; therefore in a year of low crop-load there may be less competition among sinks, unlike to what is expected in a high crop-load year. This also is supported by results about fresh weight measured in 1-year-shoots (growth of the year), 2-year-old branches and fine roots at each date (active growth), which, generally speaking, obtained lower values for the high crop-level year (D3, Table 3). The focus on these types of structure is because perennial branches are the framework (architecture) of tree, in this way, have a minimal demand of assimilates as a slight growth present (Naschitz et al., 2010). Park (2011) in persimmon and Monerri et al. (2011) in sweet orange trees agreed that, during the period from fruit development until harvest (persimmon) and during winter (sweet orange) in the leaves, shoots, old woods and roots, the low strength of fruit as sink allowed a greater accumulation of reserves. In this study, was observed the greatest accumulation of starch in young structures (current season's growth and 2-year-old branches), in the light crop-level year (D1) compared to the high crop loaded year (D3) (Table 4). Naschitz et al. (2010) stated that a low demand for carbon enhances the accumulation of starch, first in leaves and then in roots and other woody tissues, while high carbon need induces soluble sugars synthesis. Moreover, Monerri et al. (2011) observed that the levels of carbohydrates in the leaves of low crop loaded trees were double than in the high crop trees, in agreement with our findings, where LCL leaves registered 115.9% more starch concentration than in HCL one month after harvest

(Figure 3). However, as the same results obtained by Wünsche et al. (2005) in 'Braeburn' apple tree, at the end of the season no significant differences were found between the two levels of cropping (Figure 3).

As it has been reported in previous works, the starch accumulated in woody tissues is almost hydrolyzed during the dormancy period, for the further availability for new growth, bud break and fruit set of the following year (Figure 6 B, 7A - B). Then, the increase of soluble carbohydrates in woody branches towards the end of the season (D2 and D4, Figure 6A) is probably related to i) the mobilization of above ground carbohydrates reserve into the root systems and old wood, and ii) the hydrolysis of starch stored in the same tissues. Short-old spurs structure showed the smallest decline in the transition between after harvest and leaves fall (D1-D2; 6.5%), but the highest increase in soluble carbohydrates in both years. According to Naschitz et al. (2010) and Goldschmidt and Golomb (1982), the carbohydrates reserves in the tree framework (old wood) is not directly utilized, unlike what happen in current season shoots and roots. In this way, starch found in these structures can be viewed as a reserve available to support and maintain the growth of other tree organs; this might point, a higher soluble carbohydrates concentration obtained at January. Keller and Loescher (1989) stated that this behavior would be expected because older tissues have a higher volume of dead cells.

In the flower buds, the increase of total and average fresh weight between October and January (Table 3, Figure 4), could be explained by the increase of soluble carbohydrates (Figure 5), which could be related to a mechanism of freezing tolerance. According some authors, during cold acclimation, an increase of intracellular osmotic potential occurred, due to this accumulation of soluble sugars and amino acids (Marquat et al., 1999). During dormancy release (from January), buds become high sink tissues, increasing the active transport of soluble carbohydrates, which are metabolized to provide energy and carbon storage, which will be used for growth metabolism and inducing budburst (Lacointe et al., 1993; Marquat et al., 1999; Bonhomme et al., 2009; Marafon et al., 2011; Ito et al., 2012) (Figure 5, dates D2 and D4). Although, there was an increase in fresh weight in the two seasons assessed, the number of flower buds was significantly different at January (Figure 4), before dormancy release, which could explained the alternate bearing observed between the seasons (Figure 4).

In respect of the root systems, unlike that the other reports have pointed (Goldschmidt and Golomb, 1982; Monerri et al., 2011), this experiment showed a great storage of starch, either into coarse and fine roots, at October of high crop-level year (D3, Figure 6A – B). One would expect that after harvest, in a year with low fruit-level, a greater accumulation of reserves in roots occurs, being these the most active organ, as it has been reported for other species (Goldschmidt and Golomb, 1982; Lopez et al., 2007; Monerri et al., 2011); however, this did not happen in this experiment (Figure 7A – B). This could be explained, at least partially, because a small sub-sampled should be considered from the whole root system to determine a specific concentration, therefore, there is a risk of obtain a value not entirely representative (Lopez et al., 2013). Despite this contradictory outcome, it was observed that the coarse roots showed greater storage capacity, in contrast with fine roots where the starch concentration was lower, in agreement with the results described by Regier et al. (2010) for poplar trees. This type of roots (fine) are so-called 'feeder roots', and their main function is absorption of water and mineral nutrients. Therefore, they are relatively short-lived and remain unaffected by the growing conditions of the season, as also observed by Monerri et al (2011).

5.5 Conclusions

The results of this work confirmed that in pear trees cv. 'Abbé Fétel' the dynamic of interconversion between starch and soluble carbohydrates during the dormancy period occurred, at different ranges, in different organs: buds, wood and roots, which could be influenced by several factors. The increase of soluble carbohydrates before the release of dormancy, according where it happen (type of organ), suggested different functions: translocation of soluble carbohydrates or synthesis to metabolites. The level of crop-load had a major influence on the concentrations of starch compared to soluble carbohydrates, after harvest (October); however, at the end of the dormancy period (January) these differences disappeared. On the other hand, the importance of the retention of active leaf area for a longer time was shown by the different sampling dates, which showed that leaves on late November continued to produce assimilates.

Differences in the number, total and average fresh weight on flower buds at dormancy period, as well also, in starch and soluble carbohydrates in all organs evaluated could

explain the alternate bearing observed in this experiment. However, as no measurements were realized at budbreak and flowering, further investigations are needed to clarify this phenomenon.

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5.7 Figures and tables

Table 1. Yield performance of 'Abbé Fétel' pear trees evaluated in 2012 and 2013 seasons.

Harvest season	Fruit (n./tree)	Yield (kg/tree)	Fruit weight (g)
2012	17 b	4.4 b	250 a
2013	65 a	10.9 a	167 b
Signif.	***[y]	***	***
Crop-load 2012 (only)			
Low (LCL)	10 b	2.4 b	249
High (HCL)	25 a	6.3 a	252
Signif.	***	***	**n.s.**

Small letters in the vertical way indicate significant differences. [Y], significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

Table 2. Leaf measurements: number/tree, total weight/tree, average leaf weight, leaf area/tree, LAI, dry matter (DM) and water content (WC) regarding the levels of crop-load in the 2012 season (HCL and LCL), and dates of sampling (D1 and natural leaves fall).

	Leaves number	Total leaves weight	Average leaf weight	Leaf area/tree (canopy)	LAI	DM	WC
	n.	kg	mg	m^2		%	%
Crop load (CL)							
HCL	5959 a	1.59 a	267.2	7.81 a	3.10 a	57.6 b	42.4 a
LCL	4395 b	1.20 b	275.0	5.44 b	2.16 b	60.3 a	39.7 b
Signif.	**[y]	***	**n.s.**	***	***	*	*
Sampling date (SD)							
After harvest (D1)	5065	1.67 a	333.0 a	6.48	2.57	47.4 b	52.6 a
Natural leaves fall	5289	1.12 b	209.2 b	6.76	2.68	70.5 a	29.5 b
Signif.	**n.s.**	***	***	**n.s.**	**n.s.**	***	***
Interaction CL x SD	**n.s.**	**n.s.**	**	**n.s.**	**n.s.**	**n.s.**	**n.s.**

Small letters in the vertical way indicate significant differences. [Y], significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

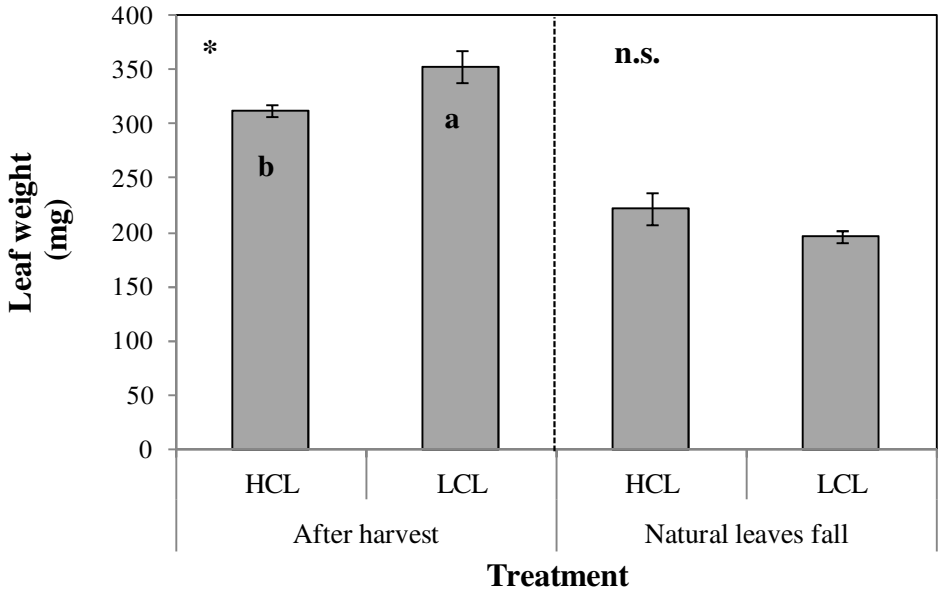

Figure 1. Interaction between crop-load (HCL and LCL) and sampling date (after harvest and natural leaves fall) for average leaf weight (mg). Mean values ± S.E. Small letters indicate significant differences. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

