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Studies on Non-destructive Detection of Fruit Maturity and on Postharvest Physiology of European Plum (*Prunus domestica* L.)

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Abbreviations

°Brix	Degrees Brix (a refractive index measure of g solute /100 g solution
°C	Degree Celsius
µl	Microliter
1-MCP	1-methylcyclopropene
ACO	1-Aminocyclopropene-1-carboxylic acid oxidase
ACS	1-Aminocyclopropene-1-carboxylic acid synthase
Anth	anthocyanins
AVG	Aminoethoxyvinylglycine
'C. Lepotica'	'Cacanska Lepotica'
Cars	Carotenoids
Chl	Chlorophyll
ChlF	Chlorophyll fluorescence
DAFB	Day after fruit bloom
EPR	Ethylene production rate
et al.	et alii (and others)
FAO	Food and agriculture organization
FFS	Final fruit set
Flav	flavonols
FRF	Far red fluorescence
FW	Fruit weight
g	gram
GC	Gas chromatograph
h	Hour
ha	Hectare
HCL	High crop load
HD	Harvest date
kg	kilogram
L	liter
LCL	Low crop load
LFR	Leaves/fruit ratio
MCL	Middle crop load
mg	Milligram
min	Minute
ml	milliliter
ND	Non-destructive
NO	Nitric oxide
ORAC	Oxygen Radical Absorbance Capacity
PAs	Polyamines
ppm	Part per million
RF	Red fluorescence
SAM	S-adenosylmethionine
SD	Standard deviation
SSC	Soluble solids content
TA	Titratable acidity
USDA	United States Department of Agriculture
UV	Ultraviolet

1. Introduction

1. Introduction

Fruits constitute an important component of human diet. They play a vital role in human nutrition because they supply the necessary growth factors essential for health. These kinds of food are classified as functional or nutraceuticals as a result of their high polyphenolic composition and related antioxidant capacity (Cook and Samman, 1996; Lombardi-Boccia et al., 2004; Jacob, 2012) as well as of their content of dietary fiber, which all together has beneficial health effects. Among these functional foods are plums consumed as fresh or dried fruits (prunes). Their consumption was found to be associated with a decrease in circulatory and digestive issues, decreases in chronic degenerative diseases, (Joshpura et al., 2001; Kim et al., 2003a, b) as well as a reduction of risk of hypertension (Beals et al., 2005).

Despite the fact that plums and prunes are recognized by the USDA (2002) as the fruits with the highest level of ORAC (Oxygen Radical Absorbance Capacity) content (5770 ORAC units per 100 grams), followed by raisins and blueberries, plums and prunes have also 4.4 times higher antioxidant capacity than apples. Nevertheless, plums and prunes consumption has remained relatively constant or even declined for many decades due to off-flavor and quality, which are the results of improper fruit ripening (Crisosto et al., 2008).

Appearance and eating quality are two of the most important factors influencing consumer's acceptance of a product. And although, the eating quality of fruit, especially stone fruit, has become a very important quality trait in the past few years (Crisosto et al., 2002), there is lack of reference data on organoleptic, nutritive and functional quality parameters most especially regarding the European plum (Crisosto et al., 2004; Diaz-Mula et al., 2008). Therefore, research on fruit quality for specific plum cultivars is highly needed (Crisosto et al., 2004; Usenik et al., 2008), to ensure superior fruit quality that is appealing to the markets.

Fruit quality as well as the maturity indices are greatly influenced by genetic background of cultivar and rootstock, environmental and climatic conditions (which vary from season to season), and cultural practices (fertigation, irrigation, pruning, thinning, harvest time determination). Stone fruit quality cannot be improved after harvest, it can only be maintained. And although, understanding the role of preharvest factors in fruit quality is a key in achieving premium fruit quality (Crisosto et al., 1997; Hewett, 2006; Crisosto and Costa, 2008), little research has been done on the effect of preharvest factors (maturity) on the postharvest life (external and internal quality and storage life) of stone fruit.

1. Introduction

The decision concerning maturity at harvest time, ripeness, and quality are mostly dependent on objective and subjective visual attributes of the fruit's external appearance, which is usually based on fruit size, weight, firmness, total soluble solids, titratable acidity, ground color, and starch breakdown. The techniques used to determine maturity indices and fruit quality ranging from destructive (traditional) (Crisosto, 1994; Watkins, 2003) to non-destructive methods (Kawano, 1994; Costa et al., 2000; Jha and Matsuoka, 2000; Peirs et al., 2001; Abbott et al., 2010; Nicolaï et al., 2014). Many of the non-destructive techniques for quality parameters have been developped based on the detection of various physical properties that correlate well with certain maturity indices. Non-destructive methods that can be carried out quickly, allows to analyse a large number of samples and perform replicate measurements on the same samples during different stages of maturation as well as during postharvest life (Betemps et al., 2012).

The degree of maturity at harvest is the most important factor, which determines the final fruit quality as well as storage life after harvest (Kader, 1999). It is still difficult to define (Usenik et al., 2008) but it is of absolute importance that optimum harvest maturity is well defined to reduce postharvest losses and to ensure fruit attains 'acceptable' eating quality after storage (Taylor et al., 1993; Crisosto et al., 2004; Guerra and Casquera 2008; Singh et al., 2009). Proper prediction for harvest maturity will also allow producers to plan well for harvesting and marketing in advance and capitalize on labor productivity. Therefore, to ensure optimal quality, there is need for markers (maturity indicators) which will help to determine the stage of maturity precisely (Abdi, et al., 1997).

The anthocyanins are the main phenolic compounds in the skin of plum fruit, especially red and purple cultivars (Diaz-Mula et al., 2008; Treutter et al., 2012). The anthocyanins increase sharply during the third stage (stage III) of fruit development. The focus of this study was to link the changes in chlorophyll fluorescence (a new spectroscopic method based on the screening chlorophyll fluorescence by phenolics present in plum fruit outer layer) and fruit development during maturation on-tree (Agati et al., 2007). Of special interest was to study the relationship between anthocyanins measurements by fluorescence-based-sensors and fruit maturity. These measurements could help in developing a maturity index marker by determining harvest date precisely and/or in developing prediction models, which all together may help producers in planning harvesting strategies.

Fruit ripening is a sequence of biochemical processes, which transform a physiologically mature but inedible fruit into an edible one. Generally, it is known that ethylene plays a key role in inducing ripening processes, especially in climacteric fruits (Streif et al., 2010). Long time, plums are classified as climacteric fruit during ripening. However,

recently some plum cultivars are classified as suppressed climacteric fruit (Abdi et al., 1997, 1998) because the level of ethylene is low compared to normal conditions and emerges during the later parts of the ripening process. To avoid oversimplification by classifying the fruit species either as as climacteric or non-climacteric (Abdi et al., 1998) there is need to understand the fundamental of fruit ripening behavior and events, which are associated to managing the ripening stage.

Studying the fruit behavior during ripening is the main key to manage the postharvest life, especially for stone fruit as they are one of the most perishable fruits. A further one is to retard fruit ripening by minimizing the biochemical process of riping by means of inhibition of ethylene biosynthesis or its action (Larrigaudiere et al., 2009). Many treatments, exogenous applicated as for example 1-methylcyclopropene (1-MCP), aminoethoxyvinylglycine (AVG), polyamines (PAs), and nitric oxide (NO), have been found to be effective in delaying fruit ripening in many fruits (Khan and Singh, 2008; Khan et al., 2009).

The main purpose of this research primarily was to use non-destructive tools for detecting fruit maturity on-tree and its relation to different maturity indices that impact them uniformly over the seasons and cultivars. Knowledge of the changes in fruit maturity attributes during the time course of fruit growth and development could be useful in developing objective maturity indices, not only for harvest but also for postharvest management.

Moreover, this aforementioned objective could then be related to ripening potential and eating quality, which could also be reliably used in a prediction model to determine optimal harvest dates of plum fruits, and as a useful tool for studying the effects of different agricultural practices, like thinning, and the effects of rootstocks on the fruit quality.

The second main objective of this study was to analyse the postharvest behavior of different European plum cultivars during ripening at normal conditions as well as in cold storage. The effect of maturity stage on the postharvest behavior and fruit quality of plum cultivars. Finally, studying the impact of 1-MCP treatment on physiological and biochemical of European plum cultivars fruits.

2. Literature Review

2. Literature Review

2.1 Plum fruit development and ripening

2.1.1 Introduction

In fruit science, the family *Rosaceae* is of great importance and plays a dominant role. It includes the pome fruits, apple and pear, the berries, blackberry, raspberry, and strawberry as well as all stone fruits (peach, plum, apricot, nectarine, cherry and almond). The fruits of the *Rosaceae* family are characterized by distinct physiological and nutritional characteristics. In particular, these fruits are rich in polyphenolic components, such as anthocyanins, vitamin C, and fiber. Thus, there has been a lot of research into the functional properties of these widely-consumed stone fruits and berries (Paliyath et al., 2008).

Stone fruits are a diverse group, mostly of the genus *Prunus,* with characteristic of lignified endocarp, a fleshy mesocarp and a thin edible exocarp. The European plum, *Prunus domestica* L. and the Japanese plum, *Prunus salicina* L., are considered the most dominant species among a number of 19 to 40 plum species, which have more than 6000 plum cultivars (Hedrick, 1911; Blazek, 2007).

The origin of the European plum is in the area around the Caspian Sea. Crane and Lawrence (1934) suggested that the hexaploid species emerged from two ancient wild plums, which were the diploid cherry plum, *Prunus cerasifera* Ehrh, and the tetraploid blackthorn *Prunus spinosa* L., thus, the product of this cross-breeding was cultivated and propagated, and carried westward to Europe and later to other continents by the immigration of Europeans to other countries, including the Americas.

The history of prunes also dates back thousands of years, since prunes were used as a ready-snack for centuries (Stacewicz-Sapuntzakis et al., 2001). Some genotypes of the numerous plum cultivars were dried and preserved by desiccation in the sun or in a warm oven for continuous consumption after the harvest season. The virtues of prunes were extolled for aiding in digestion and curing mouth ulcers.

2.1.2 Importance of plum

Plum is considered as one of the most important temperate zone fruits. It is ranked as the 4th most produced fruit as it comes after apple, pear and peach. At the same time, it is ranked on the 2nd position in terms of harvested area (FAOStat 2013) (Table 2-1). The largest producer over the world is Asia (more than 70 %) followed by Europe, North Amertica, South America, Africa and Australia. The plum has been the first species among all fruits to attract the human interest (Faust and Surànyi 1999). The immense diversity of

attract the human interest (Faust and Surànyi 1999). The immense diversity of plum, its widespread all over the world and its adaptability to varying conditions make it a very important fruit to investigate not only at present, but also for future development (Blažk, 2007).

Fruits constitute an important component of human diet and play a vital role in human nutrition by supplying the necessary growth factors essential for maintaining normal health (high sugars, organic acids, minerals and vitamins), as well as functional components including dietary fiber and secondary metabolites with health beneficial effects such as phenolic compounds (Kader, 2011). Among these functional foods (Pennington, 2002) are prunes and plums. They are recognized by USDA (2002) as the highest fruit in ORAC (Oxygen Radical Absorbance Capacity) content (5770 and 930 ORAC units per 100 grams for prunes and plums, respectively) followed by raisins and blueberries. Moreover, plums can contain 2-15 times more phenolics and up to 26 times the antioxidant activity of either peaches or nectarines. It also demonstrated 4.4 times higher total antioxidant capacity than apples despite apples being one of the most commonly consumed fruits in the human diet (Wang et al., 1996; Byrne et al., 2008).

Plum fruits and related *Prunus* species are considered important international commodities due to their broad consumer acceptance and the variety of preparations, including canned, dried, fresh, processed for cooking use or distilled into brandy (Diaz-Mula et al., 2008; Okie and Ramming, 1999). The consumption of these fruits has been associated with the decrease in chronic degenerative diseases and circulatory and digestive issues. These effects are a result of their high polyphenolic composition and related antioxidant capacity (cook and samman 1996; Lombardi-Boccia et al., 2004). In addition, these components are contributing in sensory qualities such as taste, color, and flavor in fruits, vegetables, and beverages (Cao et al., 1997; Vinson et al., 2001; Kim et al., 2003a, b).

Plums are rich in a variety vitamins including vitamins C, B1, B2 and A, which are essential for numerous functions in human body (Stacewicz-Sapuntzakis et al., 2001). They also rich in minerals, which are present in physiologically significant amounts including iron, magnesium, copper, zinc, manganese, and potassium. The amount of iron, that is bioavailable in plums, is also among the highest in fruits and vegetables.

Tab. 2.1: Production of plum crops worldwide in 2012 (FAOStat, 2013).

Region	Area harvested[1)] (ha)	Production[1)] (tonnes)	Yield[2)] (kg/ha)
Africa	46,730	326,472	6986
Asia	1,875,850	7,295,359	3889
Central America	14,917	65,379	4383
Europe	511,467	2,279,138	4456
Australia & New Zealand	3,362	17,500	5205
North America	33,906	231,764	6836
South America	45,047	486,437	10798
World (Total)	2,531,479	10,702,774	4228

[1)] Aggregate (includes official, semi-official or estimated data; [2)] Calculated Data;

2.1.3 Fruit development and ripening

2.1.3.1 Fruit growth

After pollination and fruit set, the fruit continues in growing and developing to maturity with several degrees of cell division and expansion. At fruit set (except for parthenocarpic fruits), many of the fruit tissues become meristematic and active carbohydrate sinks. In some fruits, like currants and blackberries, cell division is completed at pollination but in most of other fruits, cell division continues for a short time after pollination. Exceptionally, as in avocado, the cell division continues during the growth cycle of the fruit. Fruit can increase in mass or volume by 100-fold or more from fertilization to maturity. However, in most of fruit species, the greatest contribution in fruit size is due to the expansion in cell size (Chalmers and Van Den 1975; Valero et al., 2010).

Fruit growth, from full bloom to harvest, represents a quantitative process, which leads to an increase of fruit weight and volume (James et al., 1989). Fruit growth on the tree can be followed by physical measurements such as fruit diameter, length, weight (fresh or dry), and volume (Fig. 2-1). In general, the evolution of the previous parameters shows a simple- or double-sigmoid curve depending on fruit type. A double-sigmoid curve is characteristic of stone fruits during fruit development and ripening (*Prunus* spp.) including plums and some berries (Tonutti et al., 1997). In this double-sigmoid curve, four distinct stages (S1-S4) are established (Chalmers and Van Den, 1975). The first exponential growth stage (S1), is characterized by cell division and elongation; S2 shows slow or no fruit growth but the endocarp hardens to form a solid stone (pit hardening); S3 is the second exponential

growth phase due to cell enlargement, while in S4 the fruit growth rate decreases and fruit ripening occurs (Diaz-Mula et al., 2008). Trainotti et al. (2003) divided S4 into two sub-stages: sub-stage S4-1 where fruit reaches its full size with changes in fruit color and without any changes in ethylene production, while in the second sub-stage S4-2, fruit continues to ripen in an ethylene-dependent manner.

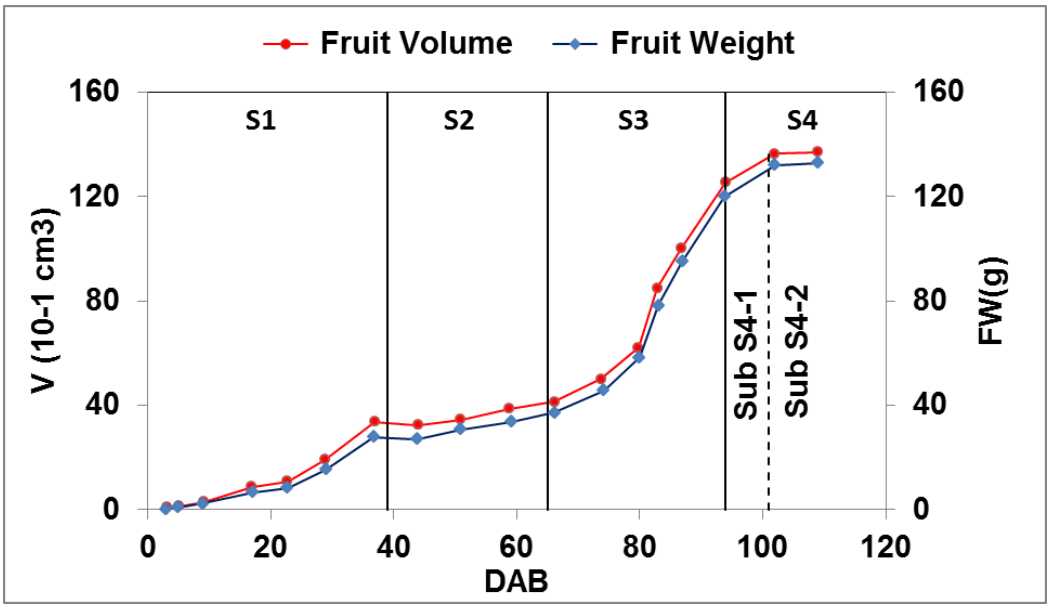


Fig. 2.1: Peach fruit growth curve (volume (V) and fresh weight (FW)) at early (E) fruit development (from 3 to 17 DAB) and stages S1 (from 23 to 37 DAB), S2 (44 to DAB), S3 (from 74 to 87 DAB), and S4 (from 94 to 102 DAB). (Redrawn from Lombardo et al., 2011)

There are many factors that affect the fruit growth and the development from fruit set to fruit ripening, mainly cultivars and environmental conditions (temperature, light, and soil nutrients). In Diaz-Mula et al. (2008) fruit growth and development on-tree was investigated for eight Japanese plum cultivars. It was found that the necessary days to achieve the commercial ripening stage ranged from 112 to 185 days for 'Golden Japan' and 'Angeleno' plum cultivars, respectively. The differences in full bloom dates were very close (from 2-14 March) for the same cultivar. Moreover, fruit development period (FDP) can be affected by temperature during early fruit development in spring, where a higher temperature during this period reduced FDP (Lopez, et al., 2007; Lopez and DeJong, 2007; Wert et al., 2007).

2.1.3.2 Fruit maturation and ripening

Fruit ripening refers to the composite of processes that occur in the later stages of fruit maturation and through the early stage of senescence, which lead to physiological, biochemical and structural changes in the fruit (Kader, 1999; Giovannoni, 2004; Toivonen,

2007). In recent years, it has been demonstrated that ripening of fruits can be genetically controlled (Brummell, 2005; Symour et al., 2008).

From a horticultural point of view, fruit ripening represents a sequential process of genetic, hormonal, and environmental events. Those events lead to dramatic changes in color, texture, flavor, and aroma of the fruit, which make the fruit acceptable or desirable for human consumption (Weatherspoon et al., 2005). Besides, fruit ripening is considered as one of the most important objectives of horticultural industries. The understanding of fruit ripening helps in harvesting fruit at the optimal maturity stage and controlling the rates of these changes related to fruit ripening, in order to extend the fruit postharvest life and provide the consumer with an acceptable product.

From the point of view of physiology, fruits are divided into two categories, climacteric and non-climacteric, based on the fundamental differences in their ripening patterns (Biale, 1964). Climacteric fruits are characterized by their increasing respiration and ethylene biosynthesis rate during ripening. On the other hand, non-climacteric fruits show no dramatic changes in respiration rate, while the ethylene production rate remains at a very low level (Barry and Giovannoni, 2007). The understanding of the biochemistry and molecular biology of the ripening process of the fruit might help in developing biotechnological strategies for extending of shelf life and quality of the fruits (Paliyath et al., 2008). Thus, physiological behavior of the fruit has got a great importance in the postharvest biology and technology.

2.1.3.2.1 Physiological and biochemical changes during maturation and ripening of plum fruit

2.1.3.2.1 a) Ethylene production and respiration rate

Generally, plums are classified as climacteric fruit with a very distinct rise in ethylene production and respiration rates during ripening. Examples of that are the Japanese plum cultivars (*Prunus salicina* L.) ‘Pioneer’, ‘Sapphire’, ‘Gulfruby’, ‘Beauty’, ‘Santa Rosa’, ‘Black Star’ and ‘Black Diamond’ (Abdi et al., 1997; Serrano et al., 2003; Singh and Khan, 2010) and European plum cultivar (*Prunus domestica* L.) ‘President’ (Valero et al., 2003). On the other hand, some other cultivars have been found to have suppressed climacteric phenotype (Abdi et al., 1997; Serrano et al., 2003), such as ‘Shiro’, ‘Rubyred’, ‘Songold’ and ‘Golden Japan’. This behavior was also reported from some cultivars of apple (Sfakiotakis and Dilley, 1973) and pears (Downs et al., 1991).

These suppressed climacteric plum cultivars produce ethylene during the latter stage of the ripening process with low levels of hormone when compared to normal climacteric ones. They also show respiration rates, which are 15-500 times less than that of climacteric

(Abdi et al. 1997; Khan and Singh, 2010). They also ripen slowly and exhibit better storage potential than non-climacteric fruits.

In the last two decades, some investigations have been done at the biochemical and molecular genetic levels on ethylene perception and transduction in a few plant species, Arabidopsis (Chang et al. 1993), Tomato (Giovannoni, 2001). Recently, the molecular mechanisms involved in ripening of plum fruit have been studied by El-Sharkawy et al. (2007, 2008, and 2009) in an attempt to understand the factors regulating the differences in date and rate of ripening among plum cultivars. They characterized four ethylene perception and signal transduction components (EPSTCs) during development and ripening of early and late plum fruit cultivars, including two ETR1-like proteins (Ps-ETR1 and Ps-ERS1), a CTR-like protein, and an ethylene-responsive element-binding factor (ERF). They concluded that there are clear differences between early and late plum cultivars in the mRNA accumulation patterns of the isolated ethylene response components. Their results indicate that, early cultivar exhibited typical climacteric fruit pattern accompanied by sharp increases of the four transcriptional levels in an ethylene-dependent manner. On the other hand, late cultivars showed a suppressed-climacteric pattern with slight increase in ethylene production related to ripening, where, the accumulation of the Ps-ETR1mRNA was ethylene independent and Ps-ERS1 mRNA was expressed at low and constant levels. Moreover, the authors suggested that the ethylene signaling is not the only essential signal, which contributes to fruit ripening but there might be more signaling pathways that are as crucial as that of ethylene.

2.1.3.2.1 b) Fruit color development

Change in fruit color is the most obvious signal of maturity as well as, it is an important external feature of fruit quality (Wills et al., 2007: Usenik et al., 2008). It is often the standard criteria that consumers use to determine, whether a fruit is ripe or unripe.

Plum fruit color during fruit maturation and ripening changes from green to red or yellow or purple depending on the cultivar. The color changes are due to degradation of chlorophyll by the enzyme chlorophyllase (Dangl et al., 2000), and coincide synthesis of the characteristic pigment for each cultivar. The color of plum fruit is mainly contributed by anthocyanins and carotenoids. In general, anthocyanins concentration increase during ripening in a range of pink, red and purple colored fruit while the carotenoids are responsible for color in yellow to red (Diaz-Mula et al., 2008; Valero and Serrano, 2010).

Anthocyanins are one of the sub-groups of flavonoids that are primarily found in red grapes, berries, and blue-black plums (Prior and Cao, 2000). In particular, anthocyanins may serve as natural colorant sources and even potential substitutes of synthetic food colorants

due to their attractive orange, red, and blue hues (Cevallos-Casals et al., 2006). Since color is the most important indicator of maturity and quality in many fruit species, and it is mainly influenced by the concentration and distribution of various anthocyanins, these compounds possess an important functional role (Usenik et al., 2009).

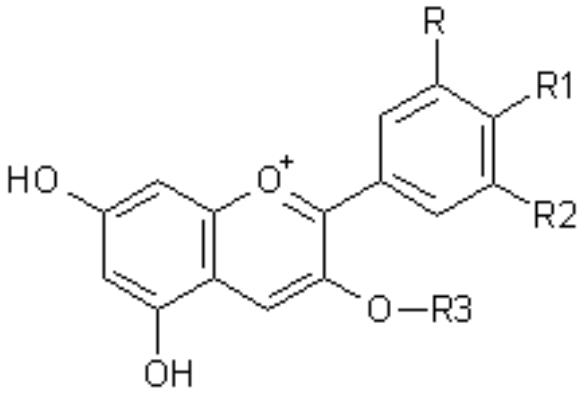
Generally, anthocyanins are composed of an anthocyanidin (aglycone form) bound to one or more sugars at different hydroxylation sites (Treutter, 2006; Usenik et al., 2009). The anthocyanins in many fruits are based on six common anthocyanidins Fig. 2-2, which are pelargonidin, peonidin, delphinidin, cyanidin, petunidin, and malvidin (Kotepong et al., 2011). The sugars that are commonly linked to anthocyanidins are glucose, galactose, rhamnose, and arabinose. These structures are further modified through the addition of other compounds, which can be bound to the sugar moieties such as methyl groups, acetic acid, propionic acid, caffeic acid, or malonic acid. Cyanidin is the most abundant aglycone in approximately 90 percent of anthocyanin-containing fruits.

Despite the limited literature concerning the anthocyanins composition in European plums, Japanese plums have been characterized by a number of anthocyanins, including cyanidin 3-glucoside, cyanidin 3-rutinoside, cyanidin 3-galactoside and cyanidin 3-acetyl-glucoside (Wu and Prior, 2005). On the other hand, study by Treutter et al. (2012) showed that the main anthocyanins in European plum were glycosides of cyanidin and peonidin.

Diaz-Mula et al. (2008) studied the development of plum fruit color on-tree in eight Japanese plum cultivars. They found that the responsible pigments for fruit color are anthocyanins and carotenoids in red/purple and orange/yellow cultivars respectively. The changes in color mostly happened after the beginning of the third stage of fruit development (stage III) and during fruit on-tree ripening. The anthocyanins and carotenoids sharply increased during this period and there is high correlation with skin color. The same trend has been found in both of fruit skin and flesh. Cyanidin 3-glucoside followed by cyanidin 3-rutinoside are the main anthocyanins while *β*-carotene is the major carotenoid in plum depending on the cultivar.

Similar results were obtained by Serrano et al. (2009) on cherry fruits, where the increase in anthocyanins occurred in stage III, which corresponds to maximum fruit growth rate. Moreover, the main anthocyanins were cyanidin 3-rutinoside and cyanidin 3-glucoside. The changes in fruit color continued after harvesting but were slower than changes during on-tree ripening.

Also, in another study on European plum cultivars Usenik et al. (2008) investigated the influence of maturity stage on the development of fruit color and they concluded that the concentration of total anthocyanins significantly increased during ripening on the tree. The main anthocyanin in this study was cyanidin rutinoside followed by peonidin rutinoside.



Anthocyanin	Color	R1	R2
Pelargonidin 3-glucoside	Orange	H	H
Cyanidin 3-glucoside	Orange-red	OH	H
Delphinidin 3-glucoside	Blue-red	OH	OH
Peonidin 3-glucoside	Orange-red	OCH_3	H
Petunidin 3-glucoside	Blue-red	OCH_3	OH
Malvidin 3-glucoside	Blue-red	OCH_3	OCH_3

Fig 2.2: The structure of the most common anthocyanins in fruits, which the three aromatic rings comprise the basic anthocyanidin (aglycone structure) that is then complemented by a sugar moiety (R3), which are arabinose or glucose or galactose.

Besides anthocyanins as a main component of fruit color, plum and other stone fruits have other predominant phenolic compounds such as neochlorogenic, chlorogenic acid (Kim et al., 2003; Treutter et al., 2012) and proanthocyanidins (Treutter et al., 2012). However, it is expected that fruit color correlated to total phenolic content or antioxidant capacity (Tomás-Barberán et al. 2001). That plum with greenish skin color had lower levels of total phenolic compounds compared to plum with red and purple color was demonstrated through the study by Rupasinghe et al. (2006) on nineteen European plum genotypes and one Japanese selection. These studies confirm that fruit color could be good indicators for external, internal, and nutrional fruit quality.

2.1.3.2.1 c) Soluble solids content

Soluble solid contents (SSC) in fruit include reducing-sugars and other carbohydrates, organic acids and amino acids (Wills et al., 1989). SSC has a remarkable influence on the sensory attributes (Ackermann et al., 1992; Hudina and Štampar, 2005), the flavor of fruit (Usenik et al., 2010) and fruit taste and consumer acceptance (Crisosto et al., 2007).

As fruit mature, the sugars become the main component of the soluble solids (Wills et al. 1989). Sugar accumulation is considered an early event during fruit growth in most of fruits (Valero and Serrano, 2013). The accumulation is mainly due to translocation of assimilates from photosynthetic leaves.

The main sugars in most *Rosaceae* species are fructose, sucrose, glucose and sorbitol (Fourie et al., 1991; Brady, 1993). The predominant accumulated sugar is dependent on species, where glucose is the major sugar in grape and fructose in mango, cherry and citrus while sucrose is the main sugar in stone fruit including apricot, peach, nectarine and plum (Valero and Serrano, 2013).

Plums are considered a rich source of sugars. They have very little starch and no sucrose at the immature stage (Khan and Singh, 2010) but by ripening, sucrose increases and exceeds the reducing sugar contents. The relative amounts of fructose, glucose, sorbitol and sucrose are differing from one cultivar to another. Singh et al. (2008) studied the influence of harvest date and maturity stage on sugars and organic acid in early (‘Blackamber’), mid (‘Amber Jewel’) and late (‘Angeleno’) Japanese plum cultivars. They found that fructose was the major sugar followed by glucose, sorbitol and sucrose.

Based on another study on four European plum cultivars by Usenik et al. (2008), the authors concluded that, in general, glucose is the predominant sugar followed by sucrose, fructose and sorbitol ranging from 38.2 to 115.0, 21.2 to 71.9, 19.1 to 34.8 and 3.5 to 27.8 g/kg FW, respectively. But in ‘Cacanska Najbolja’ cultivar the highest concentration was in sucrose followed by glucose, fructose and sorbitol.

On the other hand, in another study on Slovenian plum cultivars Usenik et al. (2007) found that sucrose was the major sugar (ranging from 37.4 to 53.6 g/kg FW), followed by sorbitol (34.0 to 50.7 g/kg FW), glucose (29.8 to 41.8 g/kg FW) and fructose (19.1 to 34.8 g/kg FW).

SSC at the fruit ripening stage depends on plant species and cultivars. Although, the SSC is used as a maturity index, it is also one of the most important quality parameters because of its direct association with eating acceptability (Crisosto et al., 2006). It is ranged from 10 to16 % in plum cultivars (Valero and Serrano, 2013).

2.1.3.2.1 d) Acidity

Unripe plum fruits are extremely acidic due to accumulation of many organic acids. Total acidity of fruit is directly influenced by the composition of different organic acids (Crisosto et al., 2006). The most important organic acids in fruits are malic, citric, tartaric, quinic, oxalic, fumaric and succinic acid. In general, malic acid is considered the predominant acid in plum fruits at maturity followed by shikimic and fumaric acid in European plums

(Usenik et al., 2008). In Japanese plum, Singh et al. (2009) found that the main acid is malic acid followed by succinic, tartaric and citric acid.

Malic acid is synthesized in the cytoplasm and then stored in the vacuoles. Although malic acid has similar metabolic connection like citric acid to Krebs cycle, they are greatly different with fruit development (Wu et al., 2002). The taste of fruit acidity is not only dependent on the total acidity, but also on the type of organic acids, which play an important role in determining fruit acidity (Valero and Serrano, 2010). Crisosto et al. (2002) concluded that the consumer acceptance for some peach cultivars is related to RTA (Ripe Titratable Acidity), because malic acid plays a critical role in the perception of acidity as a dominant acid at fruit maturity.

Malic acid declines during maturation and ripening in plum (Crisosto et al., 2007) and apples (Ackermann et al., 1992) as well as during storage. They suggested that the decline in acidity is a result of a dilution effect due to the mass increase during the cell growth phase and a rise in respiration.

2.1.3.2.1 e) Softening

Softening of fruit occurs during maturation and ripening. It involves a wide range of metabolic events including loss of turgor pressure, physiological changes in the membrane composition, starch degradation, and changes in the cell wall structure (Valero and Serrano, 2013). In plums as in other climacteric fruits, the solubilisation and depolymerisation of pectins and hemicelluloses of the cell wall are considered the most important changes, which contribute to compositional and structural changes in cell wall carbohydrates (Khan and Singh, 2010).

Generally, a number of enzymes have been associated with fruit softening, such as polygalacturonase (PG), pectin methylesterase (PME), α- and β-galactosidase and pectatelyase. All of these enzymes are based on multi-gene families, with a subset of one or more gene family members regulating the cell wall modification processes associated with fruit ripening. Nevertheless, other enzymes can also contribute to the softening process, such as rhamnogalacturonase, arabinase, cellulases (EGases), mannases and expansins (Goulao and Oliveira, 2008).

Based on the pattern of softening, fruits are classified into two categories: those that soften greatly to a melting texture as they ripen due to swelling of the cell walls (such as peach, strawberry and plum) and those that soften moderately, without cell wall swelling, and they are characterized by a crisp fracturable texture like apple.

Fruit species and ripening stage at harvest are important factors determining the rate of softening. However, it is interesting to point out that different cultivars from the same

species could also show different behavior in rate of softening. An example for this was given in Serrano et al. (2007), where it was showed that 'Black Diamond' plum exhibited much higher firmness loss (85 %) than 'Golden Japan' plums (28 %).

2.2 Fruit quality and maturity indices

2.2.1 Harvest maturity

Maturity is defined as "that stage at which a commodity has reached a sufficient stage of development that after harvesting and postharvest handling, its quality will be at least the minimum acceptable to the ultimate consumer" (Reid, 1992). Harvest maturity is that stage when fruits should be picked so that in the following marketing chain they will remain of high quality. The degree of maturity at harvest is the most important factor that determines the fruit quality as well the period for which fruit can be stored without losing quality. Picking of the fruits either earlier or later than proper harvest maturity subjects the fruit to physiological disorders. Immature harvested fruits are subject to shriveling, mechanical damage and inability to achieve premium quality standards required by consumers. On the other hand, ripe and overripe harvested fruits will be too soft or mealy with tasteless flavor and cannot reach consumers (Kader, 1999; Vanoli and Buccheri, 2012). Generally, a compromise between an earlier and a late harvest has to be reached to achieve the premium quality for consumer and in the same time extend postharvest life for marketing.

2.2.2 Maturity indices

Up to now, it is not easy to precise the optimal harvest date due to the high diversity in plum genotypes (Casquero and Guerra, 2009) and the lack of maturity indices in plums especially the European plum (Usenik et al., 2008). Maturity index is defined by Kader (2011), as a measurable character that its changes is correlated with fruit maturity and can be used to indicate when a commodity should be harvested for a specific purpose. This implies that, the maturity index of a fruit provides an indication of its stage of development or maturation. These indices are important to trade regulation and marketing strategy as well as to the efficient use of labor and resources (Crisosto, 1994).

Generally, maturity indices for harvest can be either subjective or objective. While some indices are qualitative or semi-quantitative, others are quantitative or measured using varying levels of sophisticated instrumentation (Reid, 2002).

Maturity indices must be measurable, simple, readily performed in orchard or packinghouse. They must be achievable by inexpensive equipment and should be non-destructive (ND). Moreover, they should be objective and must be consistently related to the quality parameters of the commodity (Crisosto, 1994; Vanoli and Buccheri, 2012). Research

for objective determination indices has occupied the attention of many horticulturists working on wide range of fruits for many years. However, the number of satisfactory indices that have been suggested is still few, and for most fruit this research is still very limited (Vanoli and Buccheri, 2012).

Many maturity variables of fruits attempt to provide adequate assessment of maturity stage for optimum eating quality. Each species, or even each cultivar, should have its own maturity index, which assumes a characteristic value depending on the product destination. These variables, viz. ground color, firmness, acidity, soluble solids, carbon dioxide and ethylene production rate, are useful tools for defining fruit quality attributes (Watkins, 2003). They are also based on the quality traits that interpret the gradual changes in fruit ripening (Garry et al., 2008).The rate of change of these maturity variables dependents on the physiological and biochemical changes that occur during maturation and ripening.

Therefore, clear knowledge of fruit maturity indices and their relation to fruit quality is necessary in order to assist growers in making decisions with regard to fruit handling practices. The reviewed literature covers maturity indices, biochemical and physiological changes that occur in fruit during maturation and ripening (stated previously). It puts special emphasis on harvest maturity variables, factors related to fruit quality and the limitations that meet these standard indices.

2.2.2.1 Ground color

Change in fruit color is the most obvious signal of maturity (Wills et al., 2007). It is one of the main standards that consumers use to determine whether a fruit is ripe or unripe. Crisosto and Day (2011) stated that fruit skin color is widely used for determining harvest date in plum in California, where a color chip guide is used for determining minimum maturity for some cultivars. This holds also for peaches and nectarines, where the ground color of fruit is strongly related to fruit maturity, sensory attribute and eating quality. Thus, it is also used for determining maturity (Mitchell et al., 1990).

In recent decades, great interest has been placed to develop new plum cultivars with darker skin color. In these cultivars, red or dark color is developed several weeks before harvest date, making it impossible to use the color as an index maturity (Crisosto et al., 1997).

The usage of the fruit ground color as a maturity index faces other challenges aside from the earlier developing color, where it is influenced to some degree by the environment independent of maturity. Ground color may be greener at optimum harvest in trees that have a lot of leaves per fruit that have high nitrogen levels (Little and Holmes, 2000). Furthermore, increased levels of nitrogen accompanied by high night temperatures will improve the

Chlorophyll concentration in fruit peel that gives fruits higher green ground color at harvest maturity compared with normal conditions. Kader and Mitchell (1989) mentioned that the penetration of light into the tree canopy affects the degree of fruit color. Therefore, the position of fruit on the tree also affects the fruit color. Additionally, cultivar, clone and nutritional supply influence the fruit color (Watkins, 2003).

Usenik et al. (2008) conducted a study on the development of on-tree fruit color during maturation and ripening of four European plum cultivars. They concluded that estimation of fruit color is not suitable for all plum cultivars as a maturity index. They also suggested that, as opposed to as Japanese plum and apples, there is need to involve any measurable parameters to determine the fruit maturity in European plums.

2.2.2.2 Firmness

Flesh firmness is one of the most used indices of fruit maturity and ripening. It determines the potential of plum fruit during postharvest life (Menniti et al., 2004). Fruit firmness is highly correlated to the overall quality of the fruit (Wills et al., 1989). Also, its changes have proven to be a reliable way to describe the changes in fruit ripening, making it a good way to predict bruising damage (Crisosto et al., 2001).

Crisosto (1994) suggested that flesh firmness can be used as a maximum maturity index, which is the stage at which the fruit can be harvested without suffering bruising damage during postharvest handling, while ensuring a good quality. The need for firmness as a maturity index especially increases with plum cultivars, where skin ground color is masked by full red or dark color development before maturation (Crisosto and Day, 2011).

Flesh firmness decreases during the maturation and ripening. Early plum varieties are usually less firm at the minimum maturity time than late season varieties (Kader and Mitchell, 1989). Moreover, fruit firmness can be affected by size and cultivar.

2.2.2.3 Soluble solids content and titratable acidity and SSC/TA ratio

Soluble solids content (SSC) increases during plum fruit maturation and ripening, while total acidity (TA) decreases. Hence, they are considered among the most important maturity indices. Moreover, they are also considered from the most important quality parameters because of their direct association with eating acceptability. Using SSC as a sole maturity index or using TA is limited by many factors such as: variations among cultivars, season, and production region (Kader and Mitchell, 1989; Crisosto, 1994). Additionally, fruit at different positions within the canopy induces great differences in SSC (Mitchell et al., 1991). The SSC/TA ratio has been considered to be a more reliable parameter for plum

ripening than SSC or TA alonebecause the ratio increases during ripening (Casquero and Guerra, 2009; Khan and Singh, 2007).

Crisosto et al. (2007) illustrated the interaction between Ripe Soluble Solids (RSSC) and Ripe Titratable Acidity (RTA) and their relation to consumers' acceptability for high-acid early dark plums. They concluded that the mean degree of liking by consumers decreased significantly as RTA increased.

2.2.2.4 Ethylene production

As stated previously, ethylene is a naturally synthesized plant hormone that plays a key role in initiating fruit ripening during the later stage of fruit maturation (Watkins, 2003). Those changes leads to develop characteristic attributes of flavor, texture, aroma, and loss of astringency for every cultivar, which all contribute to optimum eating quality (Weatherspoon et al., 2005).

According to Watkins (2003), ethylene is actually used as a main deciding factor in terms of harvesting decisions, especially in apples. However, this may not be always reliable because this parameter can be significantly influenced by factors such as the production region, the position of orchards within that region, the cultivar, and the growing season (Vendrell and Larrigadiere, 1997; Watkins, 2003). Due to this limitation, such a maturity variable will need to be used in conjunction with other maturity indices when predicting harvest maturity for optimum eating quality.

Moreover, Gomila et al. (2011a) mentioned that the ethylene production had the greatest correlation with DAFB (days after full bloom). But, the results showed that the changes observed in ethylene production during the last period were not highly correlated with firmness values. It is suggested that other complementary maturity indices should be taken into account.

2.2.3 Non-destructive techniques for evaluating fruit quality and maturity indices

The term quality is generally defined as the degree of excellence of a product or its suitability for a specific purpose (Abbott, 1999). On the other hand, the quality could be defined from a product's perspective differently than from a consumer's perspective, since the quality comprises many properties and characteristics. Up to now, most maturity indices are destructive, time consuming, expensive and do not present the variability in fruit quality attributes (Agati et al., 2007). In last decades, many non-destructive techniques have been developped for evaluation of internal and external quality properties as well as the ripening stage (Kawano, 1994; Costa et al., 2000; Jha and Matsuoka, 2000; Peirs et al., 2001; Nicolaï et al., 2007, 2014). These methods allow the extension of measurements on a high number

of fruits, or even on all fruits in the field. They also allow the repetition of the analysis on the same fruits, which allows monitoring their physiological evolution either on-tree, after harvesting in cold storage or even during marketing (Abbott, 1999; Nicolaï et al., 2007, 2014; Ben-Ghozlen et al., 2010a). Those methods can also provide a common language among researchers, producers and consumers. Furthermore, it can be very useful for developing models of changes in quality attributes during postharvest storage.

The decision concerning maturity at harvest time, ripeness and quality are mostly dependent on objective and subjective visual attributes of the fruit's external appearance. Such attributes are usually based on fruit size, weight, firmness, total soluble solids, titratable acidity, ground color and starch breakdown. Therefore, mostly the non-destructive techniques for quality parameters have been developped based on the detection of various physical properties that correlate well with certain maturity indices. Nevertheless, the maturity index should ensure a minimum acceptable eating quality and a long storage life (Crisosto, 1994). Therefore, the non-destructive methods may be designed to mimic human testing or at least be statistically related to human perceptions and judgments to predict quality categories.

Non-destructive techniques can mainly be classified into three categories (Abbott, 1999; Nicolaï et al., 2014):

1) Electromagnetic techniques: examples include spectroscopic methods, which can be used in evaluation of fruit appearance, and X-ray, which can be used in detection of internal disorders. Non-destructive sensors based on chlorophyll fluorescence, which was used in this study have be used in evaluation of fruit maturity (Agati et al., 2005; Ben-Ghozlen et al., 2010a), drought stress (Elsayed et al., 2011) and nitrogen status in plant (Tremblay et al., 2012).

2) Mechanical techniques: techniques that are related to texture and firmness, such as Durofel, are used in determining firmness nondestructively based on the deformation of fruit surface. This was done using metal probes (Crisosto et al., 1997) and vibration-based techniques (Nicolaï et al., 2014).

3) Electrochemical techniques: techniques that can be used for volatile aroma assessment. Electrochemical techniques correlate with ripening of fruit and vegetable as well as ethylene, especially in climacteric fruits, such as gas detector sensors and electronic noses.

