Promoting Young Apple Tree Growth after Planting in Water Limited Areas

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CHAPTER 1.

GENERAL INTRODUCTION

1.1. The importance of young apple tree growth in Uzbekistan

The economy of Uzbekistan depends heavily on the growing of cash crops. The share of agriculture in the country's GDP is 25%. Traditionally, agricultural production in Uzbekistan has been dominated by cotton and wheat and is less diversified than that of other countries. However, it is gradually moving away from cotton towards the cultivation and marketing of fruits and vegetables and other higher-value crops. The production of fruits such as apples, cherries, and peaches are increasing rapidly, and many new plantations have been established. Intensive high-density orchards were first introduced in the Samarkand region. In 2011, these high-density orchards covered 639 hectares, while in 2015, they had increased to 5,817 hectares (Ostonakulov et al., 2016).

In order to fully and efficiently exploit the export potential of the agricultural sector through increased production and the expansion of the range of fruits and vegetables being grown, two companies, "Uzbekoziqovqatholding" and "UzAgroExport," have taken over the main management tasks as well as the direction of the promotion and the handling of specialized fruit and vegetable exports.

Fruit production has grown rapidly in Uzbekistan over the past five years. Grapes and apples dominate, with apple production coming in second to grapes (Figure-1.1.1.). One of the reasons for this expansion is that it is taking place on land that is being converted from cotton. This expansion of fruit and vegetable production, fruit in particular, because of the initial capital outlays and the time it takes for a vineyard or orchard to mature, calls for the careful nurturing of the newly planted vines and trees. This is, of course, holds true for new apple orchards.

Since tree growth and the emergence of healthy branches after planting is vital for early fruiting; careful tree management is necessary for the promotion of tree growth. Uzbekistan has one of the driest climates in Central Asia (Hu Zengyun et al., 2016). Uzbekistan's hot and dry summers puts its apple industry at a disadvantage in the very competitive global apple marketplace. For example, if red-skinned apples are not covered with netting, the fruit will not color well but will instead burn, resulting in faster deterioration. Meanwhile, crop yields are relatively low compared to those in orchards in temperate climates. Finally, modern technologies necessary for the production, storage, sorting, and packaging of apples are costly, since all the equipment has to be imported. Finally, the amount of rain that falls during the growing season in Uzbekistan is insufficient for commercial apple production and irrigation systems are still being developed.

The availability of branched (feathered) nursery trees and the possibility of harvesting a crop in a tree's second or third year are critical components of high-density apple planting systems (Tojnko S., and Cmelik Z., 2004). The use of feathered trees combined with high planting densities and minimal pruning can result in significant improvements in the yields of new orchards over the first five years after planting. Higher numbers of feathers at planting result in higher yields in the second and third years (Robinson and Stiles, 1995). As the benefits of highly feathered trees were discovered, it became necessary to develop nursery management techniques to stimulate lateral branch development. In Uzbekistan, however, most nursery trees do not have these small feathered branches. Moreover, young trees do not grow branches after planting under dry conditions. For this reason, promoting branch growth is crucial for early fruit production in this dry growing region.

The typical domesticated apple is an interspecific hybrid, usually designated as *Malus* × *Domestica* Borkh (Korban and Skirvin, 1984). The 'Fuji' cultivar is a 'Ralls Janet' × 'Delicious' cross that was bred in 1939 (Smith, 1971; Kikuchi et al., 1997). In 1962, the name 'Fuji' was given to the cultivar by researchers at the Horticultural Research Station in Morioka, Japan. The name 'Fuji' comes from the town of Fujisaki (Aomori Prefecture, Japan), where the cross was originally made. 'Fuji' is one of the best known apples globally, not just in Japan (D.C.FERRE 2003). 'Fuji' has a long storage and shelf life (7-11 months) and retains its taste and texture (FAO 2018). There are still problems achieving good coloration. Fruit quality differs, depending on the rootstock and climate, and it tends towards vigorous branch growth (Graham 1982).

Rootstocks play an important role in sustaining stable tree growth and controlling tree shape in the early fruit-bearing stages of ‘Fuji’ and other young apple trees. Soejima et al. (1998) reported on the benefits of Marubakaido (*Malus prunifolia* Borkh. v*ar. Ringo* Asami), a semi-vigorous rootstock for apple trees that is widely used in Japan. Major advantages of Marubakaido are its very good anchorage and early and heavy production. Soejima et al. (2010) also studied the dwarfing rootstock JM7 (‘Marubakaido’ × M.9), a rootstock included in the JM series of rootstocks. They found that growth intensity is similar to M.9 and that it is easy to graft by hardwood cutting.

M.9 is currently the most popular dwarfing rootstock used by commercial apple producers in many parts of northern Europe. At maturity, scion trees grown on M.9 are of a size ideally suited to the high-density plantings now favored by many apple growers. “The trees crop precociously and abundantly and, at similar crop loadings, mean fruit size is generally more prominent than that produced by trees on other popular rootstocks, such as MM.106 or M.26” (Webster and Hollands, 1999). Information regarding the different rootstocks gathered during this Ph.D. research are provided in Table 1.1.1.

For the above reasons, M.9 is widely used in modern orchards in Uzbekistan. For growers and plantation owners to invest in high-density plantings, rootstock selection alongside effective irrigation practices is essential for healthy young apple tree growth.

1.2. Water resource limitations in Uzbekistan

In Uzbekistan, the amount of arable land is limited and the amount of yearly precipitation (200-350 mm) is low. Usually, rainfall occurs in late autumn, winter, and early spring, with little or no rainfall from May to October. This amount of moisture is not enough for the production of most crops; therefore, all commercial fruit orchards in Uzbekistan are irrigated. Production areas that do not require irrigation are found only in mountainous areas with yearly precipitation of around 700 mm (Ostonakulov T.E. 2010).

Water is critical to the physiological functioning of an apple tree. Water is the most significant component of the tree by mass, and nearly all growth processes can be inhibited by improper irrigation practices. However, the essential role of water does not mean that water is always scarce. Irrigation as well as the use of ground cover and other ways of managing an orchard's land surface can contribute to the soil moisture necessary for tree vigor and the overall productivity of an apple orchard (Thomas J. Tworkoski and D. Michael Glenn, 2008).

After planting, intelligent irrigation practices and the amount of water applied to young apple trees helps promote their early growth. Four ways of irrigation are used in fruit orchards in Uzbekistan. These are floor, pool type, flooding and furrow irrigation. Newly planted orchards are normally irrigated with 500 m^3/hectare, whereas in fruit bearing orchards it is 800-1000 m^3/h (Narzieva and Ostonakulov, 2010). According to Aripov (2013), fruit tree water use per hectare is 600-800 m^3 in gray soils and 400-450 m^3 in rocky soils.

The growing process of young apple trees commences from the lower part of the tree and continues on to the upper part. When they are not being watered directly, the growth of young roots occurs through the intake of water from soil moisture. Therefore, controlling soil moisture levels is vital for growers raising young apple trees.

1.3. Soil moisture retention substances

Sufficient soil moisture and substances used to control the amount and stability of water retention can enhance fruit tree root development and the growth of the upper part of a young tree. There are several soil moisture retention substances on the market: *Hydretain ES Plus* (Hydretain, Inc.), a water retention substance that is a blend of organic hygroscopic and humectant components, has been shown to hydrate the soil effectively. *Hydretain ES Plus* contains sugar alcohols, polysaccharides, and neutral salts of alpha-hydroxy propionic acid. On the other hand, a further study reported that *Hydretain ES Plus* and other humectants had no observable effect on soil water retention in drought-tolerant Coleus 'Wasabi' during the plant growth stages (Greenwell et al., 2017).

Super Sorb C is a superabsorbent hydrogel polymer composed of hydrophilic polymers with water retention properties. They can retain water a hundred times their weight. Although these substances were used in the United States as a water-retaining substance in agriculture, they were developed in Japan in the mid-1970s for personal care and hygienic products. (Kabiri et al., 2003).

Menedael is a plant root vitality element, widely used in Japan, that aids root regrowth. *Manedael* is a vitalizer that improves a plant's health and growth. It can also help to vitalize a weak plant. *Menedael* is an aqueous suspension that includes iron in the form of ions. The ions are absorbed immediately to help in plant growth. It works by increasing the intake of water and nutrients and stimulates photosynthesis.

Glutain (amino acid "γ-PGA" manufactured by *Bacillus* natto) and *Kalpak 66* "ROYAL INDUSTRIES" Co, Ltd (Made in Japan) help retain water in the soil and promote root water absorption (www.kalpak.co.jp/pdf/grutan/pdf).

1.4. Technologies for managing apple tree physiology

Controlling soil water content and irrigation practices plays an important role in improving the performance of young trees. The introduction of modern timesaving measurement technologies that do not disturb the young trees could also improve work efficiency in newly- or recently-planted high-density orchards.

Apple trees and their branches sprout many buds during the dormant season. The growth and physiological characteristics of these buds are different and there are several types, such as growing and non-growing, and flower and non-flower buds. It is crucial to know which buds are which on young apple trees before or after planting because knowing so increases work efficiency and productivity. Work in an orchard involves being able to forecast the number of branches so that the grower can prune out the branches and flower buds which will emerge on a tree. This is necessary because the grower needs to know the future location of the fruits in order to do the pruning that will result in proper tree shape. It takes a lot of experience and time for growers to understand this, not to mention the conditions unique to their orchards, in order to carry out the appropriate actions. Therefore, the way to better facilitate the efforts of growers is to introduce technologies that do not require much time and do not cause damage to the fruit, the tree or the orchard.

Non-destructive testing includes a broad range of techniques that are used in science and technology industries to evaluate a material, component, or a system's properties without causing damage. According to Crowley (2020), the visible region of the spectrum of electromagnetic radiation identified by a visible near-infrared spectrometer is typically considered to be made up of wavelengths ranging from 400 nm (violet light) to between 700 and 800 nm (red light). Manley and Baeten (2018) have noted that, "the essential origins of NIR spectroscopy include the production, reporting, and understanding of spectra resulting from the interaction of electromagnetic radiation with an object." Osborne (2000) reported that "the infrared (IR) region comprises that part of the electromagnetic spectrum in the wavelength range between 780 and 100,000 nm and is divided into near-IR, mid-IR, and far-IR subregions; the NIR region covers the wavelength range from 780 to 2500 nm".

In this study, the nondestructive testing method utilized was with a visible/near-infrared spectrometer. The measuring range of this spectrometer runs from 640 nm to 1050 nm at intervals of 2 nm. This wavelength range is used to estimate the plant water index (R900/R970) (Peñuelas et al., 1997), to detect apple bruises at between 730-830 nm (Luo et al., 2012), and to grade apples for firmness and soluble solids content (450-1050 nm wavelength range) (Mendoza et al., 2014).

1.5. Purpose of the Ph.D. research

The aim of the research was to identify methods of promoting apple tree growth after planting under conditions of limited water availability. The following issues were addressed during the Ph.D. research period:

1) The relationship between rootstock development, irrigation and soil moisture levels and branch growth after planting as well as how branch growth is affected by the planting season.

The studies that were referenced earlier all focused on ways to promote young apple tree growth. However, the synergy between rootstock, watering, and soil treatments (water retention substances) has not yet been examined. The experiments in this phase of the research examined the interaction between rootstock (Ma and Jm7) and water and soil treatments and the impact of this interaction on young apple tree growth, as well as the implications these interactions have for farm management practices in areas with limited water resources.

The soil and water management of young trees after planting for the promotion of tree growth was also addressed. Examined were a number of substances that maintain soil moisture levels necessary for tree growth when water is scarce. Measurements were made to calculate soil water levels in order to control soil moisture content.

2) To determine the effects of winter planting on root growth and the changes in young trees at different moisture levels. Until now, there has been no detailed research or experiments conducted regarding root growth related to the physiological changes in apple trees planted in early winter.

A further aim of this study was to examine the root growth in one-year-old ‘Miyabi Fuji’, comparing semi-vigorous Marubakaido (Ma) (*Malus prunifolia* 'Ringo') with dwarfing M.9 rootstocks, under cold winter and other physical growing environmental conditions. How the planting season and the environmental conditions after planting affect root growth, shoot growth and tree architecture were also studied.

3) Non-destructive measurements that detect bud condition and help forecast shoot or leaf bud or flower from non-flower before bud burst.

To ensure good future growth, it is crucial to know where new branches will appear on newly-planted apple trees before bud burst without disturbing their physical growth. Therefore, the effects of some of the indicators of young apple tree growth under different conditions were examined as well as and how water and non-destructive testing could be utilized in the orchard to distinguish growing from non-growing and flower from non-flower buds. This could help the grower plan his future orchard management endeavors.

1.6 Outline of the Ph.D. research

1.6.1 The role of irrigation, soil treatments and rootstocks in the growth of young apple trees was studied. The effects of two rootstocks (‘Marubakaido and JM7’), moisture saturation levels in the soil (normal/soil moisture levels irrigated to 70% and dry/soil moisture levels irrigated to 50%) and soil humectants (Glutain plus Kalpak 66, Hydretain ES Plus, Menedael and Super Sorb C) on the lower and upper parts of young apple trees (cv. *Miyabi Fuji)*, planted in spring and harvested in late autumn were studied (Chapter 2).

1.6.2 The effects of winter planting (January through May) on root growth and moisture content on each particular part of young apple trees (cv. *’Miyabi Fuji’*) were examined. Additionally, wintertime tree physiological changes were observed by employing nondestructive testing measures (OMT-NIR-M1 SPECTROMETER (Chapter 3)).

1.6.3 The role of non-destructive measurements of the physiology of young apple tree buds (growing and non-growing) during different phases of apple tree growth was studied. The physiology of the buds, growing and non-growing, on spring-planted young apple trees was tested (Chapter 4).

1.6.4 Application of a visible/near-infrared spectrometer in identifying flower and non-flower buds on ‘Fuji’ apple trees during winter dormancy was examined and changes in the chlorophyll content of the buds over their winter dormancy was measured (Chapter 5).

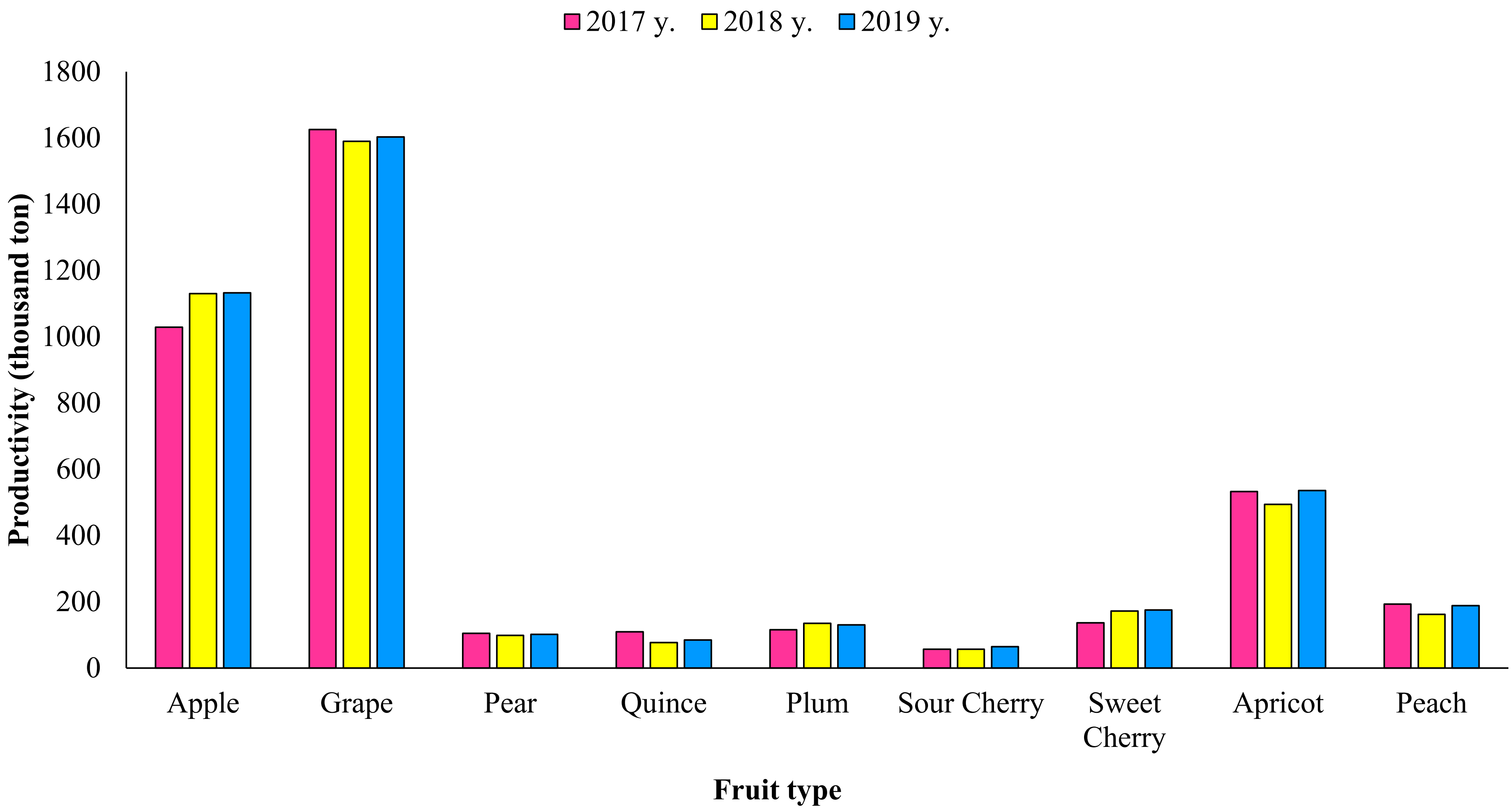


Figure-1.1.1. Statistical indicators for seed and drupe fruit production in Uzbekistan in 2017, 2018 and 2019 (thousand tons).