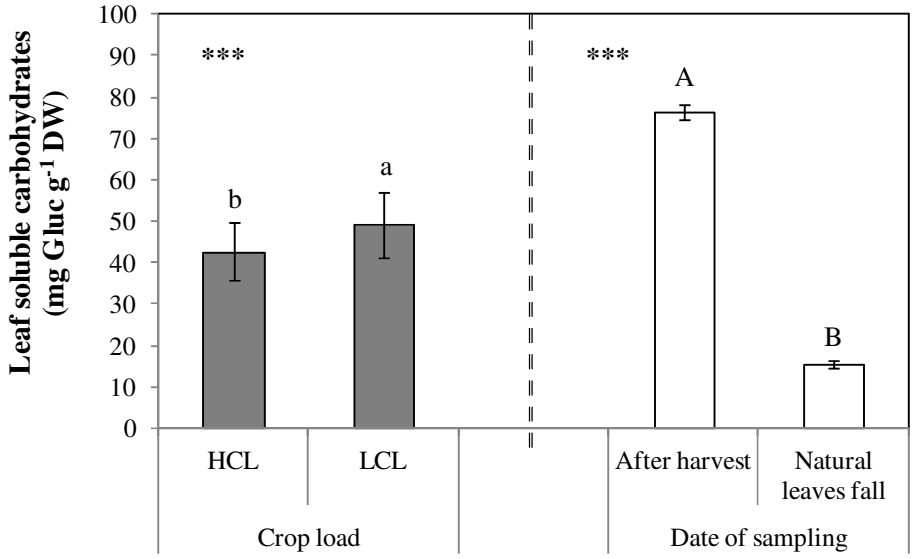

Figure 2. Leaf soluble carbohydrates concentrations (mg Glc g^{-1} DW) for each factor evaluated, crop load: high and low level, and date of sampling: after harvest and natural fall leaves. Mean values ± S.E. Letters indicate significant differences within each factor. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

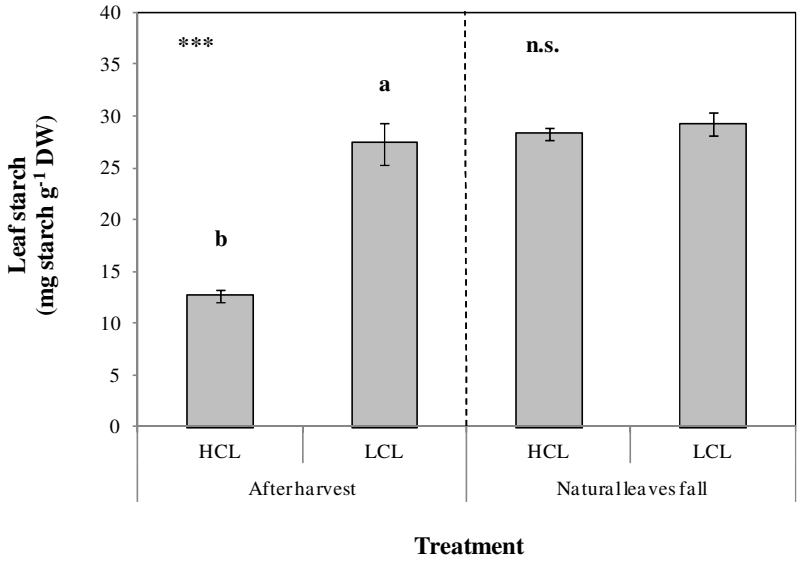

Figure 3. Interaction between factors of crop-load (HCL and LCL) and date of sampling (after harvest and natural leaves fall) for leaf starch concentration (mg starch g^{-1} DW). Mean values ± S.E. Small letters indicate significant differences. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

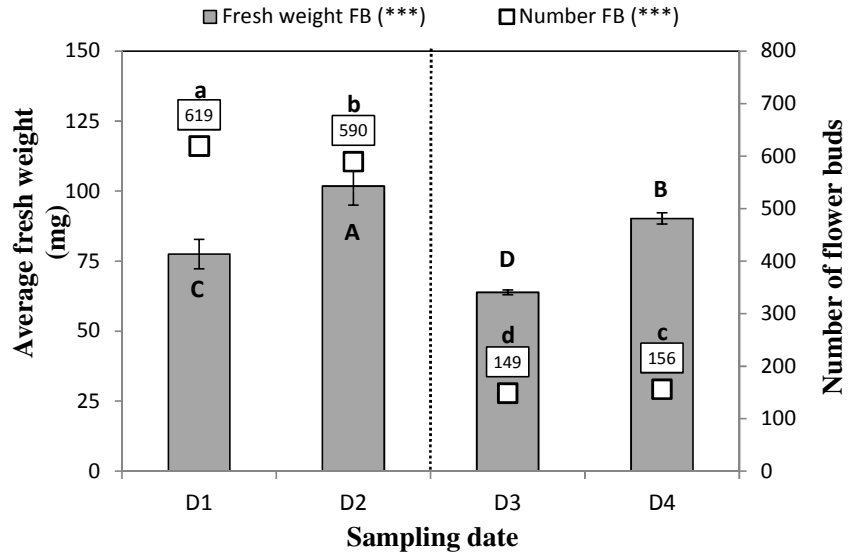

Figure 4. Average fresh weight (grey bars) and number (white square) of flower buds at different sampling dates. Mean values ± S.E. Small letters indicate significant differences among sampling dates for flower buds number, while capital letters for average fresh weight. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

Table 3. Fresh weight of all organs (above and underground) of pear trees cv. 'Abbé Fétel' evaluated at different dates during the experiment: after harvest of 2012 and 2013 (D1 and D3), and during dormancy of 2013 and 2014 (D2 and D4).

		Fresh weight								
Organs	unit	D1		D2		D3		D4		***Signif.***
Wood										
1-year	g	534.0		528.7		421.5		310.0		**n.s**[y]
2-year	g	357.8	**A**	415.4	**A**	255.9	**AB**	153.2	**B**	***
3-year	g	365.2		282.8		315.9		280.6		**n.s**
4-year	kg	3.4		3.0		3.1		3.3		**n.s**
Spurs	g	305.3		197.1		260.1		172.2		**n.s**
Trunk	kg	8.0		8.9		9.4		9.4		**n.s**
Flower buds	g	48.0	**A**	60.0	**A**	9.5	**B**	14.1	**B**	***
Roots										
Coarse (> 2 mm)	kg	2.2	**A**	1.7	**AB**	1.3	**B**	1.2	**B**	***
Fine (< 2 mm)	g	111.0		107.1		64.6		91.0		**n.s.**

Values are means of six replicates. Capital letters in the horizontal way indicate significant differences. [Y], significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

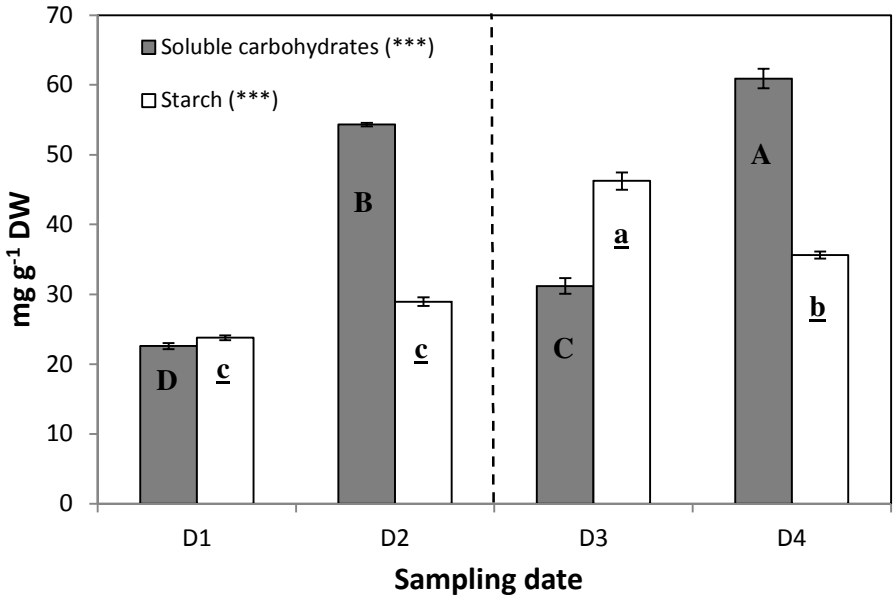

Figure 5. Starch (white bars) and soluble carbohydrates (grey bars) concentrations (mg g^{-1} DW) in flower buds at different sampling dates (2012-2013). Values mean ± S.E. (n=6). Capital letters indicate significant differences among dates for soluble carbohydrates, while small letters for starch.

Table 4. Increase of soluble carbohydrates and decrease of starch concentrations, expressed as percentage (%), between after harvest and dormancy intervals, for both assessment year (D1 and D2; D3 and D4).

	% Increase of soluble carbohydrates		% Decrease of starch	
	D1 - D2	D3 - D4	D1 - D2	D3 - D4
Wood				
Brindle-type shoots	56.0	55.5	34.2	21.8
2-yrs-old	51.2	59.5	36.9	24.3
3-yrs-old	75.2	64.1	34.7	19.5
4-yrs-old	55.5	60.1	32.0	27.8
Short-old spurs	93.6	74.2	6.5	32.7
Roots				
Coarse (> 2 mm)	5.5	48.8	33.0	62.6
Fine (< 2 mm)	10.9	28.5	(+)18.0	65.7

(+): % increase.