Recently, several studies have been done to develop non-destructive techniques based on spectral indices (Visible/Near Infra-red, fluorescence, etc,). This was done to study the relationships among spectral indices, fruit quality attributes and maturity indices in olive (Agati et al., 2005), grapes (Agati et al., 2007; Ben-Ghozlen et al., 2010a, b), pears (Gomila

et al., 2011b) and apple (Betemps et al., 2012). By studying the absorption spectra of fruits, it was found that carotenoids (Cars) have a maximum absorption at around 480 nm (from 400 to 500 nm), anthocyanins (Anth) from 530 to 550 nm, chlorophyll-a (Chl-a) peaks at around 680 nm, Chl-b at 630 nm and both Chls also absorb between 400 and 500 nm, affecting Cars and Anth absorptions (Vanoli and Buccheri, 2012).

Therefore, the measurement of spectra could help in detecting the changes in pigments in fruits and vegetables. These measurements of spectra, which are related to specific single pigment, can be linked to fruit ripening providing a good quality or maturity index (Zude et al., 2011). One of these techniques is the chlorophyll fluorescence excitation technique, which was first used for assessment of UV-absorbing compounds on leaf outer layer (Bilger et al., 1997). This technique was recently developed to new optical sensors dedicated to detect anthocyanins non-destructively in fruit skin in olives and in grapes (Agati et al., 2005, 2007) and also flavonoids in apples (Betempe et al., 2012).

The chlorophyll fluorescence (ChlF) excitation technique that was used in this study is based on the screening of chlorophyll fluorescence excitation by phenolic compounds localized in the outer layer in fruit skin. Therefore, the available light for excitation will be reduced. By comparison of ChlF signals at different excitation wavelengths, chlorophyll and the UV-absorbing phenolic compounds *in situ* can be evaluated (Agati et al., 2007).

Agati et al. (2007) assessed anthocyanins (Anth) non-destructively in grape by measuring chlorophyll fluorescence (ChlF) excitation spectra and found that ChlF signal decreased with increasing in Anth content. By comparing the ratio between the fluorescence excitation spectra (log FER) for two excitation wavelengths, 540 and 635 nm (absorbed and non-absorbed by Anth, respectively) can be non-destructively determined absolute quantitative of Anth. There is a good correlation (r^2= 0.92) between Anth values assessed by destructive and non-destructive methods.

A similar study by Bin-Ghozlen et al. (2010) was conducted on grapevine to evaluate Anth non-destructively during maturation from veraison to harvest, by Multiplex, optical sensor based on chlorophyll fluorescence technique. They also compared the non-destructive method with destructive quantification in grapevines that were produced in different locations. They concluded that the accumulated Anth measured by Multiplex from veraison to harvest was in a good correlation (r^2 =0.88) with wet chemistry. Therefore, the Anth Multiplex data can be transformed in units of mg/L.

On the other hand, Betemps et al. (2012) used the same sensor for evaluation of ripening and quality attributes in ‘Fuji’, ‘Golden Delicious’ and ‘Granny Smith’ apple cultivars. They found that the Anth and flavonols (Flav) indices were higher in fruits in sunny side compared with shady side. On contrast, Chl index was higher in shady side than for the

sunny side. Moreover, they found a fine linear regression between multiplex values and destructive analysis for Anth, Flav, and Chl. They found also a negative correlation between the apple sugar content and the chlorophyll fluorescence in the far-red spectral band. Finally, they concluded that multiparametric fluorescence based sensors can provide a good non-destructive marker of ripening and fruit quality attributes.

2.3 Fruit ripening and 1-methylcyclopropene (1-MCP)

Ripening of fruit is a series of biochemical events, which transform a physiologically mature but inedible fruit into an edible, tasty product (Streif et al., 2010). The main consequences of this behavior are a reduced shelf life and a decrease in the quality parameters, such as skin and mesocarp color changes, autocatalytic ethylene production, sugar accumulation, occurrence of decay and fruit softening (Trainotti et al., 2007). This period can be extended by minimize biochemical processes. Thus, the regulation of ripening is an extremely important factor to extend fruit shelf life and to optimize fruit quality during postharvest.

Ethylene plays a key role as a plant hormone. It is responsible for coordinating and initiating ripening events in climacteric fruit (Abdi et al., 1998; Bapat et al., 2010). It triggers the processes of ripening and senescence. The ripening of climacteric fruit can be delayed by ethylene inhibitors (Liu, et al., 2005).

There are a number of approaches to manipulate the rate of maturation and ripening ranging from preharvest application on the tree to application of postharvest physical treatments (Toivonen, 2007). Plant growth regulators are a well-studied group of compounds that have been successfully used to manipulate maturation and ripening processes for the purpose of improving fruit quality and extend postharvest life (Klein and Goldschmidt, 2005).

2.3.1 Ethylene Biosynthesis

Ethylene is a very simple hydrocarbon, a natural plant hormone, and exists in the gaseous state under normal physiological conditions. It regulates many aspects of plant growth, development, and senescence. Ethylene is also biologically active in trace amounts, and its effects are commercially important (Yang and Hoffman, 1984; Abeles et al., 1992). Normally, the ethylene production rate by plant tissue is low, but it increases in certain stages of plant growth, such as seed germination, fruit ripening, leaf senescence and abscission (Abeles et al., 1992; Bleecker and Kende, 2000). Ethylene production can also be induced by environmental stresses, such as physical wounding and cutting, chilling injury, drought and flooding (Bleecker and Kende, 2000). There are many important physiological consequences, which are correlated to an increase of ethylene production.

The biosynthesis of ethylene occurs through a relatively simple metabolic pathway that has been extensively studied and well documented in plants (Yang and Hoffman 1984; Kende 1993). Ethylene is synthesized in plant tissues via the conversions of the amino acid L-methionine into S-adenosylmethionine (SAM) catalyzed by the enzyme SAM synthetase. In the second step, SAM is converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by removal of MTA, catalyzed by the enzyme ACC Synthase (ACS) (Yang and Hoffman, 1984) and the final step is the conversion of ACC (CO_2 and HCN are removed) to ethylene, catalyzed by ACC oxidase. Oxygen is a necessary cofactor for the third step of biosynthesis. ACC can also be converted in malonyl-ACC by the enzyme ACC N- malonyl- transferase Fig. 2-3.

ACC synthases, which are encoded by a multiple gene family, generally represent the rate-limiting enzymatic step in the pathway of ethylene biosynthesis (Yang and Hoffman, 1984). This ACS has a very short half-life and its activity is regulated by several environmental and internal factors, such as wounding, drought stress, flooding and auxin biosynthesis (Abeles et al., 1992; Kende, 1993). Besides its role in formation of ACC, ACS is also involved in catalyzing the conversion of SAM into 5-methylthioadenosine (MTA). While the ACC oxidase is generally not the rate-limiting point in ethylene biosynthesis (Yang and Hoffman, 1984), although tissues that show high rates of ethylene production, such as ripening fruit and senescing flowers, show increased levels of ACC oxidase and mRNA biosynthesis (Kende, 1993).

However, methionine is found at quite low, nearly constant concentrations in plant tissues, including those that produce large amounts of ethylene, such as ripening fruits (Abeles et al., 1992). Since methionine is the sole precursor of ethylene in higher plants, tissues with high rates of ethylene production require a continuous supply of methionine. This supply is ensured by methionine recycling via the Yang cycle (Yang and Hoffman, 1984). Not all the ACC found in the tissue is converted to ethylene. ACC can also be converted to a nonvolatile conjugated form, JV-malonyl ACC, which does not break down and seems to accumulate in the tissue.

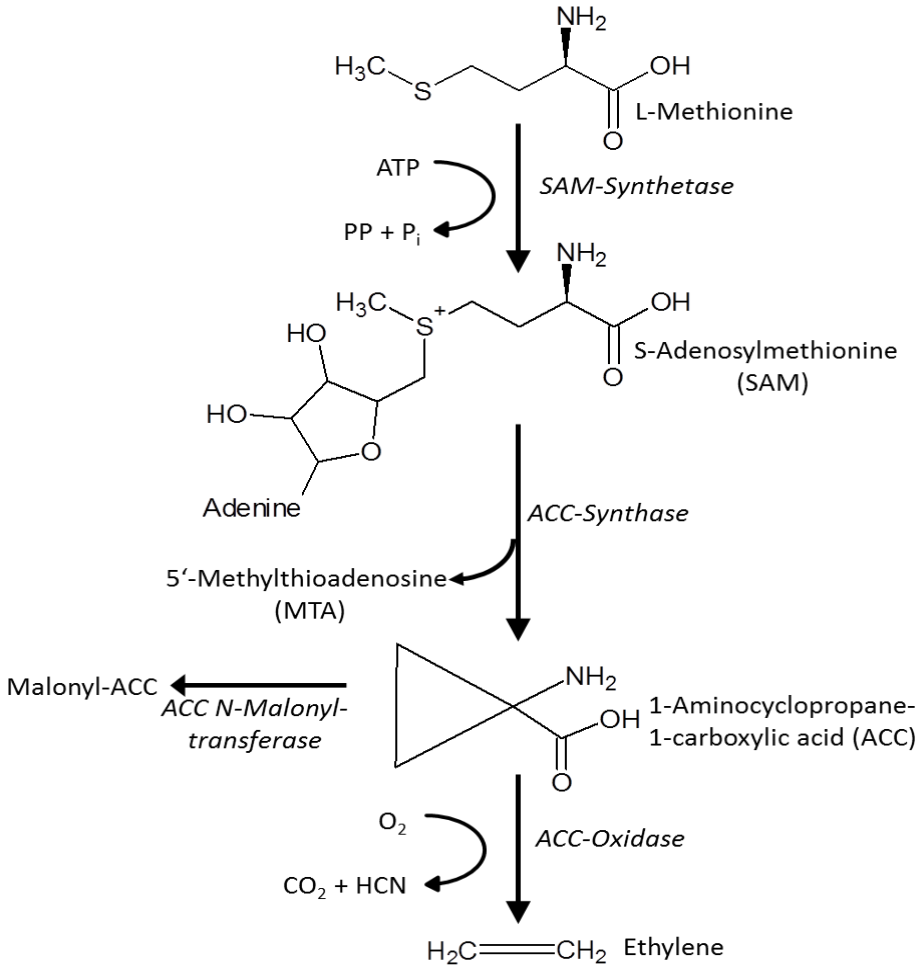


Fig. 2-3: Ethylene biosynthesis.

2.3.2 Ethylene inhibitors

During the few past years, a number of approaches for delaying ripening in many fruits have involved inhibiting ethylene production and its action. Several inorganic and organic compounds were introduced that highly affect either ethylene biosynthesis or its action. Thus, these compounds had massive impact on the fruit ripening behavior, especially climacteric fruits, by retarding fruit ripening (Larrigaudiere et al., 2009).

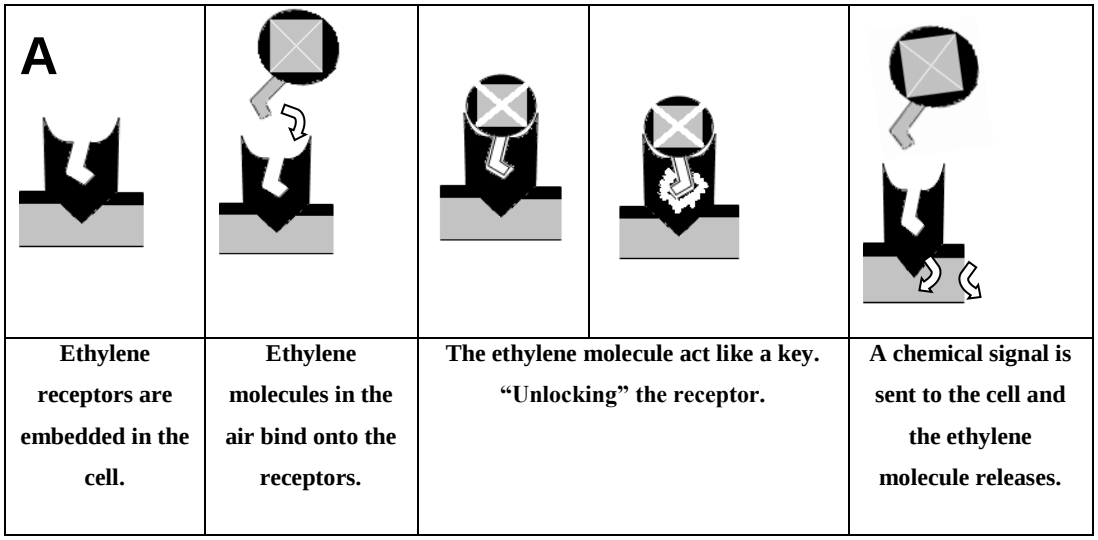
Two types of inhibitors for ethylene are established. Yang and Hoffman (1984) reviewed the first type of inhibitors, which is the synthesis inhibitor. There are two classes of synthesis inhibitors. The first class competitively inhibits ACS, such as aminoethoxyvinylglycine (AVG) and aminoethoxyacetic acid (AOA). The second class inhibits ACC oxidase (ACO) activity, such as cobalt and α-aminoisobutyric acid. These compounds inhibit the ethylene production but do not protect the commodity from exogenous ethylene. On the other hand, the second type is inhibiting ethylene action such as silver thiosulfate (STS) and 1-methylcyclopropene (1-MCP). These compounds are more specific

than the first group because they block the ethylene receptors. Thus, they protect the plant tissues from endogenous and exogenous ethylene (Sisler and Serek, 1997).

2.3.2.1 1-Methylcyclopropene (1-MCP)

1-MCP has been found to be the most effective application as an ethylene inhibitor. It is discovered by Edward Sisler and Sylvia Blankenship (Blankenship and Dole, 2003) and it is a gas with molecular weight of 54. It is an inhibitor of ethylene action and has been widely used to improve shelf life and quality of plant products. In addition, this product has been used by scientists to make advances in understanding the role of ethylene in plants (Sozzi and Beaudry, 2007). 1-MCP has a planar molecular structure with a methyl group attached at the double bond. Generally, strained compounds tend to bind to electron donor compounds, such as copper, to relieve the strain (Sisler and Serek, 1999). It proposed that MCP inhibits ethylene action by competing for the sites of binding on the ethylene receptor. This binding presumably is the mechanism of 1-MCP action. Where, the affinity of the receptors for 1-MCP is approximately 10 times greater than for ethylene. Therefore, 1-MPC is very active at much lower concentrations compared with ethylene (Binder and Bleecker, 2003). 1-MCP also influences ethylene biosynthesis in some species through feedback inhibition (Blankenship and Dole, 2003).

Blankenship (2001) described the way, how the 1-MCP acts as an ethylene inhibitor. The ethylene attaches to the receptor as a "key" fitting in a "lock", with ethylene. When ethylene attaches to the receptor, ethylene unlocks and opens a door. A cascade of events then takes place leading to fruit softening, leaves yellowing, or flowers shedding. However, 1-MCP is also able to bind to the ethylene receptor, and it also can act as a "key" that goes into the "lock", but it is unable to turn and "open the door". When 1-MCP is in the "lock", it is not possible for the ethylene to enter the lock. Hence, the 1-MCP stops the "lock" from turning, so the door cannot open (Fig. 2-4): Therefore, 1-MCP inhibits ethylene action in the plant tissues.



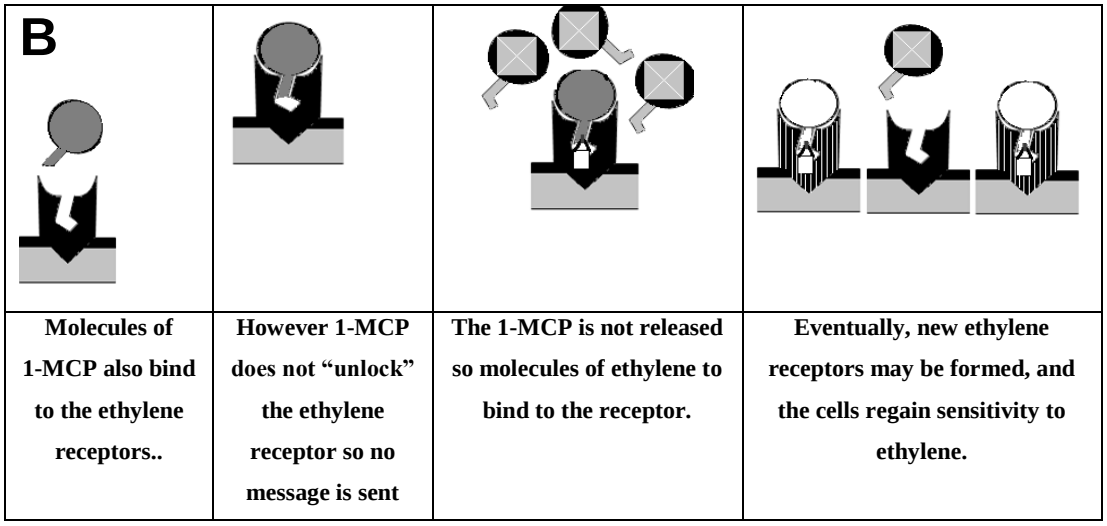


Fig 2-4: The mechanism of 1-MCP acting, (A): How the ethylene molecule is binding the receptors 'Unlocks' and leads to a chemical reaction in the plant tissue, (B): How 1-MCP inhibits ethylene action by binding its receptor and the chemical reaction does not occur (Redrawn from Jenny Bower Dept. of Pomology, UC Davis).

2.3.2.1.1 Physiological and biochemical responses to 1-methylcyclopropene (1-MCP)

The effect of 1-MCP on the physiological behavior has been studied in many fruits. 1-MCP applications have been shown to reduce respiration and ethylene production rate in a number of climacteric fruits such as Japanese plum (Abdi et al., 1998; Khan and Singh, 2007), apple (Fan et al., 1999a, b), apricot (Fan et al., 2000; Dong et al., 2002), pears (Trinchero et al., 2004) and European plum (Valero et al., 2003).

The application of MCP on avocado (Jeong et al., 2002) and on persimmon fruits (Harima et al., 2003) showed a significant delay in the rise of ethylene production. On the

other hand, 1-MCP has no effect on respiration rate in nectarine (Dong et al., 2001) and in apricot (Dong et al., 2002). This variation in response to1-MCP in apricot might be due to fruit maturity, cultivar or some other unknown factors.

Abdi et al. (1998) studied the effect of 1-MCP on the climacteric Japanese plum cultivars 'Gulfruby' and 'Beauty' and on the cultivars 'Shero' and 'Rubyred', which show suppressed climacteric behavior. They reported that 1-MCP has the potential to control the ripening of plum fruits, both, climacteric and suppressed-climacteric cultivars. They found also that a single application of 1-MCP is enough to delay the ripening in suppressed type while continuous low doses would be required for climacteric cultivars. Another study on 'Tegan Blue' plum by Khan and Singh (2007) concluded that the 1-MCP postharvest application inhibited ethylene production and respiration rate.

On the contrary, the non-climacteric fruits pineapple fruit produced more ethylene after 1-MCP treatment (Selvarajah et al., 2001). On the other hand, Tian et al. (2000) reported mixed effects on strawberry. While the application of 2 ppm of 1-MCP on early harvested strawberry fruits reduced softening, color changes and respiration rates, it had less effect on late harvested fruits.

The effects of 1-MCP on ethylene biosynthesis have been studied on the molecular and biochemical levels in many fruits. 1-MCP treatment of peach fruit has no effect on ACC synthase (ACS) gene expression or activity, but inhibit ACC oxidase (ACO) gene expression compared to control (Mathooko et al., 2001).

Another study by Liu et al. (2009) was conducted on persimmon. They found that 1-MCP inhibited gene expression for ACS and ACO as well as ethylene biosynthesis. While in banana, 1-MCP application delayed ACS and ACO gene expressions compared to untreated fruit (Nakatsuka et al., 1997). Moreover, in 'Tegan Blue' plum (*Prunus salicina* L.), 1-MCP suppressed or stopped ACS and ACO activity in fruit skin and pulp depending on its concentration (Khan and Singh, 2007).

2.3.2.1.2 Effect of 1-MCP on fruit quality

The effect of 1-MCP application on fruit quality has been studied in many fruits. 1-MCP treatments result in a delay in color changes in many fruits as avocado (Feng et al., 2000) or plum (fruit skin and pulp) (Khan and Singh, 2008; Manganaris et al., 2008). It also effectively inhibited the ethylene degreening in 'Shamouti' orange (Porat et al., 1999), banana (Sisler et al., 1996) and apple (Fan and Mattheis, 1999). However, 1-MCP has no effect on degreening of 'Oroblanco' pummelo grapefruit (Porat et al 1999).

Impact of 1-MCP on volatile production has been detected in many apple varieties. Production of volatile alcohols and esters are reduced or totally inhibited with 1-MCP

treatments in ‘Fuji’ and ‘Gala’ (Fan and Mattheis. 1999b, 2001) and ‘McIntosh’ and ‘Delicious’ (Rupasinghe et al. 2000). Similar results were obtained on plum. So, Abdi et al. (1998) suggested that the recovery treatment with propylene restored aroma production in plum fruit. On contrast in pears, 1-MCP application has no effect on volatile production in ‘d’Anjou’ cultivar (Argenta et al., 2003).

The effect of 1-MCP application on fruit firmness has been observed in many fruit crops. Although, 1-MCP delayed softening in most fruits, in avocado for 4.4 days and mango for 5.1 days (Hofmann et al., 2001) other species were not affected firmness in apricots (Fan et al., 2000).

Plum fruit softening is significantly retarded in ‘Angeleno’ Japanese plum by 1-MCP treatment after storage at low temperature. Moreover, 1-MCP application could extend fruit shelf life and replace CA (Controlled atmosphere) storage for short or medium storage period (Menniti et al., 2006). Similar results have been obtained by other studies, which examined the effect of MCP application on softening of plum fruit (Salvador et al., 2003; Valero et al., 2003, 2004; Khan and Singh, 2007, 2009).

On the other hand, 1-MCP treatment has no effect on the retention of fruit firmness in some other fruit species, such as orange (Porat et al., 1999). Moreover, the application of 1-MCP with higher concentrations shows a greater degree of softening in treated strawberry fruits than in the non-treated control fruits (Tian et al., 2000).

However, the 1-MCP treatments suppressed the enzymes involved in fruit softening such as Exo-PG, Endo-PG, PE and EGase in plum fruit (Menniti et al., 2004; Khan and Singh, 2007, 2009).

The influence of 1-MCP application on soluble solids contents (SSC) and total acidity (TA) has been studied in many fruits. Generally, the 1-MCP treatments have no effect on SSC in many fruits such as plum and apricot (Dong et al., 2002; Salvador et al., 2003), orange (Porat et al., 1999), apple (DeEll et al., 2002) and banana (Jiang et al., 2004). However, there are other studies pointing out an increase in soluble solids content by applying 1-MCP in plums (Valeo et al., 2004) and some apple cultivars such as ‘Delicious’ and ‘Empire’ (Watkins et al., 2000).

On the other hand, the loss of TA has been delayed by using 1-MCP application in many fruits, plums (Valero et al., 2003; Salvador et al., 2003; Khan and Singh, 2007), pineapples (Selvarajah et al., 2001), peaches (Liu et al., 2005) and some apple cultivars (Fan et al., 1999a, b). However, the 1-MCP application has no effect on TA in some other species such as apricot (Dong et al., 2002) and orange (Porat et al., 1999) and are inconstant with some other apple cultivars (Watkins et al., 2000).

3. Material and Methods

3.1 Non-destructive detection of fruit development and ripening

3.1.1 Plant material

The present investigations were carried out during 2010/12/13 summer seasons on five European Plum (*Prunus domistica* L.) cultivars, grown at the experimental orchard of the Unit of Fruit Science, Technische Universität München at Freising-Weihenstephan. These cultivars were:

- 'Katinka' (mid early,well balanced sugar/acid ratio, strong aromatic, medium blue and flesh freestone),
- 'Cacanska Lepotica' ('C. Lepotica') (mid early, more sour sugar/acid ratio, dark blue, strong aromatic and flesh freestone),
- 'Topfive' (mid early, well balanced sugar/acid ratio, strong aromatic, blackish blue, and flesh freestone),
- 'Haganta' (late cultivar, more sour sugar/acid ratio, strong aromatic, blackish blue and flesh freestone) and
- 'Hoh 4517'

These cultivars were cultivated in 2005, vase-shaped pruned, planted at a spacing 1.5*3.0 m and grafted on Myrobalan rootstock (*Prunus cerasifera* L.) (Vigorous to very vigorous), Wavit, Wangenheims (dwarf to semi dwarf), GF (*Prunus persica* L.**Prunus amygdalus* L.) (moderate vigorous), Ishtara (*Prunus cerasifera* Ehr.**Prunus salicina* Lindley), (moderate vigorous) and Fereley (semi dwarf) rootstocks. Plant fertilization, irrigation and protection were carried out in accordance to the recommendations for orchards in this region.

3.1.2 Treatments and Measurements

3.1.2.1 Crop load

The purpose of this experiment was to determine the effect of crop load on fruit development and quality attributes. It was carried out during 2010/12/13 seasons on five cultivars as described above. About 3–4 weeks after flowering and fruit set, 40–60 branches (one year old at least) were marked for each cultivar. Thereafter, fruits on every branch were counted and lengths of branches were measured. Primary fruit set per cumulated branch length (primary + secondary) was calculated Figs. 3-1 and 3-2.

Afterwards, branches were categorized to three crop load levels (low, moderate and high) in 2010 in ‘Katinka’, ‘C. Lepotica’, ‘Topfive’ and ‘Haganta’ cultivars and two crop load levels (low and moderate) in ‘Hoh 4517’. Branches with fruit set less than 70 % in ‘Katinka’, ‘C. Lepotica’, ‘Topfive’ and ‘Hoh 4517’ and 60 % in ‘Haganta’ were assigned to middle and low crop load levels. Branches with fruit set higher than this percentage were categorized as high crop load´ level. In ‘Hoh 4517’ breeding clone did not have enough fruit set for high crop load.

In 2012 and 2013 seasons the crop load was divided only into two levels (low and moderate) and the effect of rootstocks were included. For each crop load level, 15–20 branches were selected, where each cultivar/rootstock/ crop load combination has at least 3 branches (in 2012 and 2013 seasons). Fruits were thinned in low and moderate crop load to proper number as indicated in Tab. 3-1:

Tab. 3.1: Fruits no./m in ‘Katinka’, ‘C. Lepotica’, ‘Topfive’, ‘Haganta’ and ‘Hoh 4517’ cultivars in 2010, 2012 and 2013 seasons after thinning.

Treatments	‘Katinka’	‘C. Lepotica’	‘Topfive’	‘Haganta’	‘Hoh 4517’
Low crop load (LCL)	25	20	20	15	25
Middle Crop load (MCL)	50	40	40	30	50
High Crop Load (HCL)	70–100	70–100	70–100	60–100	70–100

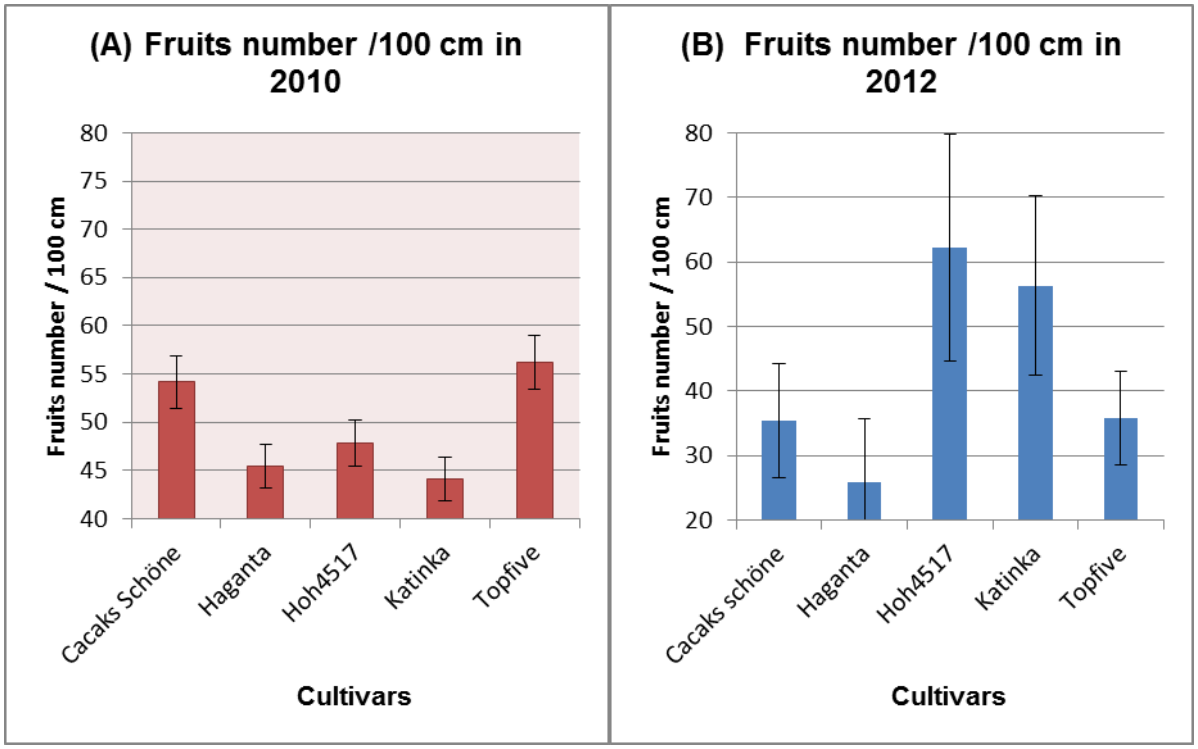


Fig. 3.1: Average of fruits number set /100 cm in 'Katinka', 'C. Lepotica' ('Cacaks Schöne'), 'Topfive', 'Haganta' and 'Hoh 4517' plum cultivars in 2010 season (A) and 2012 (B), after 20–25 days of full bloom. n = average of fruits number for (40–60 branches); values are the mean ± standard deviation (SD).

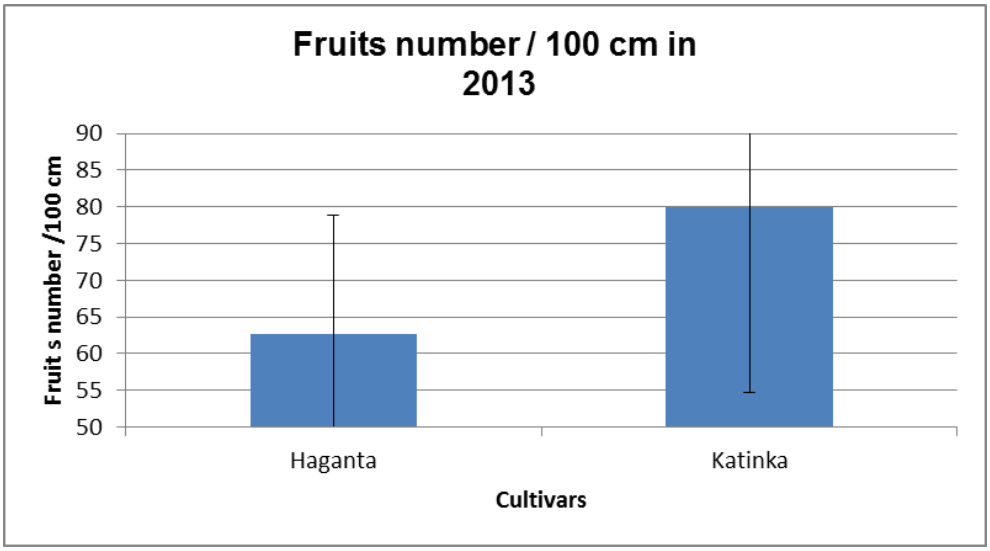


Fig. 3.2: Average of fruits number set /100 cm in 'Katinka' and 'Haganta' plum cultivars in 2013, after 20–25 days of full bloom. N = average of fruits number for (20–40 branches); values are the mean ± standard deviation (SD).

3.1.2.2 Fruit development measurements

For measuring the fruit development during fruit growth and maturation season, fruits diameters and lengths, as well as non-destructive Chlorophyll (Chl), Flavonols (Flav) and Anthocyanins (Anth) were measured.

3.1.2.2.1 Fruit diameter and length

Using a Vernier caliber connected to a notebook, measurements of diameter and length were started after 4 weeks from fruits thinning. In 2010, 100–150 fruits on 10 branches (10–15 fruits each branch) for every crop load were marked (300–450 fruits / cultivar). In 2012 and 2013 variety/rootstock /crop load combinations were included (at least 50 fruits for each variety/rootstock/crop load combination).

Fruit diameters were measured weekly and fruit lengths were measured biweekly. These measurements were performed from June, 16^{th,} July, 9th and June, 18th for 2010, 2012 and 2013, respectively, until harvesting date for each cultivar.

3.1.2.2.2 Non-destructive measurements of Chlorophyll, Flavonols and Anthocyanins

For monitoring of the development of fruit color, as well as studying the effects of crop load and rootstocks, during the fruit development and maturation, data were recorded for color development during the three seasons by the handheld flourimetric sensor, Multiplex 3. At the beginning of blue coloring, in 2010, 100–150 fruits were selected and marked for every crop load level (250–300 each cultivar) for measuring of Chl, Flav and Anth until ripening stage.

These measurements were as shown in Table (3-2), carried out during 2010 on ‘Haganta’ and ‘Hoh 4517’ only. The measurements were started from August, 12th until September, 27th and 28th for ‘Haganta’ and ‘Hoh 4517’, respectively, and they were performed once every week.

In 2012 season, the measurements were carried out on all cultivars under study (‘Katinka’, ‘C. Lepotica’, ‘Topfive’, ‘Haganta’ and ‘Hoh 4517’) and besides studying the effect of crop load, the effect of rootstock was also included. About 300 fruits were selected and marked for every cultivar, 75 fruits for every cultivar/rootstock combination divided into two crop loads (low and middle), and 35–40 fruits for each cultivar / rootstock / crop load combination.

These measurements were performed twice a week starting from July, 17th in ‘Katinka’, July, 19th in ‘C. Lepotica’ and ‘Topfive’, and August, 9th in ‘Haganta’ and ‘Hoh 4517’

until harvesting dates in August, 1st, 13th and 22nd in 'Katinka', 'C. Lepotica' and 'Topfive', respectively, and September, 18th in 'Haganta' and 'Hoh 4517'.

In the third season 2013, only two cultivars were included in this study, 1st one is the early cultivar 'Katinka' and 2nd the late cultivar 'Haganta' with two crop loads (low and middle) and grafted on four rootstocks, as in 2012. The measurements were started from July, 19th and August, 21st until August, 13th and September, 27th for 'Katinka' and 'Haganta' cultivars, respectively.

Handheld Multiplex 3 is a non-destructive fluorescence-based optical sensor (Multiplex, FORCE-A- Orcay, France). It has a handheld battery and operated optical sensor consisting of four excitation light-emitting diode (LED) sources in the UV-A (six UV-light sources at 370 nm), blue (one LED at 460 nm), green (one LED at 516 nm) and red (one LED at 637 nm). LEDs are pulsed sequentially at 476 Hz with 20 ms per flash.

It has three channels of detections in the yellow, red and far-red spectral regions. These two last detection bands at 680-690nm (red fluorescence, RF) and 730-780 nm (far-red fluorescence, FRF), respectively, corresponded to the two emission peaks of Chlorophyll (Cerovic et al. 1999).

Since the optimal localization of anthocyanins and flavonols in epidermis and outer hypodermal cells enables them to screen part of green excitation, light travelling towards the chlorophyll in inner hypodermal layers (Betemps et al., 2012). By increasing anthocyanins concentration, the green excitation decreased comparing with the red excitation light. The anthocyanins (Anth index) is obtained by comparing ChlF (chlorophyll fluorescence) in green light (absorbed by anthocyanins) and red (non-absorbed by anthocyanins as a reference).

The anthocyanins index is calculated as Anth index = log (FRF_R / FRF_G), the Anth index hence increases with increasing the anthocyanins content in fruit skin. Similarly for flavonols, where the Flav index is calculated as a log (FRF_R / FRF_UV), which is comparing between ChlF in UV light (absorbed by flavonols) and red light (non-absorbed by flavonols). On the other hand, chlorophyll index (Chl index) is calculated as a simple ratio between far-red fluorescence (FRF) and red fluorescence in red light (FRF_R / RF_R).

The sensor was insensitive to ambient light and could be used in orchard because of being the LED sources pulsed and synchronized to detections. Generally, its flash illuminates 8-cm-diameter surface (50 cm^2) but it was adapted for the plum fruit diameter. The distance from LEDs and detectors was 10 cm. The acquisition time for a single measurement is 1 sec and each measurement consisted of 500 flashes of four colors (UV, blue, green and red). The collected data was visible in a real time display and stored on a secure digital card for further analysis.

Tab. 3.2: Number of fruits, measuring times, beginning and ending of Multiplex device measuring for different European plum cultivars during 2010, 2012 and 2013.

Cultivar	Season	Fruit no.	Measuring times /week	Beginning of measuring	End of measuring
'Katinka'	2010	–			
	2012	250–300	1	July, 17	August, 01
	2013	300–350	2	July, 19	August, 13
'C. Lepotica'	2010	–			
	2012	250–300	1	July, 19	August, 13
'Topfive'	2010	–			
	2012	250–300	1	July, 19	August, 22
'Haganta'	2010	200–250	1	August, 12	September, 27
	2012	250–300	1–2	August, 09	September, 18
	2013	150–200	2	July, 19	September, 27
'Hoh 4517'	2010	150–200	1	August, 12	September, 28
	2012	250–300	1–2	August, 09	September, 18

3.1.2.3 Fruit analysis

In general, most of the fruits were harvested in ripening stage on a single day, except some cases, where fruits were harvested on two different dates, due to early-ripened fruits that were early harvested. Harvested fruits were directly transferred to postharvest laboratory of the Unit of Fruit Science, Technische Universität München at Freising-Dürnast to analyze fruit weight, diameter, non-destructive (Chl, Flav and Anth), soluble solids content (SSC) and total acidity (TA).

Fruit weight was evaluated with a digital balance. Fruit diameter and length were determined with a digital Vernier cliper connected to a notebook. Soluble solid concentration (SSC in % Brix) was determined using digital bench refractometer (TTR95n, TEC++, Germany). Titratable acidity was determined on fruits juice samples (of 20 plum fruits each), using 5 ml of juice diluted in distilled water until 50 ml, and microtiterated by NaOH 0.1 N to the endpoint of pH 8.1. Titration was performed using an automatic titration (DL22 F&B, Metteler-Toledo, Switzerland). Acidity was calculated as (g malic acid/100ml). The soluble solid content/acidity ratio was calculated as well as the soluble solids content (SSC) / acidity (TA) ratio.

3.2 Effect of leaf number/fruit ratio on fruit quality

3.2.1 Plant materials

This experiment was carried out during 2010 summer season to study the relationship between number of leaves and fruits on the fruit quality on four European Plum (*Prunus domestica* L.) cultivars, grown at the experimental orchard of the Unit of Fruit Science, Technische Universität München at Freising-Weihnstephan. These cultivars were 'Katinka', 'Cacanska Lepotica' ('C. Lepotica'), 'Topfive' and 'Haganta'.

30 branches (spurs and one year old fruity branches) were selected and marked for every cultivar. Leaves and fruits were counted about four weeks after fruit setting and leaves/fruit ratio (LFR) was calculated. Based on the LFR, the branches were classified into two categories (high and Low). Data in Fig. 3-3, shows primary LFR, which was calculated at the beginning of the experiment.

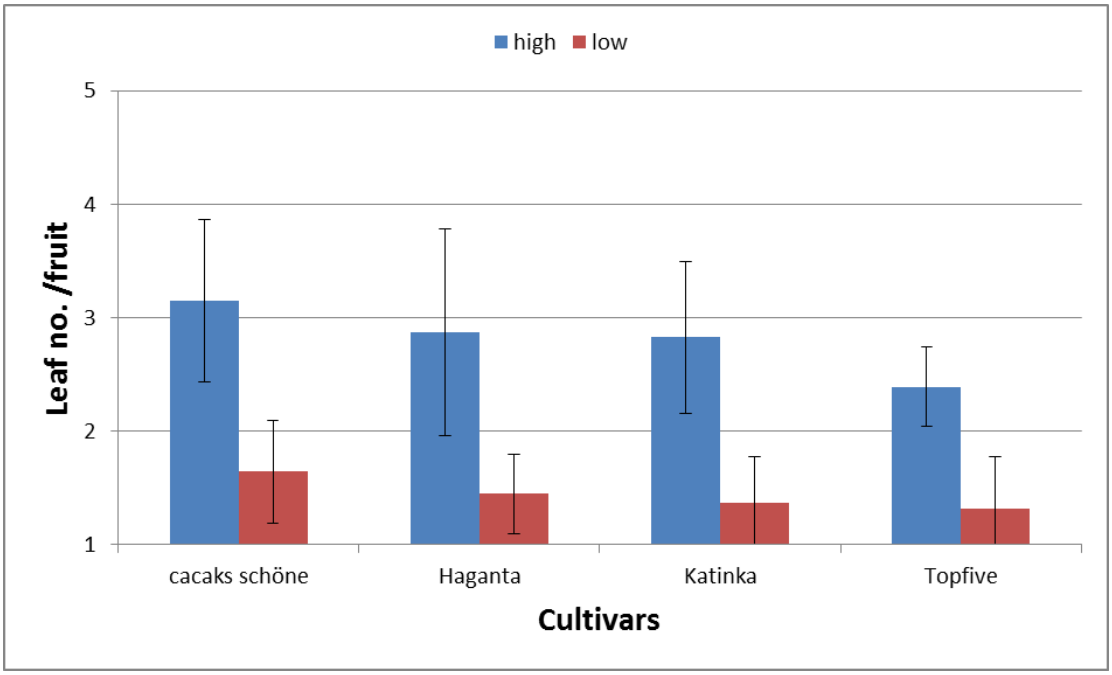


Fig. 3.3: Leaf number per fruit in 'C. Lepotica' ('Cacaks Schöne') 'Haganta', 'Katinka' and 'Topfive' plum cultivars. Values are the mean ± standard deviation (SD).

3.2.2 Fruit analysis

Harvesting was done in a similar setting as the 1st experiment (section 3.1.2.3), and the same measurements were carried out.

3.3 Detection of physiological and biological behavior of European plum fruit during the post-harvest phase and impact of 1-MCP

3.3.1 Plant material

This experiment was carried out in three seasons 2011, 2012 and 2013. In the 1st season 2011 was carried on a total of five late European plum (*Prunus domistica* L.) cultivars and one breeding clone and only 'Haganta' cultivar in 2012 season but in 2013 season was carried on four cultivars, two early ('Katinka' and 'Hanka') and two late cultivars ('Haganta' and 'Haroma'). All cultivars are grown on experimental orchard of the Unit of Fruit Science, Technische Universität München at Freising-Weihnstephan.

To study the plum fruit postharvest behavior during cold storage and shelf life, , fruits were harvested in two or three picking dates except Hoh 4517 breeding clone, which was harvested in only one picking time, as shown in Table.1. The number of picking dates was dependent on the available amount of fruits in this season for each cultivar.

The harvesting was from the 6th of September until 27th in the same month (10 to 20 days before commercial harvest date). Fruits were manually harvested and transported on several cycles to Postharvet Laboratory at the Unit of Fruit Science, Technische Universität München at Freising-Dürnast.

Immediately after the collection of fruits at the laboratory, every cultivar was sorted in order to remove mechanically damaged fruits and fruits without stems. Subsequently, they were randomly distributed and divided into batches, where each batch was placed in nylon bags. The batches were pre-cooled to 2 °C immediately after harvesting and stored at 2 °C, with an RH of 90 %, except for the first batch, which was used directly to measure ethylene production rate and to analyze fruit quality attributes. Batches were removed from cold storage after 10, 20, 30 and 40 days of storage depending on the available amounts. The harvesting dates and cultivars, which were used in the 2011 experiment are presented in Table (3-3).