Source: *Stat.uz-web site.*

Table 1.1.1. Characteristics of the rootstocks used during the Ph.D. study: semi-vigorous ‘Marubakaido’, dwarfing ‘JM7’ and dwarf ‘M.9’.

Rootstock name	Origin	Parents (if known)	Remarks	References
‘Marubakaido’	Japan	*Malus prunifolia* ‘Ringo’	Very vigorous; only average induction of yield precocity and efficiency; resistant to woolly apple aphid and collar/crown rots Very good anchorage, early and heavy production, no burr knot production, crown rot resistance, wooly apple aphid resistance, tolerance to moist soil conditions, and ease of propagation by hardwood cuttings. The shortcomings, are low virus tolerance and the formation of root suckers.	(D.C.FERRE 2003); (Soejima et al. 1998).
JM7	Apple research Center, NIFTS, Japan	‘Marubakaido’ × M.9	Vigor similar to M.9 in Japanese trails; tolerant of wooly apple aphid; resistant to collar rot; easy propagation from hardwood cuttings.	(Soejima et al. 2010).
M.9 (‘Juane de Metz’)	Reselected at HRI-East Malling, UK	Chance seedling found in France	The most popular dwarfing rootstock; induces excellent yield precocity and efficiency; induces large fruit size; brittle roots; poor anchorage; some suckering, depending on scion cultivar, rootstock sub-clone and site conditions; sensitive to winter cold, poor drainage and shows some drought sensitivity; sensitive to fire blight and to woolly apple aphid; some resistance to collar rot.	(D.C.FERRE 2003).

CHAPTER 2

THE ROLE OF IRRIGATION, SOIL TREATMENTS AND ROOTSTOCKS TO GROWTH OF YOUNG APPLE TREES

2.1 INTRODUCTION

In arid and semi-arid regions of the world, access to a stable supply of water is necessary for the successful growth of apple trees, particularly for young trees shortly after planting. This is because obtaining a sufficient number of shoots on the young tree in the first growing season greatly influences future fruit-bearing capacity. Tromp (1996) found that soil temperature affects shoot growth, especially when it rises to where it enables sylleptic shoot growth. It has also been noted that notching techniques increase branching at the top of young apple trees (Greene and Autio, 1994). Arakawa et al., (2014) showed that planting season and root mass have an impact on the length of the top two shoots on one-year-old 'Fuji' that were grafted onto 'Marubakaido' (Ma) rootstocks.

Another factor that promotes shoot growth and other physical changes in young trees is the uptake of nutrients from moisture in the soil. The hot and dry conditions during the growing season in some parts of the world, where water resources are scarce, can hinder the growth of young apple trees. To alleviate these problems, the introduction of efficient irrigation practices and water retention substances that could help maintain sufficient water moisture levels in the soil is often promoted.

It has been established that sufficiently high temperatures along with adequate irrigation contribute to the improved growth of young apple trees after they are planted. Ro (2001) found that when water treatments were applied to young apple trees in soil with a moisture content levels of 50%, they showed better average shoot length than when added to those planted in soil with a water content level of 80%. Zhou et al., (2019) noted that when the soil moisture content was adjusted to 65-75% and an $N\text{-}P_2O_5\text{-}K_2O$ fertilizer mixture controlled at 20-20-10 $g{\cdot}tree^{-1}$ was added, this combination proved to be the most effective for young apple trees planted in northwest China a semiarid area. In another study, Hydretain® ES Plus

(Hydretain, Inc.), a water retention substance which is a blend of organic hygroscopic and humectant components (sugar alcohols, polysaccharides and neutral salts of alpha-hydroxy propionic acid), was shown to effectively hydrate the soil (Roberts and Linder 2010). On the other hand, a further study reported that Hydretain ES Plus and other humectants had no observable effect on soil water retention in drought-tolerant Coleus 'Wasabi' during the plant growth stages (Greenwell et al., 2017). According to Fontenno and Bilderback (1993) Super Sorb C is a water absorbent polymer that increases weight and bulk density while retaining water.

Rootstocks play an important role in sustaining stable tree growth and controlling tree shape in the early fruit-bearing process of young apple trees. Soejima et al., (1998) reported on the benefits of Marubakaido (*Malus prunifolia* Borkh. v*ar. Ringo* Asami), a semi-vigorous rootstock for apple trees that is widely used in Japan. Soejima et al., (2010) also studied the dwarfing rootstock JM7 ('Marubakaido' × M.9), a rootstock included in the JM series of rootstocks. They found that growth intensity is similar to M.9 and that it is easy to graft by hardwood cutting.

The studies cited above focused on particular elements that promote young apple tree growth. The purpose of this Chapter is to examine the interaction between rootstocks (Ma and JM7), water (70% and 50% soil moisture levels) and soil treatments, and the impact of their interactions on young apple tree growth and the possibilities these interactions offer to growers to improve their orchard management practices in areas having limited water resources. For this experiment, three test periods were chosen. Experiment 1 was conducted in 2018, Experiment 2 in 2019 and Experiment 3 in 2020.

In Experiment 1, the Menedael treatment did not affect young tree growth; increased soil moisture through irrigation flooding was not controlled as a designed irrigation condition. The experiment was repeated in 2019 (Experiment 2) without the Menedael treatment. The impact of Super Sorb C water absorption polymers was minimal. A loss of soil moisture was identified with an EM-5, a sensor device, soil treatment dominancy was not detected in Experiment 2. Experiment 3 (2020) was redesigned to show the interaction between rootstock, irrigation, and soil treatments (water retention substances), using the same amount of soil and water treatments on both Ma and JM7 rootstocks.

The results led to the conclusion that the upper part of the young apple trees showed more growth when grafted onto Ma than on JM7 and that the root systems were significantly affected by water content levels and soil treatments.

2.2 MATERIALS AND METHODS

Experiment 1 (2018)

2.2.1 Plant materials and soil treatments.

Young 'Miyabi Fuji' (a bud sport of 'Fuji' having good fruit coloration) trees were grafted onto semi-vigorous 'Marubakaido' (*Malus prunifolia* 'Ringo') rootstock and also onto dwarfing JM7 ('Marubakaido' × M.9) rootstocks and were planted on April 23, 2018. The young apple trees were placed in 11 L black plastic nursery pots that contained a mixture of one-part potting soil used for trees and two parts volcanic black soil.

Before planting, all apple saplings were scaled to the same size by cutting them to a length of 70 cm; roots were cut back to 10 cm. Four soil treatments (water retention substances) were used. One was a mixture of Glutain (amino acid "γ-PGA" manufactured by *Bacillus* natto) and the Kalpak 66 "ROYAL INDUSTRIES" Co, Ltd (Made in Japan) soil treatment. The second was the Hydretain ES Plus soil treatment. The third soil treatment was Menedael (containing iron and a plant vitality element) and the fourth was Super Sorb C, a water absorbent polymer. Solution amounts are shown in Table 2.7.

Two water treatments were used. One was at a 70% (when irrigation was applied ECH_2O EC-5-soil moisture sensor device indicator will be 100 and then become decrease over time, while it indicates 70 or more than 70, soil moisture level was based on 70% with normal irrigation) soil water level and the other a 50% (when ECH_2O EC-5-soil moisture sensor device indicator will be 50 or more than 50, soil moisture level was based on 50% with dry irrigation) soil water treatment. The irrigations were done by hand. To manage the amount of irrigation, a Decagon Device (include ECH_2O EC-5-soil moisture sensor, and data collector with Em5b EL14159 data logger, made in the USA) was installed inside the soil in the pot and data was collected with Data Trac 3.17.2 software.

Fifty-five young apple trees were used in Experiment 1. Thirty-five of them were grafted onto Ma rootstocks, the other twenty onto JM7. Of those grafted onto

Ma, five were set aside as control trees, 15 were treated with a Hydretain ES Plus (11 ml/p) soil treatment, a Glutain ×Kalpak 66 (11 ml/p) soil treatment and a Menedael (11 ml/p) soil treatment. These 20 trees were irrigated to a 70% (normal) water content level. Five of the trees were given the Super Sorb C (50 g/p) soil treatment and five were controlled at a 50% (dry) water content level.

For those grafted onto JM7, five control trees were irrigated at a 70% water content level and five were given a Super Sorb C (50 g/p) soil treatment and five were dry controlled at a 50% water content level.

In addition, the remining five trees grafted onto Ma and the remaining five trees on JM7 were examined using the glass wall method. These ten trees were planted in a box: one side of the box was glass; the other sides and bottom were iron. The top was left open for planting and irrigation. Root growth was observed using this method.

Experiment 2 (2019)

2.2.2 Plant materials and soil treatments.

The same rootstocks, planting materials and procedures that were used in Experiment 1 were used in Experiment 2. The trees were planted on April 24, 2019. Three soil humectants (water retention substances) were used. One was a mixture of Glutain (amino acid "γ-PGA" manufactured by *Bacillus* natto) and Kalpak 66 "ROYAL INDUSTRIES" Co, Ltd (Made in Japan). The second was Hydretain ES Plus. The third was Super Sorb C (water absorbent polymer). Solution amounts are described in Table 2.7.

Two water treatments were used. One was at 70% soil water level with normal irrigation and the other was at 50% (dry irrigation). As in Experiment 1, irrigation was done by hand. The management of the amount of irrigation was done with a Decagon Device and data was collected with Data Trac 3.17.2 software.

Fifty-five young apple trees were also used in Experiment 2, thirty-five on Ma rootstocks, the other 20 on JM7. Menedael was not used in this experiment.

Five trees on Ma were control trees. Ten trees on Ma were treated with Hydretain ES Plus (11 ml/p) and Glutain × Kalpak 66 (11 ml/p); these 15 trees were irrigated to a 70% water content level. Five were treated with Super Sorb C (50 g/p), Glutain × Kalpak 66 (11 ml/p) and Hydretain ES Plus (11 ml/p) with control treatments set at a 50% water content level.

As for the trees on JM7, five were controls and five were treated with Glutain × Kalpak (11 ml/p) and irrigated at a 70% water content level. Five were treated with Glutain × Kalpak (11 ml/p) and five controlled in non-treated soil at a 50% water content level.

Experiment 3 (in 2020)

2.2.3 Plant materials and soil treatments.

In this experiment, 60 trees were used, 30 on Ma and 30 on JM7. The same planting materials and procedures that were followed in Experiments 1 and 2 were used in Experiment 3. The trees were planted on April 24, 2020.

Two water treatments were used. One treatment was with normal irrigation at a soil moisture level of 70%. The other was with dry irrigation at a soil moisture level of 50%. Soil moisture levels were calculated in the following way: when the moisture sensor was utilized and after the first irrigation, the soil sample was collected randomly and weighted. Then it was dried and Owen 24 hours and again weighed. Finally, soil moisture was measured with equation 2.1, and soil moisture indicator from the display of the sensor display parameter and soil moisture level calibrated as 70% and 50%. Watering was done by hand. Changes in soil water content were measured with a Decagon (pF meter).

$$MC_{(FW_{basis})} = \frac{FW-DW}{FW} \times 100 \qquad (2.1)$$

Half of the Ma rootstocks were irrigated to a 70% water content level, the other half to a 50% water content level. The same was done for the trees grafted onto JM7. Of the fifteen trees in each of these lots, five were treated with Glutain (11 mL/p) x Kalpak 66 (11 mL/p ^{z}), five were treated with Hydretain ES Plus (11 mL/p) and the remaining five were left untreated as controls (Table 2.7).

All the trees were purchased from "HARADA NURSERY" Co, Ltd. The experiments were conducted on the campus of the Hirosaki University Faculty of Agriculture and Life Science. The experiment design is shown below in Table 2.1.

2.2.4 Preparing the experiment site

A half-covered greenhouse (5 m wide and 10 m in length) was prepared for the experiment, with a clear plastic film polyethylene cover installed at the top as a shield against forecast the rain (Fig. 2.1). The ground surface inside the greenhouse was layered with a black weed-prevention sheet. Concrete bricks were placed on top of these sheets and four boards (1.21 m × 2.44 m) were placed on top of the bricks with a spacing of 0.50 cm between each board. The potted plants were then placed on the boards. Insecticide and fungicide sprays were applied during the post-planting shoot growth period at the same intervals as they are in area orchards.

2.2.5 Preparation samples for measuring

On November 24, each tree was carefully dug up and any soil or other matter was washed away with tap water. After that, the shoots, the main trunk (including the rootstock above the roots) and the roots were separated and measured. Root volume was measured in accordance with the Archimedes principle (10) by which a 5-liter plastic cylinder was placed in a large plastic bowl and filled with water, after which each root was carefully immersed in the cylinder. The overflow was poured into a graduated cylinder to measure the root volume.

2.2.6 Statistical analysis

All of the young apple trees were headed to the same height at the beginning of the experiment, cutting them at a point 70 cm above the graft union. During the growing season, shoot growth was observed from the headed area to the point below the four or more lateral shoots from the top. The same shoot growth was observed on both Ma and JM7. Before proceeding with a statistical analysis, all shoots were designated as follows: The topmost shoot was called the "top shoot," the second, third and fourth shoots were named the "top-three shoots" and the remaining shoots were designated as "below shoots;" the combined lengths of all shoots are referred to as "total shoot length". The results of the observations of soil treatments were analyzed using a three-way ANOVA for the interaction of rootstocks, water treatments, and soil treatments, plus a Tukey test using the R studio version 1.3.1073 (© 2009-2020 RStudio, PBC) software.

2.3 RESULTS

Experiment 1 (2018)

The interaction of rootstock and soil treatment had a significant impact on total shoot length. The rootstocks significantly affected the top shoot length (Table 2.1). The top shoot on Ma rootstock was significantly longer than the top shoot on JM7 with 70% and 50% soil water level. The lengths of the top-three shoots (with the exception of the top shoot were significantly altered on both rootstocks. The top-three shoots on the Ma rootstock were significantly longer than the top-three shoots on JM7, with 50% soil water level. Total shoot length decreased markedly for Ma with 50% and 70% soil water levels in non-treated soil. This showed that rootstocks significantly affected total shoot length, with Ma shoots being longer than those on JM7 trees with 50% and 70% soil water levels in non-treated soil.

Soil treatments led to significant changes on the upper parts of the trees. “Top first shoot” length decreased dramatically when using Super Sorb C soil treatment on Ma. The Super Sorb C soil treatment caused significant decreases in “top-three shoot” length” on Ma with 50% and 70% soil water levels. The Super Sorb C soil treatment brought on decreases in length for Ma with 50% and 70% soil water levels.