Table 5. Starch and soluble carbohydrates concentrations (mg g^{-1} DW) in different types of branches: 1-year (current season's growth), 2-year-old branches, 3-year-old branches, branches 4-and-over-years old, and short-old spurs of pear trees cv. 'Abbé Fétel' evaluated at two dates: after harvest of 2012 and 2013 (D1 and D3), corresponded to a low crop-load (LCL) and high crop-load (HCL) year, respectively.

Branches	**Starch**			**Soluble carbohydrates**		
	D1 (LCL)	D3 (HCL)	***Signif.***	D1 (LCL)	D3 (HCL)	***Signif.***
	mg g^{-1} DW			mg g^{-1} DW		
1-year	101.40 ***a***	88.56 ***a***	**n.s.**	32.73 ***ab***	31.06 ***b***	n.s.
2-year	101.96 ***a***	87.73 ***a***	**n.s.**	31.74 ***b*** **A**	29.56 ***b*** **B**	*
3-year	90.36 ***ab*** **A**	70.63 ***b*** **B**	**	29.68 ***b*** **A**	25.11 ***c*** **B**	**
4-year	77.99 ***b***	68.72 ***b***	**n.s.**	32.10 ***ab*** **A**	28.78 ***b*** **B**	***
Spurs	50.19 ***c*** **B**	81.78 ***ab*** **A**	**	35.57 ***a***	36.91 ***a***	n.s.
Signif.	***	***		**	***	

Values are means of six replicates. Capital letters in the horizontal way indicate significant differences between seasons, while small and italics letters in vertical way indicate significant differences among types of bearing wood. [Y], significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

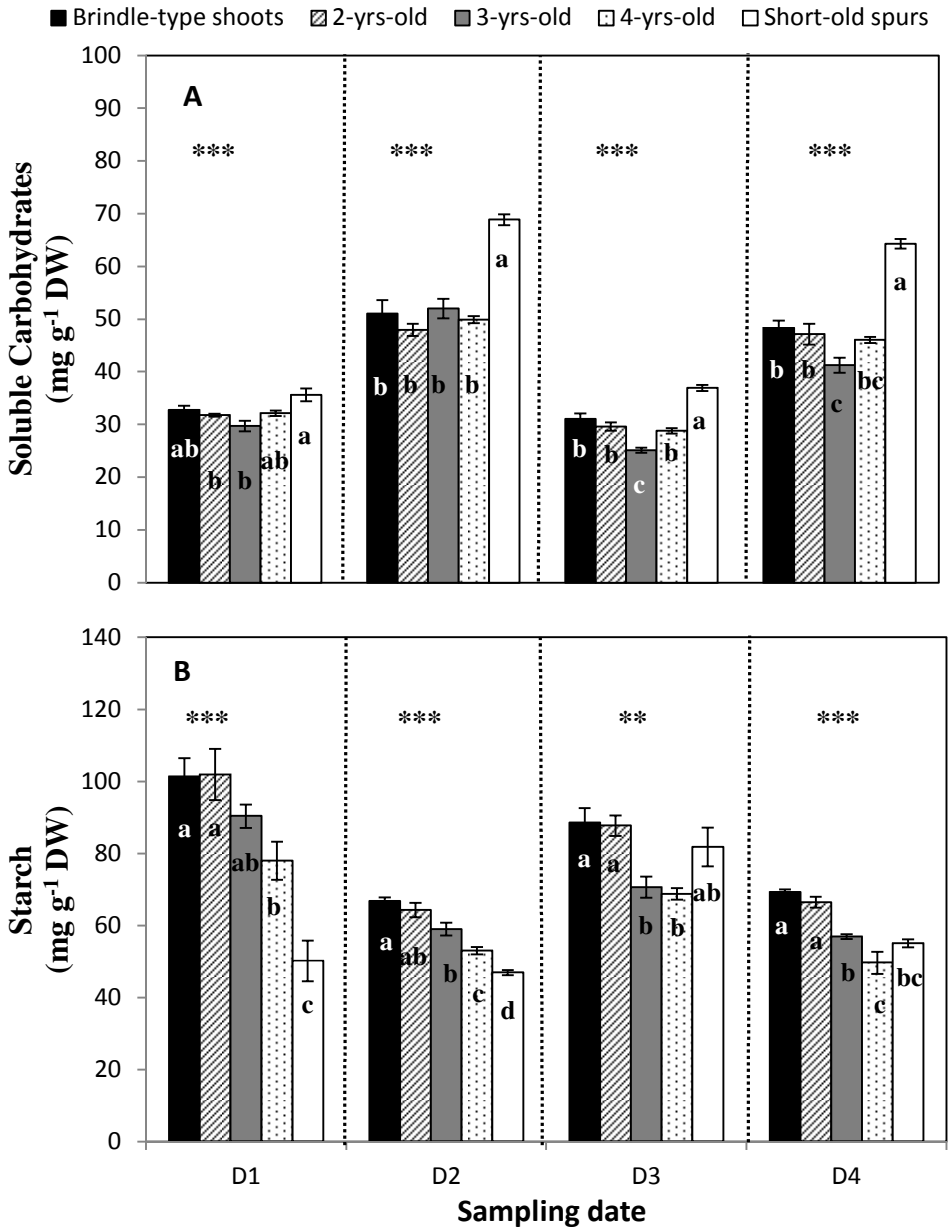

Figure 6. Soluble carbohydrates (A) and starch concentrations (B) (mg g^{-1} DW) in different aboveground woody organs evaluated: brindle-type shoots, 2-years-old branches, 3-years-old branches, 4-and-over-years-old branches, and short-old spurs. Values mean ± S.E. (n=6). Small letters in the horizontal way indicate significant differences within each date of evaluation. Significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

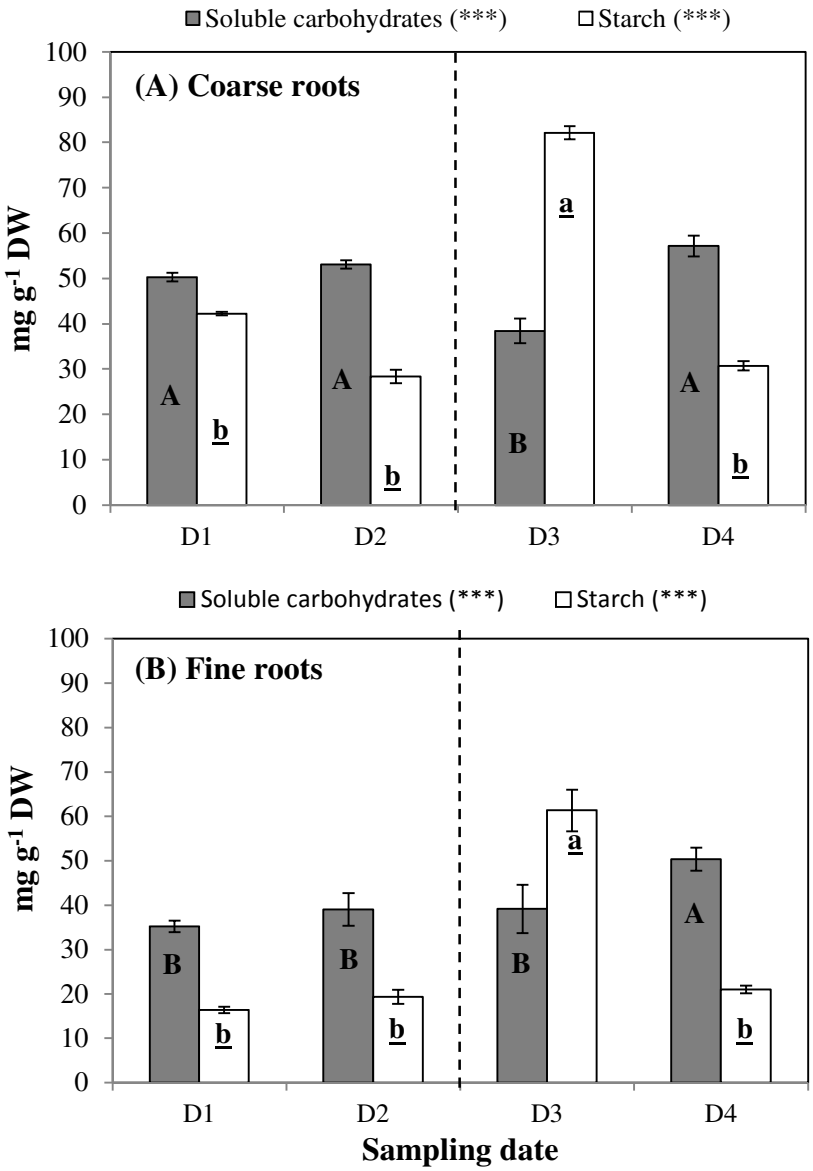

Figure 7. Starch (white bars) and soluble carbohydrates (grey bars) concentrations (mg g^{-1} DW) in coarse (A) and fine roots (B) at different dates of sampling. Values means ± S.E. (n=6). Capital letters indicate significant differences among dates for soluble carbohydrates, while small and underlined letters for starch. Significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

ROOTSTOCKS AND TRAINING SYSTEMS INFLUENCES ON NONSTRUCTURAL CARBOHYDRATES AT DORMANCY RELEASE IN ABOVE-GROUND ORGANS OF 'ABBÉ FÉTEL' PEAR TREES

6.1 Introduction

Carbohydrate partitioning and storage, have been studied taking into account the same cultivar on different rootstocks, which widely differ in growth vigor, such as in European prune (Gaudillère et al., 1992), sweet cherry (Olmstead et al., 2010), and peach trees (Caruso et al., 1997), as well as considering the effect of training systems in peach (Caruso et al., 1999), or training system-rootstock combinations in apple (Stutte et al., 1994). The transport of carbohydrates from storage organs, during late dormancy, has been also associated with the vigor of the scion-rootstock combination (Caruso et al., 1997), or the development of anatomical differences in the graft union (Olmstead et al., 2010). Incompatibility between scion-rootstock could result in an obstruction for the free movement of metabolites through this zone (Mendel and Cohen, 1967). Gur and Samish (1965), hypothesized about this incompatibility mechanism, when observed a reduced growth of the rootstock compared with that the scion, supposing this might be the result of a carbohydrate supply reduction to the rootstock. Olmstead et al. (2010) noted that the graft union of dwarfing sweet cherry rootstocks had an effect on the storage of carbohydrates, above and within the graft union, where the trunk sections of dwarfing rootstocks ('Gi5') contained higher amounts of soluble sugar respect of more vigorous rootstock ('Colt'), but lower concentrations in the rootstock. However, Mendel and Cohen (1967) noted in citrus, that the disturbance of carbohydrate translocation appears to be unrelated with incompatibility and low growth vigor in citrus.