Tab. 3.3: Cultivars and harvest dates used in experiment in 2011 season.

Cultivars	Commercial date of harvesting	Harvesting dates			
		H1 (05.09.11)	H2 (15.09.11)	H3 (21.09.11)	H4 (27.09.11)
'Haganta'	15.09.11	■	■		
'Hoh 4517'	21.09.11			■	
'Tophit Plus'	21.09.11	■	■	■	
'Hauszwetsche Wolff'	21.09.11	■	■		
'Anna Späth'	27.09.11	■	■		■
'President'	27.09.11	■	■		■

3.3.2 Physiological and chemical measurements

3.3.2.1 Ethylene production rate measurements

To study ethylene production rate in different European plum cultivars during ripening, three replicates from each of the cultivars for each analysis time were chosen, about 1 kg for each replicate. Each replicate was placed in a sealed 3 L glass jar for 2 hours at 20 °C.

Ethylene production was calculated by ethylene concentration in the gas phase of the headspace of the jars. From the headspace of every jar 1-ml gas was withdrawn by syringe over a rubber septum and injected into a Carlo Erba 4200 Gas chromatograph (Carlo Erba, Spa, Milano, Italy) equipped with aluminum column 80/100 mesh, Flame Ionization Detector (FID). The temperatures of injection, oven and detector were 175, 100 and 120 °C, respectively. The gas flow was 235 ml/min for air, 20 ml/min for hydrogen and 30 ml/min for nitrogen (carrier gas). Measurements were repeated for three or four times for every replicate. Ethylene production rate was calculated as ppm/kg/h.

3.3.2.2 Respiration Rate

Respiration rate was measured as a function of CO_2 concentration by using OXYBABY® (WITTGAS Co. Ltd., Germany) – a mobile hand held gas analyzer after calibration with standard gases. Three replicates of each cultivar, each treatment, and each sampling time were used. About 1 kg of fruit was placed in a 3 L airtight glass jar contained air as initial gas atmosphere for 2 h for each replicate. The gas samples were performed through an airtight septum and repeated three times for each replicate.

Respiration rates of plum fruits were measured by this gas analyzer as CO_2 % then later the respiration rate was calculated as ml/kg/h CO_2. Respiration rate measurements were carried out only on 'Katinka' and 'Hanka' cultivars in 2013, in addition to some limited samples of 'Haganta' and 'Haroma', which were used for measuring respiration rate by gas exchange.

3.3.2.3 Weight loss

Weight loss was measured at normal conditions room temperature 20 °C and under cold storage at 2 °C. Fruits were weighed before and after cold storage and fruit weight loss percentage was calculated (as a percentage of the initial weight), also the fruit weight was estimated during shelf life (7 days under room temperature at 20 °C).

3. 3.2.4 Fruit quality attributes

3.3.2.4.1 Soluble solids content

Fruit samples were analyzed for soluble solids concentration (SSC) and titratable acidity (TA) before and after cold storage, as well as before and after shelf life for every batch/cultivar. SSC and TA were measured as previously mentioned in the 1st experiment. The SSC/TA ratio was calculated as well.

3.3.2.4.2 Fruit skin color

Fruit skin color was studied to investigate the effect of harvesting date, maturity stage and cold storage on development of color during storage period. Two methods were used as follows:

1- Using a portable colorimeter (Minolta model, Minolta, Osaka, Japan):
   Two measurements on two opposite sides of the fruit were taken, using 25 fruits per sample. The colorimeter was calibrated using the manufacturer's standard white plate. Skin color changes were expressed by a*(+a* exhibits red, –a* exhibits green), b* (+ b* exhibits yellow, –b* exhibits blue) and L* (lightness) values.

The measurements were taken every 10 days using the same fruits along the whole time frame of taking measurements (about 40 days). Fruits were removed from cooling room and brought to room temperature (20 °C) before measuring, then they were placed back in the cooling room. These measurements were carried out on 'Haganta' for two harvest dates and on clone 'Hoh 4517' for one harvest date.

2- Using the portable handheld tool (Multiplex, described in experiment 1):
Fruits were sorted based on the maturity stage. They were sorted into two grades depending on the flavonols (Flav) reading by the Multiplex, where the Flav value in grade one was (<1 Unit) (more ripe) and in grade two was (>1 Unit) (less ripe).These measurements were carried out on 'Haganta' cultivar on two harvest dates.

3.3.2.4.3 Firmness

Plum fruit firmness was measured by a developed tool for the rapid non-destructive evaluation of firmness of soft fruit (FirmTech 2), which gently squeezes the fruits to specified force to determine the fruit firmness. For plum, FirmTech has a turntable with 12 oval shaped indentures to hold the fruits. The FirmTech was calibrated for force and fruit size by manufacturer's tools. Firmness was measured by mg/mm. Measurements were performed in 2013 for 'Katinka', 'Haroma' and 'Haganta'.

3.3.3 1-MCP treatment

Fruits were pre-cooled at 2 °C for at least 12 h before treatments. SmartFresh™ for 1-MCP (0.14 %) was applied by (Bayerisches Obstzentrum, Hallbergmoos, Germany) as a powder, which after addition of warm water (40 °C) released the active ingredient as a gas. Amount of the powder were weighed and warm water was added to obtain doses of 0.625 ppm 1-MCP. Treatments were performed in hermetically sealed plastic boxes (0,015 m^3). Duration of treatment was 24 h at 1 °C. Control fruits were treated in the same way but without 1-MCP. This experiment was carried out, in 2012, on 'Haganta'. In 2013, it was performed on 'Katinka', 'Haroma' and 'Haganta'.

3.4 Statistical data analysis

The statistical analysis was carried out by SPSS. The collected data were subjected to ANOVA analysis. Mean comparisons were performed using the Least Significant Differences (LSD) and Duncan tests to compare means at 5% probability level. The other processing of data, graphical display and standard deviation (SD) of the results were performed by Microsoft office excel.

4. Results

4.1 Non-destructive monitoring of fruit development and ripening

4.1.1 Fruit growth and development

During 2010 and 2012 the fruit size of the 'Katinka', 'C. Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517' was measured in Figs 4-1 and 4-2.

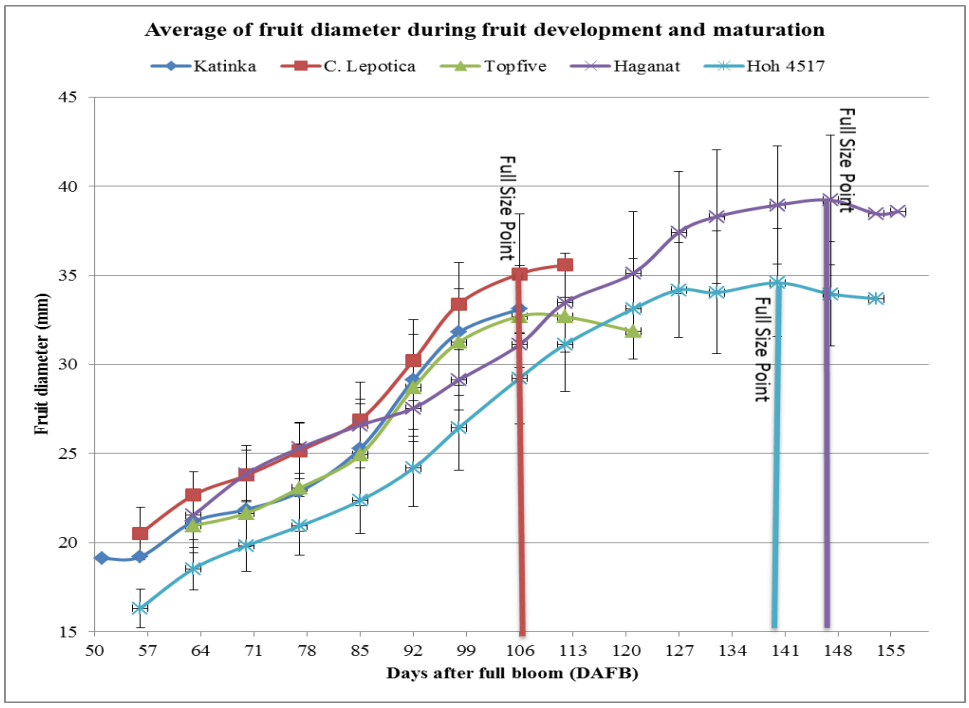


Fig. 4.1: Fruit growth of 'Katinka', 'C. Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517' plum cultivars during fruit growth and maturation period in 2010. Values on the vertical axis represent the mean of fruit diameter ± standard deviation (SD) with n = 300 to 450 fruits. Full size point is when fruits reach full size. Full blooming date was 26.04.2010 for 'Katinka', 'C. Lepotica' and 'Topfive', 22.04.2010 and 27.04.2010 for 'Haganta' and 'Hoh 4517', respectively

In the beginning fruit diameter increased slowly. After that the fruit size dramatically increased from the beginning of July 2010. Early and mid-early cultivars ('Katinka', 'C. Lepotica'and 'Topfive') reached full size (beginning of maturation) after 106 days after full bloom (DAFB), while late cultivars ('Haganta' and 'Hoh 4517') reached full size after148 and 141 DAFB; respectively, for 2010 season . In 2013, the early cultivar 'Katinka' reached full size after 94 DAFB, while the late cultivar 'Haganta' reached full size after 144 DAFB. Data in both seasons point out that fruits reached their full size around one week before harvesting date (ripening on the tree) in case of early and mid-early cultivars. In case of late cultivars,

fruits reached full size 10 to 15 days before harvesting date. An exception was found for 'Katinka' in 2010 with the fruit reaching its full size at harvesting date. The same behavior was noticed also in 2012, data is shown in Figs. 4-6 to 4-10, with exception of 'C. Lepotica'.

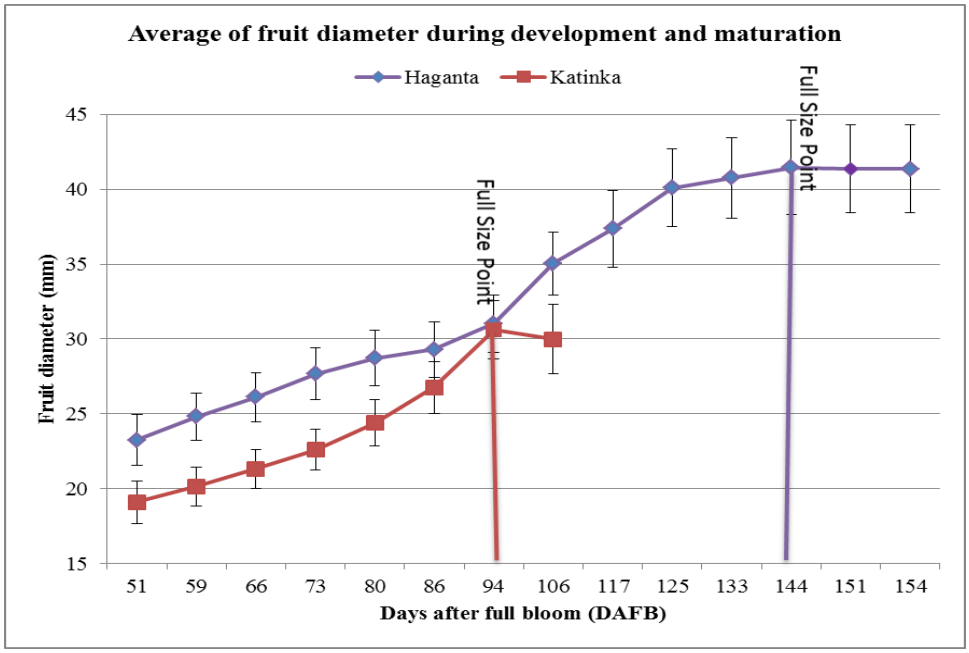


Fig. 4.2: Fruit growth of 'Katinka'and 'Haganta' plum cultivars during fruit growth and maturation period in 2013. Values on the vertical axis represent the mean of fruit diameter ± standard deviation (SD) with n = 300 to 450 fruits for each cultivar. Full size point is when fruits reach full size. Full blooming date was 28.04.2013 and 26.04.2013 for 'Katinka' and 'Haganta' respectively.

4.1.2 Correlation between fruit growth and non-destructive measurements

4.1.2.1 Anth index and fruit diameter

Non-destructive monitoring of Anth index by Multiplex (Multiplex Units) and fruit diameter showed a good correlation for 'Haganta' in 2010, 2012 and 2013 with $r^2 = 0.96$, 0.74 and 0.85 respectively. Correlation was found with $r^2 = 0.88$ and 0.81 for 'Hoh 4517' in 2010 and 2012 respectively, and with $r^2 = 0.93$ for 'Katinka' in 2013 Fig.4-3. In contrast, fruit size showed weak correlation with Anth Multiplex index by $r^2 = 0.23$, 0.47 and 0.09 for 'C. Lepotica', 'Topfive' and 'Katinka' in 2012 respectively.

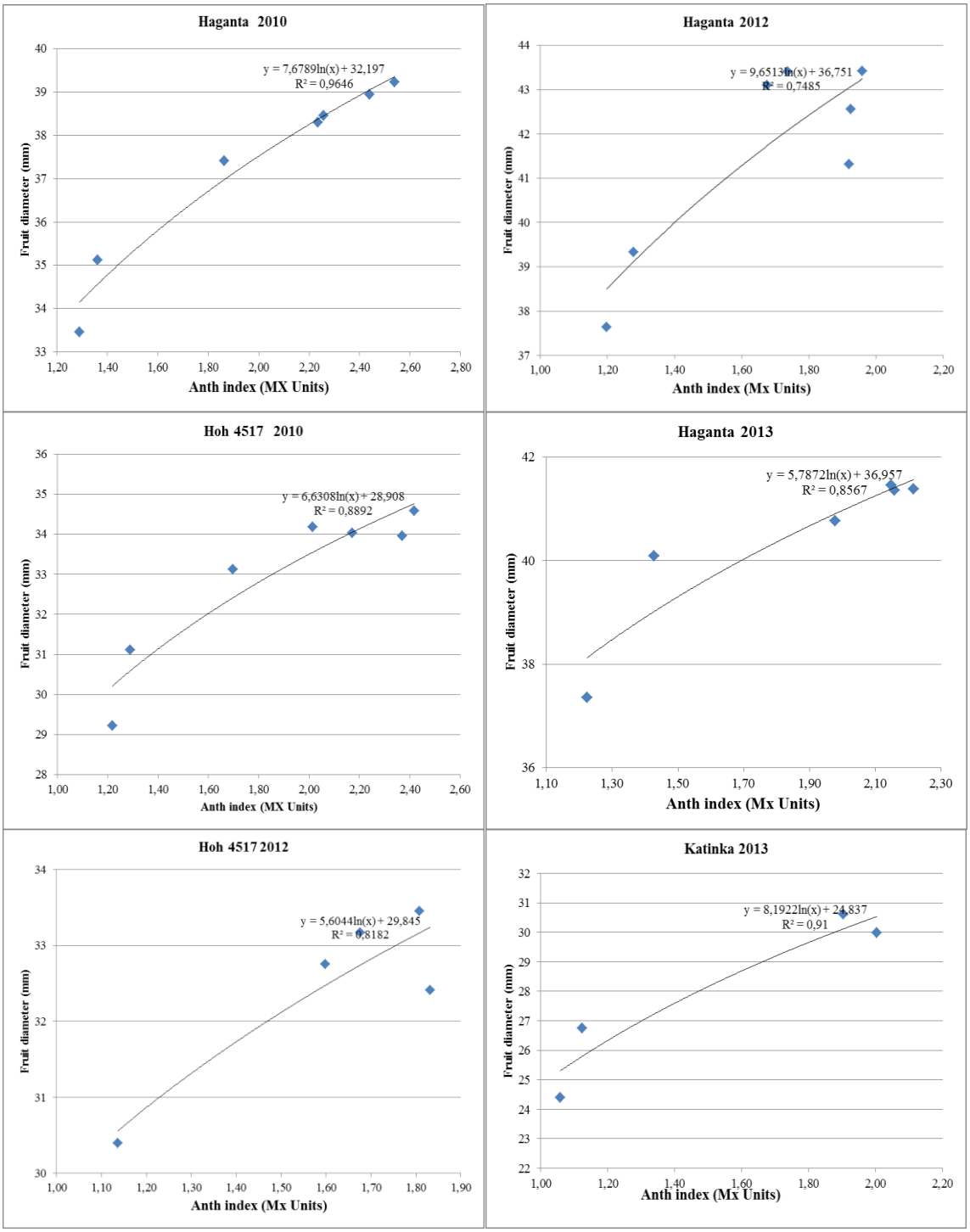


Fig. 4.3: Relationship between non-destructive Anth index (Multiplex Units , Mx Units) and fruit diameter (mm) for 'Haganta', 'Hoh 4517' and 'Katinka' during 2010, 2012 and 2013. Measurements were carried out in the same day with n = 300 fruits.

4.1.2.2 Flav index and fruit diameter

Non-destructive monitoring of Flav index by Multiplex (Multiplex Units) and fruit diameter showed weak correlation for all cultivars and all seasons as shown in Fig 4-4 for 'Haganta' in 2010 and 2012 and 'Topfive' in 2012 as examles. An exception was for 'C.Lepotica' in 2012 Fig.4-4.

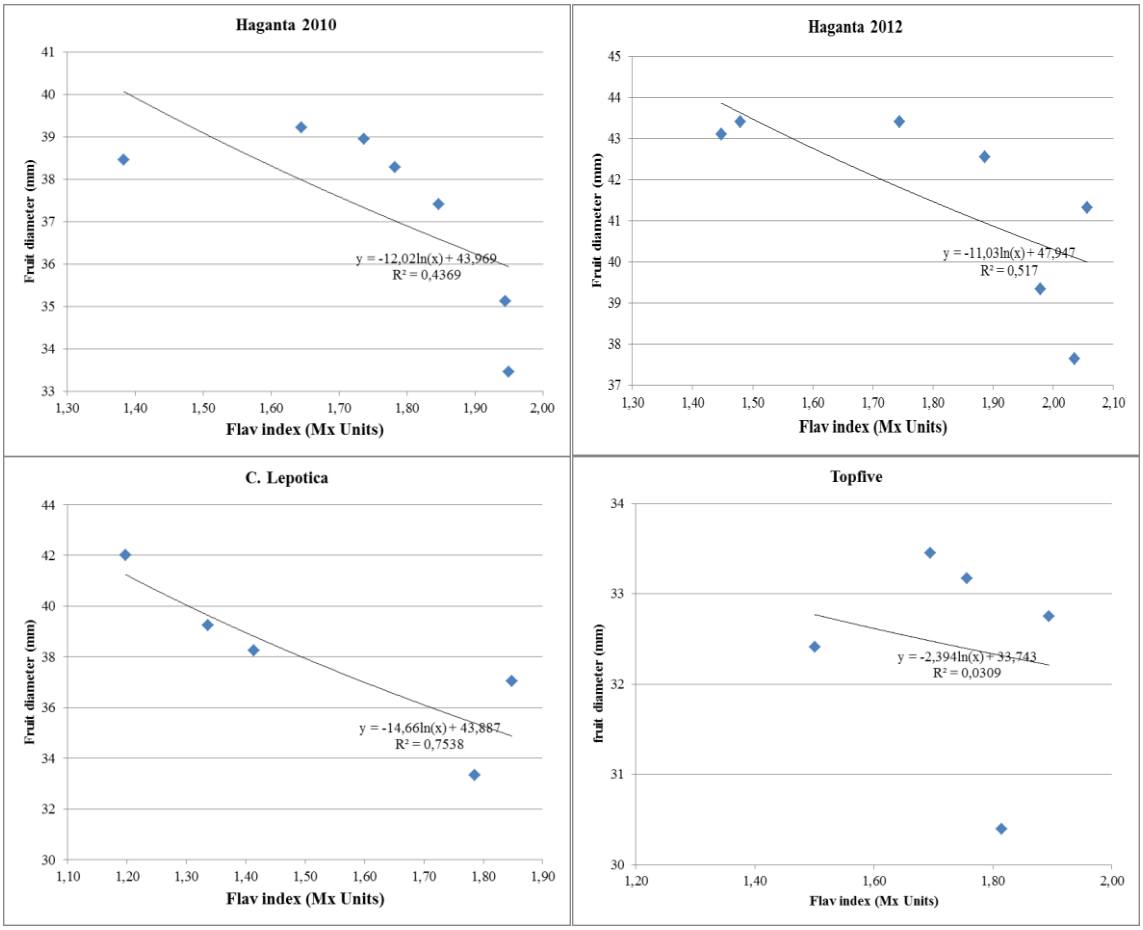


Fig. 4.4: Relationship between non-destructive Flav index (Multiplex Units, Mx Units) and fruit diameter (mm) for 'Haganta' in 2010 and 2012, 'C. Lepotica' and 'Topfive' during 2012. Measurements were carried out in the same day with n = 300 fruits.

4.1.2.3 Chl index and fruit diameter

Chl index measurement by Multiplex (Multiplex Units) and fruit diameter showed also a Weak correlation for all cultivars except for 'Haganta' in 2010 (Fig. (4-5).

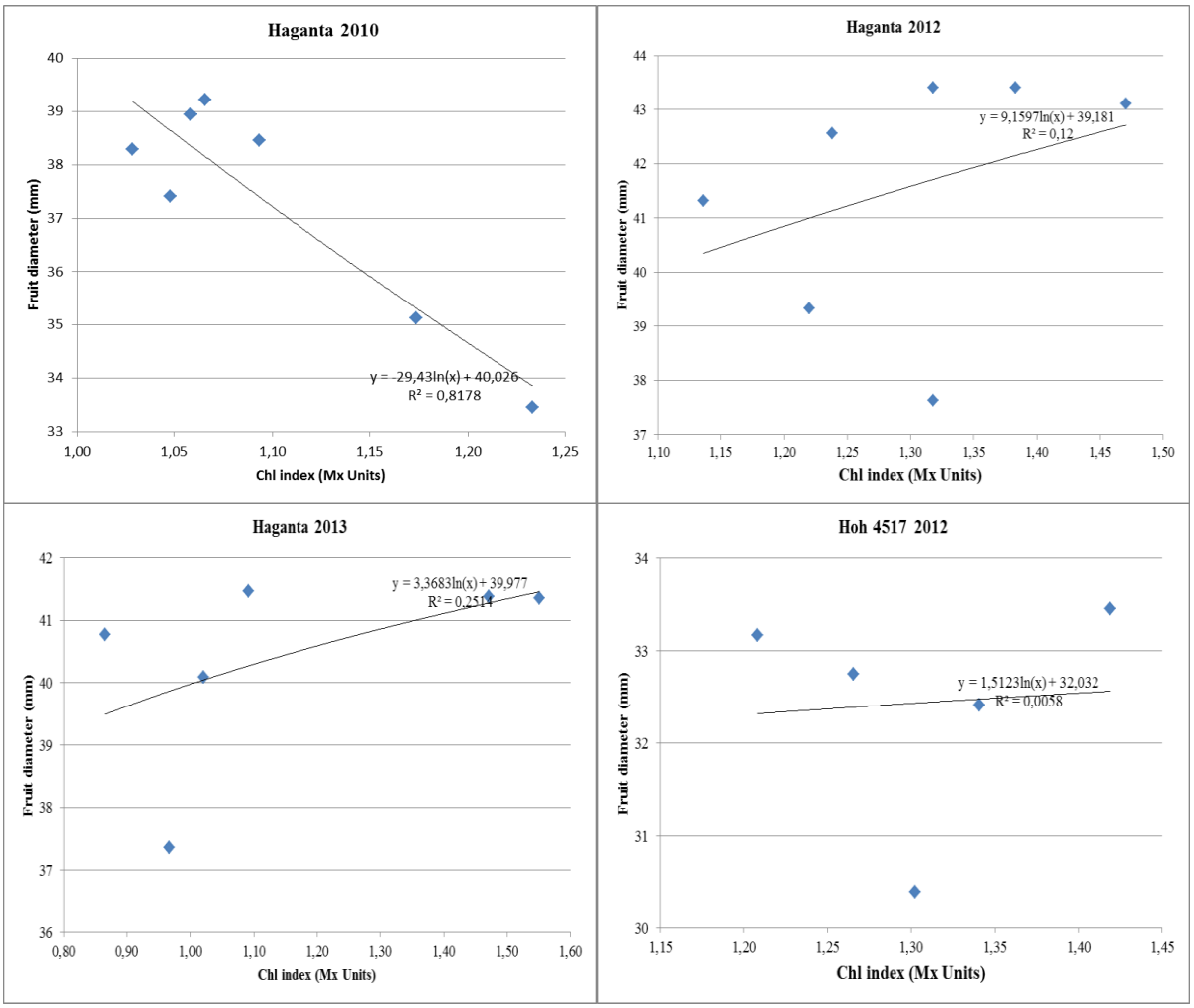


Fig. 4.5: Relationship between non-destructive Chl index (Multiplex Units, Mx Units) and fruit diameter (mm) for 'Haganta' during 2010, 2012 and 2013 and 'Hoh 4517' during 2012. Measurements were carried out in the same day with n = 300 fruits.

4.1.3 Effect of crop load

4.1.3.1 Fruit development and size

The effect of crop load on fruit development and size is presented in Fig.s 4-6 to 4-10. Fruit size was significantly affected by crop load with low crop load resulting in highest fruit size in all cultivars in the three seasons 2010, 2012 and 2013. However in 2010, the differences in fruit size were significant only when comparing low or middle crop load levels to high crop load level. No significant differences between low and middle crop load levels were found except for 'Katinka' which showed significant differences across all crop load levels. In 2012, fruit size was significantly affected by crop load only in 'Katinka' and 'Hoh 4517' but no significant differences were found between middle and low crop load levels for the other cultivars. Moreover, in 2013 season, we found significant differences in fruit diameter between crop load levels for 'Katinka' and 'Haganta'. The same behavior was found for fruit length in all seasons (data not shown). In general, the curve of fruit growth which was affected by crop load exhibited a sigmoid curve.

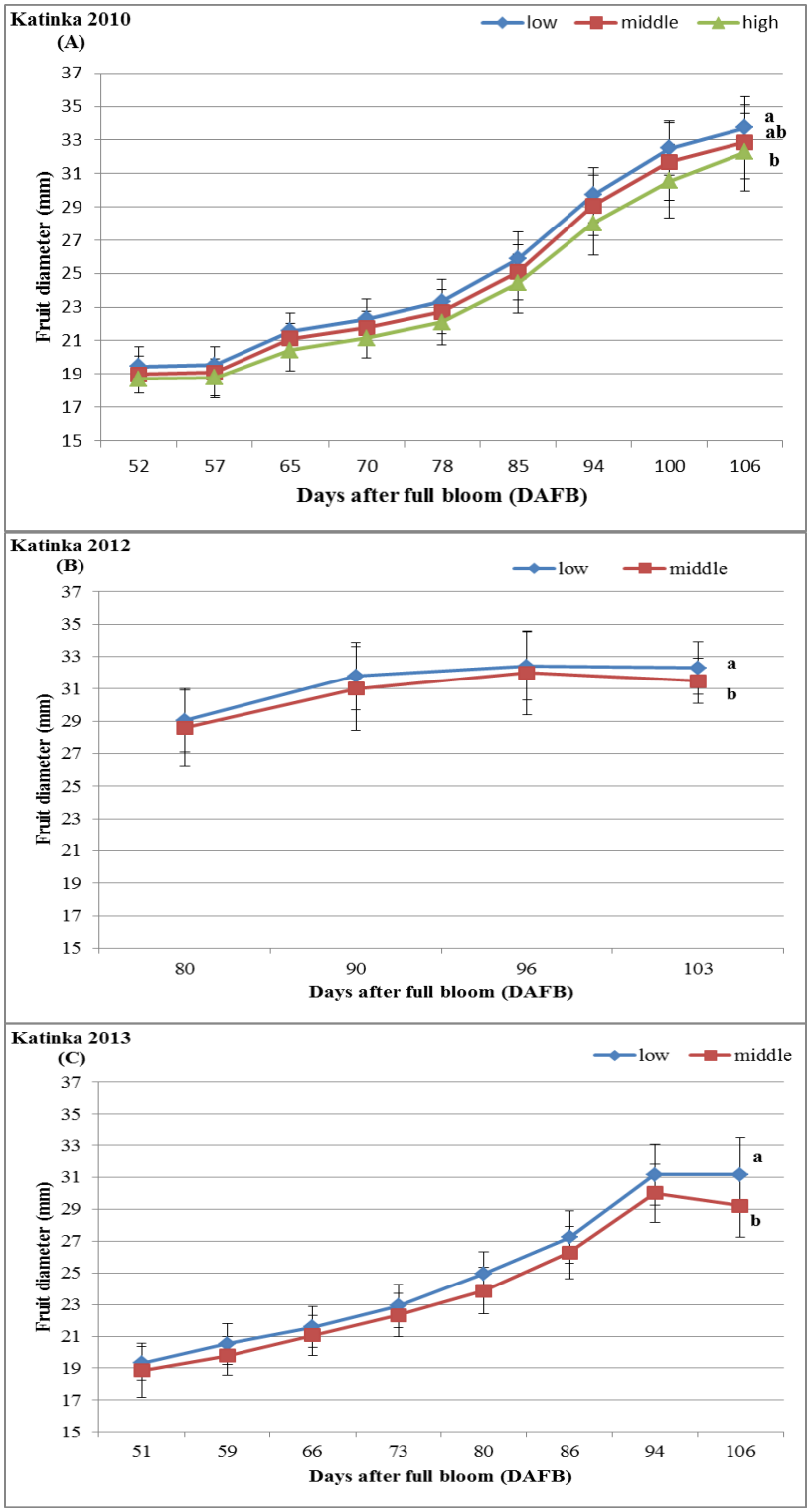


Fig. 4.6: Effect of crop load levels on 'Katinka' fruit size during growth and maturation in 2010 (A), 2012 (B) and 2013 (C) seasons. Values on the vertical axis represent the mean ± standard deviation (SD) with n = 150 to 200 fruits for each crop load: low = 25 fruits, middle = 50 fruits, high = more than 70 fruits per meter of branch. Curves indicated by the same letter, the differencesare not significant (LSD, P = 0.05) for fruits in last measuring date.

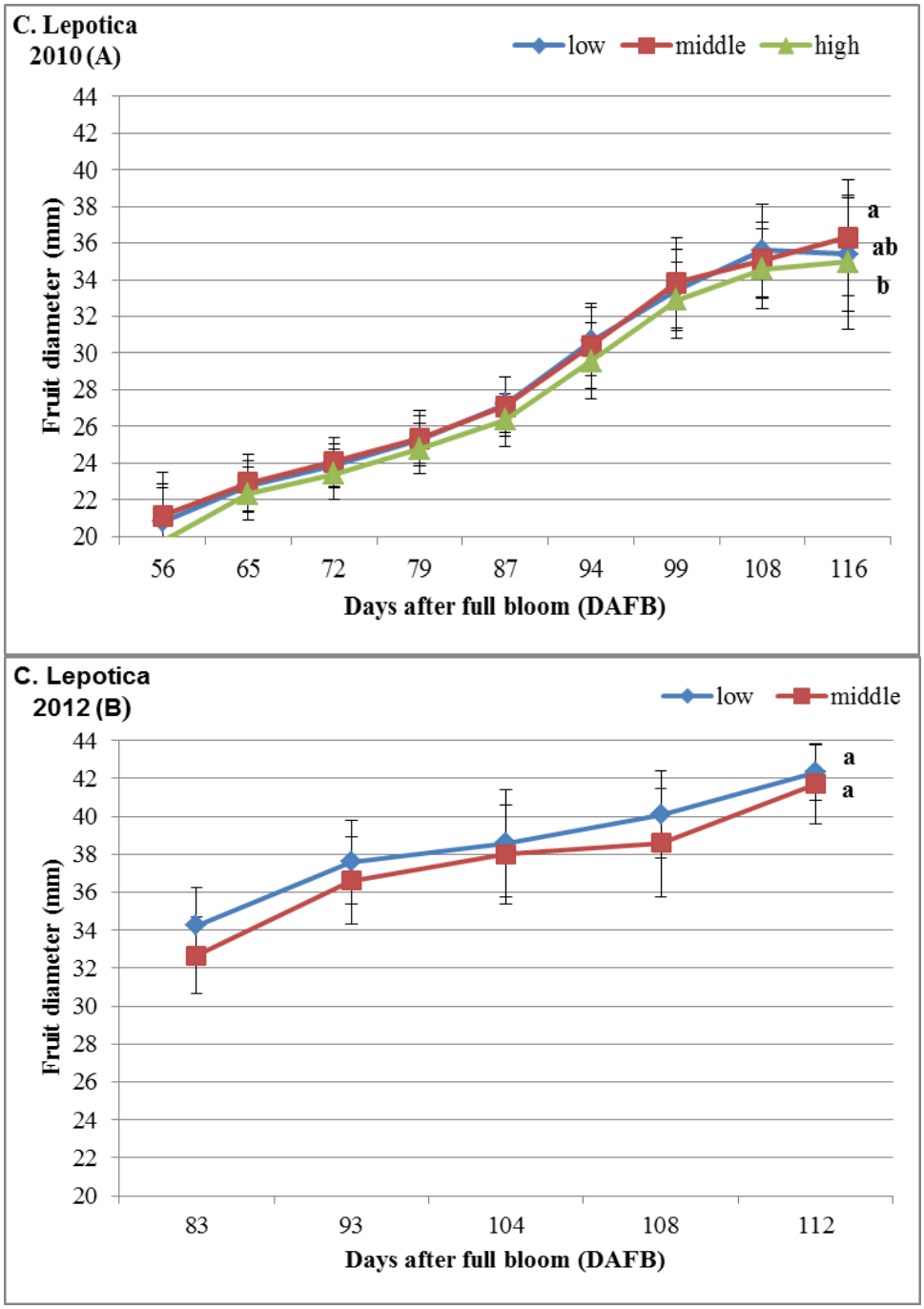


Fig. 4.7: Effect of crop load levels on fruit size during growth and maturation of 'C. Lepotica'in 2010 (A) and 2012 (B) seasons. Values on the vertical axis represent the mean ± standard deviation (SD) with n = 150 to 200 fruits for each crop load: low = 20 fruits per meter, middle = 40 fruits per meter, high = more than 70 fruits per meter of branch. Curves indicated by the same letter, the differences are not significant (LSD, P = 0.05) for fruits in last measuring date (at harvest).

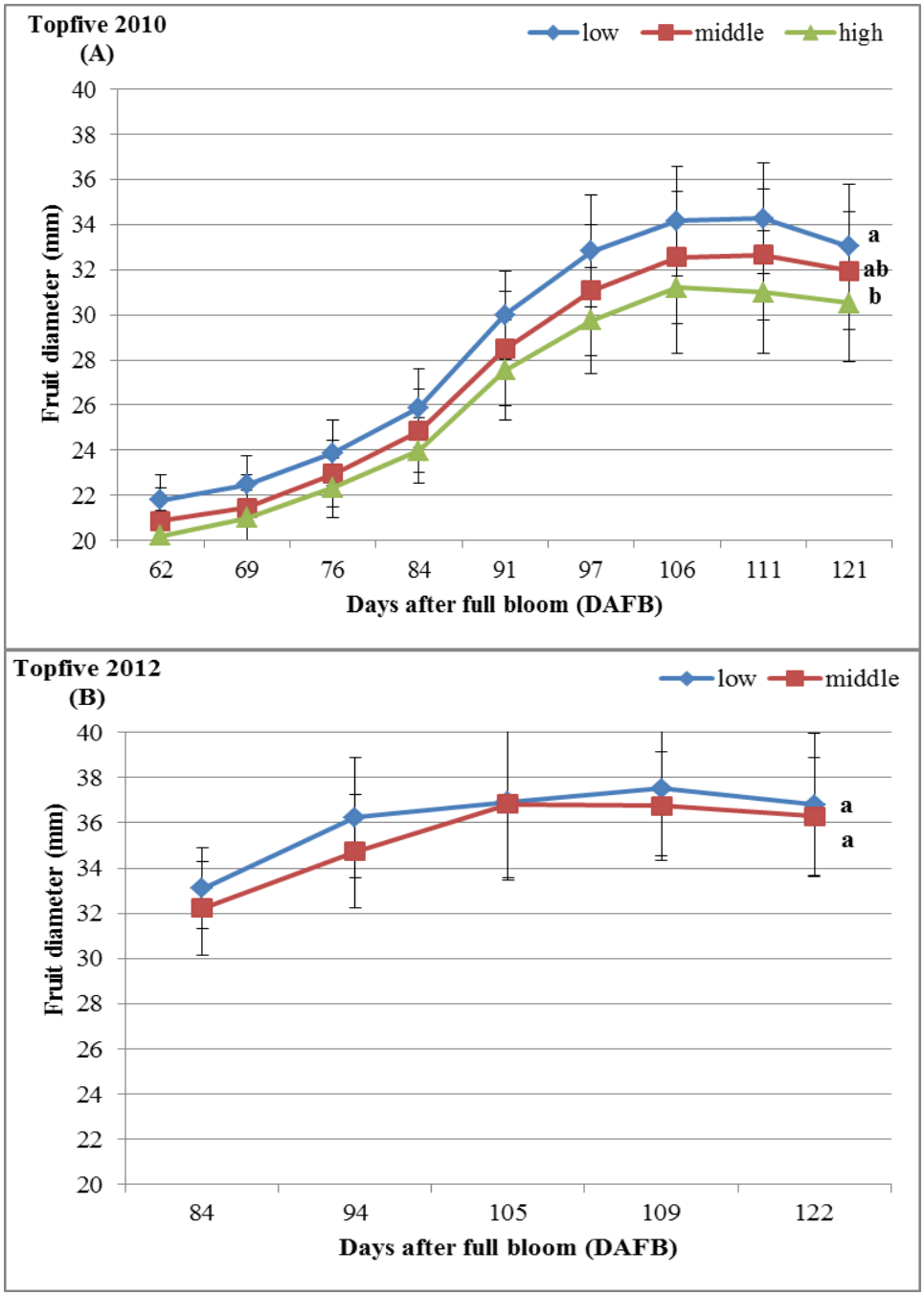


Fig. 4.8: Effect of crop load levels on fruit size during growth and maturation of 'Topfive' in 2010 (A) and 2012 (B) seasons. Values on the vertical axis represent the mean ± standard deviation (SD) with n = 150 to 200 fruits for each crop load: low = 20 fruits per meter, middle = 40 fruits per meter, high = more than 70 fruits per meter of branch. Curves indicated by the same letter, the differences are not significant (LSD, P = 0.05) for fruits in last measuring date (at harvest).

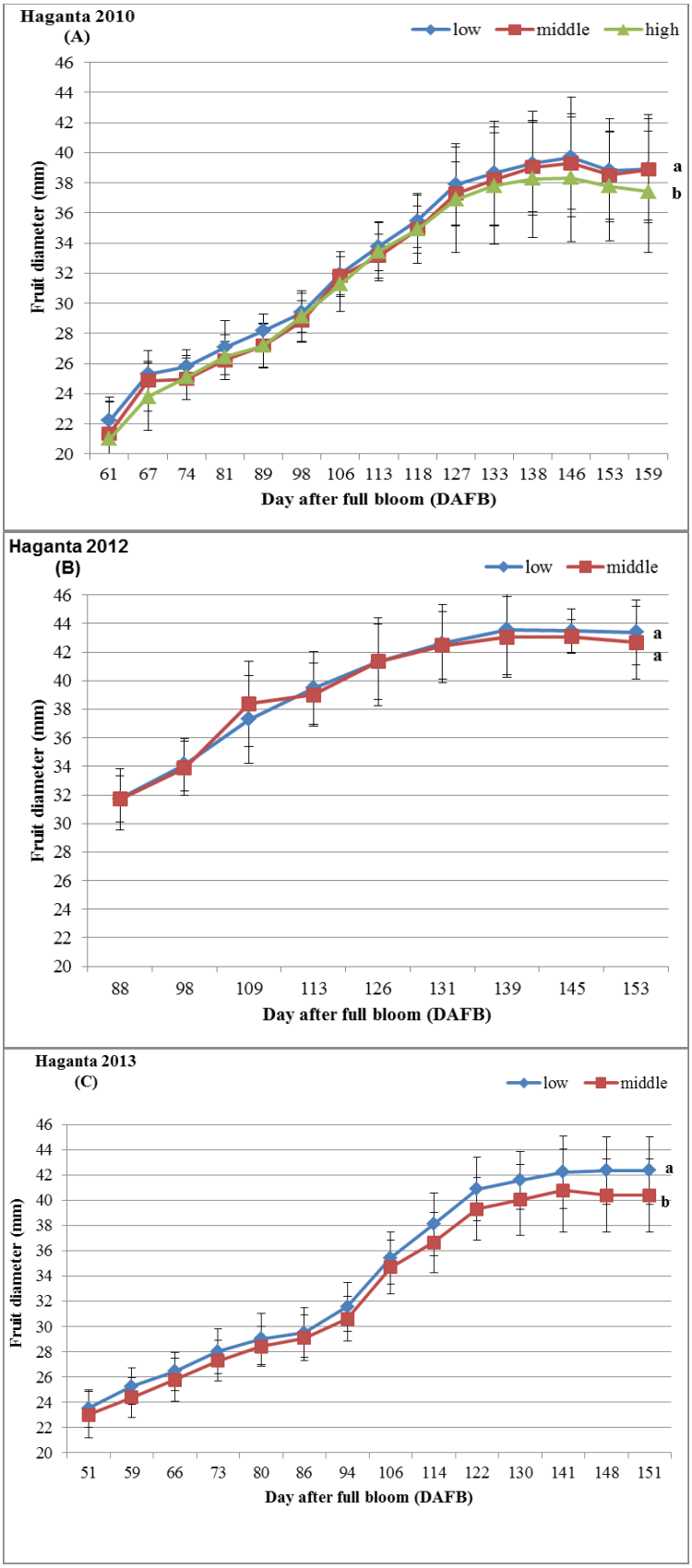


Fig. 4.9: Effect of crop load levels on fruit size during growth and maturation of 'Haganta' in 2010 (A), 201(B) and 2013 (C) seasons. Values on the vertical axis represent the mean ± standard deviation (SD) with n = 150 to 200 fruits for each crop load: low = 15 fruits per meter, middle = 30 fruits per meter, high = more than 60 fruits per meter of branch. Curves indicated by the same letter, the differences are not significant (LSD, P = 0.05) for fruits in last measuring date (at harvest).