These treatments negatively affected the upper parts of the young trees upper part on Ma with 70% soil water levels (Fig. 2.2). Top first shoot length decreased 9.5% with the Glutain soil treatment and 3.5% with the Hydretain ES Plus soil treatment, then Menedael increased 0.2% with 70% soil water levels. The Glutain soil treatment caused a 12% increase of top-three shoot length, whereas the Hydretain ES Plus soil treatment caused a 7.8% decrease, and the Menedael soil treatment a 0.1% decrease. Total shoot length increased 8.1% with the Glutain soil treatment, and decreased 5.0% with the Hydretain ES Plus soil treatment, and decreased 16.7% with the Menedael soil treatment.

The Hydretain ES Plus soil treatment significantly impacted the fresh root weight of Ma irrigated to a 70% water/moisture level and the impact was higher than with the Glutain soil treatment and the controls (Fig. 2.3). The Glutain soil treatment occurred root volume 3.7% drops and Menedael soil treatment 3.2%, and then it increased 18.7% on Glutain soil treatment on Ma with 70% soil water level.

The rootstocks significantly impacted root weight and root volume (Table 2.2). Root weight was higher on JM7 rootstock with 50% soil water level than Ma with 70% soil water level. Soil treatments affected root weight and root volume. The Super Sorb soil treatment occurred to drop root weight on 50% soil water level on Ma. Root volume decreased on Super Sorb soil treatment on 50% and 70% soil water level on Ma.

The observation of root growth on grass wall method and its screening seen on Ma rootstock Fig. 2.6 (Fig. 2.6A-first screening picture) and JM7 Fig. 2.7 (Fig. 2.7A-first screening picture). Root growth occurred for 'Miyabi Fuji' on Ma and JM7 (on May 4 in 2018 Fig. 2.6 B and Fig. 2.7 B). Root color had turned to brown before the end measurement on October 16 (on Ma Fig 2.6 C and Jm7 Fig.2.7 C).

Experiment 2 (2019)

The rootstocks significantly impacted on top first and total shoot length (Table 2.3). Soil treatments affected total shoot length, and Ma was higher than JM7 on 50% and 70% soil water level. The rootstock, water, and soil treatment interrelation significantly impacted total shoot length.

Change of shoot, trunk, and root dry weight significantly higher on Ma than JM7 rootstock (Table 2.4). Shoot dry weight was heavier on Ma 50% soil water level than JM7 50% and 70% soil water level. Trunk dry weight was heavier on Ma than JM7, 50% and 70% soil water level. Root dry weight was heavier on Ma than JM7 on 50% soil water level.

Soil water level significantly affected on top first shoot length and total shoot length on Ma (Table 2.5). Top first shoot length, the effect of soil treatment significantly impacted on Ma and Hydretain ES Plus occurred decreases of it on 70% soil water level.

Total shoot, trunk, and root dry weight significantly changed by affecting soil water level and soil treatment on Ma (Table 2.6). Soil water level and soil treatment interrelation affected root dry weight on Ma rootstock. Hydretain ES Plus significantly affected root dry weight on 70% soil water level on Ma.

Top first shoot length was higher on Super Sorb soil treatment than Hydretain ES Plus and Glutain soil treatment on Ma with 50% soil water level (Fig. 2.4). Total shoot length was 14.6% lower on Super Sorb soil treatment and 3.2% on Glutain soil treatment, but on Hydretain ES Plus soil treatment was 10.5% longer than non-treated soil.

Hydretain ES Plus soil treatment was significantly higher than Glutain soil treatment on dry shoot weight with 50% soil water level in Ma (Fig. 2.5). Trunk dry weight was 9.8% higher on Super Sorb soil treatment, and 0.4% on Hydretain ES Plus soil treatment, then 10.9% on Glutain soil treatment than non-treated soil on Ma with 50% soil water level. Root dry weight was 11.7% higher on Super Sorb soil treatment, and 8.6% on Hydretain ES Plus soil treatment, then 12.4% on Glutain soil treatment than non-treated soil with 50% soil water level on Ma.

Experiment 3 (2020)

2.3.1 Impact of rootstock, soil and water treatments on shoot growth

A three-way ANOVA revealed that trees grafted onto the two rootstocks showed significant differences in the number of shoots, total shoot length and top first and top three shoot length (Table 2.8). The number of shoots, total shoot length and the length of the top first and top three shoots were significantly greater for those on Ma than those on JM7.

Water saturation levels also had a notable impact on the number of shoots and total shoot length, but exerted no significant influence on the length of the top first and top three shoots. The greatest number of shoots were observed on Ma in soil with 70% water content levels, decreasing significantly on JM7 in soil with 50% water content.

Soil treatments greatly influenced total shoot length, although they had no significant impact on the number of shoots or the length of the top first and top three shoots. The greatest total shoot length was observed on JM7 in the trees that were taken from the pots with 70% soil water levels and non-treated soil. Total shoot length was significantly diminished on JM7 trees that were taken from the pots having water content levels of 70% and soils treated with Hydretain ES Plus. As for total shoot length variation, the Hydretain ES Plus soil treatment had the greatest impact on Ma that were grown in pots with 70% soil water levels, followed by Ma in which Glutain and Kalpak 66 soil treatments were combined with 70% soil water levels.

There were significant differences in rootstock and soil treatment interactions on the lengths of the total and the top three shoots, although there were no significant differences in the number of shoots or the top shoot length. A three-way interaction (rootstock, water and soil treatment) was observed on total shoot length. Among the different sections of the trees, the greatest impact of the treatments was observed on the number of shoots on Ma in 70% water-saturated, untreated soil, whereas the longest total shoot lengths were seen on JM7 in 70% soil water level, untreated soil. The greatest top shoot lengths were observed on Ma in 50% soil water content, untreated soil, whereas the greatest top three shoot lengths were observed on Ma 70% water-saturated, that had also been treated with Glutain and Kalpak 66.

Appearance of shoots before digging was shown in Fig. 2.8, and average shoot number was 13.7 on Ma, and 6.6 on JM7 with 70% soil water level, then 7.7 on Ma and 5.2 on JM7 with 50% soil water level.

In 70% soil water level, the Glutain soil treatment occurred to drop for shoot dry weight on Ma, and it was 14.9%, then on Hydretain ES Plus soil treatment 10.1% than non-treated soil (Fig. 2.10). Shoot dry weight was 4.2% lower on Glutain soil treatment and 0.2% lower on Hydretain ES Plus soil treatment than non-treated soil on Ma with 50% soil water level. JM7 of 70% soil water level, shoot dry weight was 6.3% lower on Glutain soil treatment, and 1.1% higher on Hydreatain ES Plus soil treatment than non-treated soil. Change of dry shoot weight on JM7 in 50% soil water level decreased 12.2% with the effect of Glutain soil treatment and decreased 13.4% with Hydreatain ES Plus soil treatment than non-treated soil.

2.3.2 Effects of treatments on trunk and shoot diameter

A three-way ANOVA showed that top shoot and trunk diameters and shoot and trunk weight were affected by the rootstock (Ma or JM7) onto which they had been grafted (Table 2.9). The top shoot diameter, trunk diameter, shoot fresh weight, and trunk fresh weight were significantly greater on Ma compared with JM7.

Water treatments significantly affected trunk diameter as well as shoot and trunk weight, although no impact was observed on the diameter of the top shoot. Trunk diameter was greater on Ma with 70% water content, but decreased significantly on Ma with 50% water content and on JM7 in both 50% and 70% water-treated soil. Shoot weight was significantly greater for trees grafted onto Ma in 50% and 70% water-treated soil, whereas no significant differences were observed for JM7 in 50% and 70% water-treated soil. There were significant differences on Ma in 50% and 70% water-treated soil when compared with JM7 in 50% water-treated soil.

Soil treatments had a significant impact on top shoot diameter and trunk fresh weight, although no significant difference was observed on trunk diameter and shoot fresh weight. Rootstock and water treatment interaction affected top shoot diameter, whereas the rootstock and soil treatments impacted trunk fresh weight and water and soil treatments affected shoot fresh weight. There were no observable changes in tree diameters due to the interaction between rootstock, water and soil treatments. Top shoot diameter and shoot fresh weight were significantly altered on Ma trees in 70% water levels in untreated soil. Trunk diameter and trunk fresh weight were significantly different on Ma in 70% water-treated soil that was followed by a Hydretain ES Plus soil treatment.

For the trees planted on Ma in soil irrigated at a moisture level of 70%, trunk dry weight was significantly higher with the Hydreatain ES Plus soil treatment than with the Glutain soil treatment (Fig. 2.11). The root dry weight of the trees on Ma dropped when using the Glutain soil treatment in soil irrigated at a moisture level of 50% (Fig. 2.12). For trees on JM7 in soil irrigated at a 70% moisture level, the trunk dry weight was 15.4% lower with the Glutain soil treatment, and 13.7% lower with Hydreatain ES Plus soil treatment than it was for those in non-treated soil. The impact of Hydretain ES Plus soil treatment on trunk dry weight was significantly lower on the JM7 trees in soil irrigated at a 50% moisture level than the Glutain soil treatment in non-treated soil.

2.3.3 Effects of treatments on root growth

A three-way ANOVA was utilized to determine the effects of rootstock, water treatments and soil treatments on root fresh weight, root volume and root-to-shoot ratio (Table 2.10). The root weight, root volume and root-to-shoot ratio were changed significantly through rootstocks. Root weight, root volume and the root-to-shoot ratio increased significantly on JM7 when compared with Ma.

Water treatments exerted a significant influence on root weight and root volume, but showed no significant difference for the root-to-shoot ratio. Root fresh weight was higher on JM7 with 70% water content and significantly higher on Ma with 50% water content. Root volume in trees grafted onto Ma in soil with 70% water content was significantly higher than JM7 in both 50% and 70% water-treated soil.

Soil treatments showed a marked impact on root weight, but no significant difference was observed for root volume and root-to-shoot ratio. Root weight was significantly greater on Ma soil irrigated to 70% moisture content level when the soil was treated with Hydretain ES Plus. Root weight for JM7 with 70% water content treated with Hydretain ES Plus was substantially lower than the root weight in trees in untreated soil.

The interaction of rootstock, moisture content levels and soil treatments showed no significant impact on root weight, root volume or root-to-shoot ratio. Rootstock, water treatment and soil treatment interactions were observed for root weight, root volume and the root-to-shoot ratio. Significant increases in root weight growth and root volume were observed for Ma in soil with 70% moisture content, treated with Hydreatain ES Plus, as well as for JM7 in soil with 70% moisture content, untreated soil. Root-to-shoot ratio increases were higher on JM7 in soil with 70% moisture content, untreated soil.

Appearance of roots after digging seen in Fig. 2.9, and average root weight was 115.2 g on Ma, and 266.5 g on JM7 with 70% soil water level, and 121.9 g on Ma and 165.4 g on JM7 in 50% soil water level.

In 70% soil water level, effect of Hydretain ES Plus soil treatment on root dry weight was significantly higher than Glutain soil treatment and non-treated soil on Ma (Fig. 2.12). Root dry weight on Ma of 50% soil water level was dramatically lower on Glutain soil treatment than non-treated soil. JM7 of 70% soil water level, root dry weight was significantly lower on Hydreatain ES Plus soil treatment than non-treated soil. Change of dry root weight on JM7 in 50% soil water level decreased 4.1% with the effect of Glutain soil treatment and decreased 23.2% with Hydreatain ES Plus soil treatment than non-treated soil.

2.4 DISCUSSION AND CONCLUSION

Experiment 1 (in 2018)

In Experiment 1 (2018) the effects of soil treatments on young 'Miyabi Fuji' apple trees, grafted onto Ma and JM7 rootstocks and planted in soil with moisture levels of 50% and 70%, were investigated. It was found that the growth of the young trees differed significantly, depending upon the rootstock. On the other hand, the soil treatments and the moisture content level for the two rootstocks showed little difference. Rootstocks, irrigation amount and soil-moisture retention treatments had no observable impact on young tree growth, at the same time significantly effect was observed which one by one of treatments. Although the same experiments were repeated in Experiment 2(2019), the Menedeal soil treatment was not used.

Experiment 2 (in 2019)

In Experiment 2 the effects of the same 50% and 70% soil moisture treatments for 'Miyabi Fuji' on Ma and JM7 rootstocks were investigated. It was found that the young trees had grown higher on Ma than on JM7, but the soil and soil moisture treatments had showed no effect on young tree growth. It is concluded that there must have been a problem with the sensors used to measure the moisture content and therefore redesigned the experiment 3 (Experiment 3).

Experiment 3 (in 2020)

In this study it was investigated the impact of rootstock and soil moisture treatments on young 'Miyabi Fuji' apple tree growth. Young apple trees are usually planted as unbranched one-year whips. According to Hull (2018), to promote the growth of new shoots, nursery trees, when planted in the spring, are usually headed 70 to 90 cm above the grafted union before planting in order to obtain a sufficient number of side branches. When this is done, three or four dominant new shoots emerge at the top. It has been observed that, when this occurs, only very short shoots grow under these top shoots (Kikuchi et al., 2003).

This phenomenon has been understood as a physical characteristic of trees having a top predominance. In this experiment, the upper three to four shoots in spring-planted trees were significantly longer than the lower shoots. Similar results have been reported by Kikuchi et al., (2003). They found that in 'Fuji', top shoot weight was the same for both pruned and unpruned shoots. While Kikuchi et al. (2003) only compared pruned and unpruned trees, during this study, were founded that the rootstock affected top shoot length on pruned trees, and that shoot length was greater on Ma with 70% water content than on JM7 with 70% water content (Table 2.8), and that top shoot length differed in soil with a moisture content of 70% depending upon the rootstock.

Our results also suggest that the impact of the rootstock on shoot fresh weight is greater on Ma with 70% water content than on JM7 (for both 50% and 70% water content). The trunk fresh weight of the young apple trees was higher on Ma with 50% water content than on JM7 with 70% soil water. These findings extend those of Campbell and Bould (1970), confirming that the number of shoots was closely related to the rootstock. In our experiment, it was not only the rootstock but also the water saturation treatments (set at 50% and 70%) affected the top parts of the young apple trees. Changes in trunk diameter and fresh weight were more pronounced on Ma with 50% water content than on JM7 (50% water content). Tworkoski and Fazio (2016) have explored the effects of environmental stress (e.g., water and nutrient availability) on the size-controlling capacity of different rootstocks. In our study, trunk growth indicated that semi-vigorous Ma rootstock with 50% soil water content was greater on JM7 dwarfing rootstocks treated with water content levels of both 50% and 70%.

Changes in the roots showed that some soil treatments had a positive impact on the fresh weight of the root (Table 2.10). In this experiment, Ma with 70% water content combined with Hydretain ES Plus showed good growth results. Our findings do not, however, support those of Greenwell et al. (2017) on the impact of humectants on plant root parameters. This study was found that root fresh weight and root volume changes occurred in trees on Ma with 70% water content in Hydretain ES Plus treated soil resulting in increased root biomass and root volume.

The healthy growth of new shoots after planting greatly influences future tree shape and initial production. It is therefore important to promote and manage root growth, even after planting, by managing water content and introducing humectants in order to using for soil. Even though this study was carried out under the half dry conditions, the results can be applied in orchards. Therefore, in the further studies are planned to implement these findings in field experiments in areas with limited access to water. These results may provide suggestions to growers in such areas as to how they might better manage their orchards and which rootstocks, which soil moisture levels and which soil water retention treatments would work best for their young apple trees.

The question of how to promote the growth of young apple trees after they are planted in areas with limited water resources was examined in this study. An experiment was designed to determine how the choice of rootstock, moisture levels in the soil and water retention treatments can be combined to promote young tree growth. Our findings led us to the conclusion that the interaction of rootstock, water levels and soil treatments affected total shoot length, root weight, root volume and the root-to-shoot ratio of young 'Miyabi Fuji' apple trees.

The fresh weight of the root was greatest for JM7 with 70% soil water content in untreated soil and for Ma with 70% soil water content treated with Hydretain ES Plus. Root volume on Ma with 70% soil water content in soil treated with Hydretain ES Plus was greater than that on JM7 with 70% soil water content in untreated soil. The interaction between rootstock, soil water content, and soil treatments was the highest on JM7 with 70% soil water content in untreated soil and the lowest on Ma with 70% soil water content in Hydretain ES Plus treated soil and on JM7 with 50% soil water content in untreated soil.