The partitioning of carbon implicates the transport of assimilates from source organs to various sinks, and their distribution, therefore the knowledge of the role of branch architecture (training system) acquire a great importance in order to understand the process of distribution of assimilates in fruit trees (Fanwoua et al., 2014). It is well known that the initial growth of shoots and fruit development are more dependent on the level of carbohydrate reserves accumulated in the previous season, in this sense different factors that influence the mobilization and distribution of these assimilated

must be considered. Priestley (1963) noted that light intensity should have none influence on the initial growth; however, the growth for an extended period in a situation of reduced light intensity, might cause a faster depletion of reserves, by extending the time needed for the new leaf surface to gain in carbohydrate resources. On the other hand, the carbohydrates flow to fruitlets and as consequence, yield, can be reduced by shading (Wünsche and Lakso, 2000). Also, smaller canopies have been associated with negative effects on storage carbohydrates and fruit quality during the growing season (Whiting and Lang, 2004). In fact, Chalmers and Van den Ende (1975) suggested that the age, or size, at which the tree optimizes dry matter partitioning to fruit, may change with orchard design and/or plant management. Therefore, training systems that maximize light exposure within production sites, generally, have higher efficiency in converting light energy into fruit than canopies with heavy internal shading (Robinson and Lakso, 1991).

In the European pear orchards, the selection of new quince rootstocks, such as dwarfing clones like MH® and Adams as well as those more vigorous like Sydo® and BA29 (Sansavini et al., 2008), and the development of new training systems like Bi-axis (Musacchi, 2008) allowed to improve the high density planting (HDP) management. New ideas regarding tree shape include plants with two or four axes, where the main objective is to split the vigor over more branches. Bi-axis, indeed, training system have the advantage to control tree growth and, as a consequence, a reduction in pruning time (Musacchi, 2008). In intensive management systems, typical of Italy and Europe, 'Abbé Fétel' is commonly grafted on quince (*Cydonia oblonga*) rootstocks, which indices an early bearing and smaller tree size (Musacchi et al., 2011).

To date, several studies have been conducted to understand the behavior of the different rootstocks and training systems, and their combinations, to improve yield and fruit quality through differentiated orchard management practices. However, there is a lack of information about the effect of vigorous or dwarfing rootstocks, and the influence of the training system on the seasonal storage of carbohydrate reserves in pear trees orchards. The main goals of this study were: 1) to analyze the relationship between starch concentrations on different bearing woods in pear trees, trained at Spindle and Bi-axis, and grafted on the same rootstock, Sydo®; 2) to compare three quince rootstocks with increasing level of vigor: Adams, MH®, and Sydo®, trained as Spindle, on the

storage of nonstructural carbohydrates (NSC), starch and soluble carbohydrates, on the different bearing woods at February (dormancy release); and 3) to evaluate the effect of bearing wood position on the tree, in the bottom and upper part of the canopy, related to the storage concentration of NSC.

6.2 Materials and methods

6.2.1 Plant material and cultivation conditions

The experiment was conducted in an experimental orchard located in Ferrara, Italy (Marborghetto di Boara, 44°51'24"N; 11°39'09"E), throughout three growing seasons (2011-2012-2013) on 6-year-old pear trees (*Pyrus communis* L.) cv. 'Abbé Fétel' (planted in 2005). Four combinations between two training systems and three rootstocks were studied (Table 1). The training systems were Spindle and Bi-axis, while the three rootstocks used were: Adams, MH®, and Sydo®.

Plant distances were 3.3 x 1.0 meters for Bi-axis and 3.3 x 0.8 meters for Spindle. Planting densities corresponded to 3,030 and 3,787 trees ha^{-1}, respectively. Orchard was set up under anti-hail nets, micro-irrigation system was adopted to provide water and nutrients and turf was periodically mowed between the cropping rows.

6.2.2 Experimental design and sampling

6.2.2.1 Harvest sampling

For all three years evaluated, at harvest time four homogenous trees for each training system-rootstock combination were selected. The fruit harvest was performed as described in Jajo et al. (2014), according two factors: a) types of woody formations, dividing them according to an age-classification in: brindle-type shoots (twigs), 2-year-old branches, 3-and-over-years-old branches (include 3-, 4- and 5-years-old fruiting wood) and, short-old spurs, and b) position in the canopy, where trees of height of

around 2.8 m was divided in two sections: low part (below 1.4 m) and high part (over 1.4 m).

6.2.2.2 Sampling of woody organs and flower buds during winter period

After harvest of 2011 and 2012, for each training system-rootstock combination, approximately 60 bearing wood structures for each type of woody formation and canopy position (above described) were traced and tagged, in order to be identified later during the winter sampling (February 2012 and 2013), for starch and soluble carbohydrates analyses of. It is important to highlight that in February, the woody formations sampled were marked according the following season (Figure 1). On the other hand, branches of 3-years-old and 4-and-over-years old were analyzed as a whole group, and were named as 3-and-over-years old braches. Moreover, at the same time of winter sampling, three subsamples of roughly 10 mg fresh weight of flower buds present on each woody formation and position were sampled. The dates of sampling corresponded to February 28 and February 15, for the 2012 and 2013 seasons, respectively.

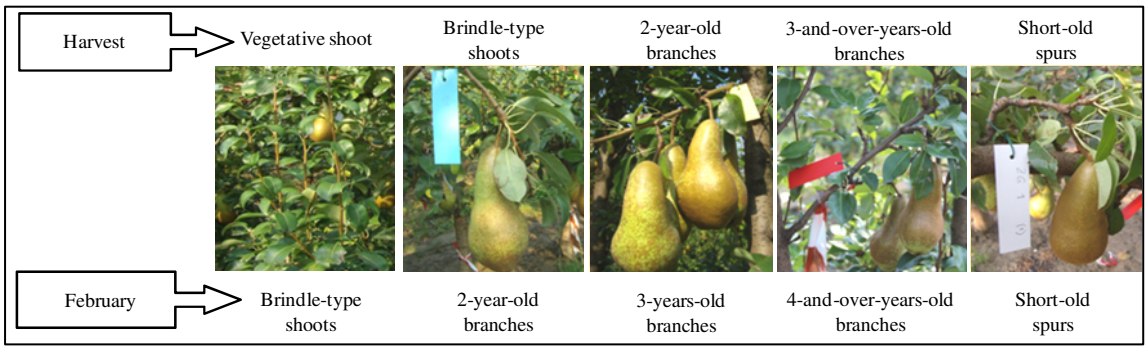

Figure 1. Diagram of the woody organs sampled in February, to determine starch and soluble carbohydrate reserves. Above to each bearing wood picture is found the name used at harvest time and under, the corresponding term used at the time of winter sampling (February), becoming the bearing wood of the following season.

6.2.3 Starch and soluble carbohydrates determinations

6.2.3.1 Preparation of material

Immediately after the separation of wood types and flower buds belonging to the different woods, three replicates, approximately of 50 g fresh weight of each bearing formations per canopy positions were sampled for starch and soluble carbohydrates further analyses. The subsamples were immediately frozen in liquid nitrogen and stored at -80 °C until analyses. Successively, samples were dried in a freeze dryer (HETO drywinner, DW3, Denmark), reweighed and ground when the dry weight was stable, till a fine powder with a mill and then stored in airtight containers at room temperature. All structures, with the exception of flower buds, were ground first in a mill (Restch SM 200, Germany) with a mesh 0.25 mm sieve, and subsequently in one (Fritsch pulverisette 14, Germany) of mesh 0.20 mm sieve, in order to standardize all samples. Flower buds were directly ground with a mesh 0.20 mm sieve.

For starch and soluble carbohydrates analyses, three replicates of 100 mg of dry weight each were used.

6.2.3.2 Determination of soluble carbohydrates concentrations

The protocol used to determine soluble carbohydrates concentrations was the same previously described in paragraph 3.2.5.2, chapter III.

6.2.3.3 Determination of starch concentration

The protocol used to determine starch concentration was the same previously described in paragraph 3.2.5.3, chapter II.

6.2.3.4 Spectrophotometer analysis

The protocol used to measure the total soluble carbohydrates and starch concentrations by spectrophotometer, was the same previously described in paragraph 3.2.5.4, chapter II.

6.2.4 Productive parameters

At harvest time, all fruit of the four trees selected for each combination were picked and separated by the different types of bearing wood (brindle-type shoots, 2-year-old branches, 3-and-over-year-old branches and short-old spurs) and position on the tree (high and low part), and then, number and weight of total fruits were recorded and expressed as kg/tree and percentage of production in each bearing wood.