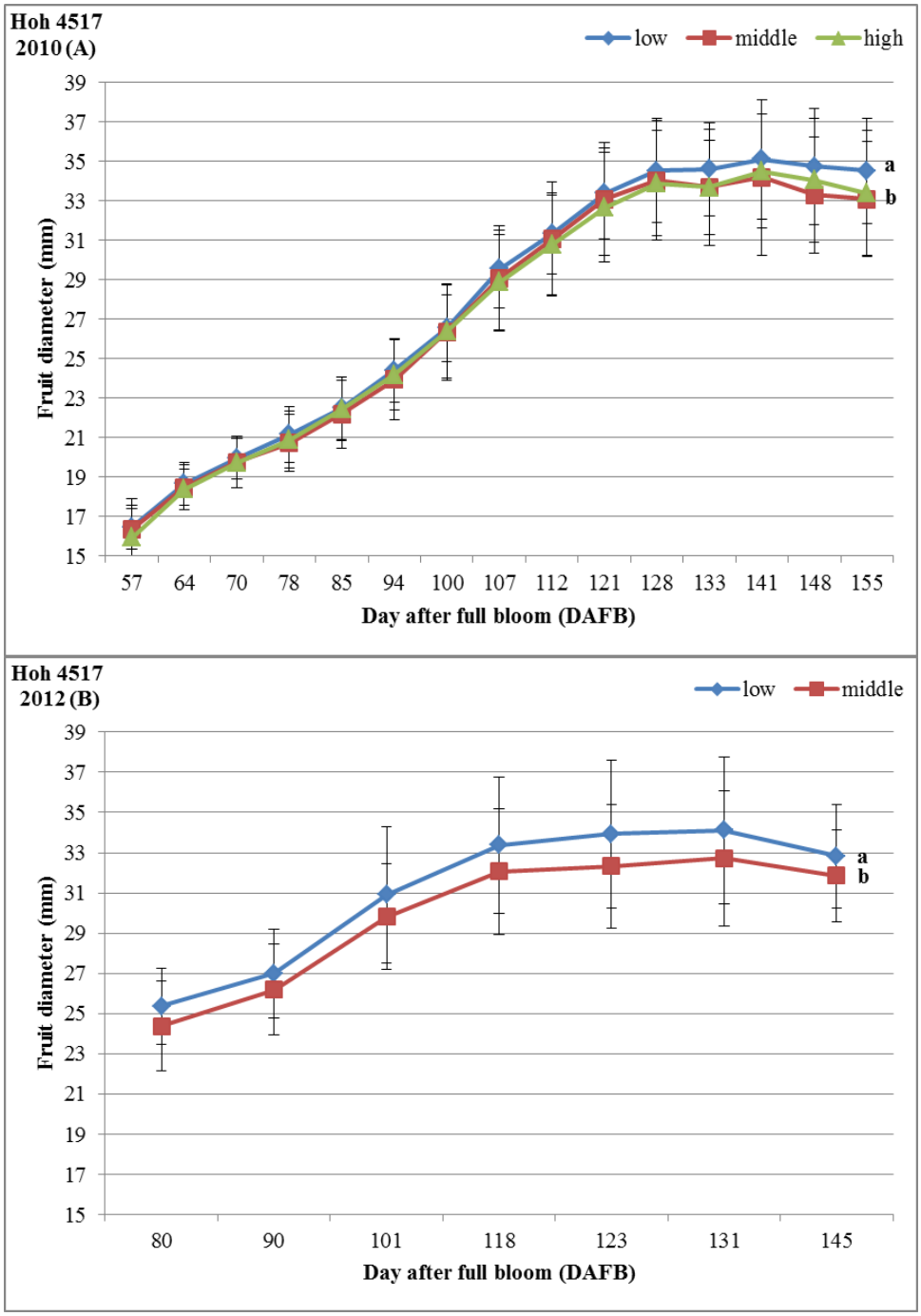


Fig. 4.10: Effect of crop load levels on fruit size during growth and maturation of 'Hoh 4517' in 2010 (A) and 2012 (B) seasons. Values on the vertical axis represent the mean ± standard deviation (SD) with n = 150 to 200 fruits for each crop load: low = 25 fruits per meter, middle = 50 fruits per meter, high = more than 70 fruits per meter of branch. Curves indicated by the same letter, the differences are not significant (LSD, P = 0.05) for fruits in last measuring date (at harvest).

4.1.3.2 Effect of crop load on fruit weight

The effect of crop load on fruit weight is presented in Tab. 4-1. Low crop load produced the highest fruit weight in all cultivars and all seasons except 'C. Lepotica' in 2010 and 'Haganta' in 2012. In these cases the middle crop load gave the highest values. However, the differences in fruit weight were not significant except for cultivar 'Katinka' and breeding clone 'Hoh 4517' in 2010 and 'Katinka' and 'Haganta' in 2013, where low crop load gave significant higher fruit weight. Middle crop load produced a significantly higher fruit weight for 'Haganta' cultivar in 2012. The biggest differences in fruit weight may be due to different climatic conditions in each season. In 2013, low crop load produced significant higher fruit weight compared to middle crop load.

Tab. 4.1: Effect of crop load levels on fruit weight (g) of plum cultivars during 2010, 2012 and 2013.

Cultivar/ Season	Date of full bloom	Date of thinning	Crop loads						Cultivar average	
			Low		Middle		High			
			FW	± SD	FW	± SD	FW	± SD	FW	± SD
'Katinka'										
2010	April 26	May, 20	26.33 a	3.01	23.79 b	4.67	23.33 b	3.66	25.11	3.92
2012	April, 20	May, 25	26.06 a	1.82	25.05 a	2.94			25.42	2.59
2013	April, 28	May, 20	28.22a	6.20	25.75b	3.25			26.94	4.80
'C. Lepotica'										
2010	April, 26	May, 20	28.17 a	4.07	30.14 a	3.09	28.31 a	3.61	29.08	3.59
2012	April, 23	May, 25	46.22 a	2.88	45.90 a	1.01			46.12	2.41
'Topfive'										
2010	April, 26	May, 20	22.89 a	6.57	22.25 a	2.41	20.44 a	4.01	21.94	4.60
2012	April, 24	May, 25								
'Haganta'										
2010	April, 22	May, 21	39.08 a	5.36	39.08 a	5.96	38.45 a	6.71	38.91	5.87
2012	April, 18	May, 26	54.49 b	3.80	61.57 a	2.37			55.67	4.46
2013	April, 26	May, 20	50.64a	4.10	45.27b	4.50			47.49	4.25
'Hoh 4517'										
2010	April, 27	May, 21	31.25 a	4.42	27.34 b	4.24	29.85 b	4.03	29.68	4.55
2012	May, 02	May, 26	27.68 a	5.66	25.13 a	4.68			26.65	5.37

Data represents the means of fruit weight (FW) ± standard deviation (SD) with n = 200 fruits for each crop load. Values with the same letter in the same row are not significant at ($P \geq 0.05$). Low crop load = 25, 20, 20, 15 and 25 fruits. Middle crop load = 50, 40, 40, 30 and 50 fruits for 'Katinka', 'C. Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517' respectively. High crop load is more than 70 fruits per meter for all cultivars except 'Hagangta' it was more than 60 fruit per meter.

4.1.3.3 Non-destructive color monitoring

The effect of crop load levels on the development of fruit skin color during fruit growth and maturation was monitored by Multiplex Fig. 4-11 to 4-15. Regarding the changes in fruit color measured by Multiplex, it was found that Chl index decreased continuously till fruit reached full size point (7 to 10 days befor harvesting in case of early and mid-early cultivars and 15 to 20 days in case of late cultivars). After that, it started to increase slightly. In contrast, Anth index either continuously increased until the same period followed by a stable phase until harvest date (on-tree ripe) or it was slightly decreasing in some cultivars. Flav was decreasing until harvest. These behaviors were found for all cultivars and during all seasons. No remarkable differences among fruit crop load levels were noticed in all cultivars during the three seasons of study. However, changes in the fruit color in low crop load level were earlier than in middle crop load level. There are noticeable differences among seasons in Chl, Flav and Anth indices for all cultivars.

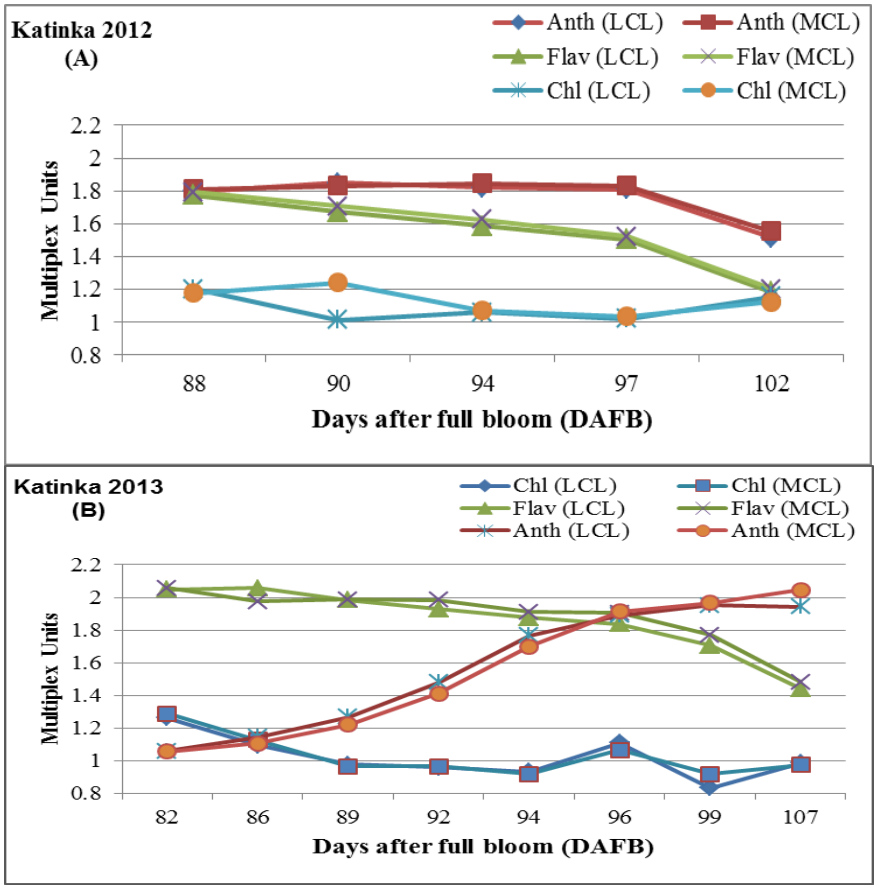


Fig. 4.11: Non-destructive monitoring of skin fruit chlorophyll (Chl), anthocyanins (Anth) and flavonols (Flav) affected by low (LCL, 25 fruit/m) and middle (MCL, 50 fruits/m) crop loads during fruit growth and maturation in 'Katinka' cultivar during 2012 (A) and 2013 (B). n = 150 to 200 fruits for each crop load.

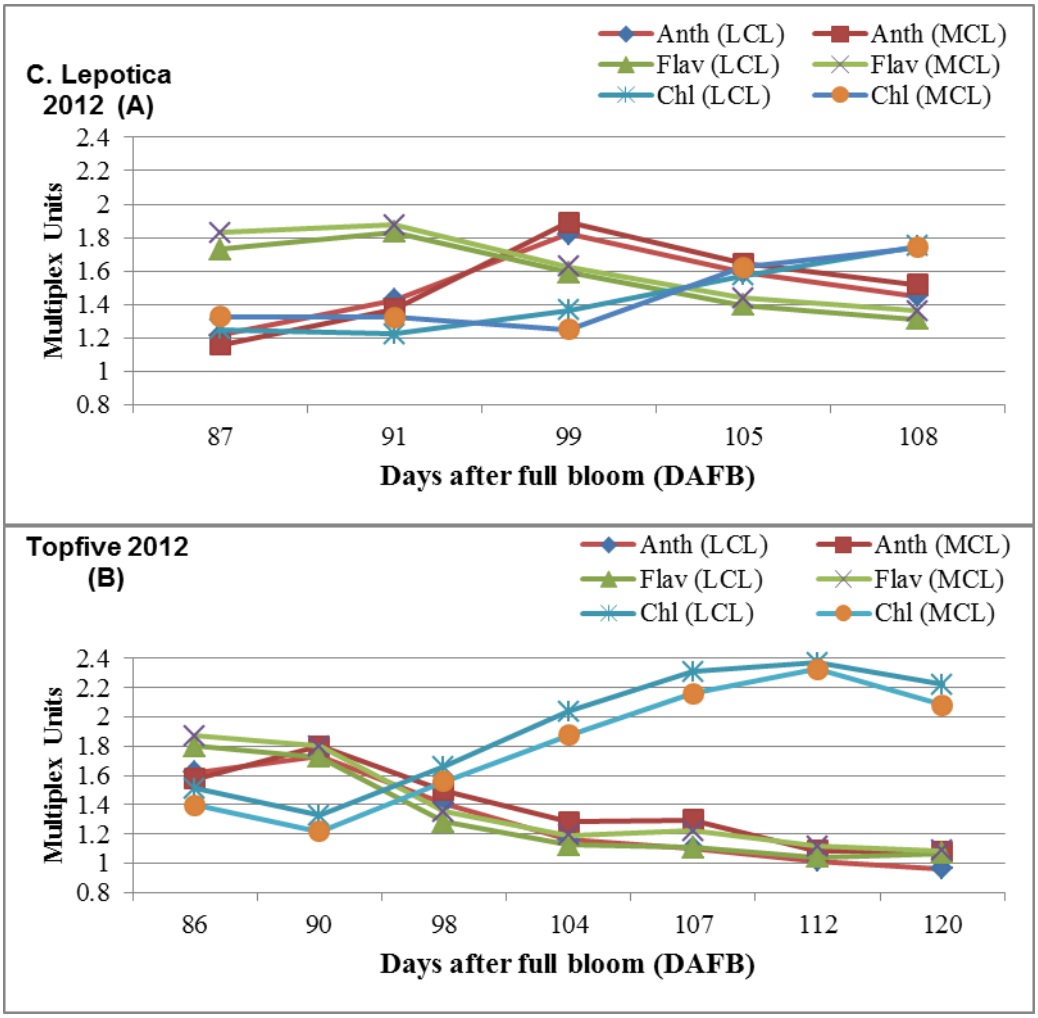


Fig. 4.12: Non-destructive monitoring of skin fruit chlorophyll (Chl), anthocyanins (Anth) and flavonols (Flav) affected by low (LCL, 20 fruit/m) and middle (MCL, 40 fruits/m) crop loads during fruit growth and maturation in 'C. Lepotica' (A) and 'Topfive' (B) cultivars during 2012. n = 150 to 200 fruits for each crop load.

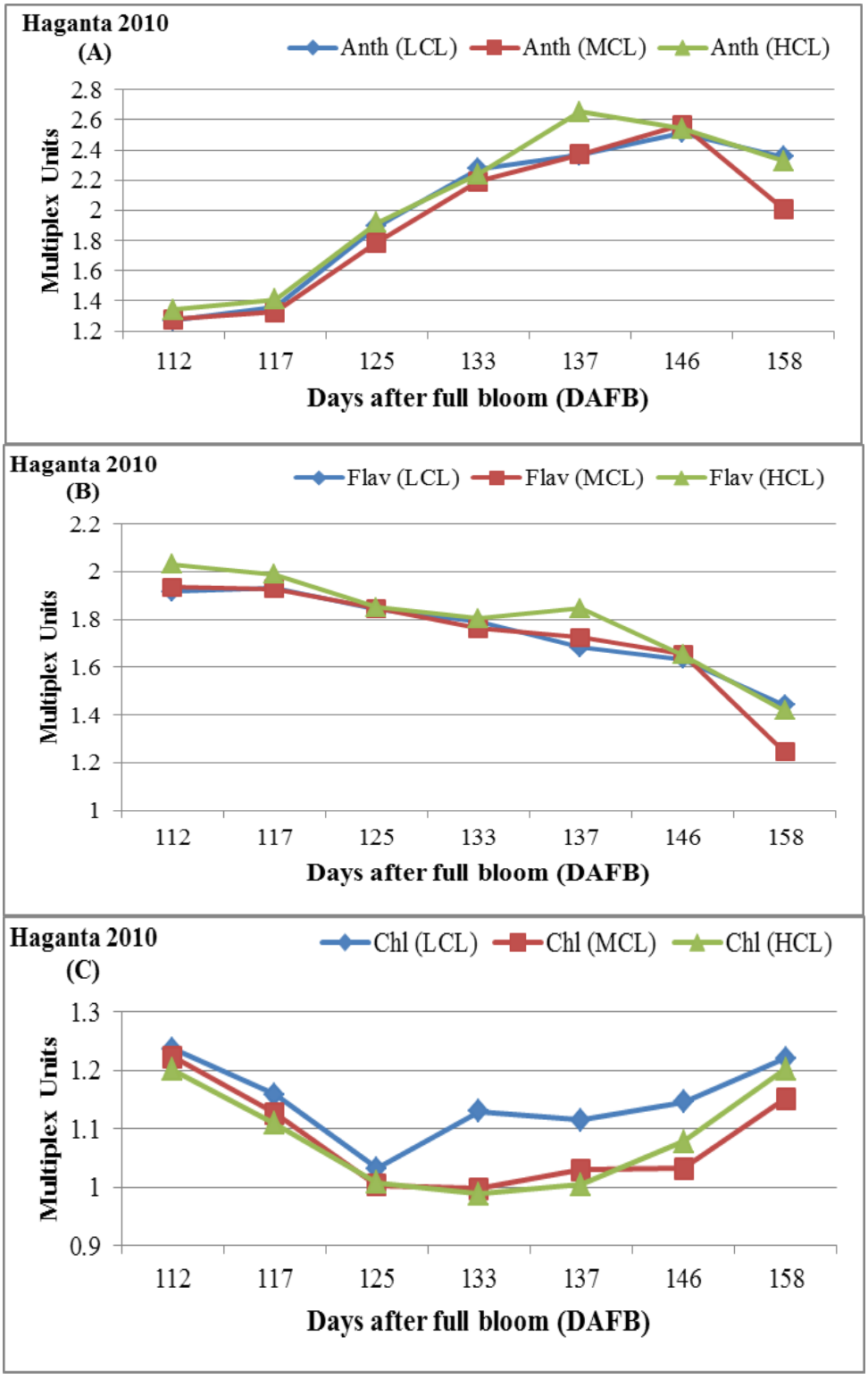


Fig. 4.13: Non-destructive monitoring of skin fruit anthocyanins (Anth) (A), flavonols (Flav) (B) and chlorophyll (Chl) (C), affected by low (LCL, 15 fruits /m), middle (MCL, 30 fruits /m) and high (HCL, more than 65 fruit /m) crop loads during fruit growth and maturation in 'Haganta' cultivar during 2010. n = 150 fruits for each crop load.

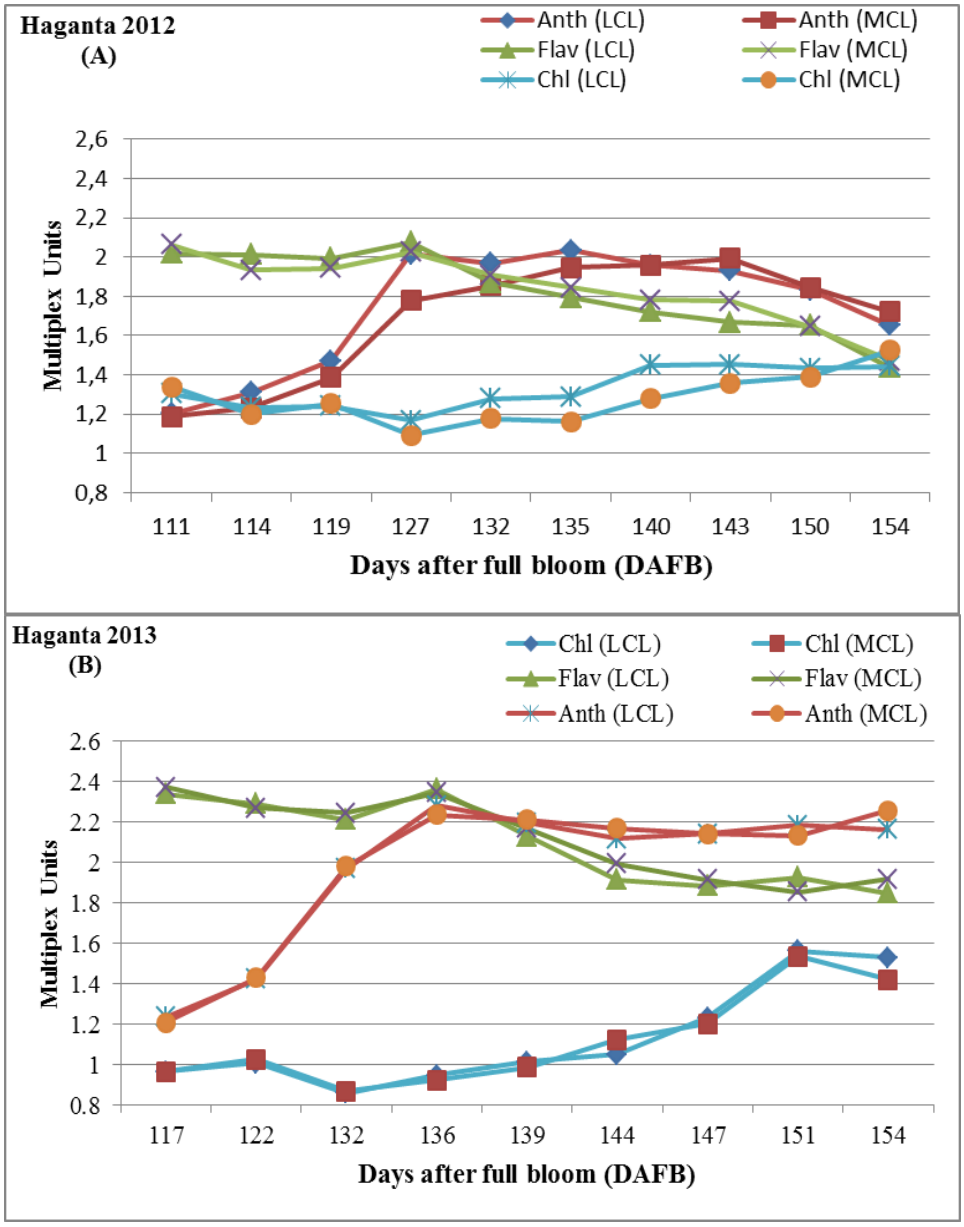


Fig. 4.14: Non-destructive monitoring of skin fruit chlorophyll (Chl), anthocyanins (Anth) and flavonols (Flav) affected by low (LCL, 15 fruit/m) and middle (MCL, 30 fruits/m) crop loads during fruit growth and maturation in 'Haganta' cultivar during 2012 (A) and 2013 (B). n = 150 to 200 fruits for each crop load.

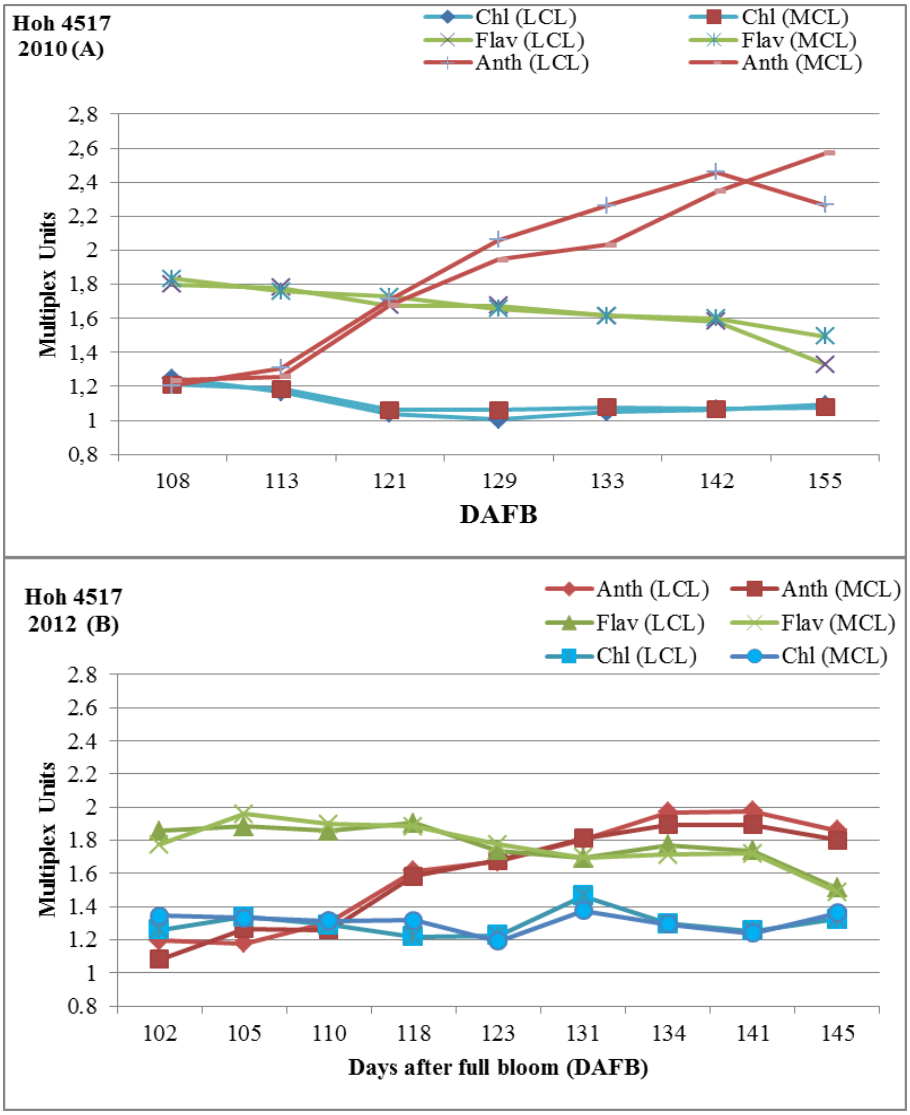


Fig. 4.15: Non-destructive monitoring of skin fruit chlorophyll (Chl), anthocyanin (Anth) and flavonol (Flav) affected by low (LCL, 25 fruit/m) and middle (MCL, 50 fruits/m) crop loads during fruit growth and maturation in 'Hoh 4517' cultivar during 2010 (A) and 2012 (B). n = 150 to 200 fruits for each crop load.

4.1.3.4 Fruit chemical attributes

Soluble solids content (SSC), titratable acidity (TA) and SSC/TA ratio in all cultivars in 2010, 2012 and 2013 seasons were measured in ripening stage as presented in Tab. 4-2, 4-3 and 4-4. The data show the effect of crop load on fruit chemical attributes in 2010 which presented different behaviors for the cultivars. For 'Haganta', 'Topfive', and 'Hoh 4517' fruit thinning had significant effects on SSC, TA and SSC/TA ratio. Significant effects were found in 'Hoh 4517' between low and high crop load levels and between low and medium crop load levels. However, no significant differences were found between medium and high crop load levels. For 'Haganta' and 'Topfive' the differences were between low and high crop load level

and between medium and high crop load levels but without significant effects between low and medium crop load. No significant effects on fruit chemical attributes were found across crop load levels for 'Katinka' and 'C. Lepotica'. An exception is acidity in 'C. Lepotica' cultivar where the high crop load resulted in the lowest acid value and low crop load in the highest. However, data presented in Tab. 4.2, 4.3 and 4.4 shows no significant effects of crop load on fruit chemical attributes in all cultivars in 2012 season. Low crop load produced the highest values in SSC but no clear trend was observed on TA. Similar behavior was noticed for 'Katinka' and 'Haganta' in 2103.

Tab. 4.2: Effect of fruit crop load on fruit soluble solids content (SSC) of 'Katinka'. 'C. Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517' during 2010, 2012 and 2013.

Cultivar/ Season	Date of full bloom	Date of thinning	Crop loads					
			Low		Middle		High	
			SSC	± SD	SSC	± SD	SSC	± SD
'Katinka'								
2010	April 26	May, 20	12.71 a	0.73	11.97 a	1.36	12.68 a	0.60
2012	April, 20	May, 25	12.93 a	0.55	12.94 a	0.47		
2013	April, 28	May, 20	12.53 a	0.52	11.83 a	0.50		
'C. Lepotica'								
2010	April, 26	May, 20	15.60 a	3.26	14.99 a	2.46	16.21 a	3.53
2012	April, 23	May, 25	14.88 a	1.37	14.44 a	0.92		
'Topfive'								
2010	April, 26	May, 20	16.67 a	4.14	15.94 a	4.07	13.77 b	2.20
2012	April, 24	May, 25	21.80 a	1.39	19.23 a	3.00		
'Haganta'								
2010	April, 22	May, 21	15.70 ab	3.53	16.87 a	4.11	15.26 b	3.05
2012	April, 18	May, 26	17.71 a	2.30	17.53 a	1.31		
2013	April, 26	May, 20	14.90 a	0.26	15.47 a	2.46		
'Hoh 4517'								
2010	April, 27	May, 21	20.43 a	3.30	17.18 b	4.21	16.82 b	3.54
2012	May, 02	May, 26	15.93 a	1.01	15.05 a	1.73		

Values represent the mean of three reading of 15–20 fruits for each one ± SD. Values with the same letter in the same row is not significant at ($P \geq 0.05$), $n = 3$

Tab. 4.3: Effect of fruit crop load on fruit titratable acidity (TA) of 'Katinka'. 'C. Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517' during 2010, 2012 and 2013.

Cultivar/ Season	Date of full bloom	Date of thinning	Crop loads					
			Low		Middle		High	
			TA	± SD	TA	± SD	TA	± SD
'Katinka'								
2010	April 26	May, 20	0.58 a	0.07	0.58 a	0.08	0.58 a	0.12
2012	April, 20	May, 25	0.67 a	0.09	0.66 a	0.09		
2013	April, 28	May, 20	0.55 a	0.05	0.51 a	0.02		
'C. Lepotica'								
2010	April, 26	May, 20	1.19 a	0.11	1.08 b	0.11	1.15 c	0.15
2012	April, 23	May, 25	1.18 a	0.22	1.18 a	0.19		
'Topfive'								
2010	April, 26	May, 20	1.46 b	0.13	1.57 a	0.12	1.57 a	0.07
2012	April, 24	May, 25	1.66 a	0.15	1.68 a	0.12		
'Haganta'								
2010	April, 22	May, 21	1.35 a	0.11	1.23 b	0.16	1.28 ab	0.22
2012	April, 18	May, 26	1.61 a	0.16	1.79 a	0.14		
2013	April, 26	May, 20	0.98b	0.20	1.19a	0.01		
'Hoh 4517'								
2010	April, 27	May, 21	1.44 a	0.06	1.43 a	0.10	1.46 a	0.23
2012	May, 02	May, 26	1.56 a	0.04	1.49 a	0.15		

Values represent the mean of three reading of 15–20 fruits for each one ± SD. Values with the same letter in the same row is not significant at ($P \geq 0.05$), $n = 3$.

Tab. 4.4: Effect of fruit crop load on fruit SSC/TA ratio of 'Katinka'. 'C. Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517' during 2010, 2012 and 2013.

Cultivar/ Season	Date of full bloom	Date of thinning	Crop loads					
			Low		Middle		High	
			SSC/TA	± SD	SSC/TA	± SD	SSC/TA	± SD
'Katinka'								
2010	April 26	May, 20	22.20 a	3.44	20.23 a	3.76	22.77 a	4.78
2012	April, 20	May, 25	19.59 a	2.39	19.85 a	2.74		
2013	April, 28	May, 20	23.13 a	2.60	23.14 a	1.79		
'C. Lepotica'								
2010	April, 26	May, 20	14.35 a	2.83	14.63 a	2.84	14.88 a	3.50
2012	April, 23	May, 25	13.03 a	2.74	12.56 a	2.28		
'Topfive'								
2010	April, 26	May, 20	12.81 a	2.91	11.30 b	2.99	9.26 c	1.45
2012	April, 24	May, 25	13.19 a	1.32	11.40 a	1.38		
'Haganta'								
2010	April, 22	May, 21	12.64 b	3.54	15.12 a	3.97	12.84 b	4.16
2012	April, 18	May, 26	10.66 a	1.84	9.78 a	0.09		
2013	April, 26	May, 20	15.75 a	3.54	13.07 b	2.49		
'Hoh 4517'								
2010	April, 27	May, 21	14.72 a	1.76	12.93 b	2.81	12.75 b	4.04
2012	May, 02	May, 26	10.17 a	0.83	10.22 a	1.97		

Values represent the mean of three reading of 15–20 fruits for each one ± SD. Values with the same letter in the same row is not significant at ($P \geq 0.05$), $n = 3$.

4.1.4 Effect of rootstocks on fruit quality parameters:

4.1.4.1 Fruit size

The effect of rootstocks on fruit quality parameters was studied in 2012 and 2013 (Fig. 4-16 to 4-18). Remarkable effects of rootstocks on fruit size of plum cultivars were found. It was observed that the changes in fruit size of all cultivars grafted on different rootstocks from the beginning of measurements till harvesting were exhibiting the classical double sigmoid curve. Rootstocks significantly influenced fruit size in both seasons 2012 and 2013. Most of the cultivars (‘C. Lepotica’, ‘Topfive’ and ‘Hoh 4517’ in 2012 and ‘Katinka’ in 2013) produced the largest fruit sizes on Myrobalan rootstock compared to other rootstocks. Contrary, ‘Katinka’ (in 2012) and ‘Haganta’ (in both seasons) produced smallest fruits on the Myrobalan rootstock while cultivars (‘C. Lepotica’, ‘Topfive’, ‘Katinka’ and ‘Hoh 4517’ in 2012 and ‘Katinka’ in 2013) produced the smallest fruits on ‘Wavit’ and ‘Wangenheims’ rootstocks. The other rootstocks ‘Ishtara’, ‘Fereley’ and ‘GF’ were in between of Myrobalan and ‘Wangenheims’ and ‘Wavit’ regarding their influence on fruit size of the grafted cultivar.

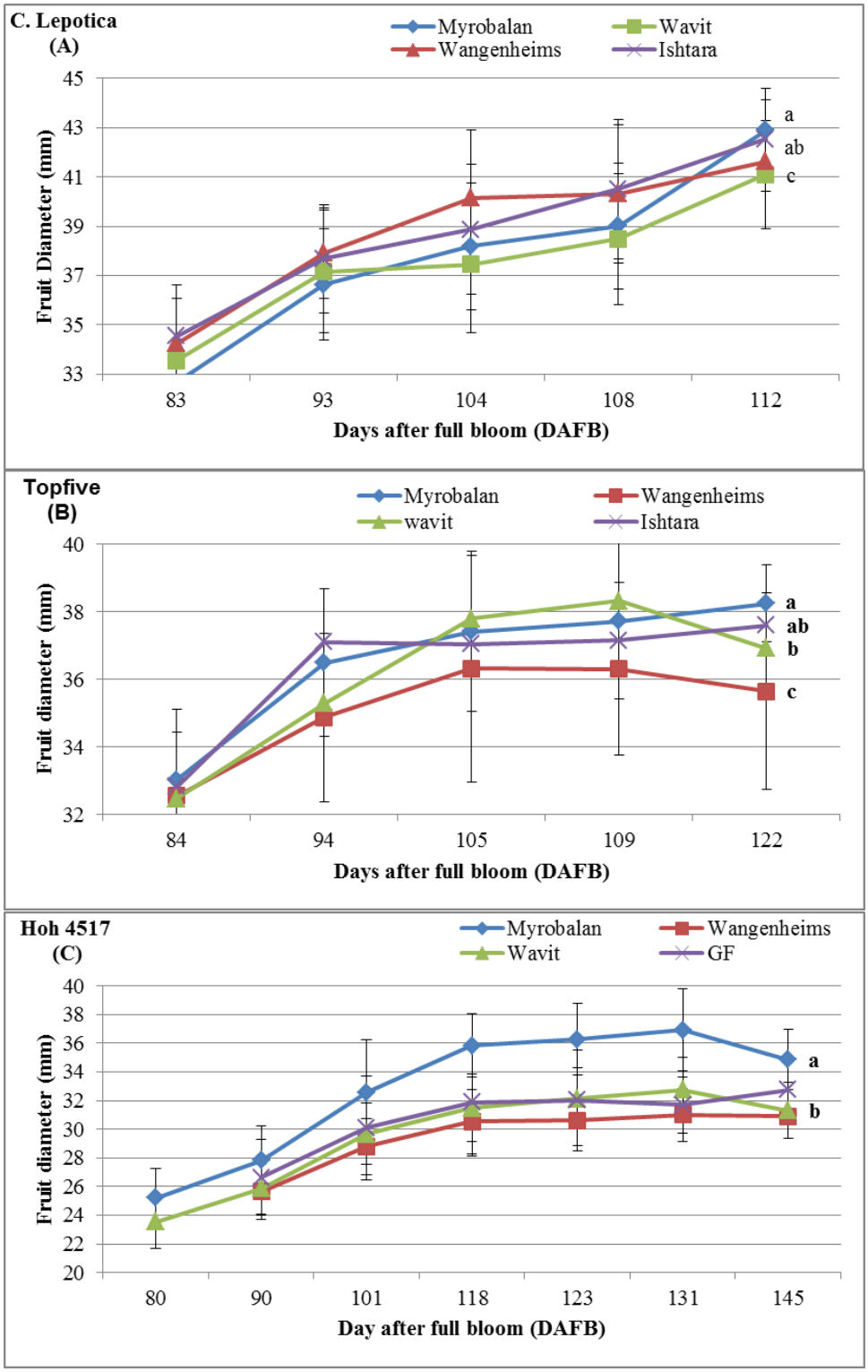


Fig. 4.16: Effect of rootstocks on fruit diameter (mm) of 'C. Lepotica' (A), 'Topfive' (B) and 'Hoh 4517' (C) cultivars during 2012. Values represent the means of fruit diameter ± standard deviation (SD) with n= 45 to 60 fruits for each combination cultivar/rootstock. The differences in fruit diameters are not significant at ($P \geq 0.05$) at harvest with curves followed with the same letter.

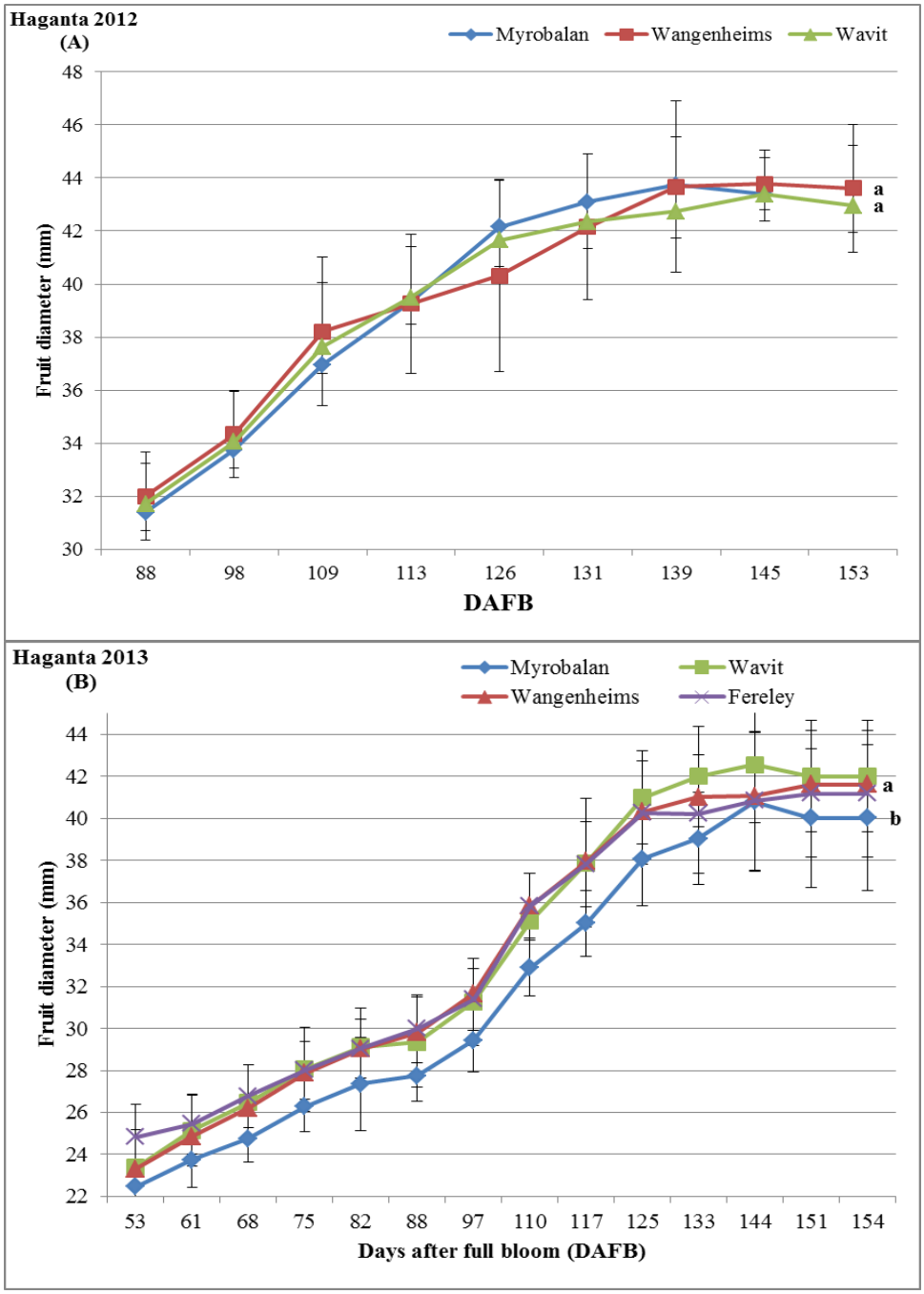


Fig. 4.17: Effect of rootstocks on fruit diameter (mm) of 'Haganta' during 2012 (A) and 2013' (B) Values represent the means of fruit diameter ± standard deviation (SD) with n= 45 to 60 fruits for each rootstock. The differences in fruit diameters are not significant at ($P \geq 0.05$) at harvest with curves followed with the same letter.

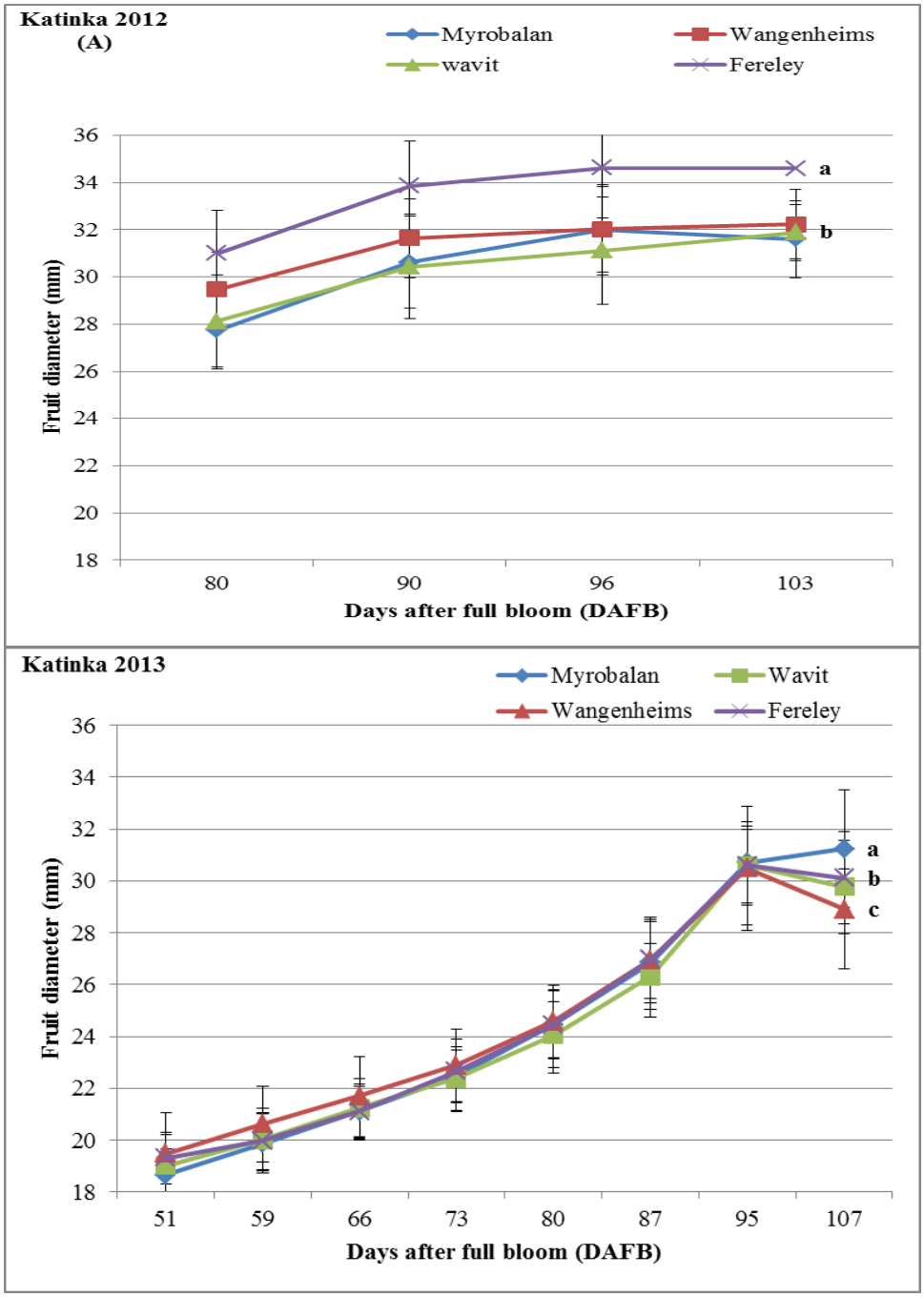


Fig. 4.18: Effect of rootstocks on fruit diameter (mm) of 'Katinka' during 2012 (A) and 2013 (B). Values represent the means of fruit diameter ± standard deviation (SD) with n= 45 to 60 fruits for each rootstock. The differences in fruit diameters are not significant at ($P \geq 0.05$) at harvest with curves followed with the same letter.