Rootstock, soil water content and soil treatment interaction were more pronounced on the dwarfing JM7 rootstock, compared with Ma, in terms of total shoot length, root weight and root to shoot ratio. Root volume and top three shoot length (rootstock and soil treatment interaction) was more pronounced on Ma with 70% soil water content in soil treated with Hydretain ES Plus and Glutain and Kalpak 66 soil treatments when compared with JM7.

2.5 SUMMARY

Young apple trees that are planted in areas with limited water resources face challenges in their early growth stages. Insufficient intake of moisture often stunts the growth of the young tree and impacts its subsequent growth. In this Chapter was investigated the interaction of semi-vigorous Marubakaido (Ma) (*Malus prunifolia* 'Ringo') and dwarfing JM7 ('Marubakaido' × M.9) rootstocks, water treatments (50% and 70% soil water content) and soil treatments (water retention substances) on young 'Miyabi Fuji' apple trees and how this interaction impacts their growth under dry climactic conditions. The development of shoots, stems and roots was analyzed. The results showed that the interaction of rootstock and water and soil treatments had a significant impact on total shoot length ($p < 0.01$), as did the interaction of rootstock and soil treatment on the length of the top three shoots ($p< 0.05$) and trunk fresh weight ($p < 0.05$). In addition, it was found that the interaction of water and soil treatments impacted shoot fresh weight ($p < 0.05$).

This study revealed that the growth of young apple trees in areas with limited water resources can be aided by providing a 70% and 50% saturation of water and soil retention treatments for young trees that have been grafted onto semi-vigorous Ma and dwarfing JM7 rootstocks. Growers in these areas should think about which rootstock to use, what soil water retention treatments that can be introduced into the soil as well the amount of water that should be applied.

Key words: 'Miyabi Fuji', rootstock, shoot growth, water treatment, water retention.

Figure 2.1 Protected greenhouse for purpose of dry condition, top half shielded with poly film and ground surface covered with black thick poly film in 2018, 2019 and 2020.

Table 2.1. Interaction between rootstock and soil treatment for 'Miyabi Fuji' apple tree's upper part growth on Marubakaido (Ma) and JM7 in 2018

Rootstock (R)	**Soil treatment (St)**	**Top 1st shoot length (cm)**	**Top three shoot length (except 1st shoot) (cm)**	**Total shoot length (cm)**
Ma	**Control 70%**	111.4 ± 3.6 bc	175.5 ± 5.9 cd	410.9 ± 29.7 c
	Control 50%	123.7 ± 7.0 c	196.1 ± 15.4 d	354.0 ± 9.8 c
	Super Sorb C 50%	87.7 ± 10.7 ab	120.7 ± 13.7 ab	272.5 ± 24.7 b
JM7	**Control 70%**	74.2 ± 2.9 a	149.9 ± 6.7 bc	227.4 ± 6.0 ab
	Control 50%	75.4 ± 3.9 a	132.0 ± 5.7 ac	235.3 ± 11.7 ab
	Super Sorb C 50%	62.4 ± 5.7 a	105.9 ± 7.6 ab	192.5 ± 8.7 a
Significance				
Rootstock (R)		***	***	***
Soil treatment (St)		**	***	***
R × St		ns	ns	*

Note: Different letter by column indicates statistically significant differences according to a Tukey test and significant levels: (**ns**) no significance, (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, (n=5).

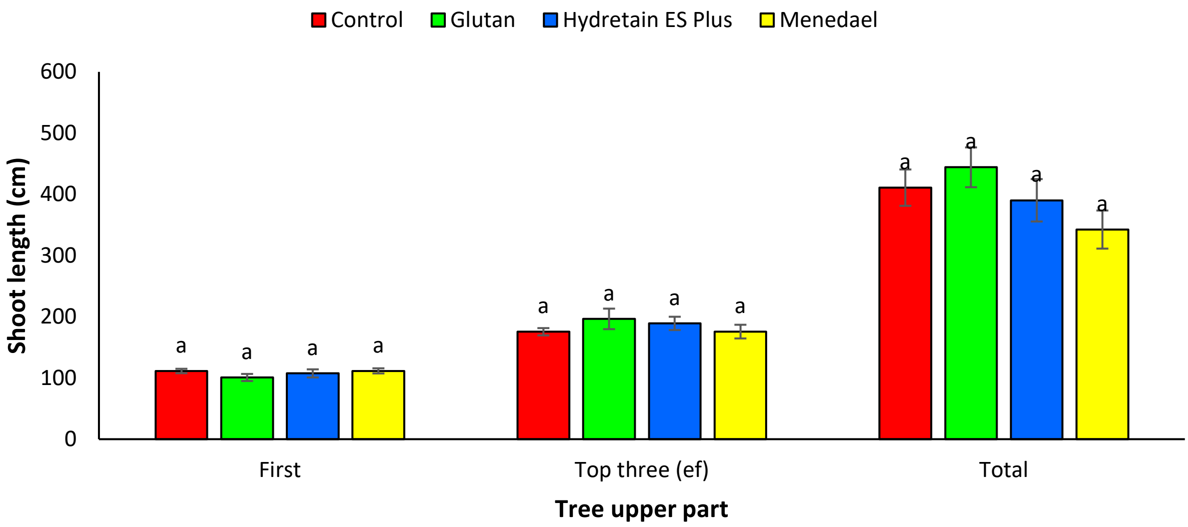


Figure 2.2 Effect of soil treatments on shoot growth for 'Miyabi Fuji' apple tree on Marubakaido (Ma) in 2018 with 70% soil water level. Ef-except top first shoot. Standard error and different letters indicate statistically significant difference among the treatments according to the Tukey test (n = 5).

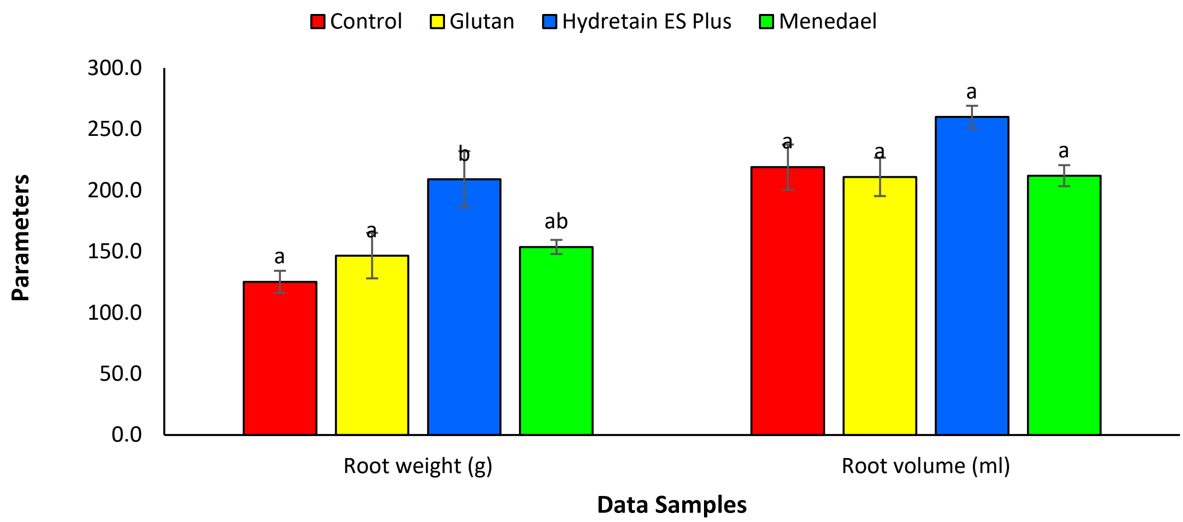


Figure 2.3 Effect of soil treatments on root weight and root volume 70% soil water treatment for 'Miyabi Fuji' apple tree on Marubakaido (Ma) in 2018. Standard error and different letters indicate statistically significant difference among the treatments according to the Tukey test (n = 5).

Table 2.2. Interaction between rootstock and soil treatment for 'Miyabi Fuji' apple tree's bottom part growth on Marubakaido (Ma) and JM7 in 2018

Rootstock (R)	Soil Treatment (St)	Root weight (g)	Root volume (ml)
Ma	Control 70%	125.2 ± 9.0 ab	219.0 ± 18.5 b
	Control 50%	171.0 ± 9.5 bc	211.0 ± 4.6 b
	Super Sorb C 50%	98.9 ± 11.4 a	122.0 ± 18.5 a
JM7	Control 70%	189.2 ± 14.8 bc	218.0 ± 19.7 b
	Control 50%	205.7 ± 18.0 c	234.0 ± 12.2 b
	Super Sorb C 50%	157.2 ± 21.1 ac	202.0 ± 21.4 b
	Significance		
Rootstock (R)		***	*
Soil treatment (St)		**	**
R × St		ns	ns

Note: Different letter by column indicates statistically significant differences according to a Tukey test and significant levels: (**ns**) no significance, (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, (n=5).

Table 2.3. Interaction between rootstock water and soil treatment for 'Miyabi Fuji' apple tree's upper part growth on Marubakaido (Ma) and JM7 in 2019

Rootstocks	Water Treatment	Soil treatment	Top 1st shoot length (cm)	Total shoot length (cm)
Ma	70%	Control	108.3 ± 4.8 ac	335.7 ± 16.3 bc
	70%	Glutain	99.0 ± 6.6 c	396.6 ± 34.9 c
	50%	Control	124.0 ± 1.9 ac	418.6 ± 29.7 c
	50%	Glutain	112.8 ± 7.6 bc	405.4 ± 21.7 c
JM7	70%	Control	81.4 ± 9.6 a	201.8 ± 25.7 a
	70%	Glutain	89.4 ± 2.4 ab	180.5 ± 17.1 a
	50%	Control	86.2 ± 10.8 ab	214.5 ± 25.9 a
	50%	Glutain	93.8 ± 5.1 ac	272.4 ± 11.5 ab
		Significance		
Rootstocks (R)			*	***
Water treatment (W)			ns	ns
Soil treatment (S)			ns	**
R × W			ns	ns
R × S			ns	ns
W × S			ns	ns
R × W × S			ns	*

Note: Different letter by column indicates statistically significant differences according to a Tukey test and significant levels: (**ns**) no significance, (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, (n=5).

Table 2.4. Interaction between rootstock, water and soil treatment for 'Miyabi Fuji' apple tree's upper- and lower-part dry weight on Marubakaido (Ma) and JM7 in 2019

Rootstocks	Water Treatment	Soil treatment	Shoot dry weight (g)	Trunk dry weight (g)	Root dry weight (g)
Ma	70%	Control	34.8 ± 1.0 bcd	69.5 ± 2.5 b	62.5 ± 4.6 bc
	70%	Glutain	40.4 ± 2.3 d	69.1 ± 1.5 b	65.0 ± 4.5 c
	50%	Control	43.3 ± 1.1 cd	72.1 ± 1.9 b	68.7 ± 3.2 c
	50%	Glutain	38.9 ± 2.7 cd	64.3 ±2.4 b	60.2 ± 6.5 bc
JM7	70%	Control	22.1 ± 3.9 ab	37.3 ± 3.3 a	54.9 ± 5.6 ac
	70%	Glutain	22.6 ± 2.6 a	41.2 ± 3.0 a	43.0 ± 3.4 a
	50%	Control	20.7 ± 4.5 ab	36.8 ± 4.6 a	36.5 ± 4.0 ab
	50%	Glutain	27.5 ± 2.9 ac	39.1 ± 3.2 a	37.6 ± 4.6 a
Significance					
Rootstocks (R)			***	***	***
Water treatment (W)			ns	ns	ns
Soil moisture (S)			ns	ns	ns
R × W			ns	ns	ns
R × S			ns	ns	ns
S × W			ns	ns	ns
R × W × S			ns	ns	ns

Note: Different letter by column indicates statistically significant differences according to a Tukey test and significant levels:

(**ns**) no significance, (***) P < 0.001, (n=5).

Table 2.5. Interaction between water and soil treatment for 'Miyabi Fuji' apple tree's upper-part growth on Marubakaido (Ma) in 2019

Water treatment (W)	Soil Treatment (S)	Top 1st shoot length (cm)	Total shoot length (cm)
70%	**Control**	108.3 ± 4.8 bc	335.7 ± 16.3 a
	Hydretain ES Plus	82.0 ± 7.2 a	406.3 ± 17.5 ab
	Glutain	99.0 ± 6.6 ab	396.6 ± 34.9 ab
50%	**Control**	124.0 ± 1.9 c	418.6 ± 29.7 ab
	Hydretain ES Plus	113.8 ± 3.9 bc	462.6 ± 40.3 b
	Glutain	112.8 ± 7.6 bc	405.4 ± 21.7 ab
	Significance		
	W	***	*
	S	*	ns
	W × S	ns	ns

Note: Different letter by column indicates statistically significant differences according to a Tukey test and significant levels:

(**ns**) no significance, (*) P < 0.05, (***) P < 0.001, (n=5).

Table 2.6. Interaction between water and soil treatment for 'Miyabi Fuji' apple tree's upper-and lower-part dry weight on Marubakaido (Ma) in 2019

Water treatment (W)	Soil Treatment (S)	Total shoot dry weight (g)	Trunk dry weight (g)	Root dry weight (g)
70%	Control	34.8 ± 1.0 a	69.5 ± 2.5 a	62.5 ± 4.6 a
	Hydretain ES Plus	43.8 ± 4.7 ab	74.7 ± 4.8 a	90.2 ± 4.8 b
	Glutain	40.4 ± 2.3 ab	69.1 ± 1.5 a	65.0 ± 4.5 a
50%	Control	43.3 ± 1.1 ab	72.1 ± 1.9 a	68.7 ± 3.2 a
	Hydretain ES Plus	53.4 ± 5.1 a	71.8 ± 3.7 a	62.8 ± 2.6 a
	Glutain	38.9 ± 2.7 b	64.3 ± 2.4 a	60.2 ± 6.5 a
	Significance			
	W	*	*	*
	S	*	*	*
	W × S	ns	ns	**

Note: Different letter by column indicates statistically significant differences according to a Tukey test and significant levels:

(**ns**) no significance, (*) $P < 0.05$, (**) $P < 0.01$, (n=5).

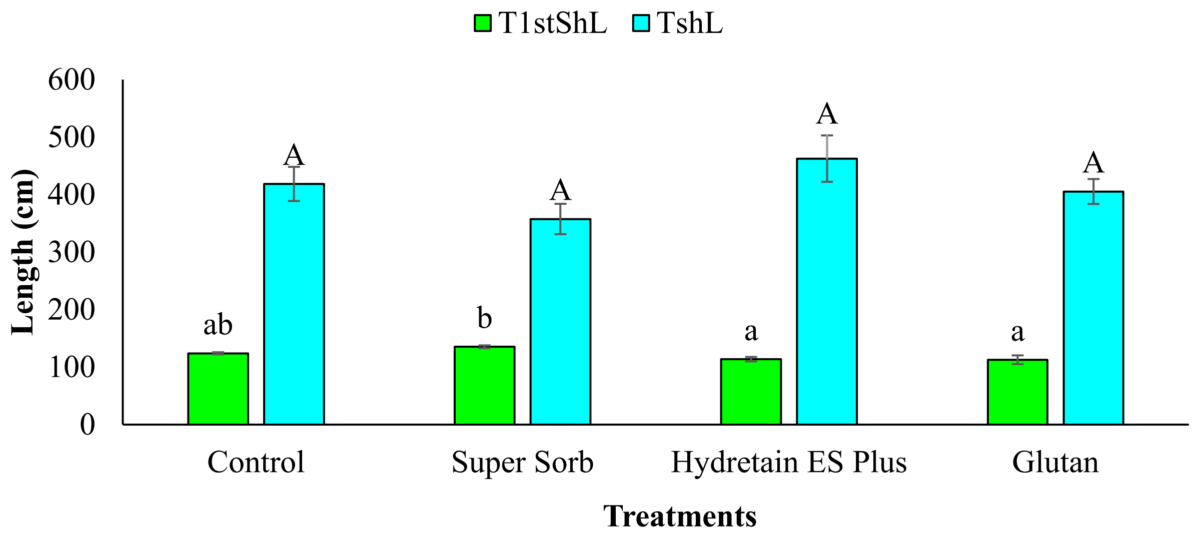


Figure 2.4 Effect of treatments on top 1st and total shoot length of 50% soil water treatment for 'Miyabi Fuji' apple tree on Marubakaido (Ma) in 2019; T1stShL– top first shoot length, TshL–total shoot length. Standard error and different letters indicate statistically significant difference among the treatments according to the Tukey test; (n = 5).