6.2.5 Statistical analysis

Statistical analyses were carried out on starch and soluble carbohydrates concentrations, and yield data according to a completely randomized design with two controlled factors (training system and rootstock). Also, canopy position, year evaluation, wood formations and interactions among them were considered. The SAS® software (Cary, NC, USA) was used and mean separation was performed by Student-Newman-Keuls (SNK) test, differences were considered significant at $p \leq 0.05$.

6.3 Results

6.3.1 Starch and soluble carbohydrates concentrations in wood and flower buds

The type of organ, regardless the training system and rootstock, had a strong effect on the starch concentration (p-value ≤ 0.0001) in February, unlike to the non-significance (n.s.) observed for soluble carbohydrates (Table 2). The woody tissues sampled presented a concentration two-fold higher than the flower buds. However, the starch

concentration in the organs was unaffected by the year of assessment (Table 2). In contrast, the values of soluble carbohydrates obtained in February 2012, were a 14.9% lower compared to those recorded in 2013. Moreover, the interactions between the type of tissue and year of assessment, was significant only for the starch, although for soluble carbohydrates were clearly evident numerical differences (Table 2).

Significant differences were found within each type of organ (wood or flower buds) when the bearing formations (brindle-type shoots, 2-year-old branches, 3-and-over year old branches, and short-old spurs), canopy positions (high and low) and the year of evaluation (2012 and 2013) were compared (Table 3). As far as the starch concentration concerned, a reduction trend, either in wood or flower buds, was detected when the age of wood formations increased (older the wood lower the starch concentration). However, for soluble carbohydrates, even if between the organs the difference was not significant, a meaningful discrimination between their amounts was observed among woody formations. The wood of short-old spurs obtained the highest value (46.51 mg g^{-1} DW), while in the flower buds the highest soluble carbohydrates concentration was reported in brindle-type shoots (44.11 mg g^{-1} DW). Regarding the effect of canopy position, a same tendency between organs was observed, where the low part showed higher amounts, both for starch and soluble carbohydrates. Furthermore, February's sampling showed significant differences between the two years (Table 3). In the 2012 lower values than those of 2013 were recorded only in woody tissues; whereas flower buds showed an opposite starch performance respect to what observed for wood, but the same for soluble carbohydrates concentrations (Table 3).

For the woody tissues, training systems, rootstocks and, combinations among them, did not induce any statistically different effect in the concentration of starch (Table 4); nevertheless, for the soluble carbohydrates significant differences ($p<0.05$) were observed between training systems. A concentration 8.1% higher in Spindle (41.82 mg g^{-1} DW) was reported compared to Bi-axis (38.69 mg g^{-1} DW). Instead, analyses on flower buds did not show significant difference among training systems. But, the effect of rootstocks and combinations were significant, either for the starch and soluble carbohydrates concentrations (Table 4). Adams rootstock presented the lowest value for starch concentration on flower buds (23.69 mg g^{-1} DW), differing statistically from the MH® and Sydo®; whereas, for soluble carbohydrates, Sydo® rootstock showed the

lowest concentration (39.99 mg g^{-1} DW). This outcome clearly reflected the results among combinations, because the same trend was maintained. In this case, Spindle/Adams obtained the lowest concentration of starch (23.69 mg g^{-1} DW), whilst the lowest soluble carbohydrates concentration was found in Spindle/Sydo® (38.50 mg g^{-1} DW), even though a lower concentration was observed in Bi-axis/Sydo® than Spindle/MH® (Table 4).

Regardless of the training system-rootstock combinations and evaluation year, in February the starch concentration were higher than soluble carbohydrates on woody organs (Figure 2A – B). However, an opposite result was obtained for flower buds (Figure 3A – B), being more significant the concentrations range of soluble carbohydrates. In woody organs in February 2013, for concentration, starch and soluble carbohydrates values obtained were higher than the respective ones in 2012. In general, brindle-type shoots were characterized by the highest amount of starch in all combinations (Figure 2B). Whereas for the soluble carbohydrates, highest amounts have been determined in the brindle-type shoots and short-old spurs (Figure 2A). A similar trend was observed in the flower buds, although there were no statistical differences for all the combinations (exception in 2012 for Spindle/Sydo®), numerically brindle-type shoots showed the highest amount of starch and soluble carbohydrates (Figure 3A – B). When comparing the two years (flower buds), an opposite dynamic was observed among the nonstructural carbohydrates, i.e., while in the 2013 flower buds on four woody categories reported a lower amount of starch than in the 2012 season, these same formations showed a higher amount of soluble carbohydrates in the flower buds.

6.3.2 Productive parameters

As shown in Figure 4, the yield per tree among the three evaluation years for each combination registered statistical differences for Bi-axis/Sydo® and Spindle/Sydo® combinations. Trees in 2012 cropped the lowest yield/tree, whereas between the seasons 2011 and 2013, no significant differences were found. Among the combination, only in the third year of evaluation (2013) significant differences were observed (Figure 4). The combination Bi-axis/Sydo® showed the highest yield/tree (17.6 kg $tree^{-1}$), being a 31.5% and 30.6% higher than Spindle/Adams and Spindle/MH®, respectively, which not

differed statistically between them (Figure 4). For the statistical analysis carried out on yield per canopy position (upper and bottom part), and year (2011-2012-2013), for each training systems-rootstocks combinations, not significant differences were found (data not shown).

Regarding the yield distribution among woody formations as percentage (%) and kg tree^{-1} for each combinations and evaluation seasons (Figure 5), the greatest yield has been harvested from 3-and-over-years-old branches, but this mainly in Bi-axis/Sydo®, Spindle/MH® and, Spindle/Sydo® combinations (Figure 5A, C, and D), which accounted approximately 50% of yield. In Spindle/Adams, only in 2011, the 3-and-over-years-old branches obtained the higher yield, whereas in the two consecutive seasons, both 2 and 3-years-old branches behaved as the most productive formations (Figure 5B). In general, the brindle-type shoots and short-old spurs have produced the lowest amount on the total yield per tree in all combinations and year of evaluations. In the three years, the yield average of brindle-type shoots accounted a 14%, 11%, 8% and 15%, for Bi-axis/Sydo®, Spindle/Adams, Spindle/MH® and, Spindle/Sydo®, respectively, while for short-old spurs corresponded to 12%, 12%, 9% and 11%, respectively.

6.4 Discussion

In woody species, the different processes during the season, as flower induction and differentiation, flowering, fruit set and fruit development and maturation are regulated by factors including supply and movement of nutrients, assimilates and endogenous hormones within the plant (Gardin et al., 2002). In February, period that precedes flowering, a higher concentration of soluble carbohydrates than starch on flower buds would be expected, as it has been observed in this trial (Table 2). Marquat et al. (1999) and Bonhomme et al (2005), both in buds of peach trees, in February, obtained a concentration of total soluble sugars roughly two-fold that of the starch. This could be explained considering the availability of assimilates to support the process of flowering and early fruit growth (Marquat et al., 1999, Bonhomme et al., 2005, Ito et al., 2012). This availability was also observed for all training system-rootstock combinations evaluated (Figure 3A – B). Ito et al. (2012), at bud break, observed an increase on

soluble sugar in shoots and flower buds, whereas a decrease of starch occurred only in shoots, which could be related to a mobilization of soluble carbohydrates from the branches to the bud. In wood organs, a major concentration of starch reserves has clearly been shown, highlighting their function of storage and support of the new growth and development tissues that will occur during the following spring season (Table 2).

The cultivar 'Abbé Fétel', according to the fruit pattern, belongs to group II (Sansavini, 2002; Musacchi, 2011), i.e., briefly, bearing on spurs on branches of two or three years old. This trial showed results in agreement with that for all combinations of training system-rootstock (Figure 5). As a consequence, it could be expected that these types of wood formations have a heavy crop-load during the season, therefore, a higher sink-source competition. In fact, in February sampling, the 3-and-over-years old branches (at harvest time corresponding to 2-years-old branches plus 3-over-years-old branches, Figure 1) showed a lower concentration of starch (Table 3, Figure 2B), although not differ statistically of 2-years-old branches. Indeed, the short-old spurs reported the lowest amount. In this regard, another explanation at this performance could be the mobilization of soluble sugars, for starch hydrolysis, from this storage pools to high demanding sites (sinks). Consequently, the brindle-type shoots (current season's growth) showed higher concentration of starch, due to a higher strength-sink respect to other types of wood. In addition, the flower buds on this formation, showed the greatest concentrations of starch and soluble carbohydrates (Table 3, Figure 3A – B). These results are in line with those obtained by Gur and Samish (1965) in pear trees. They noted that the starch concentration of both rootstock and scion decreased with the increase of age of the trees. Contrary, Caruso et al. (1999) found the highest starch concentrations in 'Flordaprince' peach trees on over-1-year-old wood components, compared to current season's shoots and 1-year-old wood, during the phase from dormancy to pit hardening; however, it is noticeable to remark, that trees were in their fourth year at the time of the study, therefore, as well as Chalmers and Van den Ende (1975) stated, the distribution of assimilates in young trees is mainly addressed to build the structure and root growth (old structures), in agreement with the results of dry matter presented by Caruso et al. (1999). It could also be said that in peach trees and in particular for an early ripening cultivar such as 'Flordaprince' the vegetative and reproductive development occur simultaneously at earlier stages, whereas on pear trees

is observed that vegetative development occurs before anthesis; in other words, the pattern of carbohydrates partitioning differ according the space-time of the phenological phases of vegetative and reproductive development between species and cultivars and, as well as, age of the orchard.