4.1.4.2 Fruit weight

The effect of rootstocks on fruit weight (g) of plum cultivars in 2012 and 2013.is shown in Tab. 4-5. Reported data show that ‘C. Lepotica’ and ‘Hoh 4517’ grafted onto Myrobalan exhibited the highest values of fruit weight. Fruits of cultivar ‘C. Lepotica’ on ‘Wavit’, ‘Ishtara’ and ‘Wangenheims’ weigh less in decreasing order . As for ‘Hoh 4517’ breeding clone, the order is ‘Wavi’t, ‘Fereley’ and ‘Wangenheims’. The significant differences were found only between the weights of fruit produced by cultivars on Myrobalan and on other rootstocks but no significant differences were found in fruit weights produced by cultivars on the rest of rootstocks other than Myrobalan. In contrast, the significant lowest fruit weight produced by ‘Haganta’ was on Myrobalan and the highest was produced on Wangenheims. The differences were not significant between between fruit weights produced by ‘Haganta’ on Wangenheims and Wavit’. ‘Katinka’ produced the highest fruit weight on ‘Fereley’ followed by ‘GF’, ‘Wangenheims’, Myrobalan and ‘Wavit’. Significant effects were due to ‘Fereley’ and ‘Wavit’ rootstocks. In 2013 season, ‘Katinka’ and ‘Haganta’ produced the highest fruit weight on ‘Fereley’ and ‘Wangenheims’ respectivly. On the other hand, the lowest fruit weight was produced by both of them on ‘Wavit’ and ‘Myrobalan’. The differences in fruit weight were not significant in 2013 season regarding the effect of rootstock

Tab. 4.5: Influence of different plum rootstocks on fruit weight (g) of 'Katinka', 'C. Lepotica', 'Haganta' and 'Hoh 4517' plum cultivars during 2012 and 2013.

Cultivar/ season		Plum rootstocks											
		Myrobalan		Wavit		Wangenheims		Fereley		GF		Ishtara	
		FW	± SD	FW	± SD	FW	± SD	FW	± SD	FW	± SD	FW	± SD
'Katinka'	2012	25.17 ab	1.54	23.06 b	2.87	26.65ab	0.28	27.78 a	1.04	27.08ab	1.72	–	
	2013	26.25 a	2.25	26.15 a	4.00	27.89 a	5.90	28.25 a	3.75				
'C. Lepotica'	2012	47.70 a	2.75	47.07 ab	3.13	44.92 b	1.31	–		–		45.23 ab	1.92
'Haganta'	2012	53.73 a	3.99	56.07 a	3.73	58.35 a	5.87	–		–		–	
	2013	43.19 a	8.50	50.59 a	5.40	49.48 a	4.90	48.70 a	8.10				
'Hoh 4517'	2012	33.41 a	4.36	25.29 b	2.05	22.54 b	1.66	25.02 b	5.55	–			

Data represent the mean of fruit weight (FW) ± SD. Values with the same letter in the same row in the same is not significant at ($P \geq 0.05$).

4.1.4.3 Fruit chemical attributes

The effects of rootstocks on the fruit chemical attributes soluble solids content (SSC), titritable acidity (TA) and SSC/TA ratio in season 2012 and 2013 are shown in Tab. (4-6 and 4-7) respectively. Regarding SSC (°Brix), fruits of 'C. Lepotica', 'Katinka' and 'Topfive' showed the highest values on Myrobalan rootstock in 2012. The differences were significant for 'Katinka' except on Myrobalan and 'Fereley' rootstocks and for 'Topfive' between Myrobalan and 'Wavit'. On the other side, 'Haganta' and 'Hoh 4517' showed the highest SSC values on 'Wangenheims' and 'Wavit', respectively.However, the differences were not significant among rootstocks. The lowest values of SSC in 2012 season were induced by 'C. Lepotica' on 'Wavit', 'Katinka' 'Topfive', and 'Hoh 4517'on 'Wangenheims', and 'Haganta' on Myrobalan. In season 2013in which only 'Katinka' and 'Haganta' cultivars were included, the highest SSC values for both cultivars were induced on 'Wangenheims'. No significant differences were found across regarding the effect of the rootstocks with 'Katinka' cultivar. On the other hand, we found significant differences were observed for 'Haganta' on 'Wangenheims' and 'Fereley' rootstocks in SSC values.

Regarding TA (malic acid g/100ml), no significant differences were found for all cultivars on different rootstocks in 2012 season except 'Topfive' cultivar which produced the highest TA value on 'GF' and 'Wavit'. The highest values were produced by 'C. Lepotica' and 'Haganta' on 'Wangenheims' and 'Hoh 4517' and 'Katinka' on 'Fereley'. On the other hand, the lowest TA values were induced by 'C. Lepotica'and 'Haganta' on Myrobalan, 'Katinka' and 'Topfive' on 'Wangenheims', and 'Hoh 4517' on Myrobalan and 'Wavit'. In 2013 season, no significant differences were found between both cultivars on all rootstocks. The highest values were produced by both of cultivars on 'Fereley', and the lowest values were produced on 'Wavit'.

Regarding SSC/TA ratio, the highest value was produced by 'C. Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517' in 2012 and 'Katinka' in 2013 on Myrobalan. The highest values for 'Katinka' were on 'Wangenheims' and 'Wavit' in 2012 and 'Haganta' in 2013 season. The lowest ratios were produced by 'Katinka' on 'Fereley' in both seasons (2012 and 2013) and 'Hoh 4517' in 2012 season and 'Haganta' in 2013 but the lowest ratios in 'C. Lepotica', 'Haganta' (in 2012) and 'Topfive' were induced by 'Wavit', 'Wangenheims' and 'GF' respectively. However, significant differences in SSC/TA ratio regarding rootstocks were found only in 'C. Lepotica'and 'Topfive' in 2012 and 'Haganta' in 2013.

Tab. 4.6: Influence of different rootstocks on fruit soluble solids content (SSC), titrable acidity (TA) and SSC/TA ratio of plum varieties during 2012.

Cultivar/	SSC (Brix %)		Acidity (TA %)		SSC/TA Ratio	
Rootstock	Mean	± SD	Mean	± SD	Mean	± SD
'C. Lepotica'	14.64	1.13	0.88	0.15	17.13	3.25
Myrobalan	15.21 a	0.43	0.81 a	0.18	19.42 a	4.08
Wavit	13.94 b	0.71	0.91 a	0.15	15.69 b	3.05
Wangenheims	14.54 ab	1.35	0.92 a	0.19	16.40 ab	4.33
Ishtara	15.03 ab	1.52	0.86 a	0.12	17.59 a	1.02
'Haganta'	17.65	1.93	1.25	0.13	13.85	1.96
Myrobalan	17.10 a	1.74	1.20 a	0.15	14.50 a	3.47
Wangenheims	19.15 a	2.44	1.35 a	0.12	13.23 a	0.13
Wavit	16.70 a	0.87	1.23 a	0.11	13.61 a	0.57
'Hoh 4517'	15.44	1.39	1.14	0.08	13.73	1.99
Myrobalan	16.10 a	1.81	1.11 a	0.04	14.90 a	2.36
Wavit	16.20 a	0.75	1.11 a	0.09	14.63 a	1.17
Wangenheims	14.55 a	1.12	1.15 a	0.12	12.85 a	2.35
Fereley	14.74 a	1.27	1.18 a	0.05	12.52 a	1.56
'Katinka'	12.93	0.49	0.50	0.07	26.45	3.34
Myrobalan	13.53 a	0.22	0.50 a	0.05	27.05 a	2.43
Wavit	12.60 b	0.24	0.47 a	0.08	27.38 a	4.90
Wangenheims	12.50 b	0.36	0.45 a	0.06	28.28 a	2.61
Fereley	13.25 a	0.21	0.56 a	0.03	23.53 a	0.78
GF	12.75 b	0.21	0.54 a	0.05	23.56 a	1.97
'Topfive'	20.86	2.35	1.25	0.10	16.81	2.10
Myrobalan	22.92 a	0.85	1.21 b	0.10	18.95 a	1.28
Wavit	22.57 a	0.11	1.37 a	0.04	16.52 ab	0.34
Wangenheims	19.04 b	2.16	1.19 b	0.06	16.06 b	2.20
GF	20.38 ab		1.38 a		14.73 b	

Data represents the mean ± SD, for each cultivar, means followed by the same letter in each column are not significant at $p \geq 0.05$ according to Duncan's Multiple Rang Test**.**

Tab. 4.7: Effect of rootstocks on fruit soluble solids content (SSC), titratable acidity (TA) and SSC/TA ratio for 'Katinka' (A) and 'Haganta' (B) during 2013.

Cultivar/	SSC (Brix %)		Acidity (TA %)		SSC/TA Ratio	
Rootstock	Mean	SD	Mean	SD	Mean	SD
'Katinka'						
Myrobalan	12.51 a	0.57	0.51 a	0,00	24.34 a	1.06
Wavit	11.83 a	0.61	0.50 a	0.01	23.47 ab	1.72
Wangenheims	12.59 a	0.17	0.53 a	0.05	23.65 ab	1.81
Fereley	11.78 a	0.08	0.56 a	0.03	21.00 b	1.02
'Haganta'						
Myrobalan	14.71 ab	0.43	1.01 a	0.09	14.74 ab	1.64
Wavit	15.17 ab	0.26	0.98 a	0.24	16.08 a	3.94
Wangenheims	16.72 a	2.15	1.17 a	0.00	14.32 ab	1.90
Fereley	14.04 b	0.93	1.20 a	0.10	11.85 b	1.77

Data represents the mean ± SD, for each cultivar, means followed by the same letter in each column are not significant at $p \geq 0.05$ according to Duncan's Multiple Rang Test**.**

4.1.4.4 Color development

The influence of rootstocks on plum fruit color development during fruit growth and maturation were detected by a hand-held non-destructive tool (Multiplex) (Fig. 4-19 to 27). Regarding chlorophyll (Chl), the highest values for all cultivars were produced on Myrobalan rootstock in 2012 season except for ‘Katinka’ which produced the highest value on ‘Fereley’ as well as ‘Katinka’ in 2013 season. For ‘Haganta’ in 2013, the highest Chl value was induced by ‘Wavit’ and ‘Wangenheims’ rootstocks. The lowest Chl values were produced by cultivars on ‘Wangenheims’ followed by ‘Wavit’ in all cultivars except ‘Katinka’ in both seasons and by ‘Haganta’ in 2013 season. In contrast, anthocyanins (Anth) values were highest in all cultivars grafted onto ‘Wangenheims’ followed by ‘Wavit’ for both seasons excluding ‘Haganta’ in 2013 season which produced the highest value on ‘Fereley’ rootstock. Similar to Anth, the content of flavonols (Flav) was influenced by rootstocks with the highest values produced by cultivars on ‘Wangenheims’ and the lowest on Myrobalan.

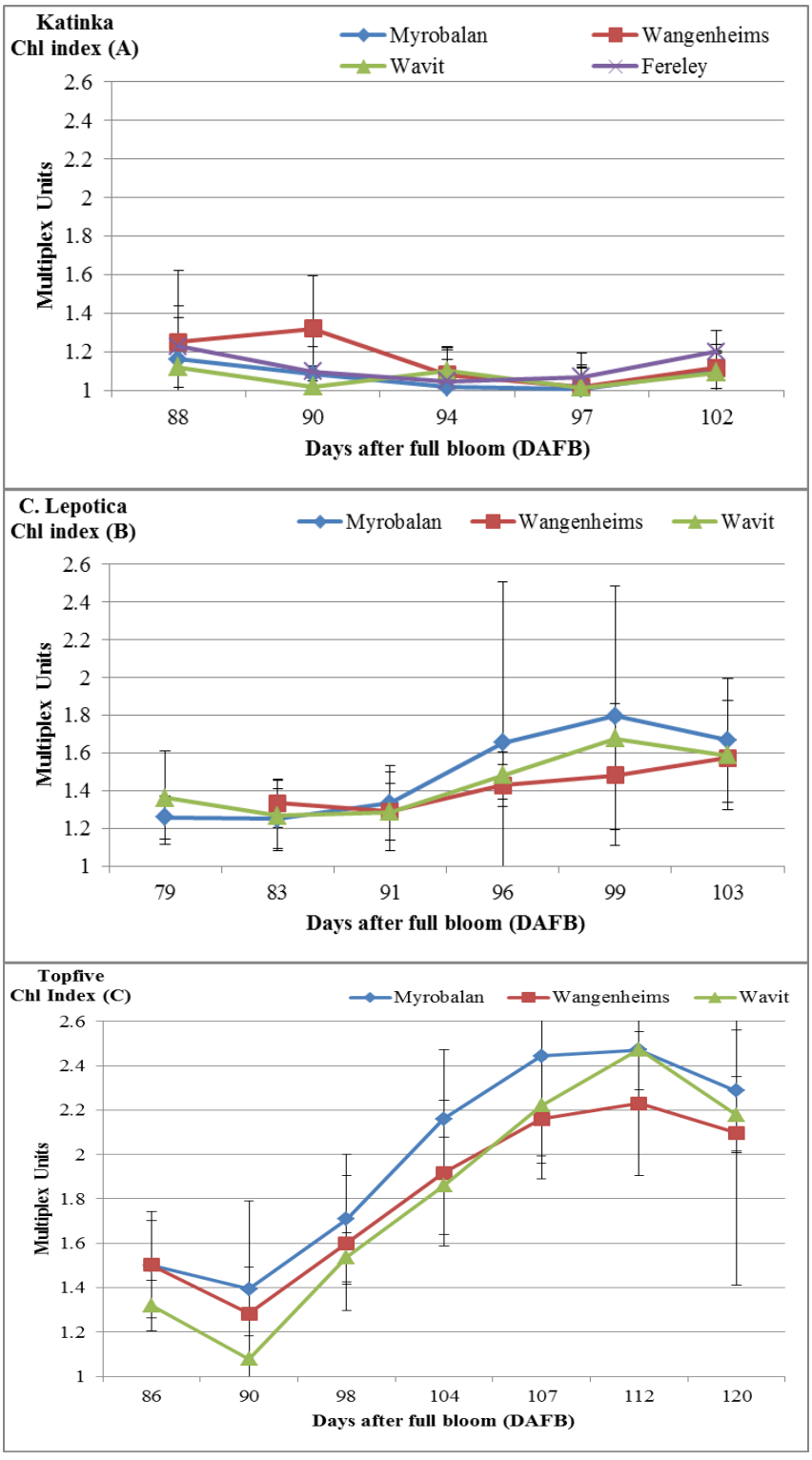


Fig. 4.19: Effect of rootstocks on 'Katinka' (A), 'C. Lepotica' (B) and 'Topfive' (C) fruit chlorophyll (Chl) concentration during fruit growth and maturation in 2012. No = 45 to 60 fruits for each rootstock.. Values are the means ± SD.

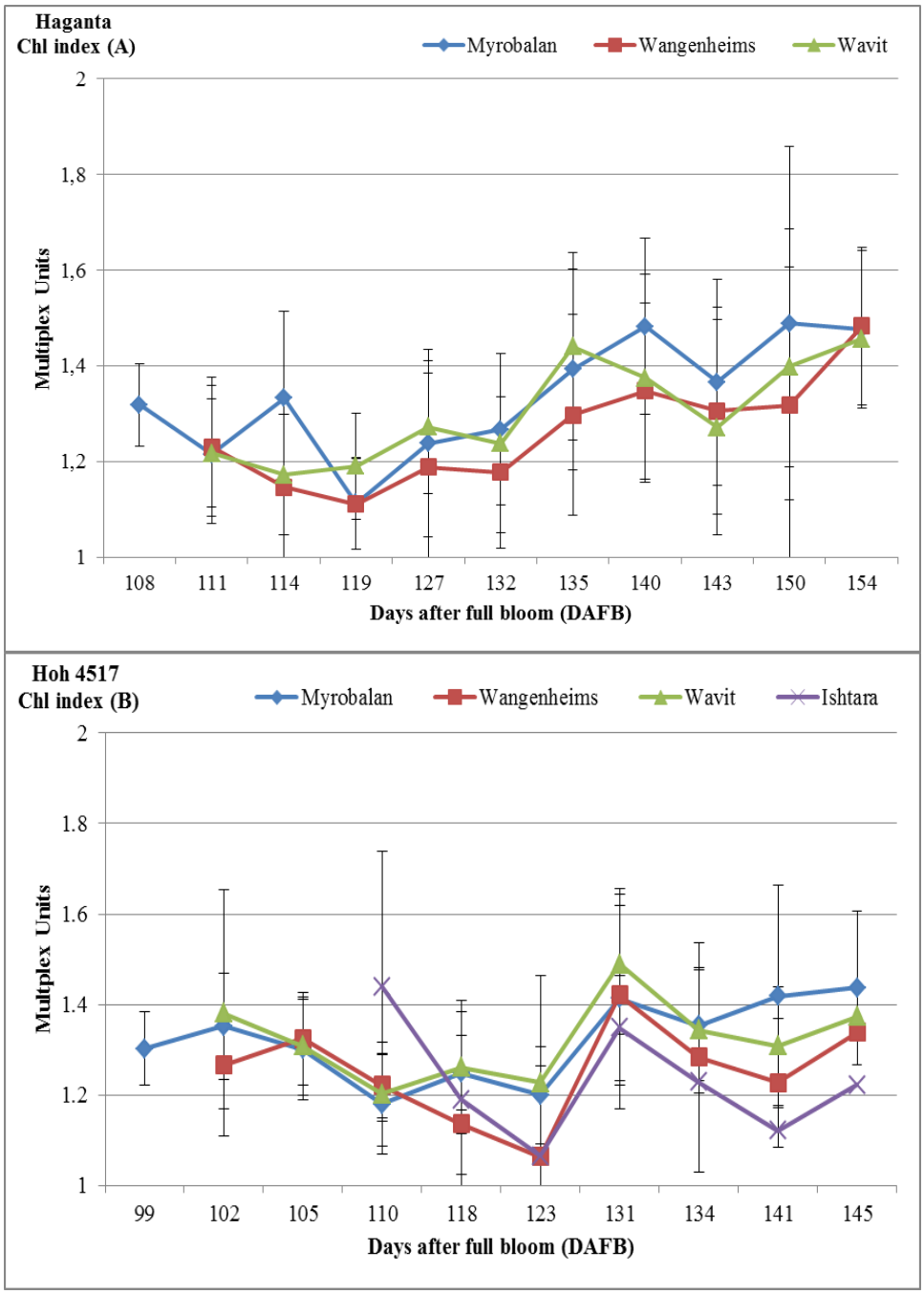


Fig. 4.20: Effect of rootstocks on 'Haganta' (A) and 'Hoh 4517' (B) fruit chlorophyll (Chl) concentration during fruit growth and maturation in 2012. No = 45 to 60 fruits for each rootstock. Values are the means ± SD.

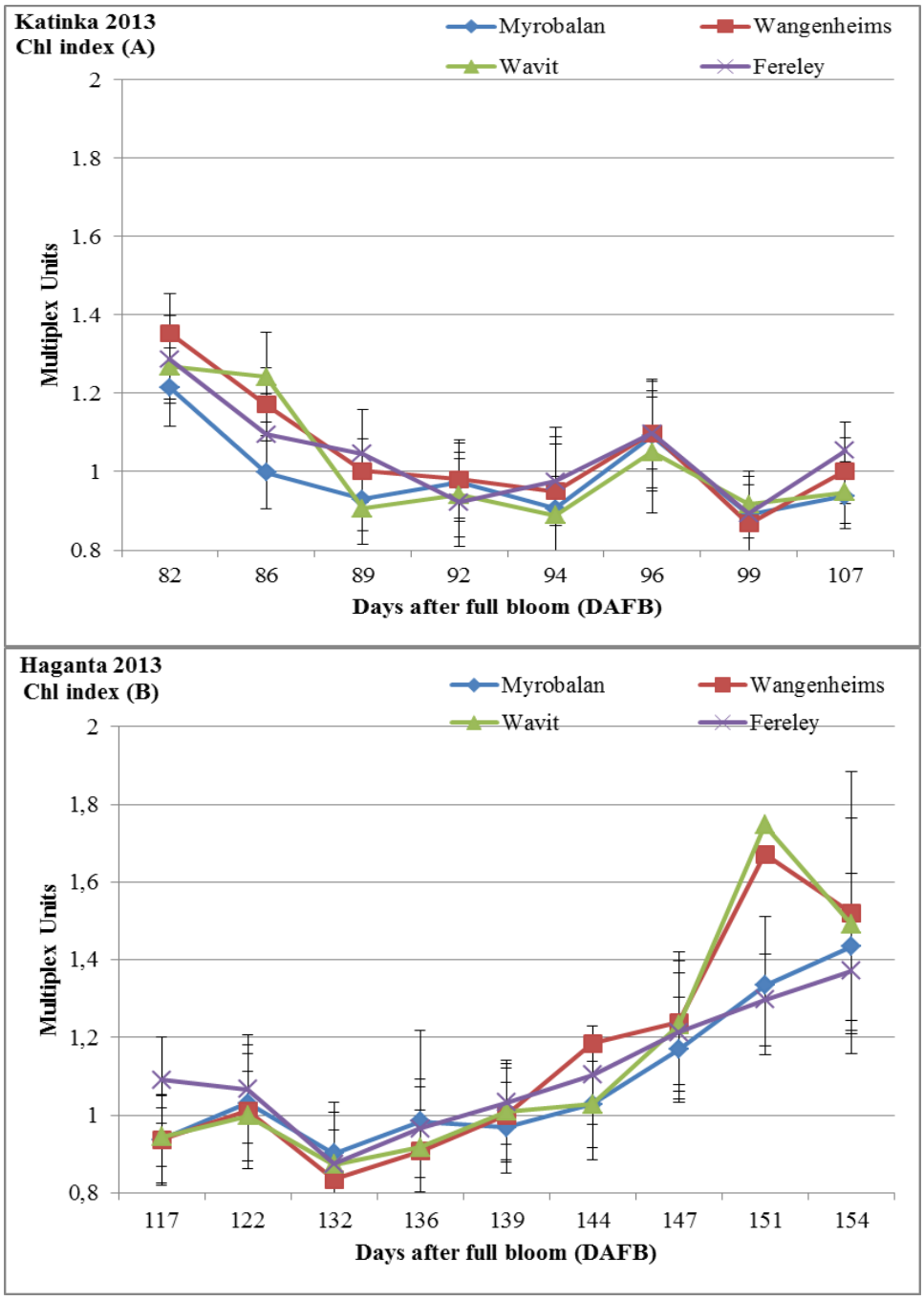


Fig. 4.21: Effect of rootstocks on 'Katinka' (A) and 'Haganta' (B) fruit chlorophyll (Chl) concentration during fruit growth and maturation in 2013. No = 45 to 60 fruits for each rootstock. Values are the means ± SD.

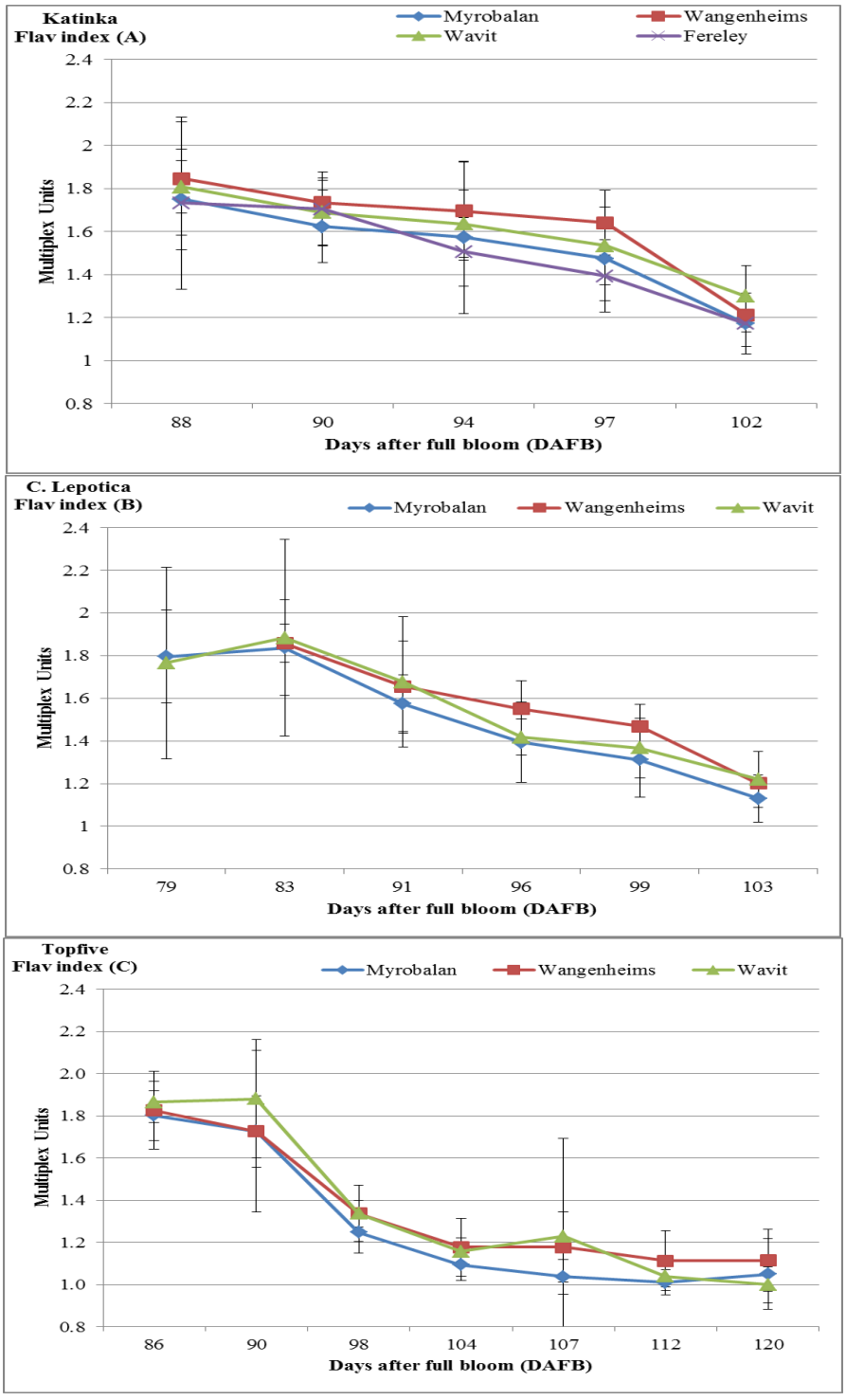


Fig. 4.22: Effect of rootstocks on 'Katinka' (A), 'C. Lepotica'(B) and 'Topfive' (C) fruit Flavonols (Flav) concentration during fruit growth and maturation in 2012. No = 45 to 60 fruits for each rootstock. Values are the means ± SD.

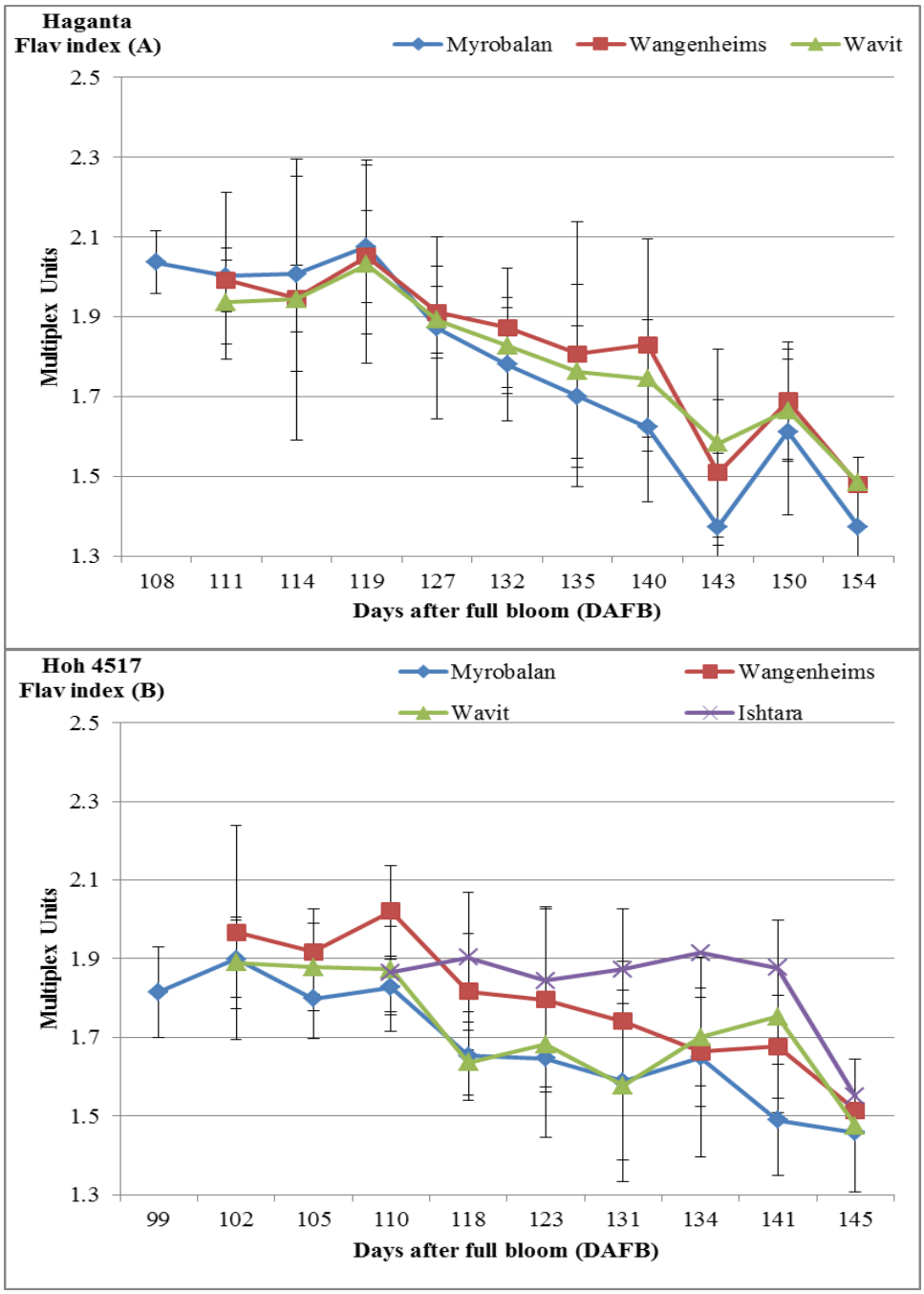


Fig. 4.23: Effect of rootstocks on 'Haganta' (A) and 'Hoh 4517' (B) fruit chlorophyll (Chl) development during fruit growth and maturation in 2012. No = 45 to 60 fruits for each rootstock. Values are the means ± SD.

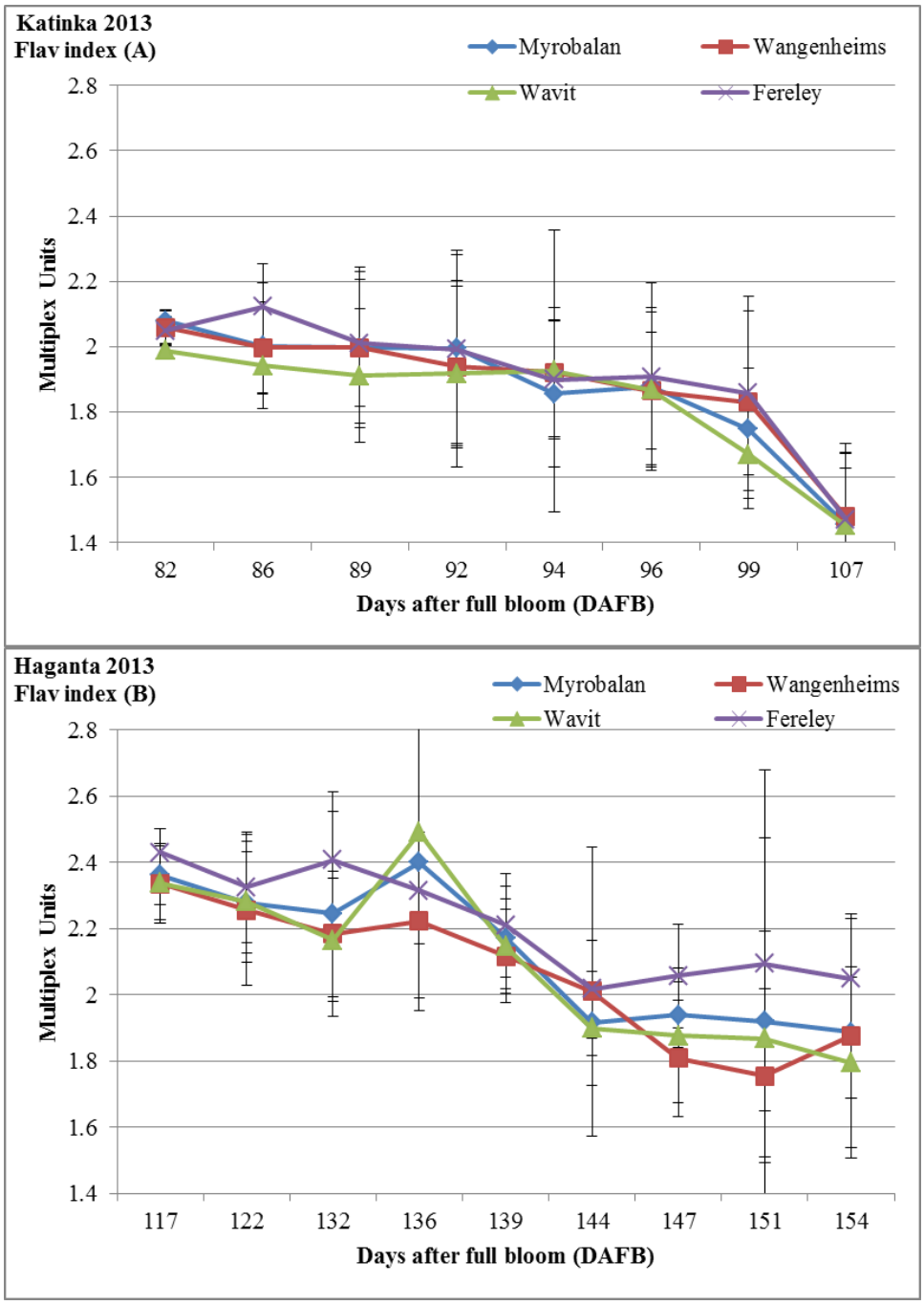


Fig. 4.24: Effect of rootstocks on 'Katinka' (A) and 'Haganta' (B) fruit flavonols (Flav) concentration during fruit growth and maturation in 2013. No = 45 to 60 fruits for each rootstock. Values are the means ± SD.

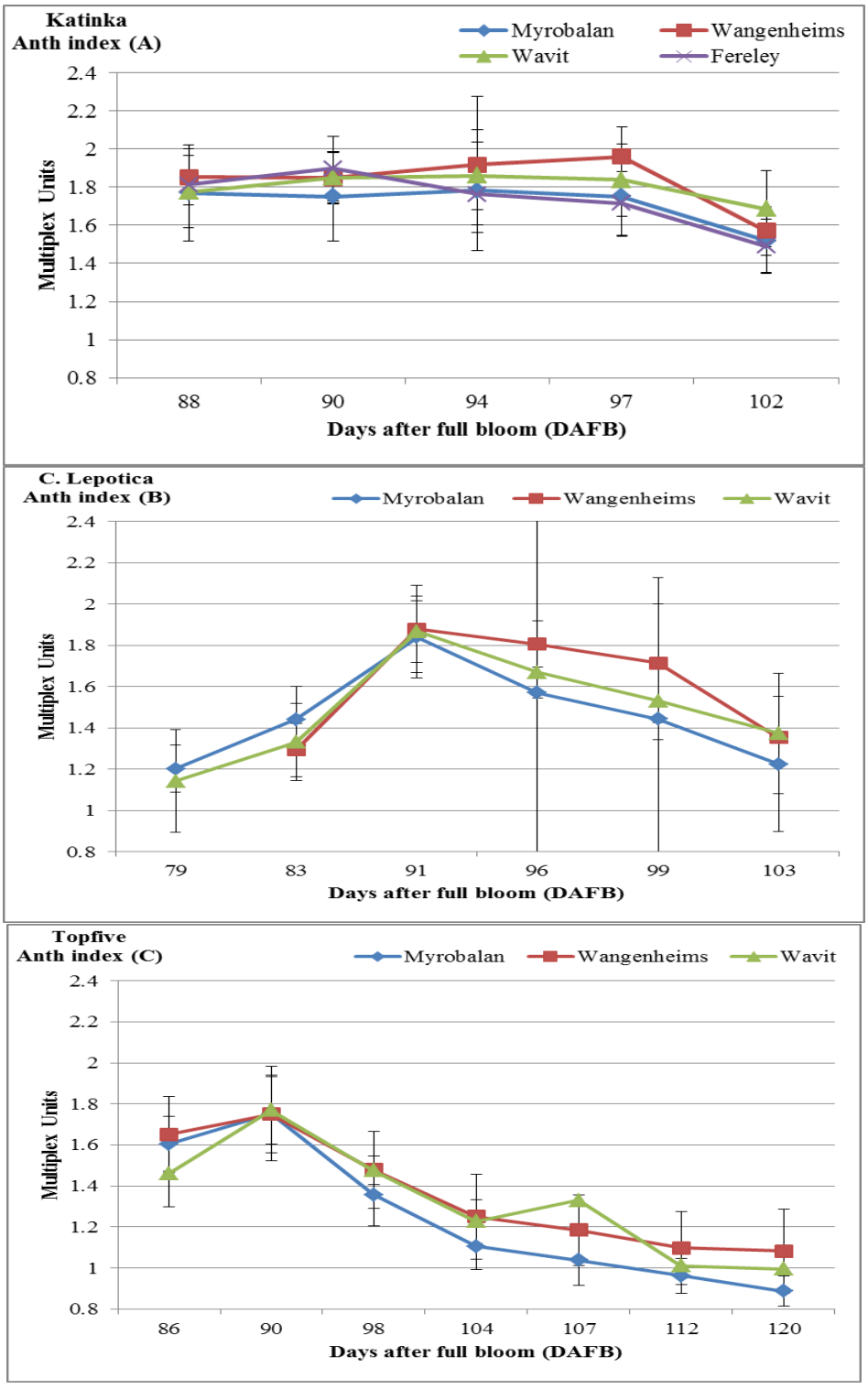
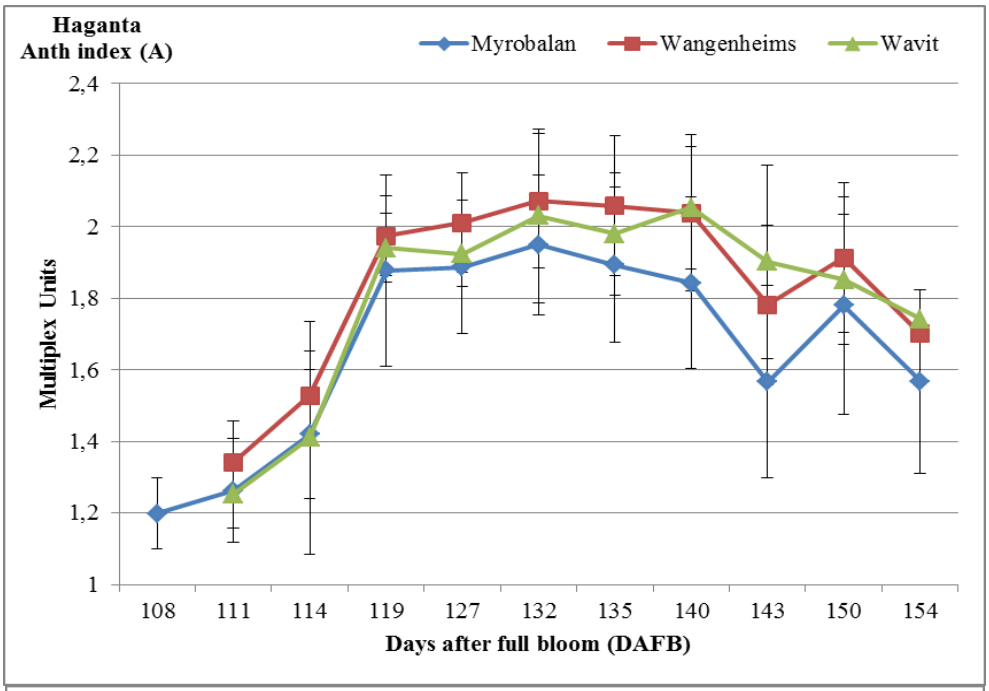


Fig. 4.25: Effect of rootstocks on 'Katinka' (A), 'C. Lepotica'(B) and 'Topfive' (C) fruit anthocyanins (Anth) concentration during fruit growth and maturation in 2012. No = 45 to 60 fruits for each rootstock. Values are the means ± SD.



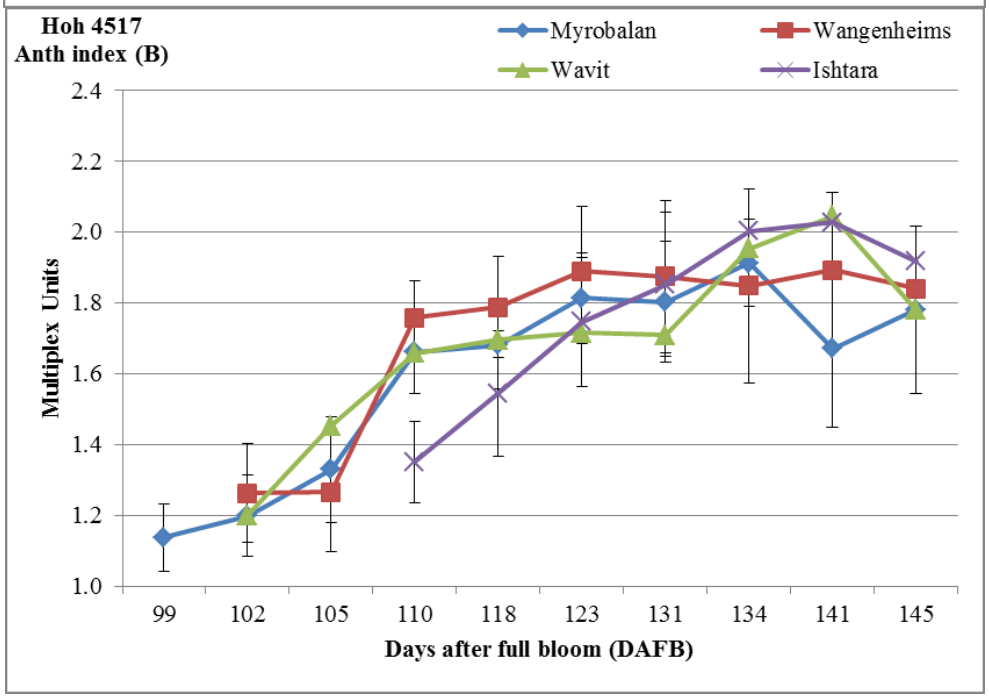


Fig. 4.26: Effect of rootstocks on 'Haganta' (A) and 'Hoh 4517'(B) fruit anthocyanins (Anth) concentration during fruit growth and maturation in 2012. No = 45 to 60 fruits for each rootstock. Values are the means ± SD.