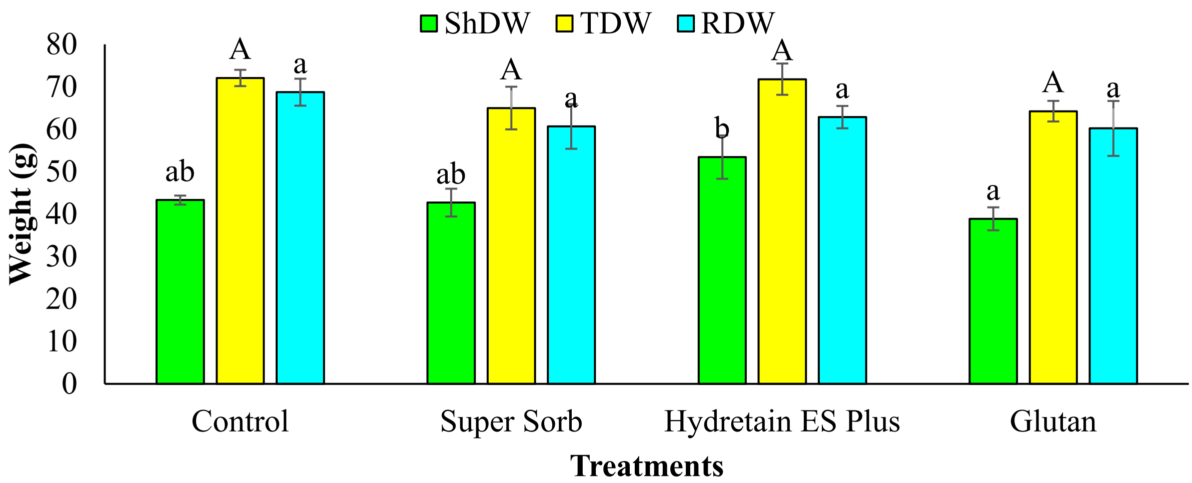


Figure 2.5 Effect of treatments on upper- and lower-part dry weight of 50% soil water treatment for 'Miyabi Fuji' apple tree on Marubakaido (Ma) in 2019; ShDW–shoot dry weight, TDW–trunk dry weight, RDW–root dry weight. Standard error and different letters indicate statistically significant difference among the treatments according to the Tukey test; (n = 5).

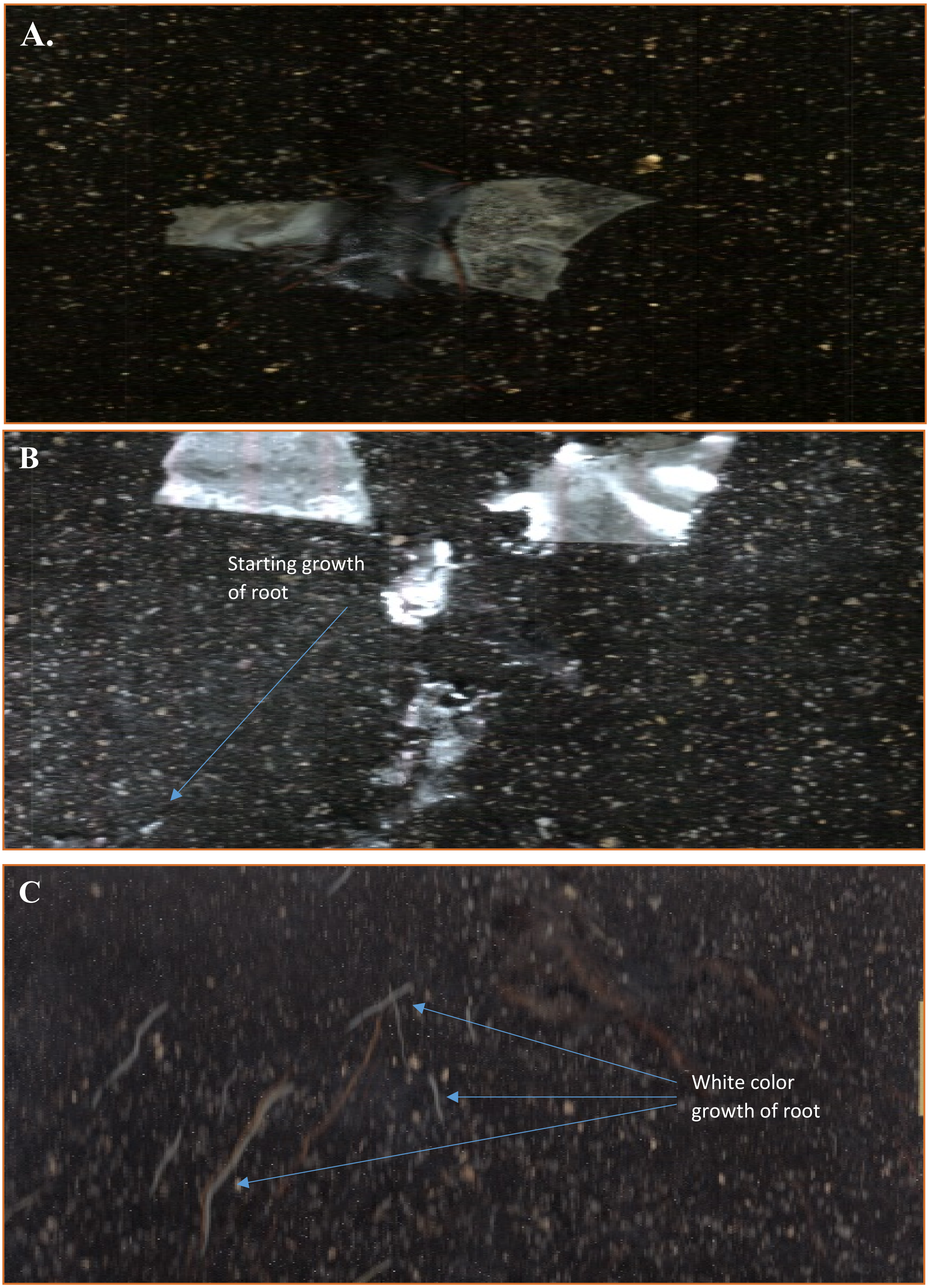


Figure 2.6 Root growing period for 'Miyabi Fuji' on Marubakaido (Ma); **A**-after planting first time root scanning April 27, **B**-first root growing May 4, **C**-condition before digging October 16 in 2018.

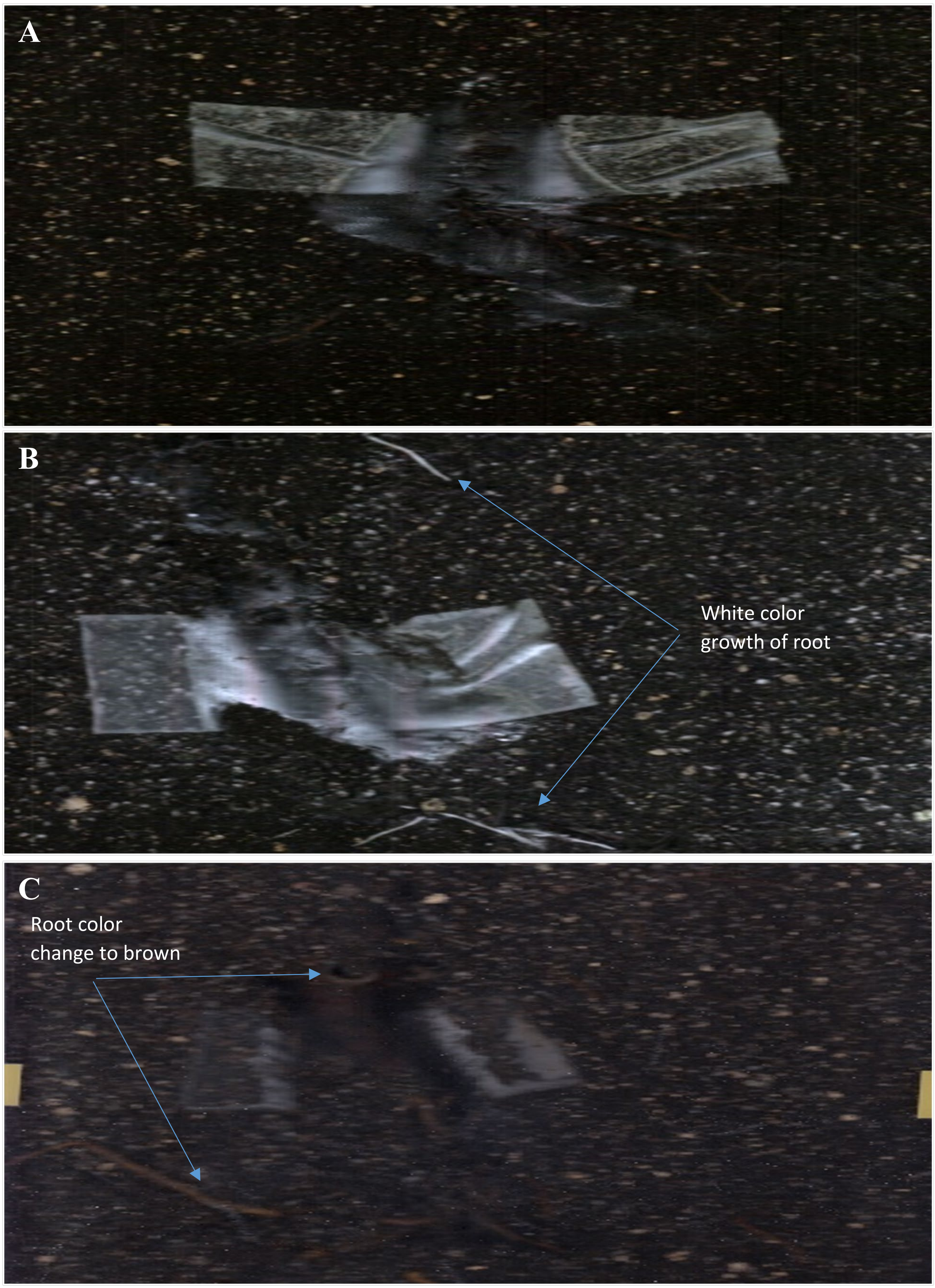


Figure 2.7 Root growing period for 'Miyabi Fuji' on JM7; **A**-after planting first time root scanning April 27, **B**-first root growing May 4, **C**-condition before digging October 16 in 2018.

Figure 2.8 Upper-part growth for 'Miyabi Fuji' on Marubakaido (Ma) and JM7 on November 23 in 2020, A-Ma 70% SWL, B-JM7 70% SWL, C-Ma 50% SWL and D-JM7 50% SWL. ASN-average shoot number, SWL-soil water level.

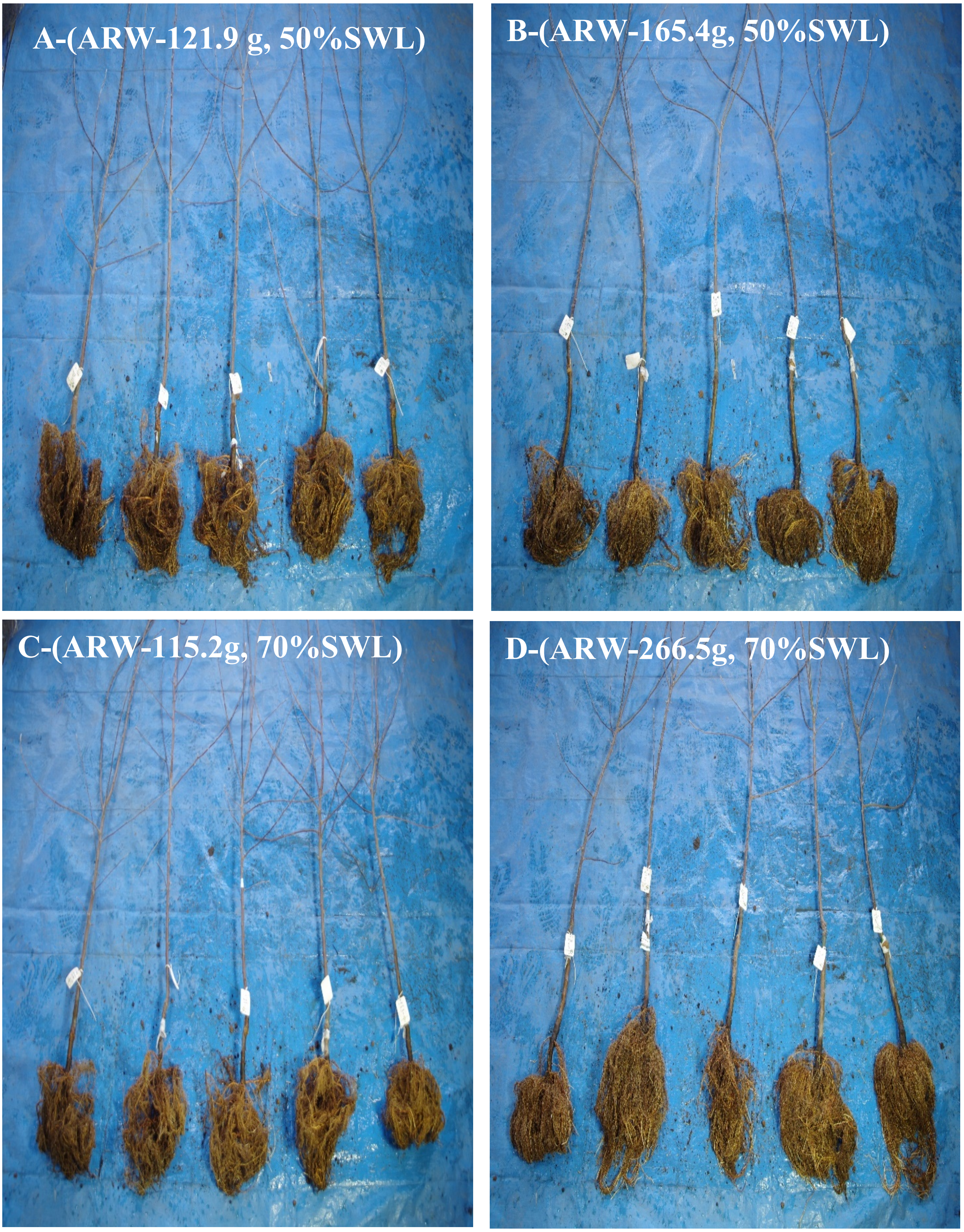


Figure 2.9 Lower-part growth for 'Miyabi Fuji' on Marubakaido (Ma) and JM7 on November 23 in 2020, A-Ma 50% SWL, B-JM7 50% SWL, C-Ma 70% SWL and D-JM7 70% SWL. ARW-average root weight (g), SWL-soil water level.

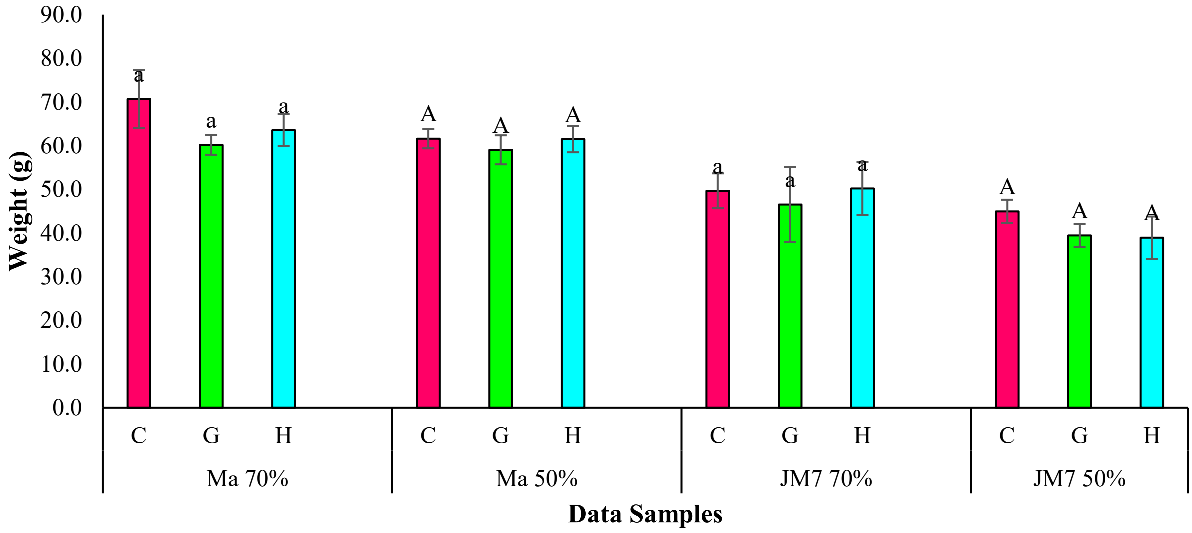


Figure 2.10 Effect of treatments on shoot dry weight for 'Miyabi Fuji' apple tree in 2020; C-Control, G-Glutain and H-Hydretain ES Plus. Standard error and different letters indicate statistically significant difference among the treatments according to the Tukey test; (n = 5).