According to canopy position, the fact that the high sections of the tree presented the lower concentrations for starch and soluble carbohydrates, regardless of organs, training system and rootstock (Table 3), could be explained by the chosen training systems. The low part of the canopy in Spindle, provides the main tree frame support, accounted by a strong set of vigorous scaffold branches (Dorigoni et al., 2011), consequently, a larger percentage of sinks sites. Gagliardi et al. (2014) reported in 'Abbé Fétel' pear trees, that the bottom canopy section showed a higher number of cluster (corymbs) compared with the upper sections, for five combinations of training systems (Bi-axis, V and, Spindle) and rootstocks (Sydo®, MH® and, Adams) evaluated, with Bi-axis registering the highest number of cluster per tree. Furthermore, Musacchi et al. (2011) observed for Bi-axis and Spindle approximately 70% of fruit production in the bottom section of the tree. In general, in the low section of a pear tree a major percentage of demanding sites (sinks) are found, and consequently, the higher concentrations of carbohydrates.

The differences between training systems for soluble carbohydrates in woody organs (Table 4), could be also attributed to the differences of branch architecture between them, owing mainly to the fact that the double axis distributes the vegetation in two axes, in order to have a high and continuous fruiting wall (Gagliardi et al., 2014), therefore inducing a lower vigor, in comparison with Spindle (Musacchi, 2008). This aspect is confirmed by the present results about yield among the three years of experimental data (Figure 4), which are in line with the studies of Musacchi (2008; et al., 2011) on pear and Dorigoni et al. (2011) in apple trees. Also, Caruso et al. (1999) observed the effect of two training systems (Y-shape and central leader) on the same rootstock in peach trees, establishing differences according to the vigor. This aspect has been related to an improvement of yield in apple orchards, due to a better light exposure (Robinson and Lakso, 1991; Wünsche and Lakso, 2000; Dorigoni et al., 2011).

Among the rootstocks the differences evidenced in flower buds, either for starch and soluble carbohydrates, showed an opposite trend between Adams and Sydo®, dwarfing and more vigorous rootstocks, respectively (Table 4), in accordance with Stutte et al.

(1994), Caruso et al. (1997) and Olmstead et al. (2010) in apple, peach and, in sweet cherry trees, respectively. These studies found, during dormancy, higher starch concentration on more vigorous rootstocks than in dwarfing ones, while the highest amounts for soluble carbohydrates were found in the dwarfing rootstocks. Whereas, Gaudillère et al. (1992) on prune trees, observed a small effect of rootstocks on starch concentration assessed before bud break, but this was practically null on soluble sugar. Also, Caruso et al. (1997) argued that the effect depends mainly on the time of the season in which sampling has been made. Moreover, Olmstead et al. (2010) suggested that as tree mature, the effect of dwarfing rootstocks on carbohydrate reserves could intensify, to a great extent, due to an increase of reproductive capacity above-ground exceeding below-ground storage capacity. Regarding the alternate bearing, this was observed on Bi-axis/Sydo® and Spindle/Sydo® combinations, whereas Spindle/Adams and Spindle/MH® did not presented great variations among the years of evaluations (Figure 4), this result clearly showed that the differences are in response to the vigor induced by the Sydo® rootstock, which is the most vigorous among those evaluated, whilst Adams and MH®, more similar in their performance, maintained a more stable production over the years. Musacchi et al. (2011) in 'Abbé Fétel' pear trees, obtained a similar finding, where Sydo® among the rootstocks evaluated was the most vigorous. Moreover, among the years of evaluations (from 3th to 5th year of planting), Adams showed the less variability in the yield (kg tree^{-1}). The competition for carbohydrates and hormonal balance has been suggested as main factors that cause to alternate bearing (Dovis et al., 2014). In evergreen species, the yield has been related to the starch concentrations during the previous dormant period (Goldschmidt and Golomb, 1982; Scholefield et al., 1985; Whiley et al., 1996a, b). Also, in several species, the starch concentration in woody organs after harvest, has been related to the factor of crop load during the growing season (Goldschmidt and Golomb, 1982; Naschitz et al., 2010; Park, 2011). In this way, it has been suggested that low level of starch stored, due to a heavy cropping, often ends in low yields in the following season, whereas high levels of starch resulting in high fruit yields. In this work, differences on starch concentrations in woody organs were found between the years (Table 3). In February 2012, the lower concentration of starch in woody organs was preceded by a high yield (2011) (Figure 4), whereas in February 2013 the starch concentration was 12% higher than the previous one, may be as a consequence of the low yield. Nevertheless, from these results we

could not ensure the regulatory role of carbohydrate reserves, being necessary to consider other parameters to evaluate (percentage of bud break and fruit set, among others).

6.5 Conclusions

To summarize, the starch and soluble carbohydrates concentrations in woody organs and flower buds of 'Abbé Fétel' pear trees, at dormancy release (February), are influenced by several factors. According to age of branches (brindle-type shoots, 2-years-old branches, 3-and-over-years old branches and short-old spurs), different concentrations of nonstructural carbohydrate reserves in organs were found. In general, as it has been reported in others works, the lowest concentrations were obtained with the increase of age of the trees. The position in the canopy, also influenced the results of starch and soluble carbohydrates concentrations, but these were mainly related to the training systems. In woody organs only the training system had an effect on soluble carbohydrate concentrations, while in flower buds, either for starch or soluble carbohydrates, these results were affected by the rootstock and training system-rootstock combinations. Also, between the years were evidenced significant differences in the carbohydrate concentrations, which could be attributed to different crop-loads levels, reflected in the differences of yields among the years of evaluation for each training system-rootstock combinations. In conclusion, from these results, we cannot determine with a sufficient grade of accuracy and certainty the role of carbohydrates in the alternate bearing shown in 'Abbé Fétel' pear trees, being necessary a future complement to these results with others researches.

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6.7 Figures and tables

Table 1. Combinations among training systems and rootstocks evaluated for this trial.

Training system	Rootstock	Plant distance (m)	Planting density (trees/ha)
Bi-axis	Sydo®	3.3 x 1.0	3,030
Spindle	Sydo®	3.3 x 0.8	3,787
Spindle	MH®	3.3 x 0.8	3,787
Spindle	Adams	3.3 x 0.8	3,787

Table 2. Starch and soluble carbohydrates concentrations (mg g^{-1} DW) according the types of organs sampled: woody and flower buds; year of assessment: February 2012 and February 2013, and their interactions.

	Starch	**Soluble carbohydrates**
Organ	mg g^{-1} DW	
Wood (W)	59.08 ± 0.71 a	40.74 ± 0.63
Flower buds (Fb)	26.53 ± 0.40 b	41.30 ± 0.44
Significance	***[y]	**n.s.**
Year		
2012	42.91 ± 1.11	37.62 ± 0.44 b
2013	43.66 ± 1.43	44.20 ± 0.51 a
Significance	**n.s.**	***
Organ x year		
W 2012	55.98 ± 0.99 b	36.72 ± 0.69
Fb 2012	29.83 ± 0.61 c	38.52 ± 0.53
W 2013	61.83 ± 0.93 a	44.32 ± 0.88
Fb 2013	23.23 ± 0.23 d	44.07 ± 0.57
Significance	***	**n.s.**

Mean values ± S.E. Small letters in the vertical way indicate significant differences. [Y], significance level: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

Table 3. Starch and soluble carbohydrates concentrations (mg g^{-1} DW) as average of two years: comparison among types of woody formation, canopy section and, year of assessment for the two organs evaluated in the trial, wood and flower buds.

Organs		Starch	Soluble carbohydrates mg g^{-1}DW
Wood	**Woody formation**		
	Brindle-type shoot	66.64 a	43.91 b
	2-years-old branches	59.19 ab	36.83 c
	3-and-over-years old branches	56.59 b	36.91 c
	Short-old-spurs	55.05 c	46.51 a
	Significance	***[y]	***
	Canopy sections		
	High	57.29 b	37.73 b
	Low	61.44 a	44.35 a
	Significance	***	***
	Year		
	2012	55.98 b	36.72 b
	2013	62.75 a	45.36 a
	Significance	***	***
	Woody formation x canopy section	**n.s.**	**n.s.**
	Woody formation x year	**n.s.**	**n.s.**
	canopy section x year	***	**n.s.**
Flower buds	**Woody formation**		
	Brindle-type shoot	30.01 a	44.11 a
	2-years-old branches	25.96 b	40.16 b
	3-and-over-years old branches	26.07 b	42.20 ab
	Short-old-spurs	24.99 b	41.56 ab
	Significance	***	***
	Canopy sections		
	High	25.98 b	37.53 b
	Low	27.35 a	44.28 a
	Significance	*	***
	Year		
	2012	29.83 a	38.52 b
	2013	23.68 b	45.49 a
	Significance	***	***
	Woody formation x canopy section	**n.s.**	**n.s.**
	Woody formation x year	***	**n.s.**
	canopy section x year	***	***

Small letters in the vertical way indicate significant differences. [Y], Ssignificances levels: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

Table 4. Starch and soluble carbohydrates concentrations (mg g^{-1} DW) as average of two years: comparison among training systems, rootstocks and combinations of training system-rootstock, for the two organs evaluated in the trial, wood and flower buds.