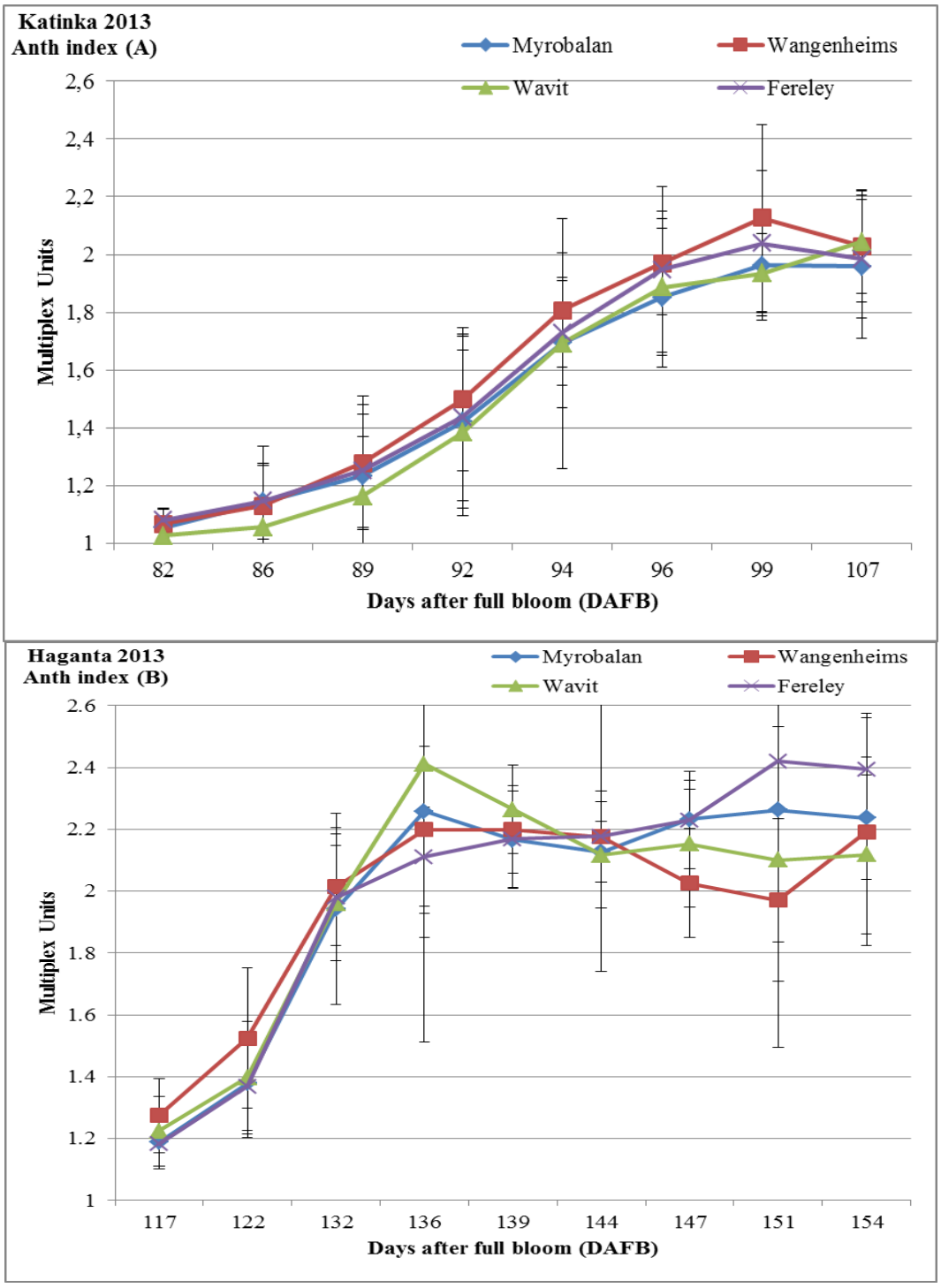


Fig. 4.27: Effect of rootstocks on 'Katinka' (A) and 'Haganta' (B) fruit anthocyanins (Anth) concentration during fruit growth and maturation in 2013. No = 45 to 60 fruits for each rootstock. Values are the means ± SD.

4.2 Effect of leaf/fruit ratio on physical and chemical attributes of plum

4.2.1 Final fruit set and fruit physical attributes

Data presented in Tab. (4.8) show the effect of leaf/fruit ratio (LFR) on physical fruit attributes. LFR has significant effect on fruit weight. If LFR has high, high fruit weight was found in all cultivars except cultivar ‘Haganta’ for which a low LFR but high fruit weight was measured**.** Final fruit set (FFS) was significantly affected by LFR with low LFR and high FFS for ‘Katinka’ and ‘Topfive’ but high FFS and high LFR in ‘Haganta’ cultivar. However, no significant effect of LFR on FFS was found in ‘C. Lepotica’. No significant effect of LFR was found on fruit diameter and length in ‘C. Lepotica’ and ‘Topfive’. In contrast, high LFR has significantly increased fruit length of ‘Katinka’ contrary to ‘Haganta’ which had low LFR but higher fruit length and diameter.

4.2.2 Fruit chemical attributes

The results in Fig. 4-28 show the effect of LFR on SSC, TA and SSC/TA ratio. There are no significant effects found on fruit soluble solids content (SSC), total acidity (TA) and SSC/TA ratio of ‘C. Lepotica’, ‘Haganta’ and ‘Hoh 4517’ cultivars. On the other hand, ‘Topfive’ showed significant effect of LFR on SSC and SSC/TA ratio but no significant one on TA.

Tab. 4.8: Average of primary fruit number, final fruit number, final fruit set, fruit diameter, fruit length and fruit weight of 'C. Lepotica', 'Haganta', 'Katinka' and 'Topfive' plum cultivars in two level of shoot length (Low and High).

Cultivar /	Prim. fr. no.		Final fr. no.		Final fr. set		Fr. diameter		Fr. length		Fr. weight	
Shoot length	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
'C. Lepotica'												
high	8.55	2.38	5.18	1.40	64.82a	24.45	35.29 a	3.46	40.73 a	3.29	31.30 a	6.33
low	9.85	2.30	5.25	1.54	52.70 a	25.65	35.26 a	3.52	40.48 a	3.53	28.92 b	6.80
'Haganta'												
high	7.71	2.05	5.43	2.71	69.82 a	28.01	38.65 b	3.62	47.76 a	3.42	42.18 b	8.15
low	8.38	1.04	3.62	1.56	43.32 b	19.05	40.24 a	3.69	48.90 a	3.68	46.57 a	10.55
'Katinka'												
high	8.44	2.65	6.11	1.76	74.96 b	18.31	32.12 a	2.49	41.46 a	2.54	30.70 a	6.51
low	9.25	2.82	8.50	3.34	90.23 a	10.93	31.49 a	2.56	40.46 b	3.03	28.28 b	4.80
'Topfive'												
high	8.50	1.40	5.64	2.44	66.09 b	26.73	31.38 a	3.42	35.98 a	2.85	24.00 a	6.30
low	9.71	2.20	7.64	2.31	80.73 a	21.40	31.43 a	3.08	36.28 a	2.76	21.98 b	5.09

Fr.: Fruit; Values are the mean of 10 replicates ± SD. For each cultivar, mean values followed by the same letter for each column are not significant P≥ 0.05 according to Duncan's Multiple Rang Test

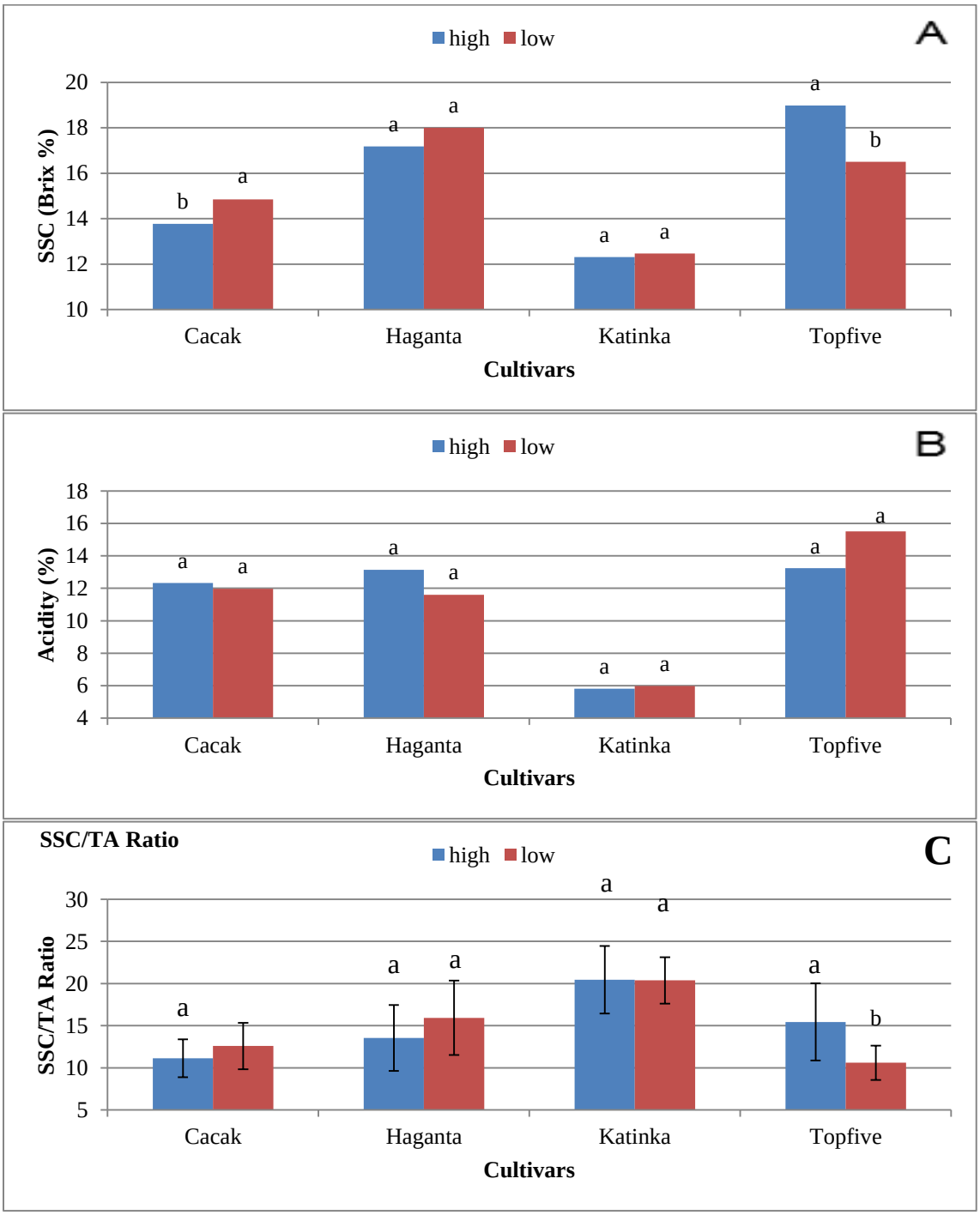


Fig. 4.28: Effect of leaf number / fruit ratio (high (2.5 to 3.2 leaves/ fruit) and low (1.5 to 1.75 leaves / fruit) on soluble solids content SSC (A), titratable acidity TA (g/100 ml juice) (B) and SSC/TA (C) of 'C. Lepotica' ('Cacak'), 'Haganta', 'Katinka' and 'Topfive' plum cultivars. Values are the means of 10 replicates, columns with the same letter for each cultivar are not significant at ($P \geq 0.05$).

4.3 Physiological behavior of European plum during postharvest and impact of 1-MCP

4.3.1 Physiological behavior of European plum during the postharvest life

4.3.1.1 Ethylene production rate (EPR) in European plum

Ethylene production rates (EPR) were measured in European plum cultivars which were harvested at different dates (Fig. 4-29 to 35). In general, most cultivars showed climacteric behaviour in EPR at all of harvest dates. Data presented in these figures show two different impacts on EPR: first, the differences due to genotypes and, second, the differences due to harvest dates.

4.3.1.1.1 Plum genotypes

Regarding the influence of genotypes, huge differences were noticed among plum cultivars in EPR during shelf life after harvest as well as after cold storage. The EPR differences in 2011 ranged from 0.5 to 10.0 ppm/kgh. At the first harvest date (first analysing time without cold storage), ‘Anna Späth’ and ‘Haganta’ cultivars produced the highest EPR values of 10.0 and 8.0 ppm/kg/h, respectively, during the climacteric period. The lowest EPR values were noticed for ‘President’ and ‘Hauszwetsche’ with 1.2 and 0.5 ppm/kg/h, respectively, in the same period. The same trend was found upon the second harvest date(first analysing time without cold storage). However, the differences among cultivars were lower compared to the first harvest date. Also, the EPR was lower in ‘Anna Späth’ than in ‘Haganta’ compared with first harvest date, where ‘Anna Späth’ EPR was higher than ‘Haganta’. The EPR in ‘Haganta’ was more than 8 fold in comparison with ‘Hauszwetsche’. In 2013, EPR ranged from 0.6 ppm/kg/h in late cultivar ‘Haroma’ to 22 ppm/kg/h in early cultivar ‘Hanka’.

4.3.1.1.2 Harvest date

The influence of harvest dates on EPR data is shown in (Fig. 4-29 to 33). There is an obvious effect of harvest dates on EPR. The reported data show that EPR was higher at earlier harvest dates in all cultivars in comparison to late harvest date. An exception is ‘Anna Späth’ at third harvest date, first analysing time (without cold storage), compared to the second harvest date, first analysing time with an EPR of 2.2 and 2.0 ppm/kg/h, respectively. For ‘Haganta’ and ‘Tophitplus’ at the second harvest date, second analysing time (stored in cold storage for 10-15days), the EPR was 8.76 and 8.19 ppm/kg/h while it was 6.67 and 3.86 ppm/kg/h in the first

harvest date, respectively. Moreover, The EPR was higher in fruit batches after cold storage in most cultivars under study compared to fruit batches before.

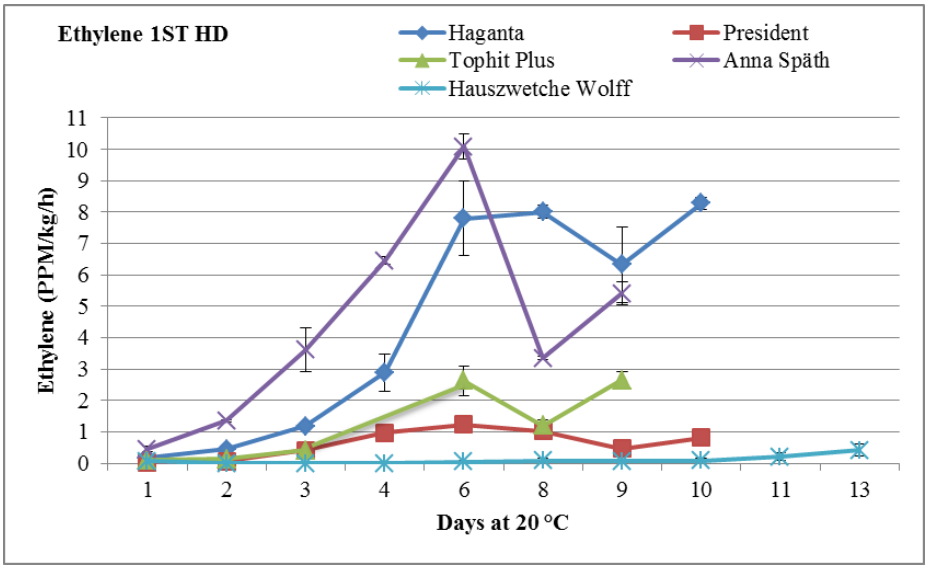


Fig. 4.29: Ethylene production rates (EPR) of plum cultivars picked at 1st harvest date (HD) (6-9-2011) during shelf life without cold storage. The values are the mean of three replicates and vertical bars represent SD.

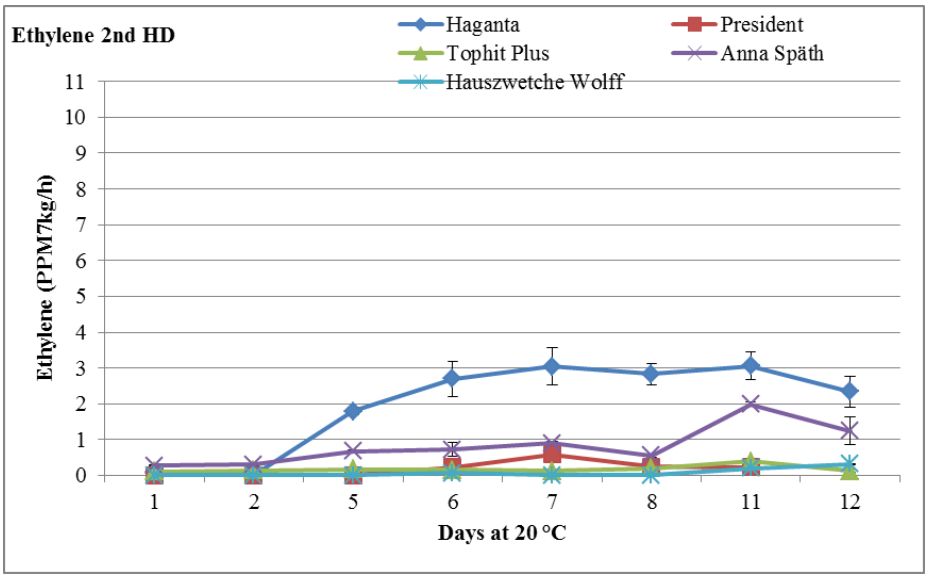


Fig. 4.30: Ethylene production rates (EPR) of plum cultivars picked at 2nd harvest date (HD) (15-9-2011) during shelf life and without cold storage. The values are the mean of three replicates and vertical bars represent SD.

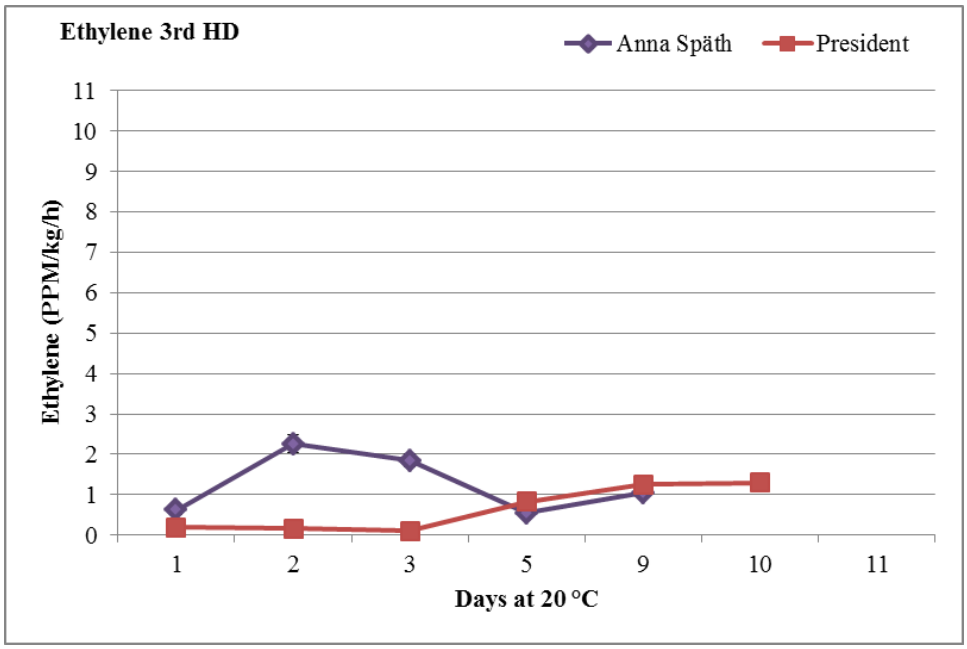


Fig. 4.31: Ethylene production rates (EPR) of plum cultivars picked in 3rd harvest date (HD) (21-9-2011) during shelf life and without cold storage. The values are the mean of three replicates and vertical bars represent SD.

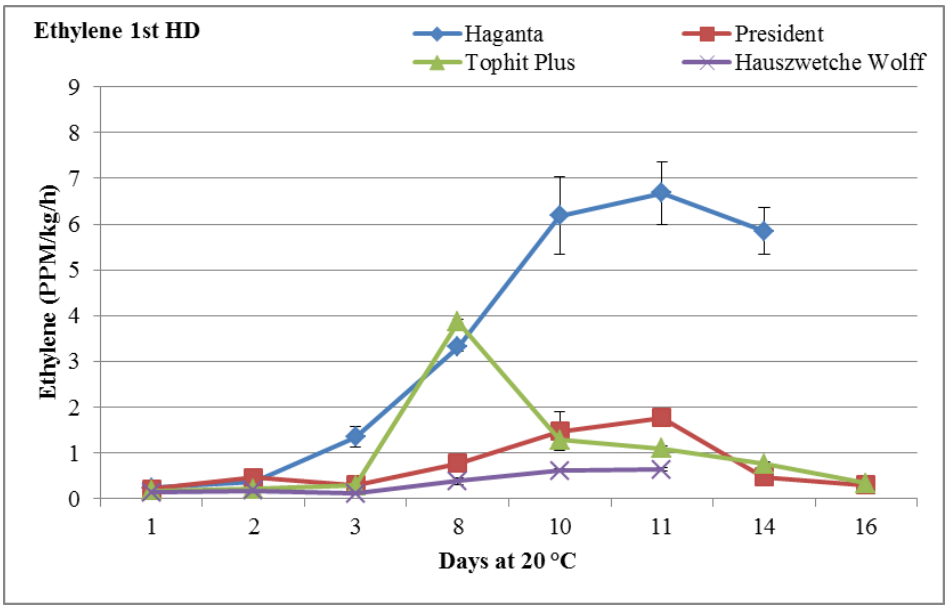


Fig. 4.32: Ethylene production rates (EPR) of plum cultivars picked in 1st harvest date (HD) (6-9-2011) during shelf life and after 10 days of cold storage. The values are the mean of three replicates and vertical bars represent SD.

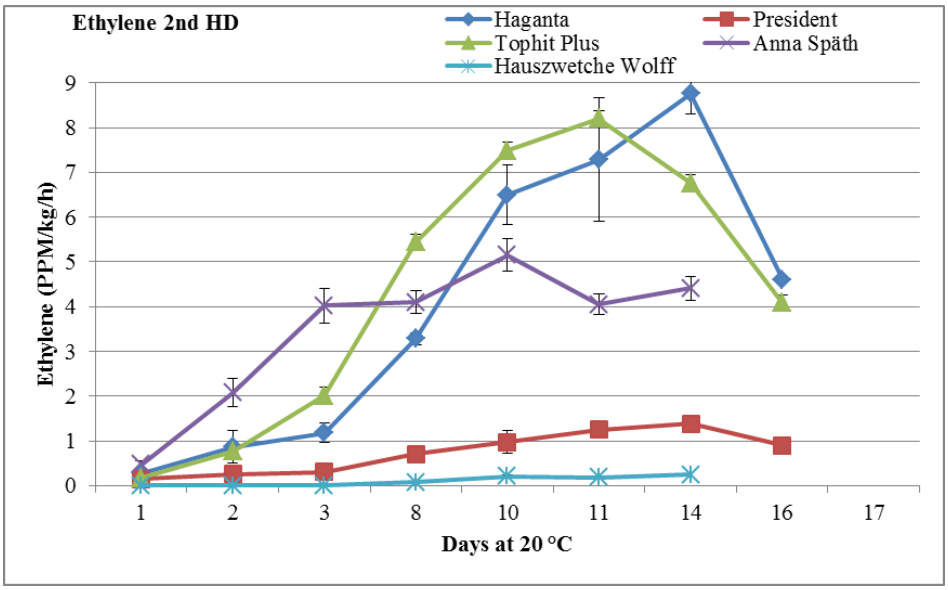


Fig. 4.33: Ethylene production rates (EPR) of plum cultivars picked in 2nd harvest date (HD) (15-9-2011) during ripening at 20 °C and after 10 days of cold storage. The values are the means of three replicates and vertical bars represent SD.

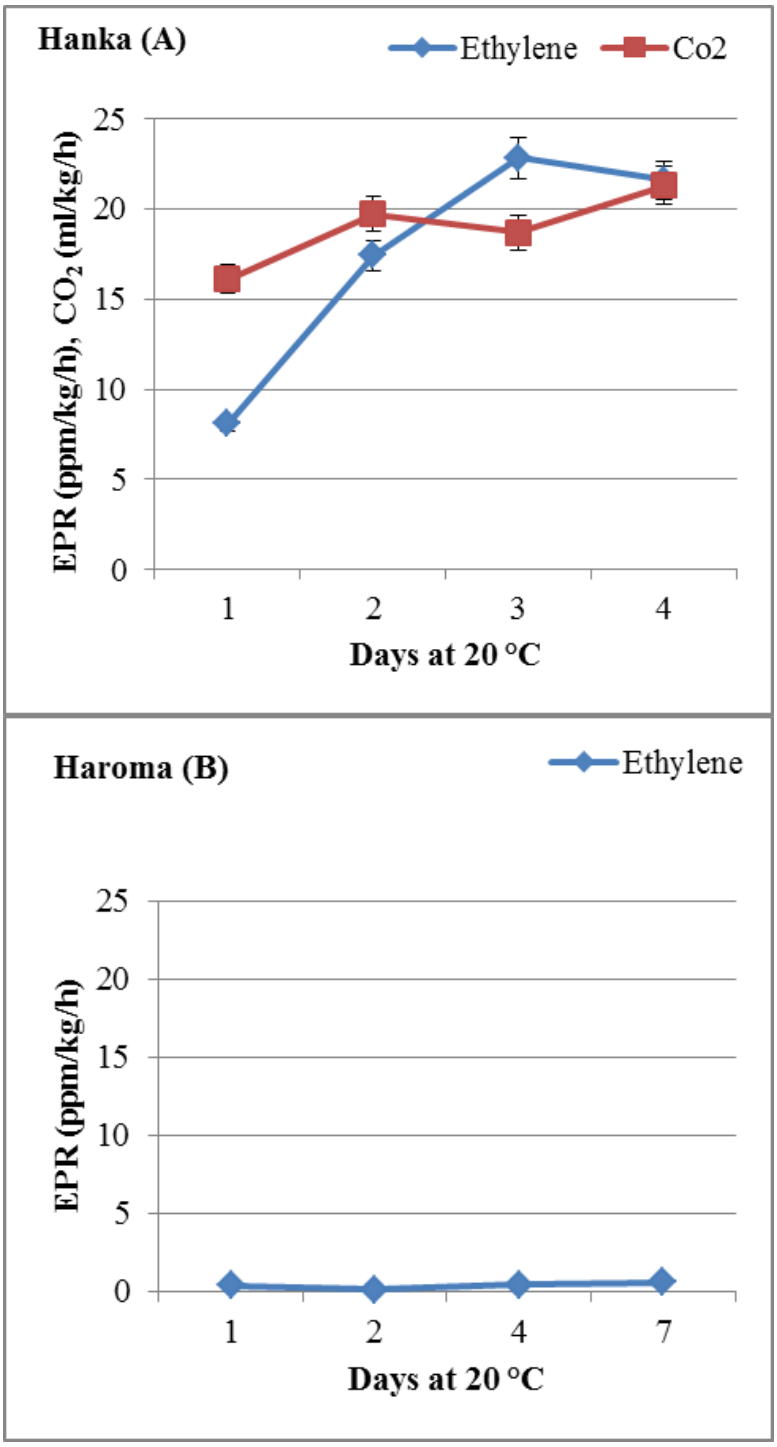


Fig. 4.34: Respiration rate (CO_2 ml/kg/h) and ethylene production rate (EPR, ppm/kg/h) in 'Hanka' (A) and 'Haroma' (B) plum cultivars during ripening at 20 °C in 2013 season. Values are means of three replicates ± SD.

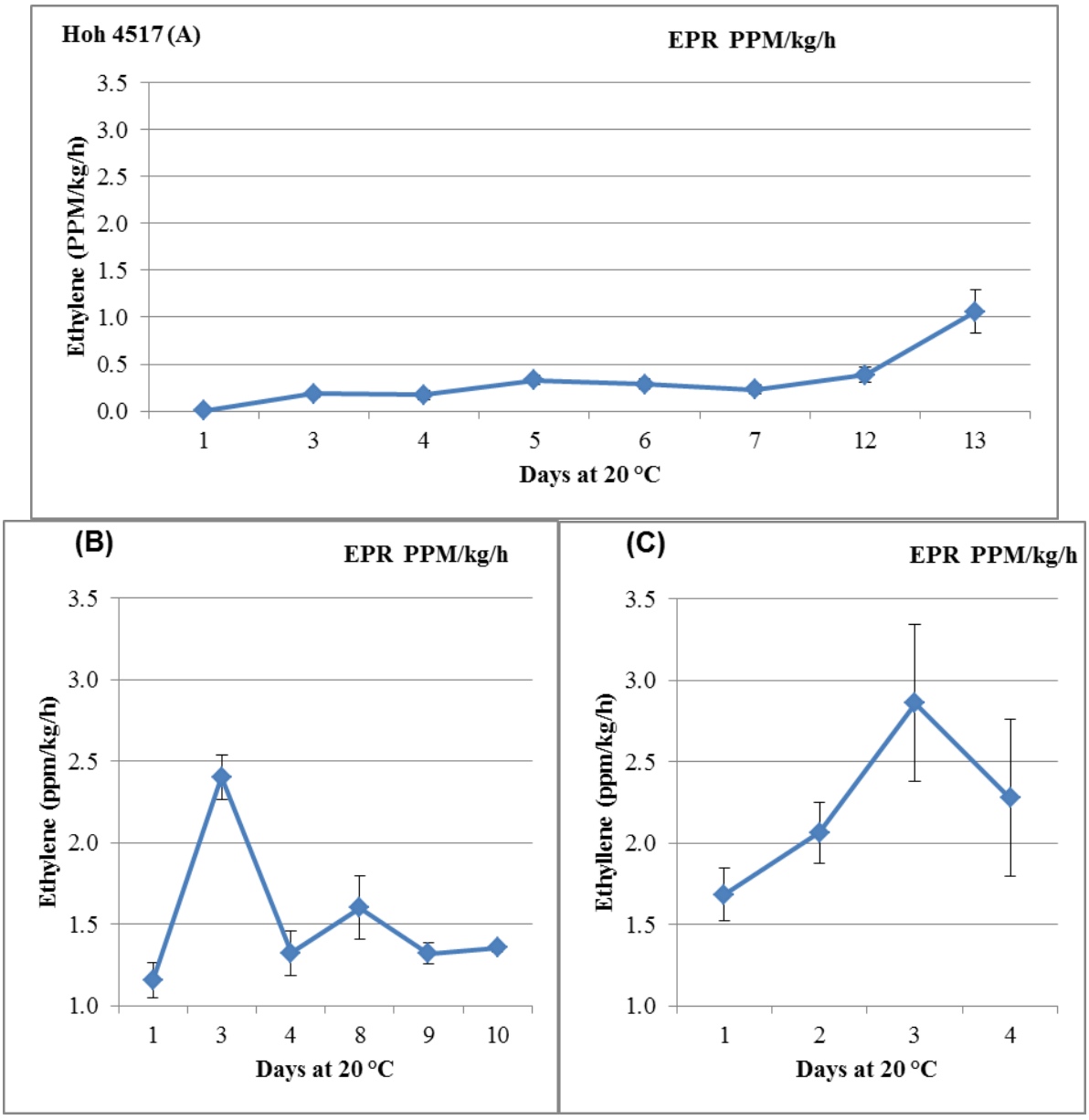


Fig. 4.35: Ethylene production rate (EPR ppm/kg/h) in 'Hoh 4517' plum breeding clone during ripening at 20 °C before storage (A), after 10 (B) and 20 (C) days cold storage at 2 °C in 2011 season. Values are means of three replicates ± SD.

4.3.1.1.3 Maturity stage

The effect of maturity stage (was determined by flavonols (Flav) values using Multiplex tool) on the ethylene production rate (EPR) during ripening at room temperature (20 °C) is depicted in Fig. 4-36. The EPR was significantly higher in maturity stage (1) with Flav values less than 1 Unit (more ripe) than in maturity stage (2) which has Flav values higher than 1 Unit (less ripe). Moreover, the EPR started to sharply increase earlier in maturity stage (1) than in maturity stage (2). On the other hand, the EPR reached the climacteric peak two days earlier in maturity stage (1) especially at the second harvest date (HD).

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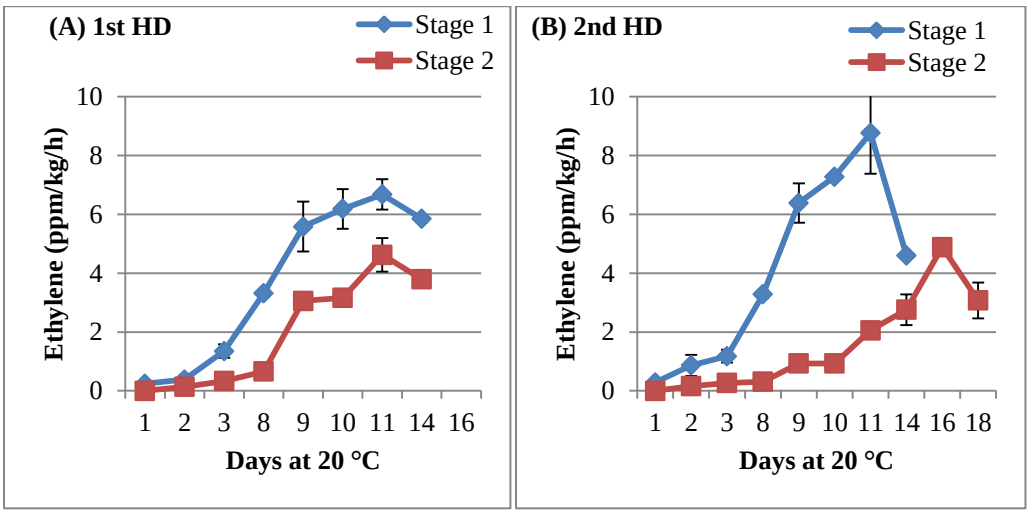

Fig. 4.36: Effect of maturity stage determined by 'Multiplex' on ethylene production rate (EPR ppm/kg/h) during ripening at 20 °C of 'Haganta' fruits at 1st harvest date (A) and 2nd harvest date (B). Stage 1 is more ripe with flavonols less than 1 Multiplex Units and Stage 2 is less ripe with flavonols more than 1 Multiplex Units. Values are means of three replicates ± standard deviation (SD).

4.3.1.2 Changes of skin color under cold storage

4.3.1.2.1 Effect of harvest date

Changes of fruit skin color, Lightness (L*), red (+a*) and blue (-b*), under cold storage as affected by harvest date and ripening stage are shown in Tab. 4-9 to 11. Skin fruit parameters were significantly affected by either harvest date or ripening stage which was determined by Multiplex in 'Haganta' plum and 'Hoh 4517'. Skin fruit color parameters markedly changed under cold storage in both cultivars 'Haganta' and 'Hoh 4517'. L*significantly decreased under cold

storage for both harvest dates for 'Haganta' but the change in L* at the 1st harvest date was higher than at the 2nd harvest date, where decreasing L* values were only significant at 4th analysing time (45 days in cold storage) in 2nd harvest date while at the 1st harvest date the decreasing L* was significant in 3rd and 4th analysing dates (30 and 45 days in cold storage, respectively). However, the differences regarding L* between the harvest dates were not significant. The same trend was observed for breeding clone 'Hoh 4517' although, the differences were not significant between 2nd and 3rd analysing time (20 and 35 days in cold storage respectively).

Unlike L*, the +a* increased in the first two weeks in fruits which were harvested at the 1st date. Thereafter, it significantly declined until the end of the storage period. In fruits of the 2nd harvest date, +a* decreased under cold storage condition from the beginning until the end of cold storage. However, the decreasing was only significant after two weeks from the beginning of storage. The differences between the harvest dates regarding the red color (+a*) were significant in 'Haganta'. In 'Hoh 4517', +a* values decreased significantly under cold storage except between 1st and 2nd analysing time (7 and 20 days in cold storage). In contrast, the blue color (-b*) was increasing in 'Haganta' fruits of 1st harvest date until the end of cold storage period although the increase was only significant at 2nd analysing time (15 days in cold storage). On contrary, the -b* values of fruits of the 2nd harvest date, decreased under cold storage, although, the decrease was not significant. However, the blue color was higher at the 2nd harvest date than at the 1st harvest date. These differences were significant especially at harvest time and the first two weeks of cold storage. At the end of cold storage the blue color was almost the same in fruits from both harvest dates. For 'Hoh 4517', the -b* values became more negative (blue color increasing) under cold storage but the increase was not significant.

4.3.1.2.2 Effect of maturity stage

The effect of ripening stage on changing of fruit skin color under cold storage are presented in (Table 4-11). In general, the reported data show the same direction of changes in color parameters affected by harvest dates as mentioned above. The L* (luminosity) was significantly higher in less ripe fruits (stage 2) than more ripe ones (stage 1). In addition, +a* was also significantly higher in stage 2 than in stage 1. It was increasing in both of the ripening stages, 1st harvest date, for two weeks and then startes to decrease but at 2nd harvest dates, the a* decreased from the beginning of cold storage. The same trend was found also with b* which decreased in 2nd harvest date in both ripening stages from the beginning to the end of cold

storage while it increased in 1st harvest date in both of ripening stages until the end of the storage. However, the differences in blue color were not significant except the 1st and 2nd analyzing times (0 and 15 days in cold storage) at 1st harvest date in both of ripening stage.

Tab. 4.9: Fruit skin color (as L*, a* and b*) measured at harvest and 15, 30 and 45 days under cold storage on different harvest dates for 'Haganta' plum cultivar.

Storage period	L*				a*				b*			
	HD1		HD2		HD1		HD2		HD1		HD2	
	mean	± SD	mean	± SD	mean	± SD	mean	± SD	mean	± SD	mean	± SD
0 day	32.31 a	3.77	31.59 a	3.38	4.44	1.76	4.16 a	1.59	–1.13	1.94	–2.79 a	2.16
15 days	32.00	3.13	31.55 a	3.87	5.31 a	1.79	3.33 b	1.51	–2.08 a	1.90	–2.66 a	1.73
30 days	31.24	2.71	31.04	2.53	4.72 b	1.74	3.26 b	1.40	–2.05 a	1.74	–2.57 a	1.68
45 days	30.85 c	2.57	30.60 b	2.35	4.08 c	1.60	3.17 b	1.56	–2.37 a	1.61	–2.39 a	1.59
Total Av.	31.60	3.13	31.20	3.13	4.62 A	1.77	3.45 B	1.55	–1.90	1.85	–2.60	1.79

N = 40 fruits, Values are the means ± SD. Means within the same harvest date (HD) and the same column (skin color element) followed by the same letter are not significant different at $P \geq 0.05$.

Tab. 4.10: Fruit skin color (as L*, a* and b*) measured 11, 20 and 35 days under cold storage for 'Hoh 4517' plum breeding clone.

Storage period	L*		a*		b*	
	mean	± SD	mean	± SD	mean	± SD
10 days	30.45 a	±3.03	3.82 a	±1.49	–0.49 a	±1.84
20 days	29.30 b	±2.29	3.57 a	±1.43	–0.73 a	±1.16
35 days	28.80 b	±2.02	2.87 b	±1.21	–0.89 a	±1.20
Total Av.	29.51	±2.56	3.42	±1.43	–0.70	±1.43

N = 40 fruits, Values are the means ± SD. Means within the same column (skin color element) followed by the same letter are not significant different at $P \geq 0.05$.

Tab. 4.11: Changes of fruit skin parameters (L*, a* and b*) under cold storage affected by harvest date (HD1: 6.9.2011; HD 2: 15.9.2011) and ripening stage.

Harvest. date (HD)	Sto-rage (days)	L*				a*				b*			
		Stage 1		Stage 2		Stage 1		Stage 2		Stage 1		Stage 2	
		mean	± SD	mean	± SD	mean	± SD	mean	± SD	mean	± SD	mean	± SD
HD1	**0**	31.55 a	3.32	33.22 a	4.10	4.11 b	1.74	4.83 bc	1.72	–1.11 b	1.88	–1.17 b	2.03
	15	31.32 ab	3.14	32.63 ab	3.02	4.94 a	1.77	5.66 a	1.76	–2.08 a	1.92	–2.09 a	1.90
	30	30.50 ab	2.42	32.04 ab	2.81	4.24 ab	1.68	5.26 ab	1.68	–2.13 a	1.59	–1.96 ab	1.90
	45	30.15 b	2.30	31.54 b	2.66	3.83 b	1.71	4.33 c	1.45	–2.21 a	1.52	–2.52 a	1.70
HD2	**0**	31.27 ab	3.60	31.86 a	3.20	3.69 a	1.47	4.56 a	1.59	–3.09 a	2.32	–2.54 a	2.01
	15	31.61 a	2.97	32.05 a	2.34	2.81 b	1.07	4.04 ab	1.67	–3.14 a	1.41	–2.14 a	1.85
	30	30.82 ab	2.70	31.28 a	2.31	2.72 b	1.24	3.88 b	1.32	–3.04 a	1.72	–2.04 a	1.46
	45	30.25 b	2.25	31.00 a	2.43	2.56 b	1.34	3.85 b	1.51	–2.69 a	1.44	–2.06 a	1.71
Total Av.		30.96	2.89	31.92	2.93	3.51	1.67	4.51	1.69	–2.49	1.83	–2.08	1.84

N = 40 fruits, Values are the means ± SD. Means within the same harvest date (HD) and the same column (skin color element) followed by the same letter are not significant different at P≤ 0.05. Ripening stages are determined by Multiplex based on flavonols (Flav index) values, stage 1 is more ripe (less than 1 unit) and stage 2 is less ripe (more than 1 unit).

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4.3.1.3 Effect of harvest dates on fruit quality attributes

Table (4-12) shows the effects of harvest dates on soluble solids contents (SSC), titratable acidity (TA) and ripening index (SSC/TA ratio) of plum fruits as well as on the behaviour of these quality attributes under cold storage and during shelf life. Generally, there was high variability among cultivars in all attributes. The average of soluble solid contents ranged from 14 to 21 °Brix for 'Anna Späth' and 'Haganta', respectively. Moreover, the acidity ranged from 0.6 to (g/100ml) malic acid for the same cultivars. However, SSC/TA ratio at harvest was in the range of 11 to 21 for 'President' and 'Anna Späth', respectively.After 7 days of ripening at room temperature (20 °C) Brix was in Range from 15.10 to 23.75 °, malic acid was found at concentrations between 0.48 and 1.09 %, and SSC:TA ratio was between 20 to 29 for soluble solids content ('Anna Späth' and 'Haganta'), acidity ('Anna Späth' and 'Haganta') and SSC/TA ('Tophitplus' and 'Anna Späth'), respectively. The harvest dates significantly influenced the SSC, TA and SSC/TA ratio. SSC markedly increased until the last harvest dates in all cultivars except 'Haganta' which SSC value slightly decreased at 2nd harvest date comparing with 1st harvest date. The highest increase in SSC values was found for cultivars 'Anna Späth' and 'President' with an increase from 12.81 and 12.70 °Brix at 1st harvest date to 16.70 and 23.80 °Brix at 3rd harvest date, respectively. Moreover, SSC increased in all cultivars at all harvest dates during ripening at room temperature (20 °C) (shelf life) except 'President' and 'Anna Späth' in the 3rd harvest date with a slight decrease in SSC. SSC increased under cold storage at earlier harvest dates especially after 10 days of cold storage followed by a decline while, at late harvest date, the SSC was decreasing under cold storage.