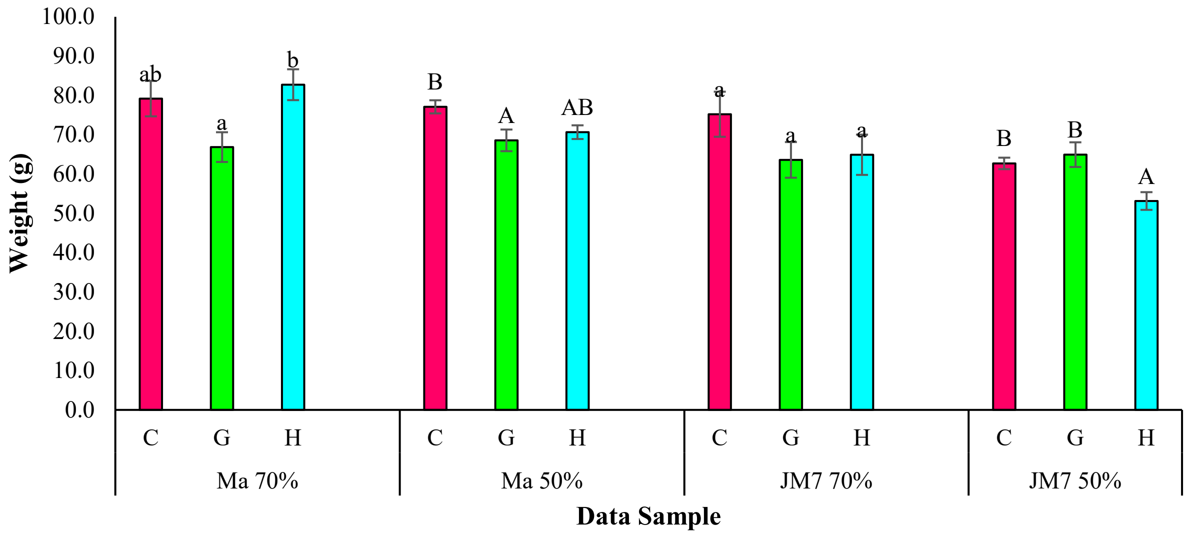


Figure 2.11 Effect of treatments on trunk dry weight for 'Miyabi Fuji' apple tree in 2020; C-Control, G-Glutain and H-Hydretain ES Plus. Standard error and different letters indicate statistically significant difference among the treatments according to the Tukey test; (n = 5).

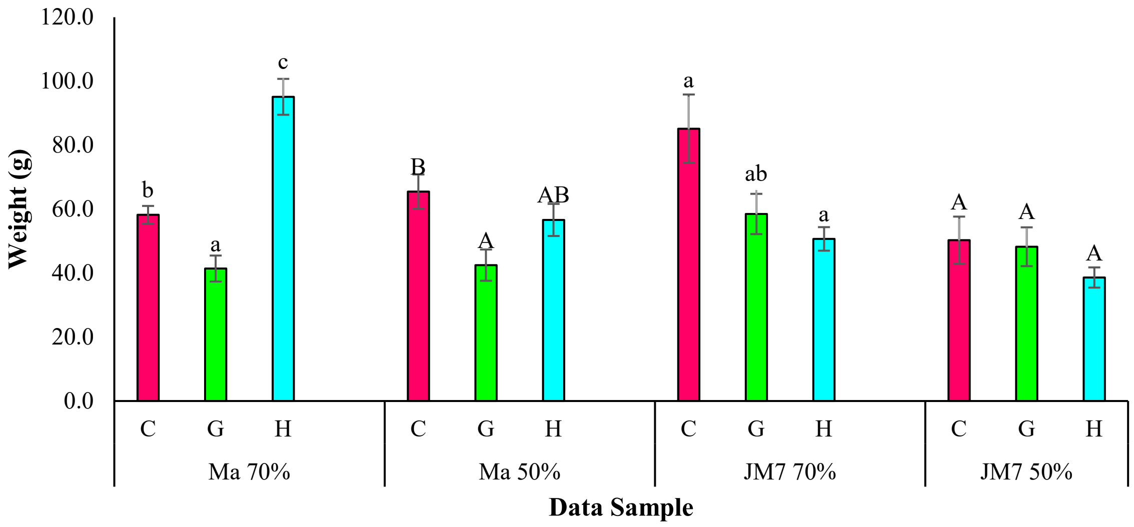


Figure 2.12 Effect of treatments on root dry weight for 'Miyabi Fuji' apple tree in 2020; C-Control, G-Glutain and H-Hydretain ES Plus. Standard error and different letters indicate statistically significant difference among the treatments according to the Tukey test; (n = 5).

Table 2.7 Experiment materials and used solutions of soil treatment for 'Miyabi Fuji' on Marubakaido (Ma) and JM7.

Rootstocks	**Water treatment**	**Soil treatment**
Ma	70%	Control
		Glutain (11 mL/p) x Kalpak 66 (11 mL/p ^{z})
JM7	50%	Hydretain ES Plus (11 mL/p)
		Menedael 11 ml/p
		Super Sorb C (50g/p)

z: mL/p – soil treatments mixed with soil and 11 mL per pot.

Table 2.8. Effects of treatments on the number of shoots and total shoot, top first shoot and top three shoot length (Means ± SE) for 'Fuji' on Ma and JM7 in 2020.

Rootstock	Water treatment	Soil treatment	Number of shoots[b]	Total shoot length (cm)[a]	Top first shoot length (cm)	Top three shoot length (cm) [a]
Ma	70%	Control	13.0 ± 0.6b	497.0 ± 25.4ac	105.9 ± 5.9ac	206.0 ±15.4ab
		Glutain + Kalpak 66	9.0 ± 1.5ab	394.2 ± 23.8a	117.7 ± 3.0 c	225.6 ± 10.8b
		Hydretain ES Plus	7.8 ± 0.6ab	630.7 ± 33.6cd	102.7 ± 4.2ac	189.7 ± 9.4ab
	50%	Control	7.2 ± 1.3ab	499.7 ± 17.5ac	124.7 ± 5.6c	189.0 ± 8.4ab
		Glutain + Kalpak 66	10.6 ± 2.7ab	397.7 ± 18.3a	113.6 ± 3.2bc	198.8 ± 7.1ab
		Hydretain ES Plus	7.8 ± 1.7ab	449.9 ± 10.0ab	118.4 ± 3.5bc	208.0 ± 17.8ab
JM7	70%	Control	6.6 ± 0.5ab	715.8 ± 48.0d	93.3 ± 4.8ab	202.9 ± 16.9ab
		Glutain + Kalpak 66	6.2 ± 1.5ab	603.9 ± 44.4cd	111.0 ± 5.9ac	167.1 ± 5.3a
		Hydretain ES Plus	6.2 ± 1.6ab	540.8 ± 29.0bc	94.1 ± 8.3ab	162.3 ± 8.0a
	50%	Control	5.2 ± 1.4a	558.8 ± 28.2bc	102.0 ± 6.8ac	192.5 ± 11.1ab
		Glutain + Kalpak 66	5.6 ± 0.7a	541.3 ± 21.4bc	103.3 ± 7.5ac	153.3 ± 10.0a
		Hydretain ES Plus	6.4 ± 0.9ab	464.2 ± 20.0ab	86.7 ± 3.5a	179.5 ± 15.0ab
			Significance			
Rootstock (R)			***	***	***	***
Water treatment (W)			*	***	ns	ns
Soil treatment (S)			ns	***	ns	ns
R × W			ns	ns	ns	ns
R × S			ns	***	ns	*
W × S			ns	ns	ns	ns
R × W × S			ns	**	ns	ns

Note: Different letters by column indicate statistically significant differences according to a Tukey test and significant levels: (NS) no significance, (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$ (n=5);
[a] From top to below second, third and fourth shoots;
[b] Only those shoots that were longer than 10 cm and shorter than 35 cm were counted.

Table 2.9. Effects of different treatments on top shoot diameter, trunk diameter, shoot fresh weight and trunk fresh weight (Means ± SE) of young 'Miyabi Fuji' apples on Ma and JM7 in 2020.

Rootstock	Water treatment	Soil treatment	Top shoot diameter (mm)	Trunk diameter (mm) [a]	Shoot weight (g) [a]	Trunk weight g)
Ma	70%	Control	11.6 ± 0.3 d	17.7 ± 0.6 de	132.6 ± 11.9c	140.8 ± 6.8bc
		Glutain + Kalpak 66	10.6 ± 0.5 cd	16.1 ± 0.3 be	110.8 ± 5.2ac	117.0 ± 7.7ab
		Hydretain ES Plus	10.6 ± 0.3 cd	18.14 ± 0.8 de	122.0 ± 6.5bc	154.2 ± 6.0c
	50%	Control	11.2 ± 0.3 d	18.2 ± 0.3 e	117.6 ± 3.7bc	141.4 ± 4.3bc
		Glutain + Kalpak 66	11.0 ± 0.3 cd	16.8 ± 0.4 cde	109.0 ± 7.3ac	119.0 ± 5.2ab
		Hydretain ES Plus	11.2 ± 0.3 d	17.2 ± 0.3 de	117.3 ± 6.3bc	130.3 ± 3.7ac
JM7	70%	Control	8.5 ± 0.6 ab	15.8 ± 0.6 bd	96.7 ± 7.5ac	136.0 ± 9.3ac
		Glutain + Kalpak 66	9.1 ± 0.5 bc	14.7 ± 0.3 abc	92.7 ± 16.5ac	119.4 ± 8.7ab
		Hydretain ES Plus	8.1 ± 0.3 ab	14.8 ± 0.6 abc	96.2 ± 11.3ac	116.9 ± 10.7ab
	50%	Control	8.2 ± 0.6 ab	14.3 ± 0.3 ab	86.7 ± 5.1ab	112.5 ± 3.8ab
		Glutain + Kalpak 66	7.9 ± 0.2 ab	14.4 ± 0.6 ab	81.9 ± 6.9ab	113.5 ± 7.8ab
		Hydretain ES Plus	6.7 ± 0.4 a	13.4 ± 0.4 a	71.8 ± 6.7a	104.3 ± 6.3a
			Significance			
	Rootstock (R)		***	***	***	***
	Water treatment (W)		ns	*	*	*
	Soil treatment (S)		*	ns	ns	*
	R × W		*	ns	ns	ns
	R × S		ns	ns	ns	*
	W × S		ns	ns	*	ns
	R × W × S		ns	ns	ns	ns

Note: Different letters by column indicate statistically significant differences according to a Tukey test and significant levels,

(NS) – no significance, (*) – p < 0.05, (**) – p < 0.01, (***) – p < 0.001, (n=5);

[a] all shoots (top, top three, below and secondary shoots).

Table 2.10. Effects of treatments on root fresh weight, root volume and root to shoot ratio (Means ± SE) for 'Miyabi Fuji' on Ma and JM7 in 2020.

Rootstock	Water treatment	Soil treatment	Root weight (g)	Root volume (ml)	Root: shoot ratio
Ma	70%	Control	115.2 ± 11.6ab	153.5 ± 17.3 ac	0.9 ± 0.1ab
		Glutain + Kalpak 66	74.7 ± 9.6a	97.5 ± 11.5 a	0.7 ± 0.1a
		Hydretain ES Plus	240.4 ± 19.3cd	253.5 ± 21.5 c	2.0 ± 0.1ac
	50%	Control	121.9 ± 10.1ab	106.5 ± 17.3 a	1.0 ± 0.1ab
		Glutain + Kalpak 66	74.0 ± 7.8a	69.1 ± 7.3 a	0.7 ± 0.1a
		Hydretain ES Plus	104.0 ± 7.1ab	109.5 ± 12.5 a	0.9 ± 0.1ac
JM7	70%	Control	266.5 ± 34.5d	237.5 ± 28.6 bc	2.8 ± 0.3c
		Glutain + Kalpak 66	181.0 ± 18.9bd	158.1 ± 21.3 ac	2.1 ± 0.3bc
		Hydretain ES Plus	147.9 ± 11.1abc	136.5 ± 22.0 ab	1.7 ± 0.4ac
	50%	Control	165.4 ± 26.2abc	141.5 ± 25.8 ab	2.0 ± 0.1ac
		Glutain + Kalpak 66	161.3 ± 33.9abc	134.5 ± 38.4 ab	2. 1± 0.6bc
		Hydretain ES Plus	127.6 ± 10.2ab	112.5 ± 13.3 a	1.8 ± 0.2ac
			Significance		
	Rootstock (R)		***	***	***
	Water treatment (W)		***	**	ns
	Soil treatment (S)		**	ns	ns
	R × W		ns	ns	ns
	R × S		***	***	**
	W × S		ns	ns	ns
	R × W × S		***	*	*

Note: Different letter by column indicates statistically significant differences according to a Tukey test and significant levels: (NS) no significance; (*) $p < 0.05$, (**) $p < 0.01$, (***) $p < 0.001$ (n=5).

CHAPTER 3

ROOT GROWTH AND BUD DEVELOPMENT IN WINTER-PLANTED YOUNG 'MIYABI FUJI' APPLE TREES

3.1 INTRODUCTION

The apple (*Malus* × *Domestica* Borkh.) is one of the world's most widely cultivated fruits (Pereira et al., 2006). Commercial apple production takes place mostly in temperate climate areas where snowfall normally falls during the winter. Young apple trees are usually planted in early spring or late autumn, although planting times differ depending on weather conditions (Arakawa et al., 2014). In recent years, the spring planting of fruit trees has become the normal practice in temperate areas as well as in more arid areas with limited water resources (Kikuchi et al., 2003).

However, the timing of the planting in water–challenged areas impacts the roots of young plants, especially those of young apple trees in certain areas of Central Asia. There, apple trees are grown under dry and hot summer conditions, whereas in winter temperatures often fall far below freezing and there is a significant amount of snowfall-generated moisture (Hu Zengyun et al., 2016). In these areas, water availability and its efficient use after planting are critical factors for favorable tree growth. Therefore, in order to determine optimal planting times and the best tree management practices, an understanding of the physiological development of newly planted trees is essential.

Most commercially grown nursery trees have weak root systems and show pruning damage, which affects shoot growth. Previous studies have reported that a healthy root system promotes shoot growth, i.e., the number of shoots and the height of trees in one-year-old apple trees (Arakawa et al., 2014). Similar root and shoot growth have also been observed in citrus trees (Bevington et al., 1985), while Budiarto et al. (2019) described the potential benefits of citrus root pruning to manage plant growth.

Arakawa et al. (2014) reported on the impact of the winter and fall planting of apples trees on root growth and the impact of the roots on shoot growth. This is in contrast to spring-planted apple trees, where root growth is delayed until after shoot growth. On winter-planted trees, the buds do not start to grow until after root growth because of the dormancy caused by the cold weather. There is no detailed research or experiments that have been conducted regarding root growth that is related to the physiological changes in apple trees that are planted in early winter.

The rootstocks onto which the young trees are grafted is also an important factor in the development of young trees after they are planted. Soejima et al. (1998) reported that Marubakaido (Ma) (*M. prunifolia* Borkh. *var. Ringo* Asami), a semi-vigorous rootstock for apple trees, is used in most of the apple orchards in Japan. The advantages of semi-vigorous Ma are its perfect anchorage, early and heavy production, resistance to burr root, crown rot, wooly aphids, tolerance to wet soil conditions, and ease of propagation with hardwood cuttings.