Organs		**Starch**	**Soluble carbohydrates**
		mg g^{-1}DW	
Wood	**Training system**		
	Bi-axis	58.33	38.69 b
	Spindle	59.71	41.82 a
	Significance	**n.s.**	*
	Rootstock		
	Sydo®	58.83	40.77
	MH®	57.58	40.85
	Adams	62.22	41.76
	Significance	**n.s.**	**n.s.**
	Combination		
	Bi-axis Sydo®	58.33	38.69
	Spindle Sydo®	59.34	42.85
	Spindle MH®	57.58	40.85
	Spindle Adams	62.22	41.76
	Significance	**n.s.**	**n.s.**
Flower buds	**Training system**		
	Bi-axis	27.02	41.47
	Spindle	26.67	42.18
	Significance	**n.s.**	**n.s.**
	Rootstock		
	Sydo®	27.39 a	39.99 b
	MH®	28.56 a	45.09 a
	Adams	23.69 b	42.96 a
	Significance	***	***
	Combination		
	Bi-axis Sydo®	27.02 a	41.47 b
	Spindle Sydo®	27.76 a	38.50 c
	Spindle MH®	28.59 a	45.09 a
	Spindle Adams	23.69 b	42.96 ab
	Significance	***	***

Small letters in the vertical way indicate significant differences. [Y], Ssignificances levels: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

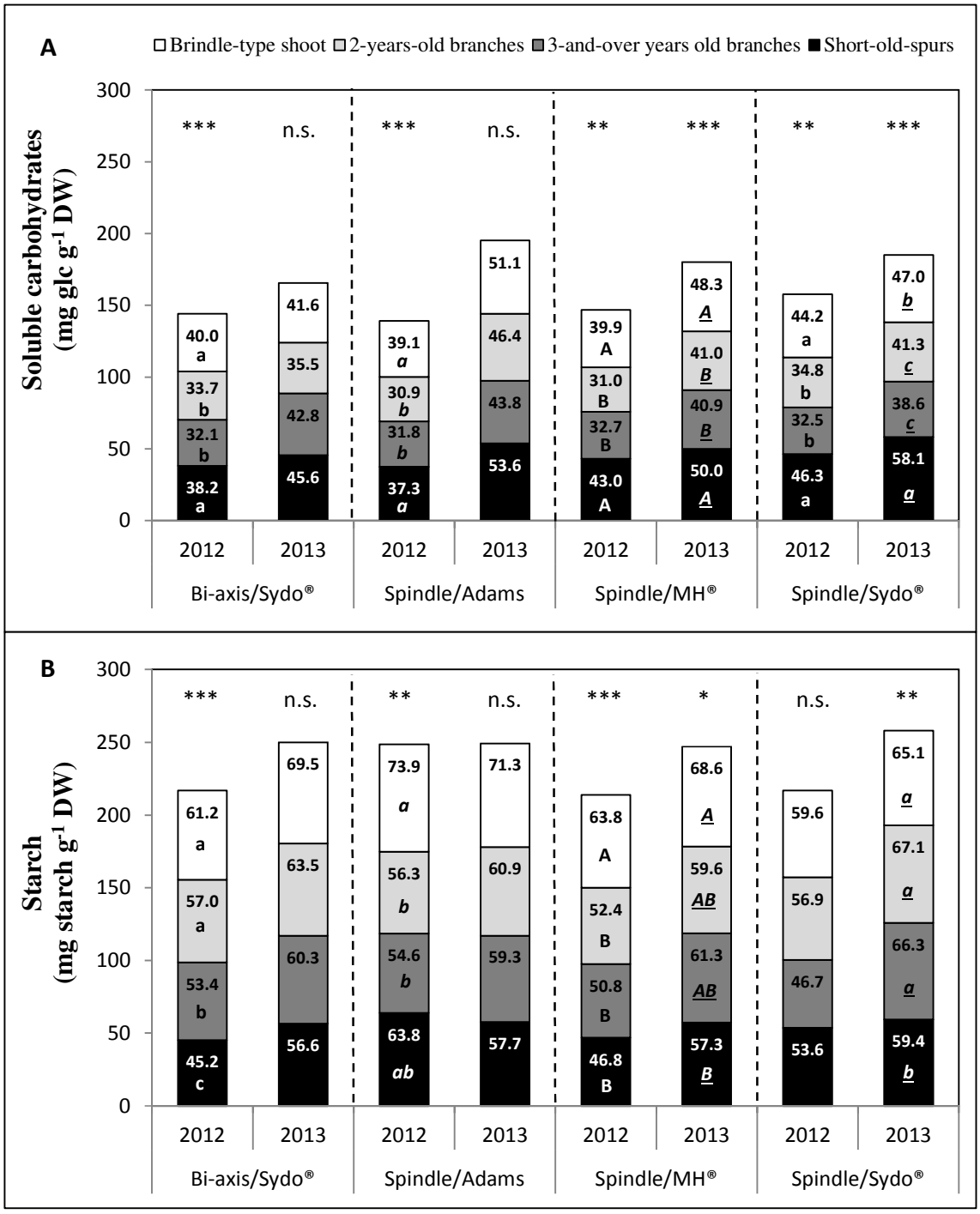

Figure 2. Soluble carbohydrates (A) and starch (B) concentrations on woody formations according to age-classification in: brindle-type shoots, 2-year-old branches, 3-and-over-year-old branches and short-old spurs, for the two-year evaluation: 2012 and 2013, and the four training system-rootstock combinations. Mean values followed a small letters in a vertical way indicate significant differences. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

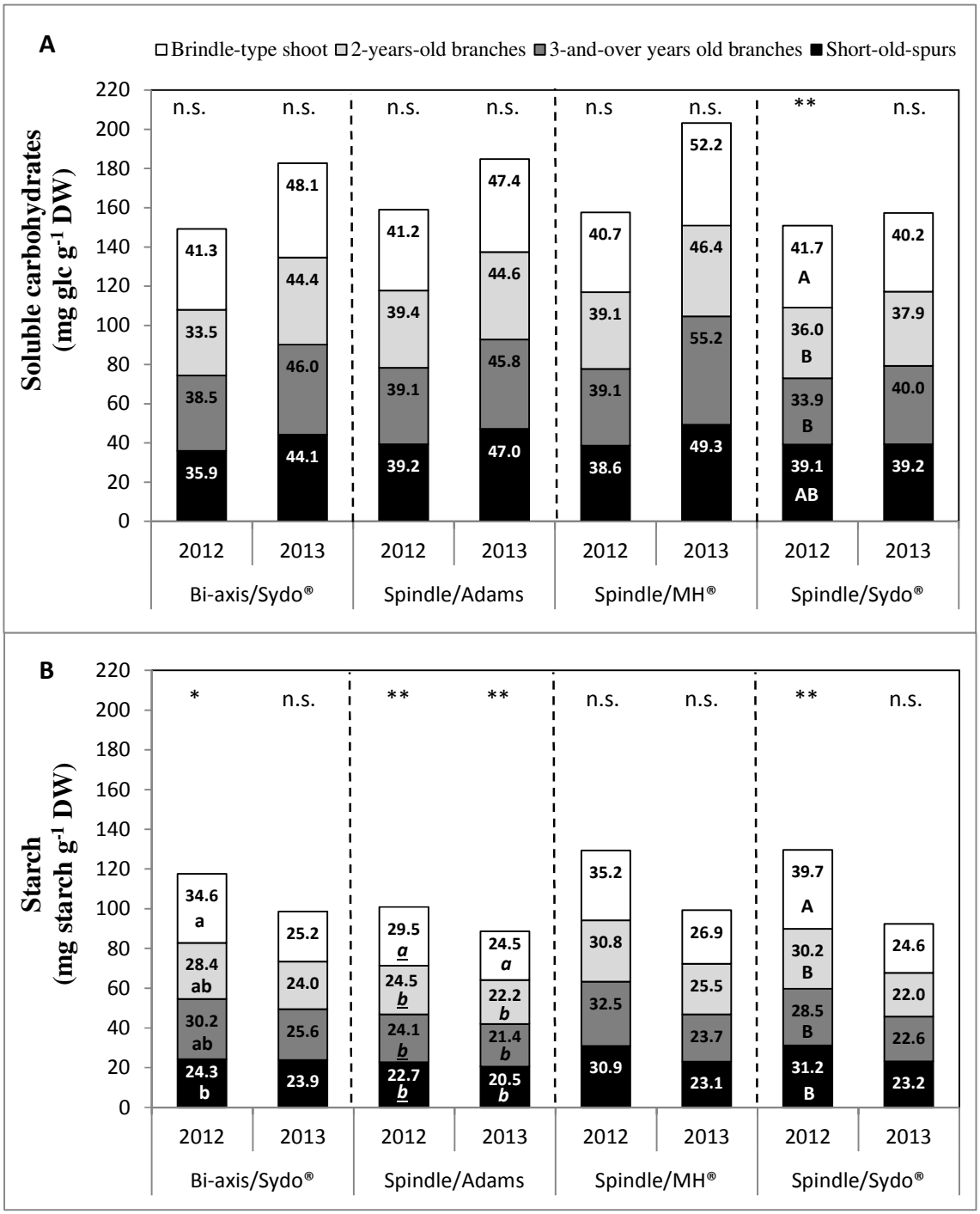

Figure 3. Soluble carbohydrates (A) and starch (B) concentrations on flower buds inserted in different woody formations according the age on bearing wood: brindle-type shoots, 2-year-old branches, 3-and-over-year-old branches and short-old spurs, for the two-year evaluation: February 2012 and 2013, and the four training system-rootstock combinations. Mean values followed a small letters in a vertical way indicate significant differences. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