Total acidity (TA) was substantially affected by harvest date, by cold storage and by ripening at room temperature (20 °C). TA decreased during ripening on the tree as well as at room temperature conditions and under cold storage especially after 10 days of cold storage. After 20 days of cold storage, TA reached an almost stable value for each cultivar at room temperature and showed almost no changed during ripening.

Ripening index (RI) or SSC/TA ratio significantly increased during on-tree ripening until the last harvest date as well as after ripening at room temperature or after cold storage. The ratio was highly significant in last harvest dates as well as last batches were removed from cold storage.

Tab. 4.12: Soluble solids content (SSC), titrable acidity (TA) and SSC/TA ratio at harvest and during storage of plum cultivars fruits picked at different dates (HD1: 06.09.2011; HD 2: 15.09.2011; HD 3: 21.09.2011)

Variety/Harvest dates (HD)	Shelf life	SSC (°Brix)			TA(g/100 ml)			SSC/TA ratio		
		Cold storage period (days)								
		0	10	20	0	10	20	0	10	20
'Anna Späth'										
HD1	**0 days**	12.81	14.40		0.85	0.84		15.16	17.25	
HD2		13.60	15.10	14.10	0.75	0.70	0.34	18.04	21.60	41.11
HD3		16.70	14.20		0.58	0.39		29.04	36.13	
HD1	**7 days**	13.90			0.43			32.03		
HD2		16.20	12.10		0.55	0.41		29.56	29.44	
HD3		15.20	n d		0.48			31.93		
'Haganta'										
HD1	**0 days**	22.05	19.95	19.90	1.53	1.50	0.85	14.45	13.26	23.54
HD2		21.65	21.05	21.25	1.42	1.35	0.85	15.24	15.66	24.97
HD3										
HD1	**7 days**	24.30	19.50	19.85	1.16	1.28	0.89	21.26	15.27	22.39
HD2		23.40	20.95	24.50	1.02	0.78	0.85	23.00	27.05	28.93
HD3										
'Hzw. Wolff'										
HD1	**0 days**		19.10			1.16			16.51	
HD2		20.50	23.00		1.03	1.12		19.96	20.50	
HD3										
HD1	**7 days**	20.40	20.00		0.99	0.68		20.52	29.41	
HD2		20.20	19.10		0.88	0.77		22.93	24.81	
HD3		20.70			0.76			27.24		
'Hoh 4517'										
HD3	**0 days**	17.00	18.30	18.00	1.02	0.69		16.75	26.72	
HD3	**7 days**	17.20	19.30		0.83	0.55		20.6	35.1	
'President'										
HD1	**0 days**	12.70	10.00		1.25	0.60		10.14	16.69	
HD2		13.50	10.90	11.30	1.21	1.05	0.76	11.15	10.39	14.89
HD3		23.80	20.20		1.05	0.73		22.69	27.63	
HD1	**7 days**	17.60	17.20		0.85	0.69		20.68	25.07	
HD2		15.60	13.30	15.20	0.98	0.64	0.71	15.89	20.78	21.41
HD3		21.40	17.80		0.73	0.68		29.36	26.25	
'Tophit plus'										
HD1	**0 days**	16.90	17.40	16.80	1.25	1.00	0.75	13.53	17.49	22.43
HD2		18.00	17.30	17.60	1.30	1.08	0.76	13.85	16.00	23.22
HD3		18.90			1.05			17.98		
HD1	**7 days**	19.00		15.80	0.96		0.65	19.90		24.16
HD2		18.90		18.40	0.94		0.73	20.17		25.14
HD3		20.10			0.94			21.31		

‚Hzw. Wolff': 'Hauszwetschge Wolff'.

4.3.1.4 Weight loss

There are remarkable differences in the percentage of weight loss among plum cultivars fruits either under cold storage or at room temperature (20 °C) and as well between 1st and 2nd harvest dates Fig. (4-37) and Table (4-13). The percentage of weight loss was substantially higher at 1st harvest date than at 2nd harvest date. Moreover, weight loss percentage in first batches was higher compared to second one for the all cultivars except for 'Haganta' at 2nd harvest date with a higher weight loss in 2nd batch than in the first one. In general, weight loss increased significantly during cold storage in all cultivars. Weight loss percentage after shelf life period was also significantly affected by the harvest dates for 'Haganta' and 'President' cultivars indicated by the weight loss at 1st harvest date compared to the 2nd harvest date. For the rest of cultivars, no differences in weight loss were noticed. High differences were observed among cultivars in fruit weight loss percentage under cold storage or during shelf life.

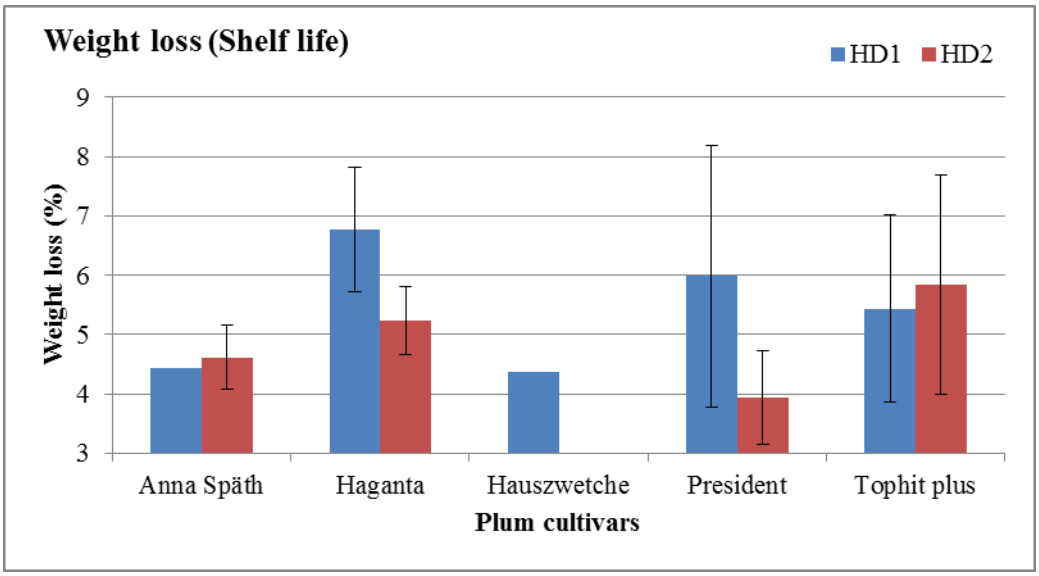

Fig. 4.37: Wight loss percentage after shelf life of plum cultivars fruits harvested at different picking dates. Values are the mean of three replicates, vertical bars present standard deviation (SD).

Tab. 4.13: Weight loss percentage of plum cultivars during cold storage on fruits collected at different harvest dates (HD1: 06.09.2011; HD 2: 15.09.2011; HD 3: 21.09.2011).

Cultivar / Harvest date	Storage period (days)				Cultivar Average	
	15 day		29 day			
	Weight loss (%)	±SD	Weight loss (%)	±SD	Weight loss (%)	±SD
'Anna Späth'						
HD2	1.13	0.22	1.68		1.32	0.36
'Haganta'						
HD1	2.79	0.51	5.47	2.86	4.57	2.62
HD2	0.83	0.07	2.93	0.14	1.53	1.09
'Hzw. Wolff'						
HD1	2.00	0.18			2.00	0.18
HD2	1.32				1.32	
'Hoh 4517'						
HD3	1.18		2.71		2.54	1.32
'President'						
HD1	0.72	0.01			0.72	0.01
HD2	0.42	0.31	1.34		0.72	0.58
'Tophit plus'						
HD1	1.31	0.12	2.27		1.63	0.56
HD2	1.10		1.70		1.40	0.42

Values are the means of weight loss pecentage (%) ± standard deviation (SD), HD: harvest date; ‚Hzw. Wolff': 'Hauszwetschge Wolff'.

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4.3.2 Effect of 1-MCP on quality of plum fruit and its behavior during postharvest life

4.3.2.1 Ethylene production rate

Postharvest application of 1-MCP significantly reduced ethylene production levels either during shelf life or after cold storage in the early cultivar 'Katinka' in 2013 and in the late cultivar 'Haganta' in 2012 and 2013 (Fig. 4-38 to 40). In general, ethylene production levels were significantly higher in untreated fruits (at least one and half fold) than in treated fruits. Moreover, the climacteric rise was delayed with a lower peak of ethylene in treated fruits except for 'Katinka' at 2nd analysing time (after 10 days in cold storage) showing an ethylene production rate slightly higher in treated fruits in 2nd day at 20 °C. Moreover, ethylene was detected neither in treated nor in control fruits of 'Haganta' 2012during first three days of ripening at 20 °C in the first batch (without cold storage) and 1st day in 2nd batch (after 15 days in cold storage). Ethylene production rate reached the climacteric peak after 12, 8 and 7 days at 20 °C for 'Haganta' 2012 in first batch (without cold storage), 2nd batch (after 15 days of cold storage) and last batch (after 30 days of cold storage), respectively., The same trend was found in 'Katinka' and 'Haganta' in 2nd season (2013).

4.3.2.2 Respiration rate

Respiration rate (CO_2/kg/h) was noticeably higher in control fruits compared to with 1-MCP treated fruits (Fig. 4-39 a, b and c). In 1st batch (without cold storage) of 'Katinka' fruits, CO_2 level has two peaks at 2nd and 4th day at 20 °C. Moreover, CO_2 reached the climacteric peak in later batches earlier than in the first ones: the respiration rate reached the peak at 3rd and 4th day for 3rd and 1st batches, respectively. In general, 'Katinka' fruits exhibited climacteric characteristics in respiration rate like the 'Hanka' cultivar (Fig.4-34).

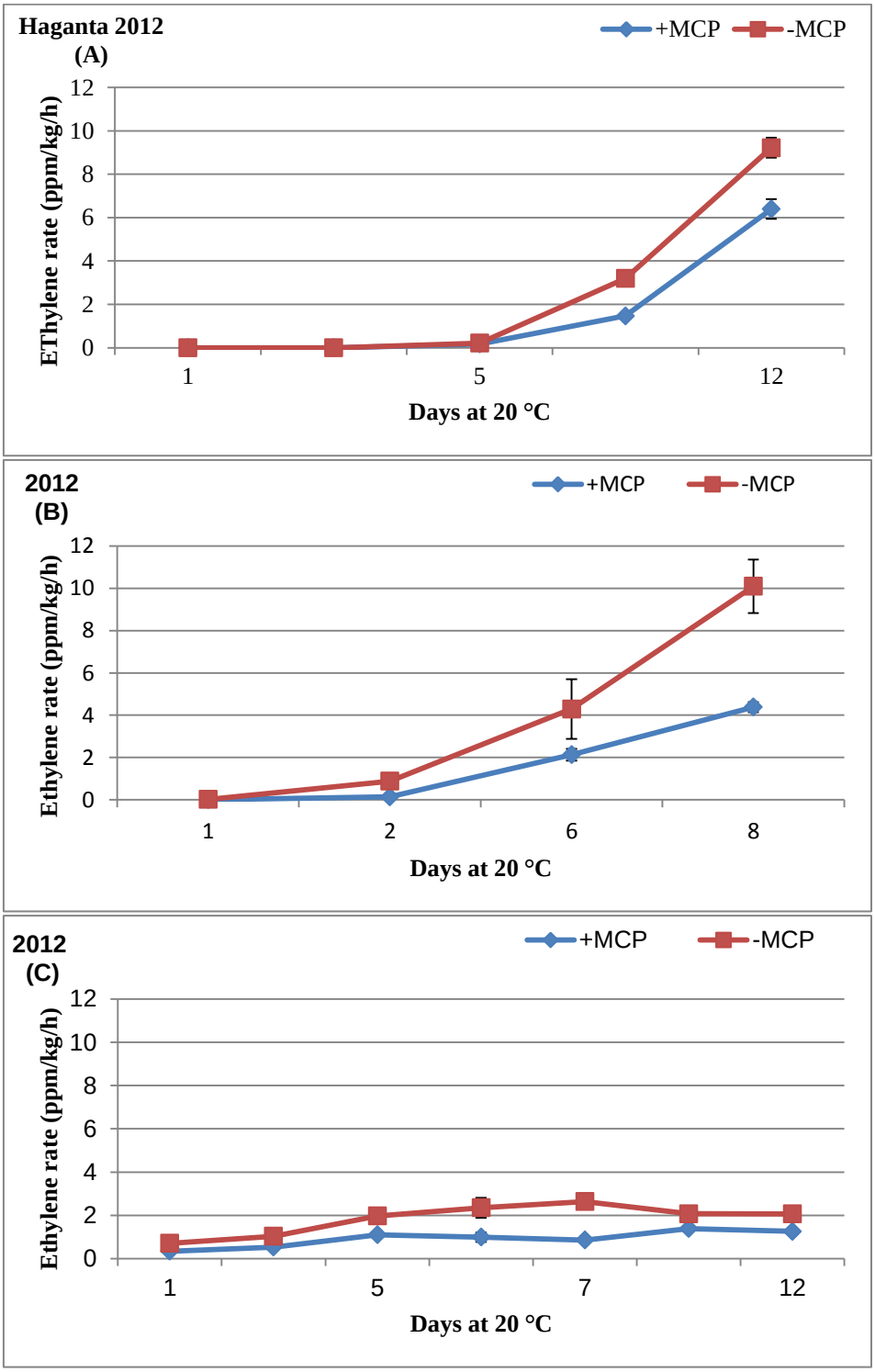

Fig. 4.38: Effect of 1-MCP on ethylene production and respiration rate of 'Haganta' plum in 2012 during ripening at 20 °C, without cold storage (A), after 10 days (B) and 20 days stored in cold storage (C). Values are mean of three replicates and vertical bars represent standard deviation (SD).

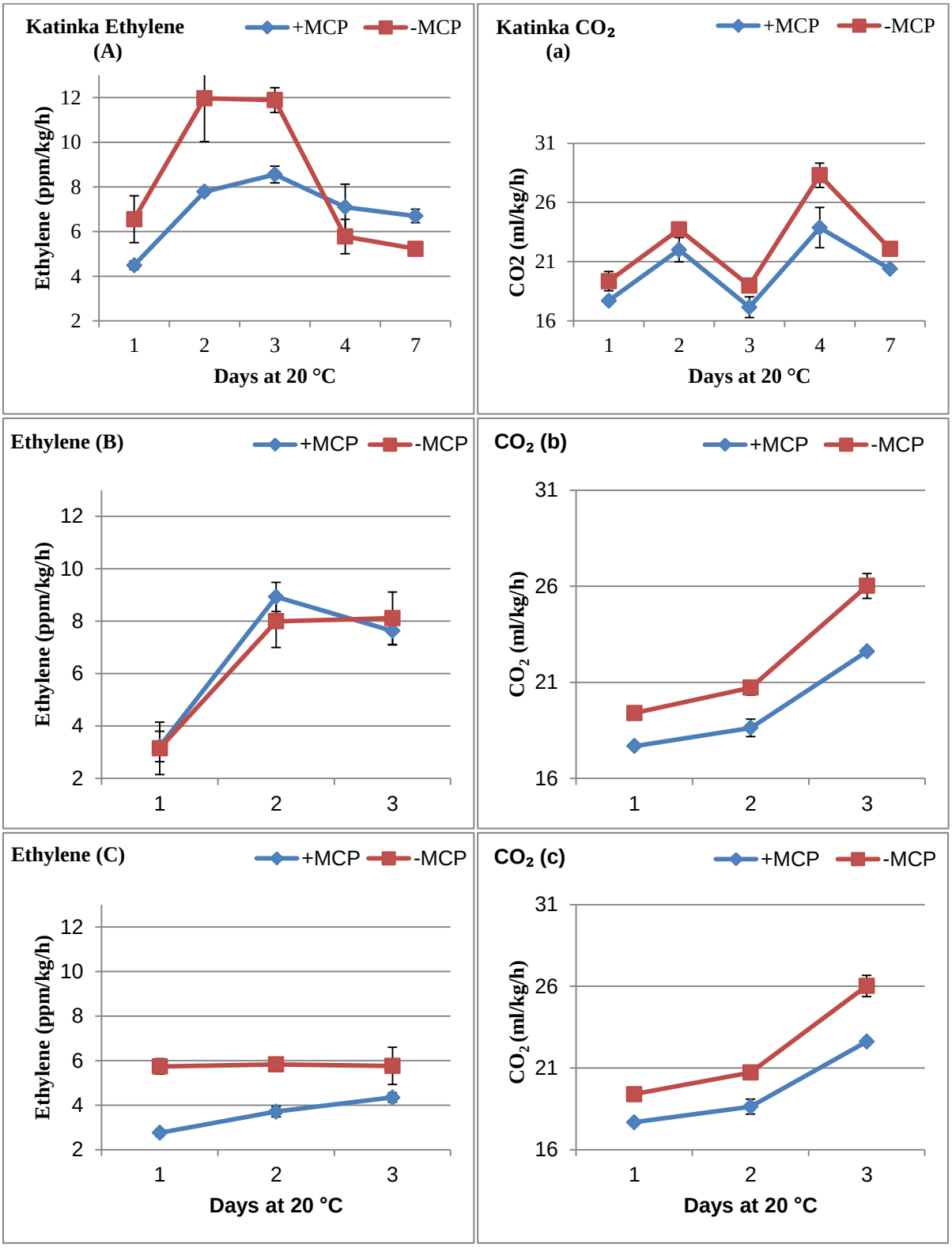

Fig. 4.39: Effect of 1-MCP in ethylene production and respiration rate of 'Katinka' plum in 2013 during ripening at 20 °C, after treating without cold storage (A), 10 days (B) and 20 days stored in cold storage (C). Values are mean of three replicates and vertical bars represent standard deviation (SD).

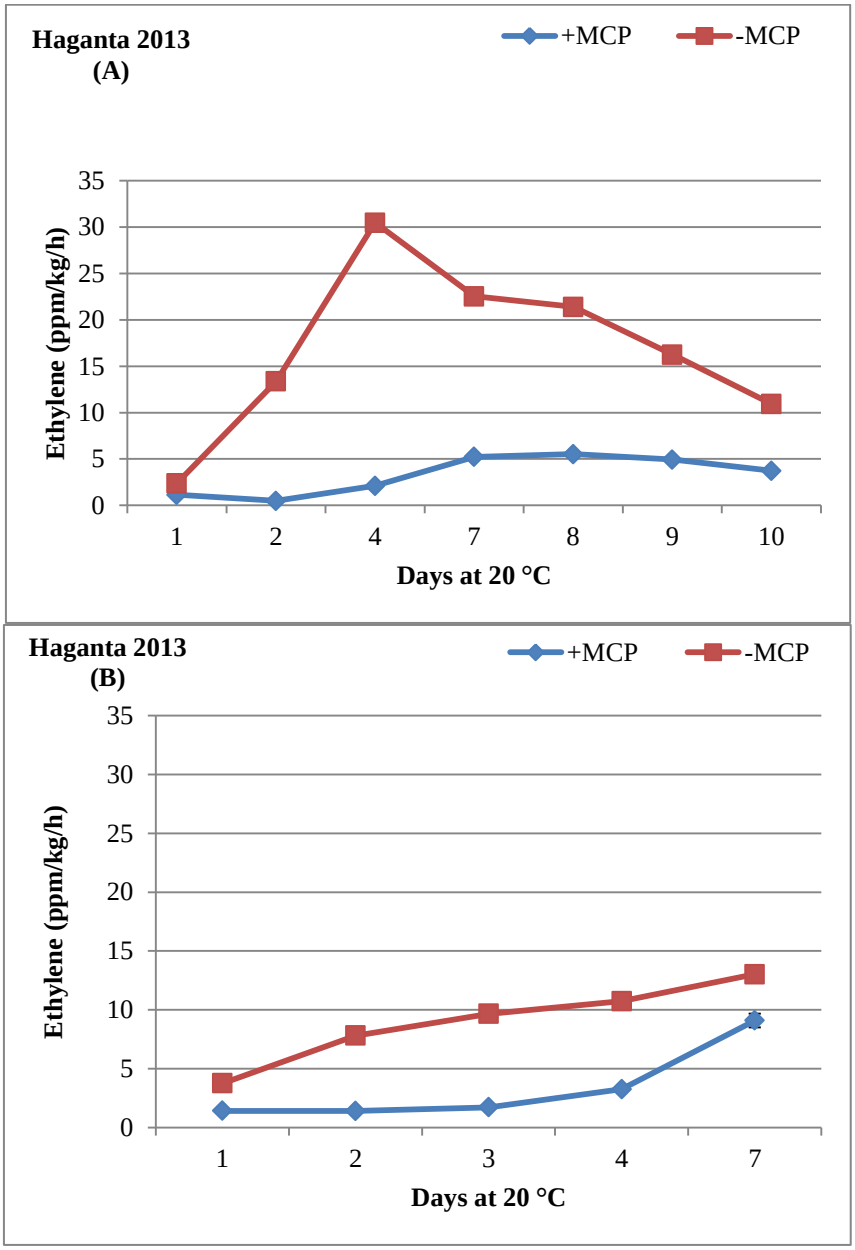

Fig. 4.40: Effect of 1-MCP on ethylene production rate of 'Haganta' plum in 2013 during ripening at 20 °C, without cold storage (A) and after 15 days stored in cold storage (B). Values are mean of three replicates and vertical bars represent standard deviation (SD).

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4.3.2.3 Soluble solids content, titratable acidity and SSC/TA ratio

The effect of 1-MCP on fruit quality data is presented in Figures 4-41, 42 and 43. The 1-MCP had significant effect on fruit soluble solids content (SSC) especially after shelf life. In treated 'Haganta' plum fruits in both seasons (2012 and 2013), SSC increased during cold storage and after shelf life but decreased markedly in untreated fruits. The same trend was found in 'Katinka' except in the last batch (after 15 days in cold storage) either before or after shelf life without remarkable differences.

Regarding acidity (TA), as shown in Fig.(4-45, 46 and 47 (B), TA decreased after shelf life in both treatments in both seasons and for both cultivars 'Katinka' and 'Haganta' except 'Haganta' at the 3rd analyzing time in control treatment showing slightly increased TA values. Applying 1-MCP treatment, there were no obvious changes of TA . The 1-MCP treatment in 2012 season gave slightly higher TA at the beginning of cold storage of 'Haganta' while no marked effect was found in the same cultivar as well as in 'Katinka' in 2013.

Ripening index (SSC/TA ratio) increased under ripening at room temperature in both treatments but it was higher in treated fruits in both seasons and for both cultivars. Exception were 'Katinka' fruits in the last batch (15 days of cold storage + 3 days shelf life) and also 'Haganta' fruits in the first batches in both seasons where SSC/TA ratio was higher in control than in treated fruits. Generally, SSC/TA was decreasing during the whole storage period in 'Haganta' cultivar unlike 'Katinka' where the SSC/TA was increasing during the storage period.

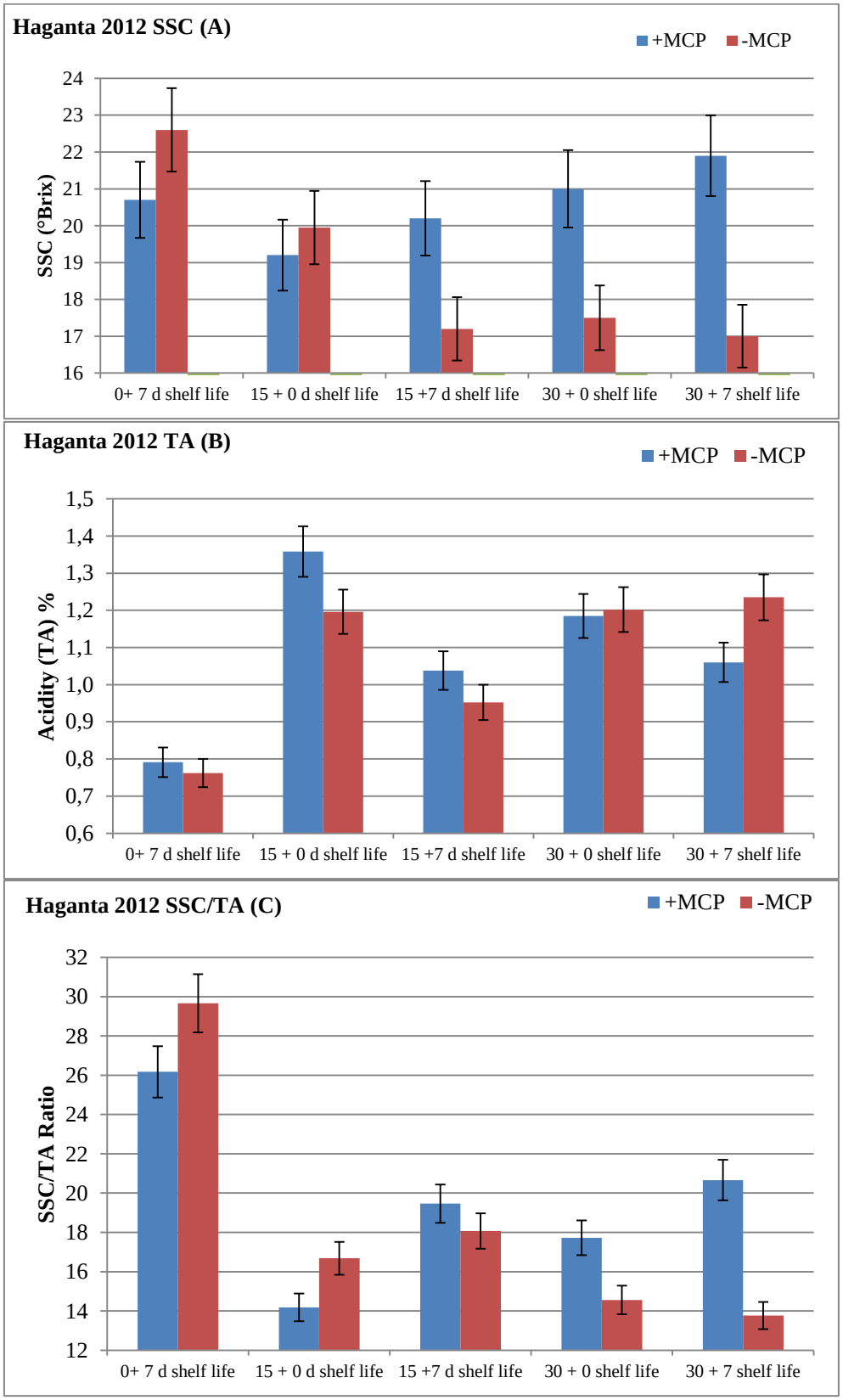

Fig. 4.41: Effect of 1-MCP on 'Haganta' plum fruit soluble solids content (SSC in °Brix (A)), acidity (TA in g/100 ml (B)) and SSC/TA ratio (C) in 2012 season, after treatment 0, 15, 30 days of cold storage followed by 0 and 7 days in shelf life (at 20 °C)

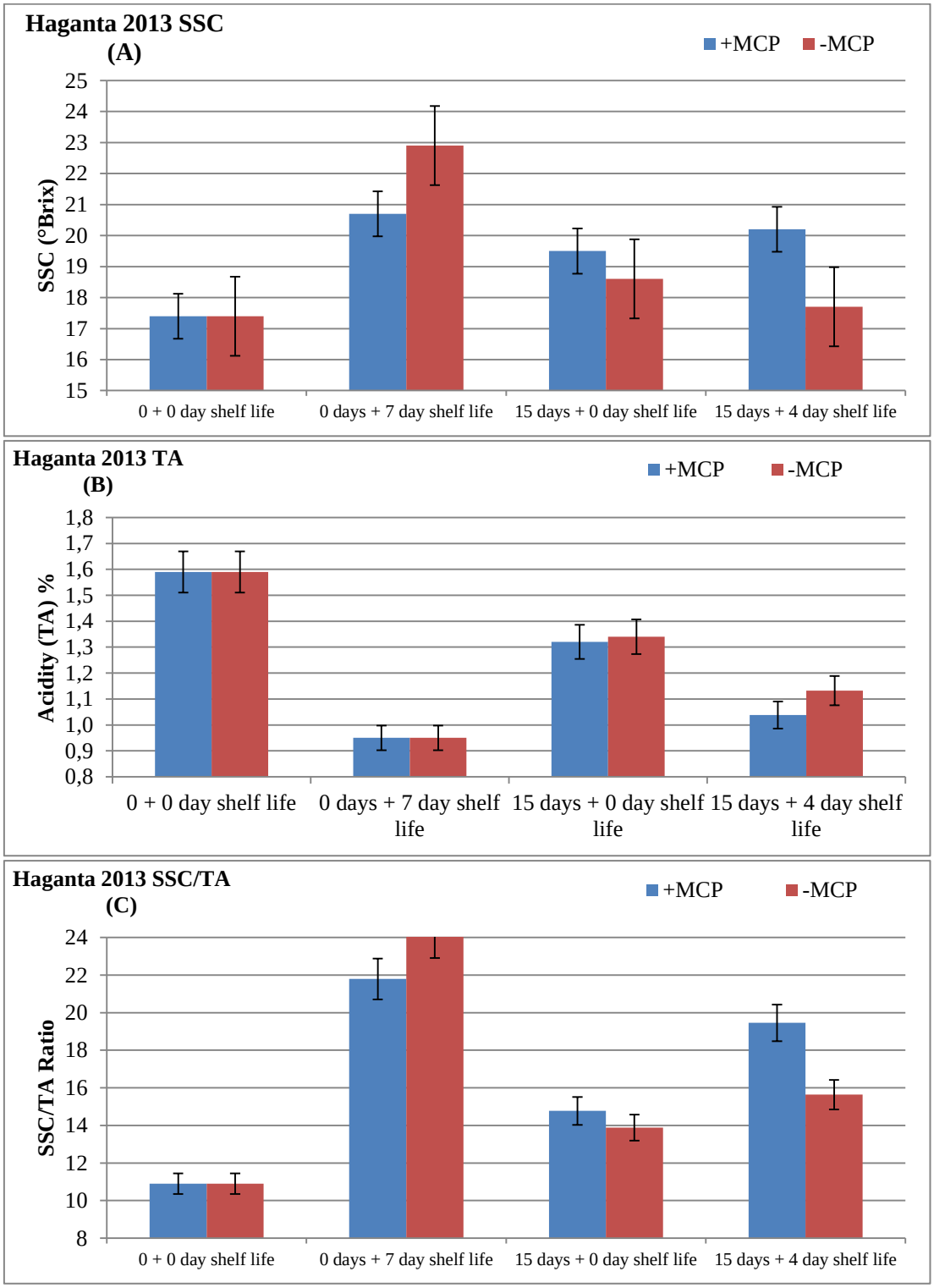

Fig. 4.42: Effect of 1-MCP on 'Haganta' plum fruit soluble solids content (SSC in °Brix (A)), acidity (TA in g/100 ml (B)) and SSC/TA ratio (C) in 2013 season, after treatment 0 and 15 days of cold storage followed by 0 and 7 or 4 days in shelf life (at 20 °C)

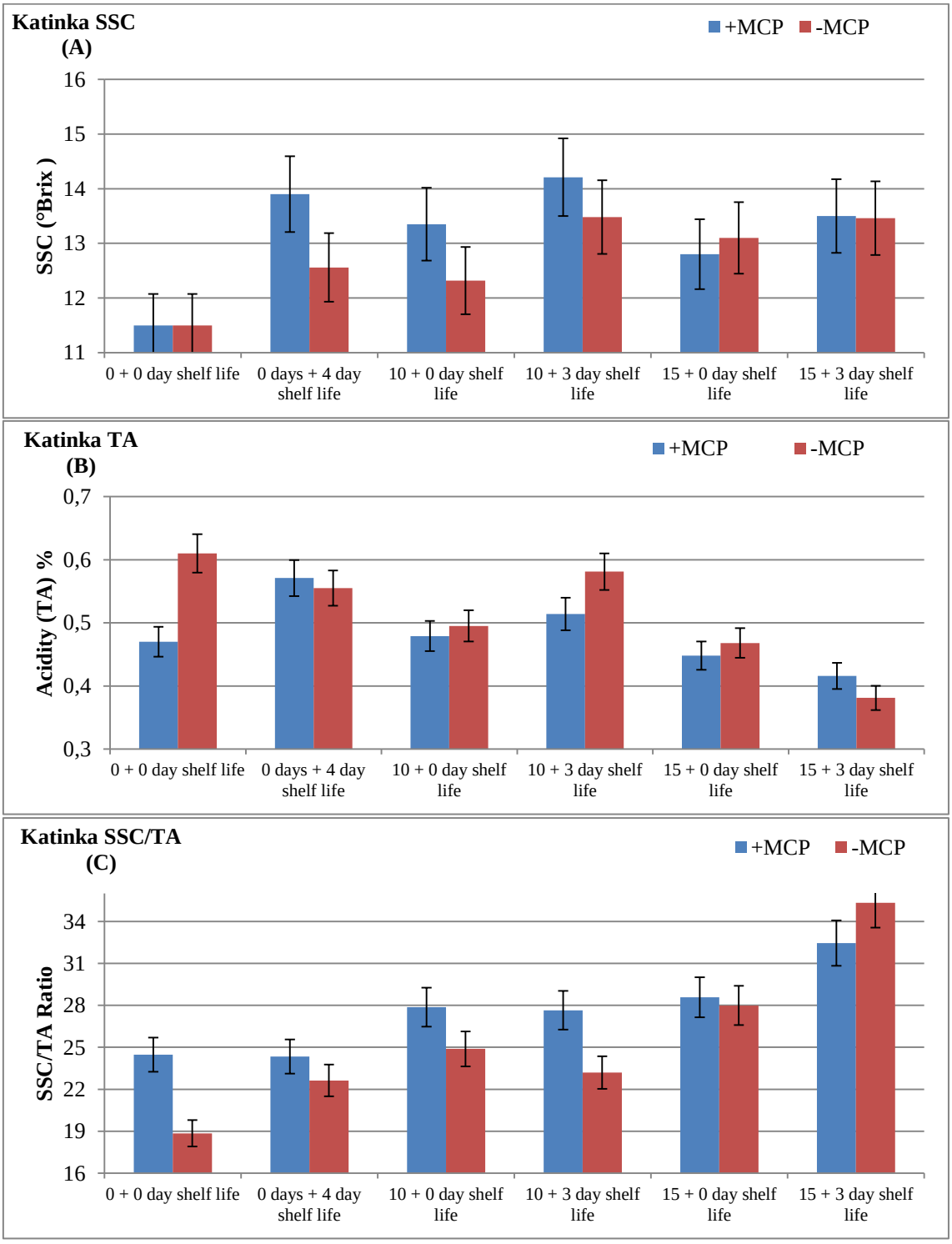

Fig. 4.43: Effect of 1-MCP on 'Katinka' plum fruit soluble solids content (SSC in °Brix (A)), acidity (TA in g/100 ml (B)) and SSC/TA ratio (C), after treatment 0, 10, 15 days of cold storage followed by 0 and 4 or 3 days in shelf life (at 20 °C)

4.3.2.4 Fruit weight loss

Fruit weight loss percentage was slightly affected by the 1-MCP treatments. The untreated control exhibited higher weight loss than 1-MCP treatment during cold storage and after transferre of fruits to to room temperature conditions. An exception was 'Haganta', 2nd batch (15 days of cold storage + 5 days at room temperature), with a decreased weight loss percentage due to the 1-MCP treatment (Fig. 4-44).

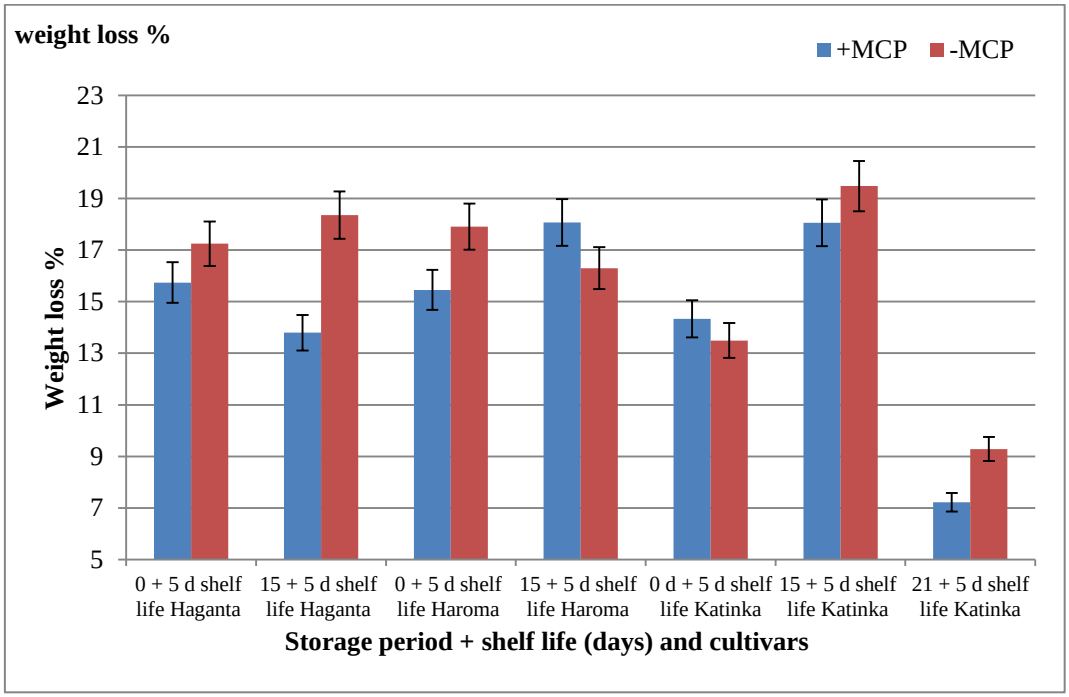

Fig. 4.44: Effect of 1-MCP on fruit weight loss of 'Katinka', 'Haganta' and 'Haroma' cultivars after treatment 0, 15 and 21 days of cold storage followed by 5 days shelf life.

4.3.2.5 Fruit firmness

The effect of 1-MCP application on fruit firmness before and after shelf life is shown in Fig 4-45. 1-MCP had marked effect on fruit firmness since control plum exhibited higher firmness than 1-MCP treated fruit. However, the differences were only significant in case of 'Haganta'. Generally, fruit firmness significantly decreased during shelf life in both treatments.

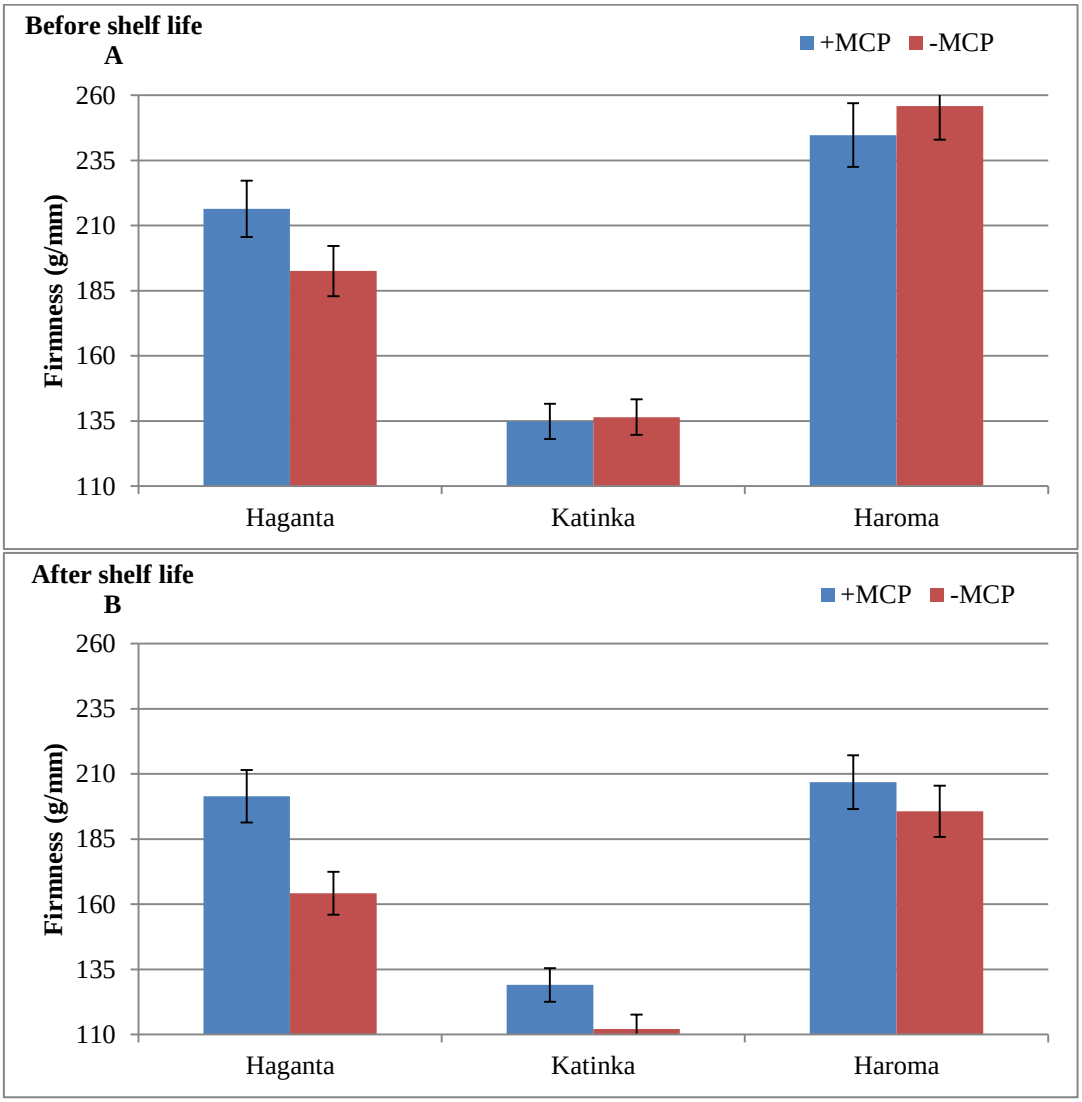

Fig. 4.45: Effect of 1-MCP on fruit firmness of 'Katinka', 'Haganta' and 'Haroma' cultivars after before (A) and after (B) shelf life (5-7 days at room temperature).

5. Discussion

5.1 Non-destructive detection of fruit development and ripening

5.1.1 Fruit development and ripening

Fruit development analysis during growth and maturation as well as non-destructive techniques were used as potential indicators of fruit maturity. Fruit growth is a quantitative process from bloom until harvest during which the size and/or the weight increases until the fruit reaches its size (James et al., 1989). To study this process, it is necessary to consider the different influences on variability such as crop load and rootstocks.