Moreover, the physiological changes that occur in the buds that appear on the trunks of the winter-planted trees are important. The buds play an important role in managing future shoot growth and tree shape. In this study, bud light absorbance and the physiological conditions of the young winter-planted trees were measured with a spectrometer. Using a spectrometer, one can also test a plant's water index (Ribera-Fonseca et al., 2019) as well as the dry matter content of a plant's fruit (Toivonen, et al., 2017). Observing the physical condition of winter-planted apple trees, especially their buds before bud burst, can be helpful to researchers and apple growers because they can more accurately forecast bud growth and the timing of bud burst.

In this chapter, the root growth of one-year-old 'Miyabi Fuji' and the impact of the physical growing environment is examined by comparing semi-vigorous Marubakaido (Ma) (*Malus prunifolia* 'Ringo') rootstocks with dwarfing M.9 rootstocks under cold winter conditions. In addition, this chapter also looks at the bud characteristics of young winter-planted 'Miyabi Fuji' apple trees. The planting season and the environmental conditions after planting affect root growth, shoot growth and tree architecture. The results showed that for trees planted in winter, root growth happens from March, with significant differences in the two rootstocks that were studied.

3.2 MATERIALS AND METHODS

3.2.1 Plant materials and experiment design

One-year-old 'Miyabi Fuji' (a bud sport of 'Fuji' having good fruit coloration) apple trees, grafted onto Marubakaido (Ma) and M.9 rootstocks, were planted on November 25, 2019. They were then observed over the winter and during the spring growing season. The experiment design was as follows: five measuring dates (January 27, February 27, March 27, April 27, and May 28), two rootstocks (Ma and M.9), and 15 young trees planted on each of the two rootstocks. All 30 trees were purchased from "HARADA NURSERY Co., Ltd, and experiments were conducted on the campus of Hirosaki University. Before planting, all apple saplings were scaled to the same size by cutting them to 70 cm; roots were pruned to 10 cm (Fig. 3.1 (A, B)).

On November 25, the young apple trees were placed in 11 L black plastic nursery pots that contained a mixture of one-part potting soil used for trees and two parts black volcanic soil. These were then placed in a specially designed hole (Fig. 3.1 (C)) that would prevent them from freezing during the winter. In mid-April, the potted trees were placed above ground. Daily temperature alterations are shown for the duration of the experiment (Fig. 3.2). The average monthly temperatures and total precipitation for the period during which the experiment was conducted are also shown in Fig. 3.2. In December, the average monthly temperature was 1.7°C and rainfall was 145.5 mm. In January, the average temperature was 0.3°C and the rainfall 101 mm. In February, the average temperature had risen to 0.5°C and precipitation was 144.5 mm. In March, the average temperature was up to 4.9°C, while the precipitation had dropped to 84.5 mm. In April, the average monthly temperature rose to 7.5°C and precipitation increased slightly to 98 mm. Finally, in May, the average temperature was 15°C and total precipitation was 54 mm.

3.2.2 Studied traits

On January 27, 2020, the first measurements were made and were repeated on the 27th of each ensuing month until May 28. The young trees were removed from their pots each time before taking the measurements. The roots were separated from the soil and washed, and their average length was measured with a caliper. The tree samples were classified into the following parts: trunks, rootstock stems, and roots. All of the parts were weighed, then dried in an oven at 80°C for 48 to 60 hours. After being dried, all samples were again weighed and measured for moisture content (MC). MC was determined using the following equation (3.1) where FW is the fresh weight of the sample and DW is the dried weight of the sample (Turner, 1981):

$$MC_{(FW_{basis})} = \frac{FW-DW}{FW} \times 100 \qquad (3.1)$$

3.2.3 Bud light absorbance

Bud light absorbance was tested with an ultra-mini visible/near-infrared spectrometer device (OMT-NIR-M1 by Optcom Co., Ltd. using SpectralRatio Version 1.1.0.1. software) for the 'Miyabi Fuji' on the Ma and M.9 rootstocks when they were measured on the 27^{th} of January, February, March and April. Measurements were not made on May 28 since bud burst had already occurred. The wavelength range that can be measured on this device is 640-1050 nm, with an interval of 2 nm. The data collected was analyzed with a PCA test using R software.

3.2.4 Data analysis

The impact of the moisture content (MC) of the roots was analyzed by a one-way ANOVA (the difference between dates) and a Tukey test. The MC of the rootstocks on the different dates were analyzed by a two-way ANOVA. New shoot length was analyzed using the Student's t-test. All of the above analyses were performed using the R studio version 1.3.1073 (© 2009-2020 RStudio, PBC) software.

3.3 RESULTS

3.3.1 Root growth

Root growth change was examined between January and May 2020 for the 'Miyabi Fuji' trees that had been grafted onto the Ma and M.9 rootstocks (Table 3.1). Observations on January 27 and February 27 did not show any root growth for either rootstock. On March 27, only root hairs and root caps less than or equal to two cm were observed. On April 27, these root hairs and root caps had grown in length to ten cm or more. Then, on May 28, vigorous growth of the primary root, the secondary root, and tertiary roots were seen.

3.3.2 Moisture content changes in separate parts of the trees

The changes in MC in the trunk and the results of the ANOVA are shown in Table 3.2. The moisture content of the trunks had increased significantly from January to May for the trees on both rootstocks. There was, however, a statistical difference between the two rootstocks; the MC for M.9 was higher than that for Ma ($P \leq 0.01$). The percentage change in MC for Ma in May was higher than that recorded in January, February, and March. The MC for the trunks of the dwarfing M.9 had increased significantly from January to April and May. There was no significant difference statistically between the rootstocks and the months in which they were measured for trunk MC.

Rootstock stem MC changes and the results of the ANOVA test are shown in Table 3.3. The MC of the rootstock stems increased from January to May on both rootstocks, and changes in rootstock stem MC changed markedly in different months during the experiment period. There was a statistically significant difference between the rootstocks; the MC of the M.9 rootstock stems was higher than that of the Ma ($P \leq 0.0001$). The Ma MC showed considerable decline from January to February, increased dramatically in March and April, and rose even more so in May. The MC for M.9 was notably higher in May when compared with earlier months. There was no statistically significant interrelation between the rootstock and the dates for rootstock stem MC.

The changes in MC in the roots and the results of the ANOVA test are shown in Table 3.4. The MC in the roots underwent considerable changes during the experiment period; MC increased from January to May for the roots on the trees on both rootstocks. There was statistical significance in the effects of the rootstocks. The root MC of M.9 was higher than that of Ma ($P \leq 0.05$). The root MC of M.9 increased significantly from January and February to March and even more so in April; then, from April to May it decreased greatly. There was no significant interaction between rootstock and the date for the root MC.

3.3.3 New shoot growth for 'Miyabi Fuji' trees on Ma and M.9

New shoot growth on the trees and the results of the T-test are shown in Fig. 3.3. The new shoots on the trees differed significantly for the Ma and M.9 rootstocks, although the growth of new shoots commenced in May for both Ma and M.9. There was a statistical difference between the rootstocks; the total number of new shoots on the Ma trees was higher than the number on the M.9 ($P \leq 0.05$).

The total number of new leaves on the shoots was significantly higher that the number of new leaves on the stems on both the Ma and the M. 9 rootstocks (Fig. 3.4 and Fig. 3.5).

The weight of the new leaves on the shoots and the stems was measured with a scale and the differences between them were analyzed with a Student T-test (Fig. 3.6). The weight of new leaves on the shoots had increased significantly more that of those on the stems.

The differences in the root fresh weight of the two rootstocks is shown in Fig. 3.7. On May 28, the fresh weight of the Ma roots showed a more dramatic increase than that of the M.9 roots. From January through April, no changes in root fresh weight were observed for the young trees.

3.3.4 Bud light absorbance

The bud light absorbance measured on the visible/near-infrared spectrometer for the Ma rootstocks in January was greater than it was in March, from 640 nm to 700 nm wavelength. The 1050 nm measurement on March 27 was greater than the 702-measurement made on January 27 (Fig. 3.8). On April 27, the 670 nm wavelength bud light absorbance measured on the visible/near-infrared spectrometer was greater than it was in March, whereas in April it was lower than in January.

Bud light absorbance measured on the visible/near-infrared spectrometer between January and February was identical for the M.9 rootstock (Fig. 3.9). It was lower in April when compared to January and March.

The light absorbance of the top bud on the tree grafted onto the Ma rootstock was lower than that on the M.9 rootstock when measured with the visible/near-infrared spectrometer (Fig. 3.10). The light absorbance of the buds did not differ in March and April for either of the rootstocks. In April, the light absorbance of the top bud, as shown on the visible/ near-infrared spectrometer, was lower than in January and in March.

The PCA percentage of explained variance for the buds on the trunks on the M.9 rootstock is shown in Fig.3.11A. The first dimension of PCA variance was 90.7% and the second dimension was 8.6%; the sum of these two dimensions was almost 100% (99.3%). The analyzed PCA plots are shown in Fig. 3.11B. The PCA analysis helped drawn a clear distinction between the February and the April spectral data for the buds.

3.4 DISCUSSION AND CONCLUSION

In cold weather areas, young apple trees purchased from nurseries are usually planted in the early winter months, just before snowfall. However, there is no detailed research or experiments that have been conducted regarding root growth related to the physiological changes that occur in apple trees planted in early winter. Our findings verified that no root growth occurred in the wintertime, January to February. The roots started to grow slowly from March to April, whereas in May vigorous root growth was observed. Van et al. (2011) reported that root growth for dwarfing M.9 occurred from early spring (December in New Zealand), although they did not check or mention wintertime root growth.

Temperature change is also vital for root growth. During the experiment period, the average daily temperature in March was 4.9 °C, which impacted root growth. Lopushinskiy and Max (1990) found that, for forest trees root growth occurs when soil temperature is 5° C or above.

The MC changes were measured in the lower and upper parts of the trees to determine the relationship between root condition and the growth of different parts of the tree. The MC of the trunk rose slowly from January to May. Root MC increased from January to April when the new roots appeared. These findings suggest that these MC changes are related to root growth and root activity (water absorption by the roots). An increase in trunk MC and rootstock stem MC may be related to cold-related damage in young trees during the spring, since it has been suggested that the cold hardiness of woody plants is related to water relations parameters (Anisko and Lindstorm, 1996).

In our study, root MC decreased when shoot growth occurred in May on the young apple trees. Diminishing root MC did not affect root growth in May and vigorous root growth continued.

Bud burst was found at the end of April (data not shown), while total new shoot length was observed at the end of May (Fig. 3.3). This suggests that, in May, the development of shoots on trees grafted onto a semi-vigorous rootstock (Ma) take longer than those on a dwarfing rootstock (M.9). Bevington and Castle

(1985) reported that root growth declined during shoot elongation for citrus trees when there were no soil temperature or water content issues.

It is essential to manage soil and water to promote root growth even after planting. It appears that both rootstock selection and winter planting are critical for root growth when young apple trees are planted in areas where moisture is provided by snowfall in the winter but suffer from a shortage of water during the non-winter months.

This research study looked into the effects of winter planting on root growth and certain physical features of one-year old 'Miyabi Fuji' apple trees during the winter and spring. The winter planting affected on root growth was found and that the MC of the trees changed from February to March. Accordingly, significant physiological changes were observed in the trees.

Hence, winter planting in areas with limited water-resources would ensure that there would be sufficient soil moisture to support root growth and encourage bud break. Therefore, in the future, it will be intended to extend the scope of our research to include water-challenged areas. These results may provide insights for apple growers in such areas regarding the most effective planting times and the impact of planting times on shoot growth and the growth of the upper and lower parts of young trees.

Moreover, it was investigated the bud light absorbance of young apple trees with a visible/near-infrared spectrometer on five equally spaced dates between from January to May. Data findings showed that in April the absorbance of light of the buds on the upper and lower parts of the trunk was lower than in January and March. The light absorbance of the buds on the middle part of the trunk were the same when measured with the visible/near-infrared spectrometer. Light absorbance of the buds on the very top of the trunk, when measured at 640-700 nm on the spectrometer, was greater for Ma than for M.9. Spectro data showed that bud light absorbance was higher on M.9 than Ma when they are measured from January through March estimated Spectro data. These findings suggest that the greater amount of light absorbance of buds at the top of the trunk of young apple trees showed that these were more physiologically active than the buds on the middle and lower parts of the one-year-old tree trunks. If the visible spectrometer readings of trunk bud light absorbance were to rise, they would like occur near bud burst, and if near-infrared spectrometer readings of bud absorbance were to decrease they might occur as a result of free water movement in the buds at the top of the trunks of the young apple trees.

3.5 SUMMARY

Determining the timing of planting is an integral part of any agricultural operation. This plays a particularly important role in water-challenged areas where soil moisture is an issue. During the winter months in these areas, there is usually sufficient precipitation to maintain adequate water content levels in freshly planted trees. However, during the summer and early autumn, there is very little precipitation. This can adversely affect young trees. In this Chapter, measurements were taken to determine root growth, bud development and other changes in the upper parts of apple trees that had been planted in the winter, instead of being planted in the spring when planting usually takes place. In this experiment, one-year-old 'Miyabi Fuji', grafted onto Marubakaido (Ma) (*Malus prunifolia* 'Ringo') and M.9 rootstocks, were examined from January through May. The results showed dramatic changes in root growth from March (average root length less than two cm before March) to May (average root length longer than ten cm) for both rootstocks. Furthermore, trunk moisture content increased over time (51.8% in January and 56.1% in May on M.9). Although root growth in the young apple trees occurred, it is unknown if root water absorption began before or at the same time as this root growth. However, root growth developed favorably because of the soil moisture generated by the winter precipitation. Satisfactory root growth and tree moisture content changes was found in the trees used in the study, leading us to conclude that winter planting could be recommended in areas where water resources are limited in the non-winter months.

Keywords: Bud growth, growing season, rootstock, water content, root growth, shoot growth.

Figure 3.1 One-year-old ‘Miyabi Fuji’ before and after planting; (A) semi-vigorous Marubakaido (Ma); (B) dwarfing M.9; (C) specially designed hole to prevent winter freezing.

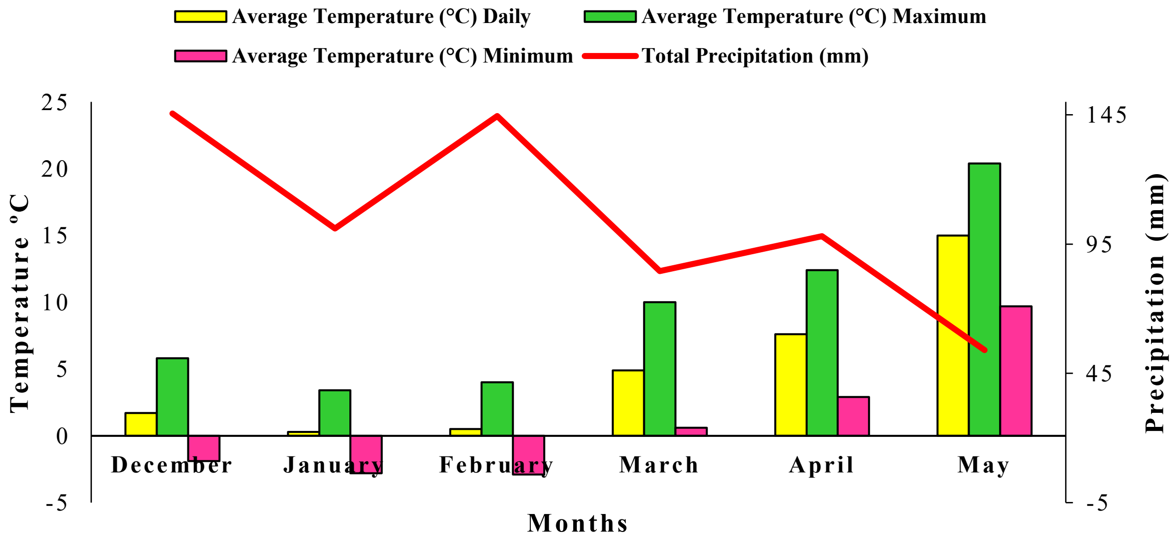


Figure 3.2 Daily, maximum, minimum temperatures and total precipitation in Hirosaki, Japan during the experiment period.