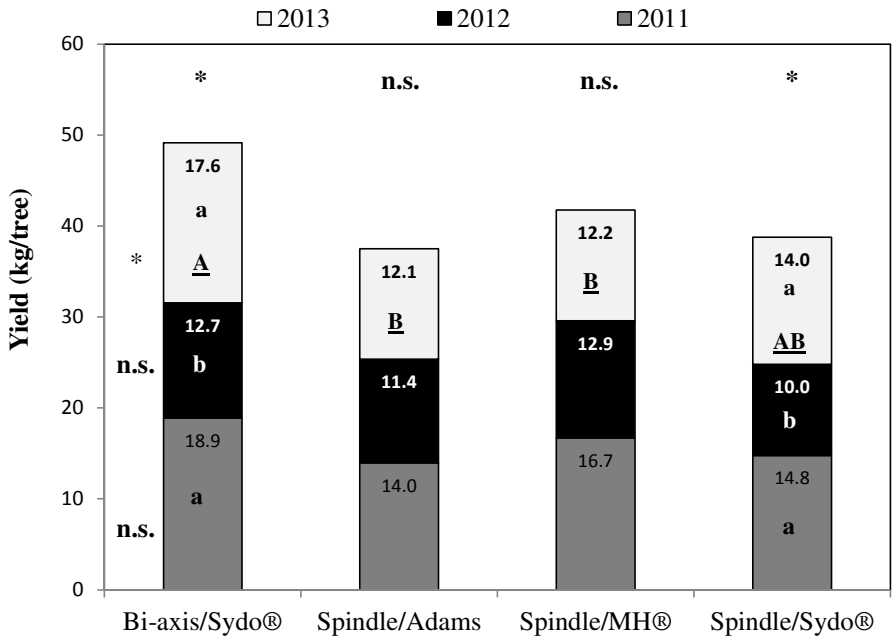

Figure 4. Yield per tree (kg/tree): comparison among three years of evaluation (2011-2012-2013) within each training system-rootstock (vertical way) and among combination (horizontal way). Mean values followed a small letters in a vertical way indicate significant differences within each combination among years, while capital and underlined letters indicate significant differences among combinations. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

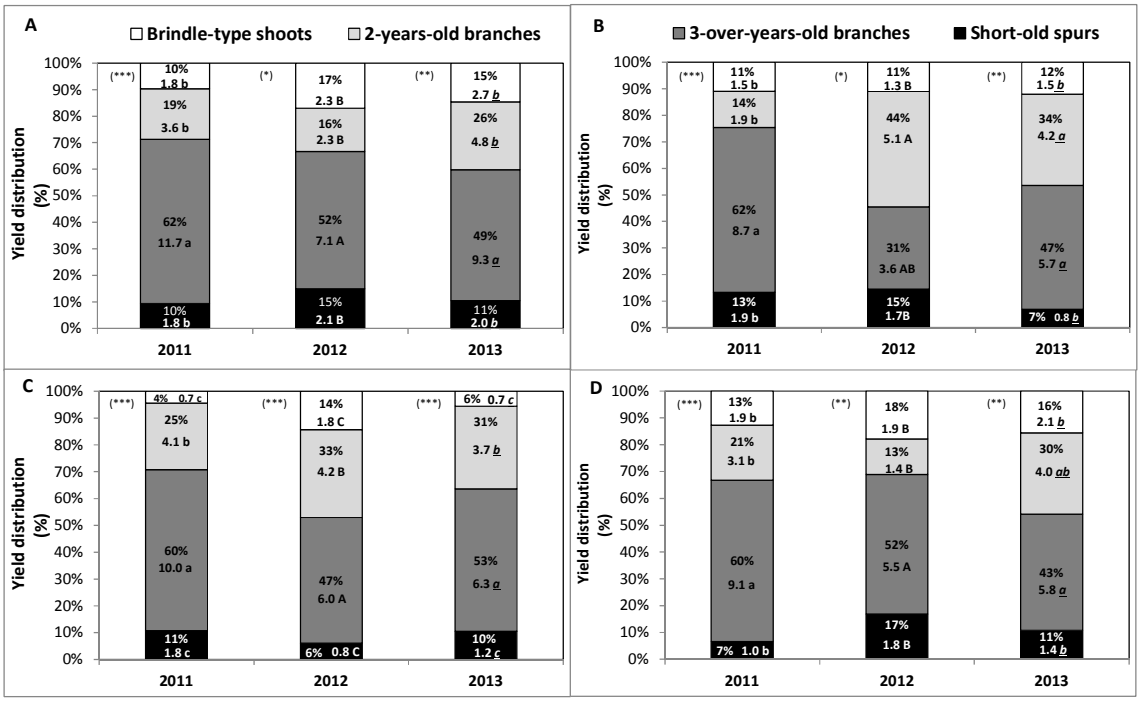

Figure 5. Yield distribution per woody formations as percentage (%) and kg/tree for each combination: Bi-axis/Sydo® (A), Spindle/Adams (B), Spindle/MH® (C) and, Spindle/Sydo® (D), and evaluation seasons: 2011, 2012 and 2013. Mean values followed a small letters in a vertical way indicate significant differences within each year of evaluation. Significance: *=p<0.05, **=p<0.01, ***=p<0.001, n.s. = not significant.

GENERAL CONCLUSIONS

This research work highlighted the behavior of carbohydrates reserves considering mainly starch, in: fruits, leaves, woody organs, roots and flower buds of 'Abbé Fétel' pear trees, at different physiological stages during the season. Factors, such as training systems, rootstocks and types of bearing wood, influencing carbohydrate reserves dynamics, in storage, partitioning and mobilization, were considered.

Starch, in fruits of 'Abbé Fétel', confirmed to follow a typical pattern of accumulation and degradation, described by other authors for different species. During its development, from earlier stages, the fruit starts to accumulate starch until several weeks before harvest. As starch degradations begin, a steady increase of soluble carbohydrates occurs. From the present results, the different types of bearing wood taken under evaluation (brindle-type shoots, 2-years-old branches, 3-years-old branches and short-old spurs) showed the same pattern of starch accumulation and degradation. As far as the rootstocks concern, no significant differences were reported for starch concentrations in the comparison between them; however, for soluble carbohydrates a slight effect was observed, mainly due to the different vigor induced by them and mainly related to their different sink's strength. On the other hand, among the years, the maximum starch concentrations, which always corresponded to the onset of its degradation, varied between the evaluation seasons. These differences could be explained mainly by mutable environmental conditions, cultural practices and, eventually by the inherent characteristic of the cultivar. The effect of crop-load, studied in other species, had a clear effect on starch concentrations; trees with a heavy crop registered lower amounts compared to those from low-cropping trees.

Moreover, the starch degradation in fruits was correlated to different fruit traits; this allowed to obtain some relationships able to explain its behavior during the season. The starch hydrolysis was highly related to fruit weight, soluble solids content, flesh firmness and I_{AD} index. Regarding the specific soluble sugars and organic acids, being glucose, fructose, sucrose and quinic acid were related to starch degradation, during fruit maturation, these parameters are reliable and accurate indicators of potential fruit quality at harvest.

This work also showed the importance to preserve a healthy well-managed canopy after harvest until the natural leaves fall. This assumption was demonstrated by the high concentrations of starch and soluble carbohydrates still found in leaves on late November, even if these leaves had already started their senescence phase. After harvest, woody organs and roots presented the highest concentrations of starch, which later on is hydrolyzed to soluble sugars. Regarding of the types of woody organs, the brindle-type-shoots, corresponding to the current's season shoot growth, stored the highest amount of starch, in October. Whereas in the others structures, the concentrations decreased, and were correlated with the increase of the age of the branches. Instead, in January (dormancy period), the structures of short-old spurs showed the highest concentrations of soluble carbohydrates, while branches from one to three and more years old, had similar amounts. These outcomes emphasized the importance and specific function of these organs to support the metabolism of the new tissues, especially in woody species that start blooming and developing fruit before a substantial canopy volume has been reached. Particular behavior was presented by the old-structures, where the soluble carbohydrates appeared not to be directly utilized; unlike to what is happening in current season shoots. In this way, starch found in these structures can be viewed as a reservoir available for future requirements of the other tree organs; this might point, the higher soluble carbohydrates concentration obtained in January. In general, in the woody organs and roots soluble carbohydrates follow a seasonal pattern, peaking just before bloom, rapidly declining during flowering and fruit set, then remaining low until mid-summer, and rising through autumn and winter.

This research provides data that can be used to define in a deeper way the effect of training system – rootstock combination on carbohydrate reserves used in pear trees. The canopy architecture of the two training systems assessed, affected the storage carbohydrates in woody organs. In February, before dormancy release, woody organs and flower buds on Spindle, obtained higher concentrations compared to Bi-axis, due mainly to the fact that the double axis splits the canopy vigor between two axes, in order to have a high and continuous fruiting wall, therefore inducing a lower vigor, in comparison with Spindle. Furthermore, the results also confirmed the different behavior of the rootstocks. Adams and Sydo®, dwarfing and more vigorous rootstocks,

respectively, showed an opposite trend, attributed to the different vigor induced by them.

The methodology developed in this work to determine the total soluble carbohydrates and starch, has allowed to analyze and to obtain, in a short period of time, a considerable amount of samples providing, at the same time, reliable and accurate values. In this way, the validation of this technique for measurement of starch values would facilitate monitoring and understanding of the effects of different treatments on the dynamics of accumulation of starch reserves.

To conclude, a better understanding of the behavior of carbohydrates reserves within the plant, regarding of: source-sink relationships, storage and distribution, as well as, the knowledge of the influence of several factors, such as: environmental conditions, cultural practices, and characteristics inherent to the cultivar, could provide relevant information to improve the different management practices used to increase the yield efficiency of crops, as well as, at critical periods, can be used as a complementary strategy in orchard management.

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