In this study, fruit growth (diameter and length) followed a double sigmoid pattern which is characteristic for stone fruits and some berries (Chalmers and Van Den, 1975; Tonutti et al., 1997). This pattern has four distinct growth stages in stone fruit. The first stage (S1) is characterized by cell division which takes about 30 days after fertilization. This stage was not detected in this study since the measurements were carried out 50 DAFB (Days After Full Bloom), Fig. 4-1 and 2. The second stage (S2) is short and may overlap with the third stage, especially in early cultivars, when the fruit shows slow or no fruit growth while the endocarp hardens to form a solid stone in S2 (Diaz-Mula et al., 2008). This period is obvious for ‘Katinka’ whose fruit size did not increase from June, 16, until June, 21. 2010. Thereafter, the fruit size increased as in other cultivars. The third stage (S3) is a period of rapid growth in the exo- and mesocarp that usually starts 4-6 weeks before harvest and is characterized by the increase of cell size that had been formed in the first stage (Zuzunaga et al., 2001; El-Sharkawy et al., 2007). During period S3, the fruit diameter has increased from 18 mm to 34 mm and from 16 mm to 35 mm for ‘Katinka’ and ‘Hoh 4517’, respectively. In the fourth stage (S4), the fruit growth rate decreases and fruit ripening starts (Diaz-Mula et al., 2008). Trainotti et al. (2003) divided S4 into two substages: S4-1 with the fruit reaching its full size without any change in ethylene production while fruit ripening continues with changes in ethylene production in S4-2. Remarkable differences among early and late cultivars have been observed in the course of this stage. In early cultivars, this period was short: the fruits reached their full size at harvest time or close to harvest date with 5 days for ‘Katinka’ as well as for ‘C. Lepotica’. ‘Topfive’ fruits reached their full size around one week before harvesting date. For the late ripening cultivars ‘Haganta’ and ‘Hoh 4517’, the time between the full fruit size and harvest date was about 10 to 15 days for the 2010, 2012 and 2013 seasons. However, there are few studies that have compared fruit development patterns of early and late cultivars of the same species (Bargioni et al., 1983; DeJong et al., 1987; Diaz-Mula et al., 2008). DeJong et al. (1987) reported that the variations between early and late cultivars

may be due to the differences in the rates of metabolic activity. It was shown that the respiration rate was higher in early cultivar ‘June Lady’ than late cultivar ‘O’Henry’. Although full bloom occurs within 10-14 days in almost all cultivars, there are large differences in fruit ripening dates. This was described for peach cultivars (Bargioni et al., 1983) and Japanese plum (Diaz-Mula et al., 2008). The differences in full blooms among cultivars ranged from 2-14 days but the differences in harvest dates were about 70 days. The differences in full bloom dates for all cultivars under study here were between 5-8 days but the difference in harvesting date reached 52 days between ‘Katinka’ and ‘Haganta’ in 2010.

In the present study, the harvest date differed among the seasons. In 2012, it was about 10-13 days earlier than in 2010 and 2013. In 2010, the harvest dates were August, 10th, 16th and 22nd for ‘Katinka’, ‘C. Lepotica’ and ‘Topfive’ and September, 27th and 29th for ‘Haganta’ and ‘Hoh 4517’, respectively. In 2012, the harvest dates were August, 1st, 13th, and 19th and September, 18th, for the same cultivars. On the other hand, the full bloom dates were in April, 26th, 20th and 28th for ‘Katinka’, April, 26th, 23rd and 30th for ‘C. Lepotica’, April, 26th, 24th and 30th for ‘Topfive’, April, 22nd, 18th and 26th for ‘Haganta’ and April, 27th, 26th and May, 2nd for ‘Hoh 4517’ for 2010, 2012 and 2013, respectively. The fruit development period (FDP) in 2012 was shorter than that in 2010with 104, 118, 154 and 146 days for ‘Katinka’, ‘Topfive’, ‘Haganta’ and ‘Hoh 4517’ in 2010 and 107, 119, 159 and 156 days in 2012 for the same cultivars but it took 113 days for ‘C Lepotica’ in both of seasons. FDP could be affected by temperature during early fruit development in spring. High temperature during this period (30-40 DAFB) reduces FDP which was shown for apple by Warrington et al., (1999) and for peach by (DeJong, 2005; Lopez and DeJong, 2007; Lopez, et al., 2006, 2007; Wert et al., 2009). Moreover, the fruit size exhibited significant differences among the seasons. For instance, the average fruit diameter of ‘Haganta’ was 39 mm in 2010 while it was 44 mm in 2012. This was a clear indication that the seasonal climatic differences have a direct effect on fruit quality attributes which was confirmed previously by Frick (1995) on pears. In our study, the changes in fruit diameter between subsequent measurements represent the changes in the fruit size during their growing period**.**

5.1.2 Creating variations in fruit development by thinning

Since consumers prefer big fruits, the fruit size is considered as an important external quality attribute that attracts consumers. Plum trees produce an excessive number of flowers which will become fruits upon pollination. When the actual fruit number exceeds the favorable one, the fruit size will be reduced resulting in a loss of fruit value (Webster and Spence, 2000). Therefore, crop load management is a critical factor for improving fruit

quality. Around 5% of flowers seem to be sufficient for producing a proper crop load of plum fruit with good quality for fresh market.

The results in chapter 4.1.3.1 Figs 4-6 to 4-10 point out that fruit thinning has improved the fruit size. Low crop loads (25, 20, 20, 15 and 25 fruits/100 cm for 'Katinka', 'C.Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517') have significantly produced the largest fruit size. These results were confirmed in three seasons for all cultivars except in 2010 for 'C.Lepotica', 'Topfive' and 'Haganta' with significant differences only between low crop load and high crop load (65-100 fruits/100 cm) while no significant differences were found between low and middle crop load (50, 40, 40, 30 and 50 fruits/100 cm for 'Katinka', 'C.Lepotica', 'Topfive', 'Haganta' and 'Hoh 4517'). These results are in agreement with the results obtained by Wells and Bukovac (1978) who found that the fruit size of 'Stanley' Plum (*Prunus domestica* L.) was well correlated with crop load. Similar results were found by Byers et al. (2003) for peach who stated that the thinning of flowers or fruitlets is an important factor in controlling the fruit size of stone fruits.

The effect of thinning on fruit weight has been studied in many fruit trees. Early fruit thinning (up to 1 month after full bloom) increased plum fruit weight (Belmans and Keulemans, 1987) without affecting the total fruit yield while thinning one month later increased the fruit size but the total fruit yield was decreased. These results support our data Tab. 4.1, a low crop produced the highest fruit weight at least in tendency but not always significant. On the other hand, in the 2nd experiment in 2010 Tab. 4-8 all differences were significant between high and low crop load. These results are in agreement with Buler et al. (2006) who found no significant differences based on fruit thinning of Japanese plum. This may be due to a late hand thinning or to the interaction with rootstock effects with more profuns differences in fruit weight (see below). Fruit weight is a quantitative parameter. Environmental conditions probably affect fruit growth and development (Kader and Mitchell, 1989; Crisosto, 1994; Corelli-Grappadelli and Lakso, 2004). In the present study, the greatest differences in fruit weight were found between seasons. The average fruit weight in 2010 was 29, 39, 30 and25 g for 'C.Lepotica', 'Haganta', 'Hoh 4517' and 'Katinka', respectively, while in 2012 it was 46, 56, 27 and 25 g for 'C. Lepotica', 'Haganta', 'Hoh 4517', and 'Katinka', respectively. The differences seem to be higher in cultivars that have big fruits such as 'Haganta' with a fruit weight of 39, 56 and 46 g in 2010, 2012 and 2013, respectively. Fruit weight could be also affected by the primary fruit set (number of fruits/m after setting). In 2010, the primary fruit set for 'Haganta' was 46% (46 fruits/m) while it was 26 and 60 % in 2012 and 2013. The competition among fruits in the early period after the fruit set (during S1) affects the fruit size and the fruit weight (Westwood, 1978) when the cell number increases due to cell division (Chalmers and Van Den 1975; Valero et al., 2010). The early thinning

could be more effective in improving fruit size and weight. The rate of fruit growth depends considerably on the crop load (Palmer et al., 1997). The increase in fruit size and weight with increased thinning intensity indicates a greater availability of nutrients for fruit growth and development (Frick, 1995; Tahir and Hamid, 2002; Peter and Abraham, 2007). Thinning may stimulate the fruit growth by affecting the rate and duration of cell division, by promoting cell enlargement, or by stimulating the production of intercellular spaces (Salvador et al., 2006).

In an earlier study (Meland, 2009) found that the final percentage of the fruit set of thinned treatment was higher than unthinned ones. Especially for heavy crop loads, the fruits drop may be heavier than this could be due to a dry season and a limited water supply.

In our study, thinning treatments improved fruit soluble solids content (SSC) in all cultivars except ‘C. Lepotica’ in 2010, Tab. 4.2, 3and 4. In 2012, no significant differences were found. Moreover, only ‘Topfive’ produced highly significant SSC with high leaf/fruit ratio (LFR) compared with low LFR in 2nd experiment in 2010, Fig. 4-28. Taqhipour and Rahemi (2010) found that SSC increases in pear fruits with thinning by ethephon due to the reduction of the fruit's volume. In contrast, other authors found that the SSC increases with light crop loads due to the enhancement of the leaf-fruit ratio reducing the competition among fruits (Taqhipour and Rahemi, 2010). Roussos et al. (2011) carried out thinning in three apricot cultivars at the pit-hardening stage. They found no significant differences in the SSC and the TA (titratible acidity) due to crop loads. In our experiment, in general, thinning levels have no significant effects on fruit TA and SSC/TA ratio except for ‘C.Lepotica’, ‘Haganta’ and ‘Topfive’ in 2010 without a stable trend. Similar results were obtained by Von Bennewitz et al. (2010) on sweet cherrywithout significant differences as well.

Our trials show that the effects of fruit thinning depend on cultivar, season and primary fruit set. According to Westwood (1992), some plum varieties do not need thinning as they will have higher “fruit drop” and lower crop load than other plum varieties that need thinning. Physiological and biochemical changes during the growing season should be taken in consideration with the waves of drop (Racskó et al., 2006). The number of successive waves and their severity depends on the intensity of the fruit set (Racskó et al., 2006). In addition, the general degree of fruit drop is highly variety dependent (Jackson, 2003).

5.1.3 Effect of rootstocks on fruit quality attributes

The effects of the rootstock on a fruit tree's adaptability, precocity, growth control, yield and fruit quality attributes are well known from previous studies (Grzyb et al. 1998; Webster, 2001; Botu et al., 2002; Hrotko et al., 2002; Botu et al., 2004; Lanauskas, 2006; Daza et al., 2008; Świerczyński and Stachowiak, 2009). However, studies analyzing the

effect of rootstocks on plum fruit quality are more recent and scarcer than those studying the behavior on different soil types or the response to soil born diseases. Daza et al. (2008) suggested the following trends of rootstocks on fruit yield and fruit quality attributes as follow: high yield was produced by cultivars on rootstocks of hybrids > peaches > plums rootstocks, high firm fruit was produced by cultivars on rootstocks of hybrids > peaches > plums rootstock, more colored fruits were produced by cultivars on rootstocks of plums > peaches > hybrids rootstpcks and high soluble solids content were produced by cultivars on rootstocks of plums > peaches > hybrids. In experiment presented here, the rootstock had an impact on the plum fruit quality attributes especially on fruit weight and fruit size (Fig.s 4-16 to 4-18 and Table 4-5). Myrobalan rootstock produced the largest fruit size and the biggest fruit weight in most cultivars. The differences were significant. These results are contrary to those of Lanauskas (2006) using Myrobalan, 'St. Julien A' (*Prunus insititia*), 'St Julien GF 655/2' and 'Marianna GF 8/1' (*Prunus cerasifera x Prunus munsoniana*), and of Świerczyński and Stachowiak (2009) using Myrobalan and a selection of *Prunus tomentosa* who found no influence of rootstocks on fruit weight. They reported that the mean of the fruit weight depended only on the cultivar.

In contrast, Grzyb et al., (1998) reported significant effects of Myrobalan, 'Wangenheims' and vegetatively propagated rootstocks ('Pixy', 'St. Julien GF 655/2') on fruit weight. These results are in line with our results. Daza et al., (2008) found also significant effects of rootstocks on the fruit weight and size of 'Pioneer' Japanese plum. From the previous studies, information about the rootstock effect on fruit weight is diverse.

In the present study, rootstocks had also significant effects on soluble solid contents (SSC). The highest SSC values were produced on Myrobalan by most cultivars (Tables 4.6 and 7). There are no significant effects of rootstocks on titratable acidity (TA) and SSC/TA ratio in most cultivars. There are diverging reports stating that rootstocks either affect fruit organic contents (Lipecki et al., 2001; Daza et al., 2008) or do not (Dziedzic et al., 2006). In a study twelve years ago, Dziedzic et al. (2006) showed significant effects of rootstocks on SSC in some seasons and non-significant effects in others.

In general, Myrobalan rootstock had good effects on quality parameters in most cultivars. It is well known from previous studies that trees grow strongly on vigorous rootstocks and that they enter the fructification period relatively late (Lang, 2000; Sitarek et al., 2007). Since the trees in the orchard of the Unit of Fruit Science were planted in 2005 and experiments were started in 2010, it could be that some trees grafted on the vigorous Myrobalan rootstock still have not yet reached the mature stage. The numbe rof fruits set after 3-4 weeks after full bloom of cultivars grafted on Myrobalan were significantly the lowest

compared with other rootstocks (data not shown). This could be the reason why the fruit weight and the SSC were higher in fruits from trees grafted on Myrobalan compared with others. At the same time, chlorophyll (Chl) detected non-destructively by Multiplex on fruits of cultivars grafted on Myrobalan was significantly higher but, contrary, anthocyanins (Anth) index was the lowest in the same fruits (see below). These results confirm the idea that vigorous rootstocks increase the fruit content of Chl and decrease the content of Anth.

From the results obtained in this study with European plums, we cannot establish a general rule. In fact, it seems that the effect of different rootstocks on fruit quality attributes differs from one year to another. It is even possible that the same rootstock would show opposite behavior in different seasons. Similar results were reported also for peaches and apricots (Egea et al., 2004). Additional studies for longer periods are necessary towards a better understanding of the influence of rootstocks on fruit quality parameters in European plums.

5.2 Non-destructive detection of kinetics of European plum fruit ripening

Fruit maturation and ripening is associated with important biochemical changes that modify color, texture, taste and other quality traits. The color changes are due to degradation of chlorophyll by the enzyme chlorophyllase (Dangl et al., 2000) and coinciding synthesis of the pigments characteristic for each fruit. The color of red and purple plum fruits is mainly contributed by anthocyanins. In this study, anthocyanins (Anth) index assessed by a non-destructive tool (Multiplex) increased in all cultivars and seasons (Figs. 4-9 to 4-13) during maturation and ripening. This result was predictable as Anth synthesis started after the beginning of S3 and increased sharply at the end of this period and the beginning of S4 as reported by destructive methods (Diaz-Mula et al., 2008; Valero and Serrano, 2010; Miletić et al., 2012).

In the present study, we used the non-destructive tool to detect anthocyanins, flavonols and chlorophyll development during fruit maturation to find a relation with ripening and optimal harvest time. The results show that changes in anthocyanins can reliably be detected. Anth index showed a good correlation with fruit size during fruit growth in some cultivars like ‘Haganta’ with $r^2 = 0.96$, 0.81 and 0.85 for 2010, 2012 and 2013, respectively (Fig. 4.3), and ‘Hoh 4517’ with $r^2 = 0.88$ and 0.81 for 2010 and 2012. On the other hand, fruit size showed weak correlation with Anth Multiplex index by $r^2 = 0.23$, 0.47 and 0.09 for ‘C. Lepotica’, ‘Topfive’ and ‘Katinka’ in 2012, respectively. However, ‘Katinka’ fruit size showed a good correlation with Anth index in 2013 with $r^2 = 0.93$. However, it is cautioned that the

exact relationships differed with the cultivars. Moreover, there is a considerable limitation of Anth index because of the occurance of a saturation effect or even the occurance of a decrease that could be found in the last stage of ripeningwhen the fruit full size point was already passed. This effect may be due to the changes in fruit structure during the season such as increasing of transparency that influence its optical properties (Ben-ghozlen et al., 2010a) or increasing pH that could shift anthocyanins absorption region (Treutter, personal communication). More work is needed to study this point that could be a key point in understanding plum fruit ripening. With solving the saturation effect, the Multiplex could eventually be used for anthocyanidins quantification as used in grape (Ben-ghozlen et al., 2010a). They reported that the anthocyanidins content can be calculated from Multiplex values as Anth (in mg/L) = Anth (Multiplex) / 0.0016.

A continuous decrease of the flavonols (Flav) index was observed for all varieties in all seasons during maturation. Generally, it is well known that phenolic acid concentrations decrease during ripening, whereas flavonoid concentrations increase (Macheix et al., 1990; Manach et al., 2004). On the other hand, other researchers reported different trends as for pears by Amiot et al. (1995) who showed three different trends of total phenolics: first one is the total phenolics is increasing with fruit ripening, second trend is the total phenolics is increasing at the beginning and subsequent decrease during ripening and the third trend is the total phenolic decrease during ripening. Similar trends were obtained by Miletić et al. (2012) for the European plum cultivar ‘Stanley’ depending by the ripening stages. The lack of a clear trend concerning total phenolics content may be due to the variations in composition of compounds that are ranked within phenols during ripening as reported by Buta and Spaulding (1997) and Raffo et al. (2002) in their studies on tomato fruits.

Generally, Flav index showed a weak correlation with fruit size during maturation with r^2 = 0.52 and 0.43 for ‘Haganta’ and ‘Hoh 4517’ in 2010, 0.47, 0.46, 0.03, and 0.51 for ‘Topfive’, ‘Katinka’, ‘Hoh 4517’ and ‘Haganta’ in 2012, and 0.52 and 0.49 for ‘Haganta’ and ‘Katinka’ in 2013, respectively. However, it showed a good correlation with ‘C. Lepotica’ cultivar with r^2 = 0.75 in 2012, Fig. 4.4.

Similar relation as for the Flav index was observed for the non-destructive index of chlorophyll (Chl). Chl index was weakly correlated with fruit growth during maturation period: r^2 of 0.25 and 0.37 for ‘Haganta’ and ‘Katinka’ in 2013 and r^2 of 0.54, 0.18, 0.005, 0.64 and 0.49 for ‘C Lepotica’, ‘Haganta’, ‘Hoh 4517’, ‘Katinka’ and ‘Topfive’ in 2012, respectively. However, a good correlation was noticed for ‘Haganta’ in season 2010.

Unexpectedly, Chl index increased slightly in some cultivars such as ‘Haganta’ and ‘Hoh 4517’ and sharply in others such as ‘C. Lepotica’ and ‘Topfive’ (Fig. 4.5). Ben-Ghozlen et al. (2010a) reported that the large content of anthocyanins in red cultivars at the later stage of maturation can induce an apparent decrease in flavonols and an increase in Chl fluorescence signatures.

In the present study, the Multiplex sensor could be used for anthocyanins, flavonols and chlorophyll development in plum fruit simultaneously in situ on-tree. Multiplex measurements showed clearly the accumulation of anthocyanins in fruits during maturation. In addition, the Multiplex sensor detected the decrease of flavonols in all cultivars during the same period. At the same time, it showed the behavior of chlorophyll during ripening in European plum which decreased at the beginning and later started to increase. This could a result of anthocyanin accumulation at the later stage of maturation (Ben-Ghozlen et al., 2010a).

It is clear from the presented data that the Multiplex Anth index and fruit size run synchronously (Fig.s, 4-1 to 4-13). The full size point (the beginning of maturation as reported by Ismail and Kender, (1974)) coincides with the highest value of Anth index in all cultivars and nearly all seasons except for ‘C. Lepotica’ in 2012. This congruency as reported in section 5.1 is longer for late cultivars like for ‘Haganta’ and ‘Hoh 4517’ than for early cultivars. Therefore, the correlation between the data derived from anthocyanin measurement by Multiplex and fruit development can consitute a useful tool for following the maturation in plum fruit.

5.2.1 Influences of cultivars and crop load as determined by Multiplex

The monitoring of anthocyanins, flavonols and chlorophyll by Multiplex in the present study showed an important variation from season to season. Anth index for ‘Haganta’ was 2.6, 2.0 and 2.2 for 2010, 2012 and 2013, respectively. This variety showed the highest anthocyanins in all seasons Fig.s 4-11 and 4-15. It seems that late cultivars showed higher anthocyanins content than earlier ones. Similar results were reported for raspberries (De Ancos et al., 2000; Anttonens and Karjalaine, 2005). Miletić et al. (2012) reported the effect of season on anthocyanins and phenolic acids for the European plum cultivar ‘Stanley’. The detection of dynamic of European plum fruit maturation by Multiplex showed precociously the fruit color development for low crop load than middle and high crop load. Similar effects of crop load on fruit maturity were detected by destructive methods in apples by Sharples (1968), Palmer et al. (1997) and Wünsche et al. (2000). They showed that fruit maturity was

reached earlier on trees with low crop load. The differences detected by Multiplex were largely in accordance with differences in fruit size which were reported in paragraph 5.1.2.

5.2.2 Influence of rootstocks on maturation of plum fruits

As mentioned previously, the differences due to the rootstocks used on fruit maturity were more obvious than the differences based on the thinning levels. Similarly, significant influences of rootstocks were detected by Multiplex, in Anth, Flav and Chl indices. Generally, the highest anthocyanins values were detected in almost all cultivars on 'Wangenheims' (dwarfing rootstock) followed by 'Wavit' (dwarfing rootstock), but the lowest were measured in cultivars grafted on Myrobalan (vigorous rootstock). A similar trend was found for flavonols. Contrary, Chl index was higher in fruits derived from cultivars grafted on Myrobalan rootstocks while being the lowest for cultivars on 'Wangenheims'. It is well known that fruit maturity is earlier if varieties are grafted on dwarfing rootstock rather than on vigorous rootstocks. In addition, fruits from trees grafted on dwarfing rootstocks have a more reddish coloration than fruits of cultivars on vigorous rootstocks (Jackson, 2003). Moreover, the results obtained by Multiplex in this study demonstrate also that fruit quality and phytochemical characteristics were significantly affected by rootstocks.

5.2.3 Physiological behavior of Plum fruits during ripening assessed by Multiplex

In the present study, 'Haganta' plum fruits classified as S1 (more ripe) or S2 (less ripe) on the basis of the Anth and Flav indicesshowed significant differences in ethylene production rate (EPR). Additionally, the climacteric ethylene peak was earlier in S1 than in S2 (Fig. 4-36). Similar studies were performed by Gomila et al. (2011a, b) on 'Williams' pear. They used spectroscopy in the Vis-NIR range and established the AD index (absorbance difference) as the difference between the average values of absorbance between two points near to the peaks of chlorophyll absorption: 677 and 722 nm. They found a high correlation between the evolution of AD during shelf life and ethylene production. They concluded that AD index could be useful to identify the physiological changes that occur during 'Williams' pear ripening with the advantage of an instant and non-destructive determination.

5.3 Physiological and biological behavior of European plum fruit during the post-harvest phase

The understanding of the biochemistry and molecular biology of the ripening process is a key point of developing biotechnological strategies for extending fruits shelf life and

quality (Paliyath, et al., 2008). Ethylene plays a significant role in regulating the ripening process of climacteric fruit. Thus, the physiological behavior of fruits has become more important in the postharvest biology and technology. In present study, the physiological behavior of some European plum cultivars was studied. Few analysis had been carried out on Prunus domestica compared to Japanese plum. This study concentrated on the ripening behavior of European plum cultivars due to the difficulty in assessing the harvest maturity and because of their short storage and shelf life. The effect of ripening stage on physiological and biochemical changes was determined during this period.

5.3.1 Ethylene production rate (EPR) in European plums

The analysis show great differences among plum cultivars in EPR behavior during ripening (Fig. 4-29 to 33). Some cultivars showed a clear climacteric behavior in ethylene production such as ‘Haganta’, ‘Anna Späth’, ‘Hanka’ and ‘Katinka’ with a EPR of 8.0, 9.0, 22.0 and 12.0 PPM/kg/h, respectively. On the other hand, some cultivars showed little changes in EPR at the end of shelf life period such as ‘Hauszwetsche Wolff’, ‘President’, ‘Tophitplus’ and ‘Haroma’ with 0.5, 1.0, 2.0 and 0.6 PPM/kg/h, respectively. These results are in accordance with those of other researchers gained on Japanese plum (Abdi, et al., 1997; Serrano et al., 2003; Singh and Khan, 2010) as they found some cultivars with typical climacteric reaction and others with suppressed climacteric reaction such as ‘Shiro’, ‘Rubyred’, ‘Songold’ and ‘Golden Japan’. Suppressed climacteric phenotypes were previously reported by Abdi et al. (1997) for some Japanese plum cultivarswhich fruits produced rather low levels of ethylene during the late ripening stage when compared to normal climacteric ones. Also, respiration rates It was shown which were 15-500 times less than that of climacteric phenotypes (Singh and Khan, 2010). Similar behavior was reported for some apple and pear cultivars (Sfakiotakis and Dilley, 1973; Downs et al., 1991).

The same tendency was found for the respiration rate of ‘Katinka’ and ‘Hanka’ at their climacteric peak with 28 and 22 ml CO_2/kg/h. More differences were reported for peach which respiration rates varied from 64 to 110 ml CO_2/kg/h at 20 ºC depending on the genotype (Crisosto and Kader, 2002).

In this study, EPR was higher in early cultivars such as ‘Hanka’ and ‘Katinka’ (Figs. 4-34 and 4-39) than in the late cultivars such as ‘President’. ‘Tophitplus’, ‘Hauszwetsche Wolff’ and ‘Haroma’ (Figs. 4-29 and 4-34). In early cultivars, ethylene production reached the climacteric peak in the course of 3 days of storage at 20 °C room temperature, while in late cultivars the ethylene peak appeared after 7 days of storage under the same conditions which indicates the onset of the climacteric stage. These results are supported by studies on

peaches (DeJong et al., 1987) . They found that early cultivars had higher and more pronounced respiration rates at the climacteric peak than the late ones. Golias et al. (2013) stated similar results on the late maturing apricot cultivars ‘Leskova’ and ‘Bergeron’ as the fruits exhibited a low rate of ethylene production while the respiration rate is about 15-500 times less than in climacteric cultivars (Abdi et al., 1997; Khan and Singh, 2010). These results support the hypothesis that suppressed climacteric cultivars ripen slowly and exhibit better storage potential than climacteric phenotypes (Abdi et al., 1997; Khan and Singh, 2010). In present study, cultivars that showed low EPR stayed longer in shelf life.

El-Sharkawy et al. (2007, 2008, and 2009) reported that early ripening cultivars exhibited a typical climacteric behavior accompanied by a sharp increase of the ethylene perception and signal transduction components (EPSTCs) during development and ripening in an ethylene-dependent manner. On the other hand, late cultivars showed a suppressed-climacteric pattern with slight increase in ethylene production related to ripening. They suggested that the differences in the accumulation levels and/or pattern of the various ethylene perception components throughout ripening of early and late fruits might be due to the variation in the levels of auxin and ethylene among the two plum cultivars. In early cultivars, auxin might be accumulated rapidly and in much higher levels during S3 which may lead to up-regulation of different transcripts and proteins associated with auxin including different ethylene synthesis, perception, and signal transduction elements (Miller et al., 1987). In contrast, late cultivars seem to produce insufficient quantities of auxin to co-ordinate the transition into ripening stage. The low level of auxin throughout the S3 stage results in minimal accumulation of ethylene-related proteins and, consequently, delay in reaching the S4 stage by the fruit. Such variations affect the capacity of the fruit to produce and to respond to ethylene which results in the differentiation in ripening behavior thereafter (Miller et al., 1987).

The physiological basis involved in the ripening process of European plum is still unclear impeding the development of technologies to enhance fruit storability.According to the respiratory pattern, fruits have been traditionally classified as climacteric or non-climacteric (Biale, 1964). The ripening of climacteric fruits is accompanied by a distinct increase in respiratory rate which is generally associated with elevated ethylene production just before the increase of respiration. After the climacteric rise, ethylene production declines significantly during the postclimacteric phase (Hoffman and Yang, 1984; Barry and Giovannoni, 2007). In these fruits, ethylene plays a key role in the physiological and biochemical changes that occur during ripening (Lelièvre et al., 1997; Giovannoni, 2001). However, non-climacteric fruits do not exhibit increases in ethylene production and

respiration rate but rather undergo a gradual decline in respiration rate during ripening (Knee et al., 1977). The classification of European plum as a climacteric or non-climacteric fruit is contradictory. While many authors classify plum cultivars as a climacteric fruit there are many cultivars exhibiting a suppressed climacteric reaction which was described for first time by Abdi et al., (1997) for Japanese plum. The results obtained in this study confirm a similar behavior for some European plum cultivars. Thus, classification European plum as a climacteric or a non-climacteric is an oversimplification since this characteristic could depend on the cultivar analysed.

5.3.2 Effect of harvest date on ethylene production rate (EPR)

In the present study, EPR was higher at advanced harvest dates after cold storage of plum cultivars. In the same way, fruits in an advanced ripening stage (Stage 1), as determined by Multiplex (Fig. 4-36), showed higher EPR than those in stage 2 (less ripe) and reached the ethylene peak earlier. On the other hand, EPR was higher in fruits which have been harvested earlier. However, plum fruit shelf life was longer in 2011 than in 2012 and 2013 as most of the harvest dates were before the optimum harvest date. This explains why EPR in 'Haganta' reached the climacteric peak earlier in 2012 and 2013 (4-12 days) while being stored in room temperature whereas in 2011, the peak was accomplished 7-14 days after storage at room temperature. However, ethylene reached climacteric peak in early cultivars ('Katinka' and 'Hanka') on the second or third date of shelf life but in late cultivars 'Haroma' and 'Haganta', it was determined on the seventh and forth day under the same conditions in the same season, respectively. Similar results were obtained by Valero et al. (2003) on the European plum cultivar 'President'. EPR was higher accompanied by the peak of EPR detected earlier in fruits which were harvested on the first harvest date than those harvested on the second date.

5.3.3 Effect of harvest date on fruit quality attributes

Harvest date significantly influenced fruit color parameters. Fruit color (-b which represents blue color) at a late harvest date was significantly higher than at an early harvest date. The change in fruit color at the second harvest date was not significant. Though, at the end of the storage period no significant differences were found between the first and the second harvest dates for the blue color due to the decrease in (-b) for late harvest date and its increase for earlier harvest dates (Table 4-9). However, fruit color on the first harvest date did not reach the blue color value of the fruits harvested on the second harvest date. Fruits harvested earlier lost their luminosity (lightness, L*) faster than those harvested late. Variation in loss of lightness may be due to the fruit wax layer which might be uncompleted in fruits harvested earlier.

Casquero and Guerra (2009) showed that the 'Green Gage' plum fruits which were harvested in early stages did not develop the specific color (greenish yellow) neither during cold storage nor during ripening at room temperature conditions. Similar results were obtained by Westercamp (1996) for the same cultivar.

SSC increased significantly at advanced harvest dates in all cultivars as well as during ripening at room temperature (Table 4-12). SSC also increased under cold storage in earlier harvest dates for most of cultivars but it was decreased in last harvest dates (close to optimal harvest date). On the other hand, TA decreased with advanced harvest dates as well as during ripening at room temperature and cold storage. The changes in SSC and TA during harvesting and cold storage resemble the results found for 'Songold' plums by Taylor et al. (1995), Westercamp (1996), and for 'Green Gage' plums by Casquero and Guerra (2009). Studies of Agulheiro-Santos and Pacheco Ribeiro (1998) on 'Green Gage' plums have not shown any significant increase in SSC after harvesting. Kluge et al. (1996) suggested that the increase in SSC after harvesting is due to water loss.

The SSC/TA ratio increased with harvesting dates as well as during cold storage and ripening at room temperature with the later dates having higher values. Similar results were obtained by Kluge et al. (1996) and Casquero and Guerra (2009) whereas contradictory results have been reported by Meredith et al. (1989) for peaches and by Guerra et al. (2008) for 'Green Gage' plum. Crisosto (1994) described the SSC/TA ratio as the most reliable parameter for plum ripening as this ratio increases during ripening and has a good relation with human perceptions of fruit quality (Taylor et al., 1993; Khan and Singh, 2007; Casquero and Guerra, 2009).

Weight loss under cold storage was higher in fruits harvested at the earlier harvest date than at the late harvest date for all cultivars as well as for the cultivars 'Haganta' and 'President' after shelf life. However, weight loss after shelf life of fruits of 'Anna Späth' and 'Tophitplus' was slightly higher when picked late compared to early harvest (Fig. 4-37 and Tab. 4-13). These results are supported by studies of Kluge et al. (1996) and Agulheiro-Santos et al. (2005) on 'Green Gage' and 'Rainha Claudia' plums. On the other side, analysis on 'Green Gage' plum showed that weight loss was greater when harvesting fruits at a late date than at an early date (Casquero and Guerra, 2009). Fruit weight loss depends mainly on loss of moisture which is regulated by epi-cuticular waxes which increase during maturation (Lau, 1992). The high weight loss in fruit harvested at an early stage of maturation may be due to a poorly developed waxy surface and cuticle (Ihabi et al., 1998; Sass and Lakner, 1998). It may explain relatively lower weight loss in fruits harvested late that have completely developed a waxy layer on their surface (Lau, 1992). At the same time, it may also explain relatively loss of luminosity (L* parameter) in early harvested fruits.

In this study, the incidence of weight loss during storage could be reduced by harvesting at a proper harvest date. Plum cultivars vary significantly in weight loss from 2.27 to 5.47% for 'Tophitplus' and 'Haganta' harvested at the first date and 1.34 to 2.93 % for 'President' and 'Haganta' harvested at the second date, respectively. The transpiration of fruits depends on skin thickness and the nature of surface waxes as reported for apples (Veraverbeke et al., 2003) which may vary considerably for different cultivars or even for the same cultivar in different years of production (Homutova and Blazek, 2006). The moisture and subsequent weight loss in fruits generally increased with the increase in storage duration (Guerra et al., 2009; Casquero and Guerra, 2009)

The present as well as previous studies confirm the importance of determining the optimal harvest date (Abdi et al., 1997; Kader, 1999; Casquero and Guerra, 2009). Weight loss and loss of lightness were higher in case of harvesting fruits at an early stage. In addition, early harvested fruits could not reach the optimum color and ripening index.

5.3.4 Impact of 1-MCP on fruit ripening and quality

The ripening process of climacteric fruits is regulated by the plant hormone ethylene. Ethylene plays a key role as a plant hormone in coordinating and initiating ripening events in climacteric fruits (Abdi et al., 1998; Bapat et al., 2010). It triggers the processes of ripening and senescence. Once the autocatalytic ethylene production starts, a wide range of both physical and chemical changes occure such as tissue softening, pigment degradation and biosynthesis of new ones and changes in sugars and organic acids composition and concentration (Giovannoni, 2001). The ripening of climacteric fruits can be delayed by ethylene inhibitors (Liu, et al., 2005).

There are a number of approaches to manipulate the rate of maturation and ripening ranging from preharvest application to the fruits to application of postharvest physical treatments (Toivonen, 2007). Current research is focused on the use of effective and non-contaminants agents to prolong the fruit storability and to extend their shelf life. The application of 1-MCP in some of European plum cultivars showed beneficial effects such as reduction of both ethylene production and respiration rate and loss of fruit weight. Fruit quality could also be maintained by what storability and shelf life could be extended.

Application of 1-MCP significantly reduced ethylene production rate (EPR) during shelf life or cold storage for the early cultivar 'Katinka' and the late cultivar 'Haganta' during seasons 2012 and 2013. Similar results with 1-MCP were obtained in several Japanese and European plum cultivars (Abdi et al., 1998; Salvador et al., 2003; Valero et al., 2003). The reduction of ethylene production during shelf life of European plum after or without cold storage may be caused by 1-MCP. One explaination is that 1-MCP might interact, compete

with or block ethylene receptors irreversibly (Blankenship and Dole 2003). The reduction of ethylene production also could be due to the suppression of key enzymes in ethylene synthesis cyrcle 1-aminocyclopropene-1-carboxylic acid synthase (ACS, EC 4.4.1.14) and 1-aminocyclopropene-1-carboxylic acid oxidase (ACO, EC 4.4.17.4) and the reduction of 1-aminocyclopropene-1-carboxcylic acid (Khan and Singh, 2007, 2009). It seems that some new receptors are formed since ethylene production started slightly to increase. However, the ability of fruits to generate new receptors depends on the fruit type as reported in previous studies for 'Royal Zee' plum (Dong et al., 2002) and apple (Watkins et al., 2000). However, 1-MCP treatment delayed the onset of ethylene production but it was not totally inhibitory as reported for Japanese plum (Abdi et al., 1998), peaches (Mathooko et al., 2001) and apricots (Dong et al., 2002).

In a previous study on Japanese plum by Abdi et al., (1998), it is reported that repeated 1-MCP applications are required for climacteric phenotypes but a single application is sufficient for suppressed climacteric phenotypes. The effectiveness of 1-MCP depends on 1-MCP doses, on the fruit ripening stage as well as on application conditions (Valero et al., 2003, 2004) but this information is still scarce for European plum.

Here, 1-MCP significantly decreased respiration rate for 'Katinka' and 'Haganta' (Fig. 4-39). These results are in accordance with findings by Dong et al. (2002), Salvador et al. (2003) and Valero et al. (2003, 2004). 'Katinka' showed a biphase pattern in respiration rate at the second and at the forth date of ripening at room temperature in treated and untreated fruits. This behavior was reported in a previous study on banana and Japanese plum by Khan et al. (2009). On the other hand, they found that treating the plum fruit with high 1-MCP doses of 2.0 ppm prevented any respiratory raise in 'Tegan Blue' Japanese plum. However, the effect of 1-MCP on respiration rate was not as pronounced as that found on ethylene production rate.

In the present study, soluble solid contents (SSC) increased in the beginning in treated and untreated fruits at room temperature and cold storage and then started to decrease in untreated control fruit. On the other hand, SSC steadily increased in 1-MCP treated 'Haganta' fruits until the end of the storage period (30 days in cold storage + 7 days in shelf life) in 2012. In 2013, it slightly decreased for 'Haganta' and 'Katinka'. SSC in 1-MCP-treated fruits was lower than in fruits untreated used as control at the beginning but at the end of storage was significantly higher than SSC in control treatment. These results confirm that 1-MCP delayed ripening in 'Haganta' and 'Katinka' European plum fruits (Valero et al., 2004; Khan and Singh, 2007; Khan et al., 2009). However, other studies point out that 1-MCP has no effect on SSC on some species such as Japanese plum and apricot (Dong et al., 2002; Salvador et al., 2003) and orange (Porat et al., 1999).

On the other hand, titritable acidity (TA) decreased in 1-MCP treated and untreated fruits both under cold storage or normal conditions. Slightly differences were noticed between treated and untreated fruits in TA. These results are in accordance with results obtained by Dong et al. (2002) for apricot and Porat et al. (1999) for orange. However, an inconsistant trend was found for some apple cultivars (Watkins et al., 2000). In contrast, 1-MCP delayed the decline of TA for European plum (Valero et al., 2003), for peach (Liu et al., 2005) and for some apple cultivars (Fan et al., 1999a, b).

Generally, SSC/TA ratio increased in 1-MCP treated and untreated fruit in both seasons. SSC/TA ratio was significantly higher in 1-MCP treated fruit by the end of the storage period for ‘Haganta’ in 2012 and 2013 but it was slightly lower for ‘Katinka’. These results are in accordance with the analysis of Martınez-Romero et al. (2003) on Japanese plumshowing that the effect of 1-MCP on SSC/TA ratio is dose-dependent for ‘Santa Rosa’ but not for ‘Golden Japan’.

Upon application of 1-MCP, weight loss was markedly reduced from 30% to 10% in ‘Haganta’ and from 10% to 7.5% in ‘Katinka’. ‘Haroma’ exhibited contradictory trends as shown in (Fig. 4-44). The effect of 1-MCP on reducing weight loss was described by many authors for plum (Valero et al., 2003) and avocado (Joeng et al., 2002). On the other hand, no effect has been detected for apricot and orange (Fan et al., 2000; Porat et al., 1999). Loss of weight is one of the most important reasons for fruit quality deterioration. Based on results of the present study, 1-MCP could be a good tool to delay weight loss during shipping and marketing of European plum.

Generally, fruits loose flesh firmness during maturation and ripening. Fruit firmness is a often appreciated sensorial attribute and is sometimes considered the main factor for fruit acceptance. In our results, 1-MCP significantly decreased the softening of fruit compared with non-treated fruits (Fig, 4-45). These results are in accordance with many authors reporting on plum (Salvador et al., 2003; Valero et al., 2003, 2004; Khan and Singh, 2007, 2009) and on some other fruit species such as avocado and mango (Hofman et al., 2001) with a 1-MCP based delay in fruit softening for 4.4 and 5.1 days, respectively, compared to control fruits. The early cultivar ‘Katinka’ has less firmness compared with late cultivars ‘Haganta’ and ‘Haroma’. This result is in the same trend with the reported study by Kader and Mitchell, (1989).

6. Summary

Fruit quality and especially eating quality of stone fruits has become very important for markets and consumers in recent decades. Pre- and postharvest factors that influence the quality of European plum were studied during three seasons (2010, 2012 and 2013) to optimize fruit quality at harvest and to maintain this quality during storage and marketing. The locations of a tree in the field and of a fruit on the tree affect the measured maturity of the plum fruit in addition to greater genetic variations in plum. For solving this problem it is necessary to understand the variation within the crop and manage its distribution and marketing accordingly. The stage of maturity at harvest is an important preharvest factor that directly affects the rate of fruit ripening and the quality following storage. Hence, a non-destructive method for monitoring plum fruit maturation was studied with the aim in determining the optimal picking time. This analysis included preharvest factors affecting fruit quality.

The non-destructive monitoring included the estimation of the fruit skin pigments anthocyanins (Anth), chlorophyll (Chl) and flavonols (Flav). The Anth index showed a good correlation to fruit size until its full size point. Thereafter, the Anth index declines for most cultivars. Therefore, the Anth index can be used to follow fruit development.

Using the non-destructive data on skin pigments it was possible to sort plum fruits according to their maturity degree. This was confirmed by their respective ripening behavior as indicated by their ethylene production. Thus, the method can be used to sort fruits after harvest with respect to their storability.

Fruit thinning experiments were made aiming in altering fruit growth and inner quality parameters. Despite of the late thinning, fruit size, soluble solids and titratable acid were mostly improved in the low crop load variant. These effects could hardly be detected by the non-destructive monitoring of skin pigmentation. In tendency, an earlier anthocyanin accumulation appeared in fruits when crop load was low.

The rootstocks have highly significant effects on fruit quality. The highest fruit size was produced by ‘C. Lepotica’, ‘Topfive’ and ‘Hoh 4517’ on Myrobalan rootstock and the lowest on ‘Wavit’ rootstock in 2012. The smallest fruit size for ‘Katinka’ in 2012 and ‘Haganta’ in 2013 was produced on Myrobalan. The results showed a significant effect of rootstocks on fruit chemical attributes. The highest SSC values were produced by ‘C. Lepotica’, ‘Katinka’ and ‘Topfive’ on Myrobalan in 2012 and ‘Haganta’ on ‘Wangenheims’ in 2013. The results

show the influence of the rootstock on the cultivar grafted. Non-destructive color monitoring by Multiplex showed higher anthocyanins (Anth index) in fruits produced by most of cultivars on dwarfing rootstocks (‘Wangenheims’ and ‘Wavit’) but the highest chlorophyll (Chl index) was produced by cultivars on vigorous rootstock (Myrobalan)

A further aim of this study was to analyze the postharvest behavior of European plum cultivars and the influence of the stage of maturity at harvest on quality changes during storage and shelf life. The influence of 1-MCP on physiological and biological characteristics of plum fruits was studied as well. The results of this work showed a clear climacteric peak in ethylene production especially for earlier ripening cultivars with 8, 9, 22 and 12 ppm/kg/h for ‘Haganta’, ‘Anna Späth’, ‘Hanka’ and ‘Katinka’, respectively, compared to late ripening cultivars which have a lower ethylene production of 0.5, 1, 2 and 0.6 ppm/kg/h for ‘Hauszwetsche Wolff’, ‘President’, ‘Tophitplus’ and ‘Haroma’. The late cultivars showed longer shelf life than the early cultivars. 1-MCP treatment of the fruits reduced ethylene production more than 30 % and delayed the climacteric peak in ‘Haganta’ and ‘Katinka’. In addition, 1-MCP delayed fruit ripening shown by a lower SSC in treated fruits at the beginning of storage and by a significant higher SSC at the end of storage. Moreover, it significantly decreased weight loss and delayed fruit softening for the cultivars under study but no stable trend with acidity (TA) was found. Based on the result obtained by this work 1-MCP is a good tool for delaying ripening the European plum fruit cultivars under study.

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Publications emerged from this work

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