Table 3.1 Root growth for 'Miyabi Fuji' on semi-vigorous Ma and dwarfing M.9 rootstocks from January through May 2020.

Months	**Root growth starting (cm)** [a]		**Parts of the root** [b]
	Ma	**M.9**	
January 27	NG	NG	All
February 27	NG	NG	All
March 27	≤ 2	≤ 2	Root cap
April 27	≤ 10	≤ 10	Root cap
May 28	≥ 10	≥ 10	All

Note: **a** – average length of new root growth;

b – the new root growth occurred in the root area;

NG – no growth;

All – primary, secondary, tertiary root and root region.

Table 3.2 Changes in trunk moisture content for 'Miyabi Fuji' on semi-vigorous Ma and dwarfing M.9 rootstocks from January through May 2020.

Rootstocks	**Moisture content (%)**				
	January	**February**	**March**	**April**	**May**
Ma	50.9 ± 0.52a	49.7 ± 0.43a	50.3 ± 0.78a	53.1 ± 0.2ab	55.8 ± 1.4b
M.9	51.8 ± 0.15a	52.1 ± 0.03ab	52.1 ± 0.05ab	53.0 ± 0.3b	56.1 ± 0.4c
	P value	**Significance**			
Rootstock (R)	0.008026	**			
Date (D)	9.297e-08	***			
R x D	0.239132	Ns			

Note: Means ± standard error and different letters indicate statistically significant differences among the months according to the Tukey test;

(*) – P ≤ 0.05, (**) – P ≤ 0.01, (***) – P ≤ 0.001, (**NS**) – no significance, (n=3).

Table 3.3 Changes in rootstock stem moisture content for 'Miyabi Fuji' on semi-vigorous Ma and dwarfing M.9 rootstocks from January through May 2020.

Rootstocks	**Moisture content (%)**				
	January	**February**	**March**	**April**	**May**
Ma	47.7 ± 0.1b	46.0 ± 0.3a	47.6 ± 0.4b	48.7 ± 0.5b	51.4 ± 0.19c
M.9	48.2 ± 0.6a	48.6 ± 0.4a	48.7 ± 0.6a	50.1 ± 0.7a	52.7 ± 0.34b
	P value	Significance			
Rootstock (R)	0.0001374	***			
Date (D)	1.281e-08	***			
R x D	0.2879832	Ns			

Note: Means ± standard error and different letters indicate statistically significant differences between the months according to a Tukey test;

(*) – P ≤ 0.05, (**) – P ≤ 0.01, (***) – P ≤ 0.001, (**NS**) – not significance, (n=3).

Table 3.4 Changes in root moisture content for 'Miyabi Fuji' on semi-vigorous Ma and dwarfing M.9 rootstocks from January through May 2020.

Rootstocks	**Moisture content (%)**				
	January	**February**	**March**	**April**	**May**
Ma	55.0 ± 3.3a	54.1 ± 1.4a	60.6 ± 1.1a	61.0 ± 1.6a	56.3 ± 5.4a
M.9	55.3 ± 19.4a	56.8 ± 11.3a	65.8 ± 9.2bc	67.4 ± 7.7c	58.7 ± 7.5ab
	P value	**Significance**			
Rootstock (R)	0.044436	*			
Date (D)	0.002475	**			
R × D	0.759174	ns			

Note: Means ± standard error and different letters indicate statistically significant differences between the months according to a Tukey test;

(*) – $P \leq 0.05$, (**) – $P \leq 0.01$, (***) – $P \leq 0.001$, (**NS**) – no significance, (n=3).

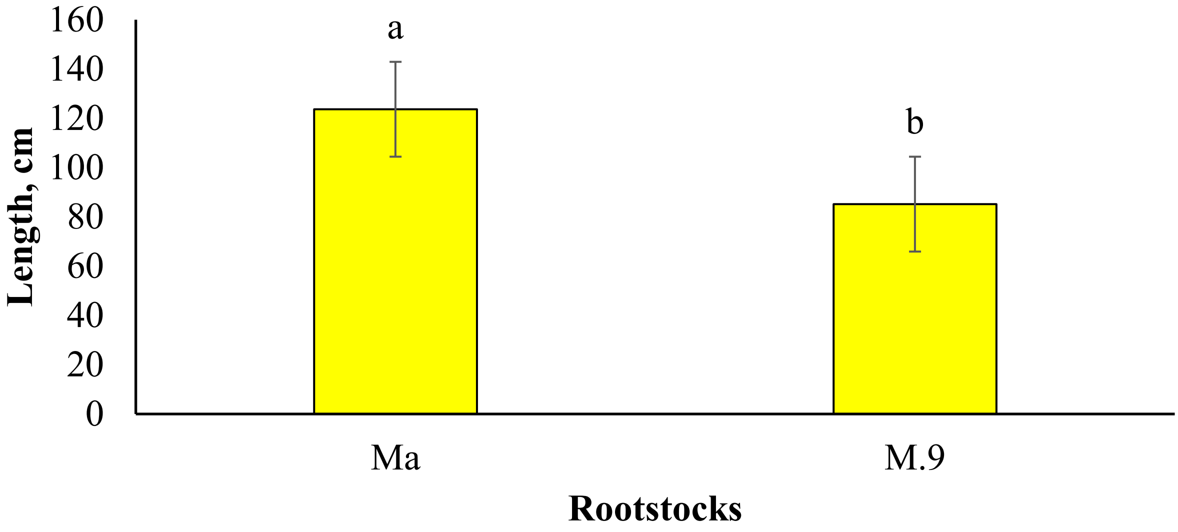


Figure 3.3 Total new shoot length (cm) for 'Miyabi Fuji' on semi-vigorous Ma and dwarfing M.9 on May 28, 2020. Means ± standard error; different letters indicate statistically significant differences between the rootstocks according to a T-test P ≤ 0.05, (n=3).

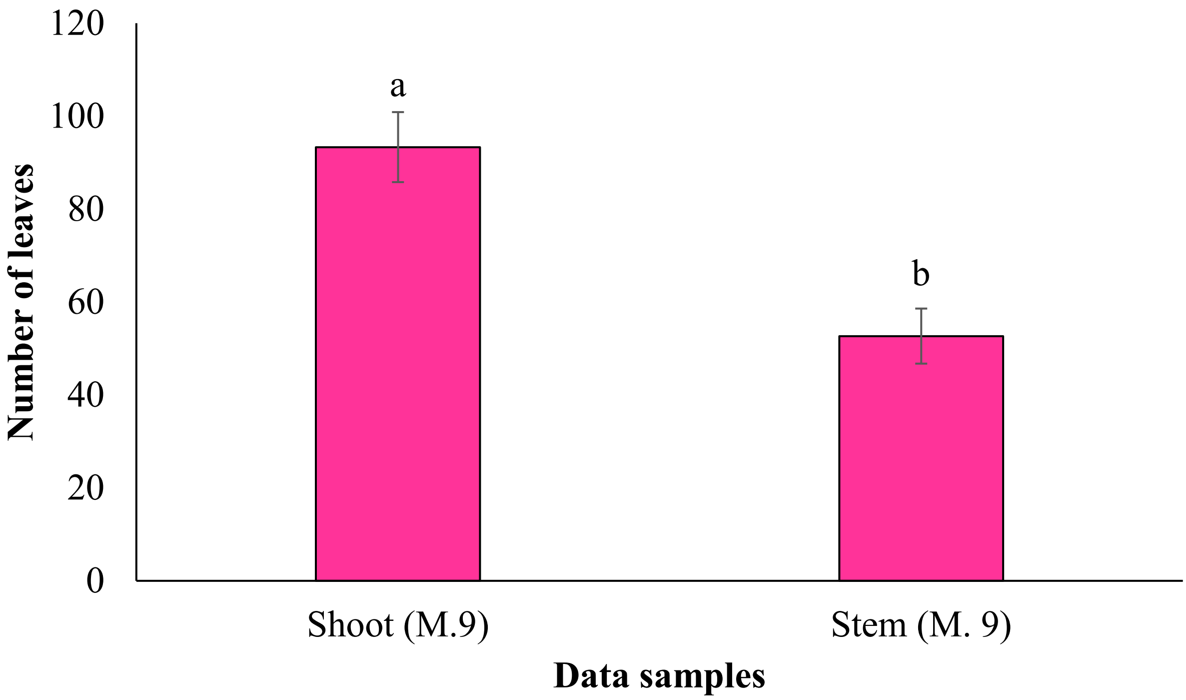


Figure 3.4 Total number of new leaves for 'Miyabi Fuji' on dwarfing M.9 on May 28, 2020. Means ± standard error; different letters indicate statistically significant differences among the rootstocks according to a T-test P ≤ 0.05, (n=3).

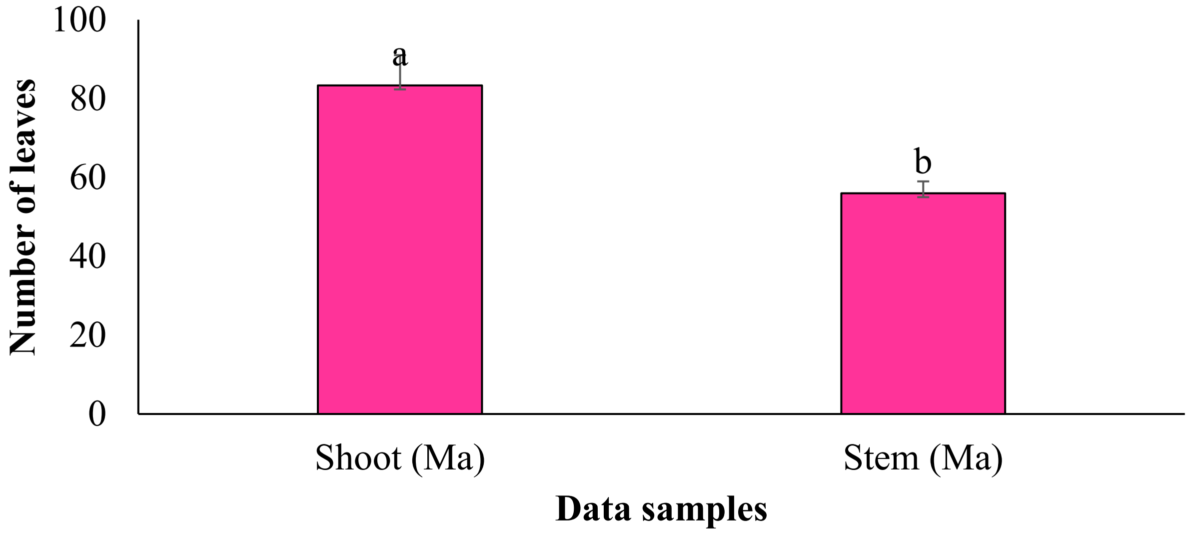


Figure 3.5 Total number of new leaves for 'Miyabi Fuji' on semi-vigorous Ma on May 28, 2020. Means ± standard error; different letters indicate statistically significant differences among the rootstocks according to a T-test P ≤ 0.05, (n=3).

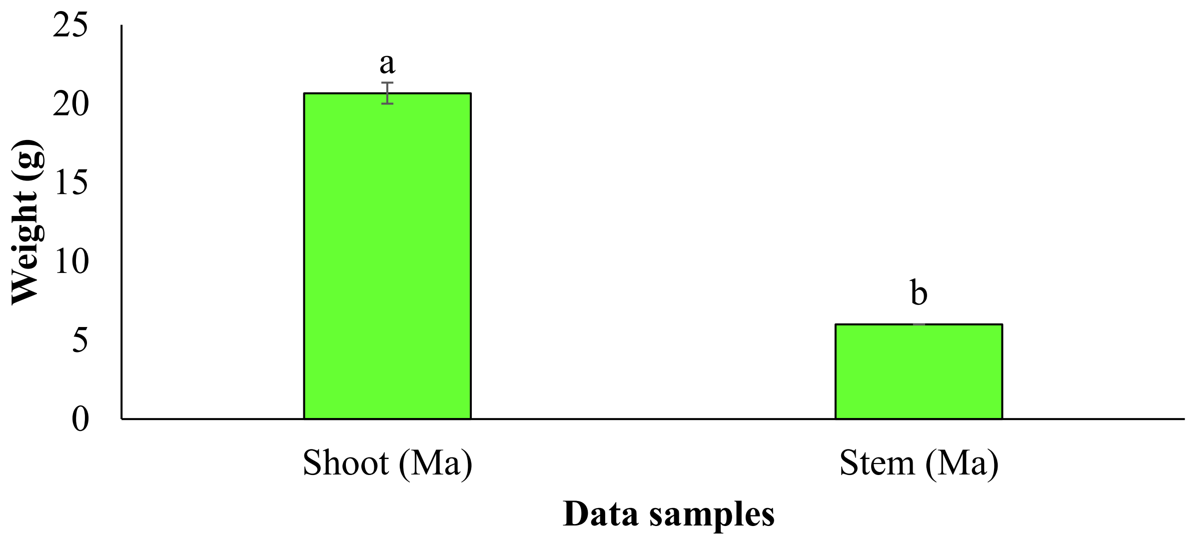


Figure 3.6 Changes in leaf weight between shoot and stem for 'Miyabi Fuji' on semi-vigorous Ma on May 28, 2020. Means ± standard error; different letters indicate statistically significant differences among the rootstocks according to a T-test P ≤ 0.05, (n=3).

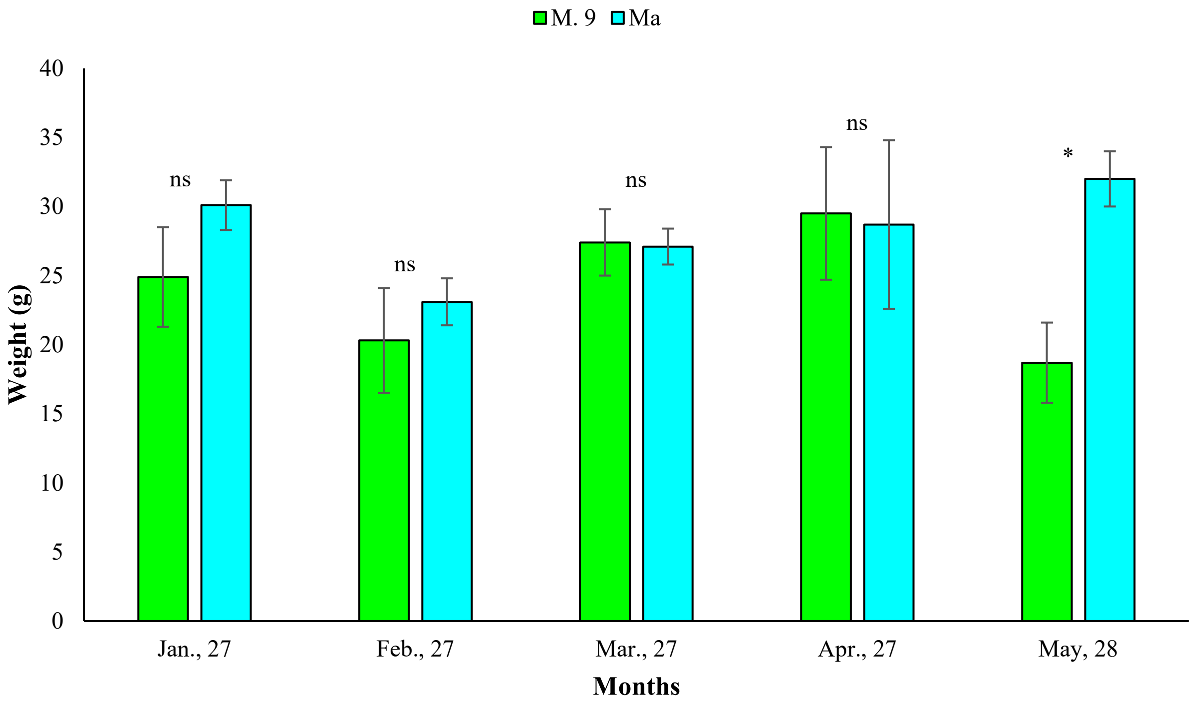


Figure 3.7 Changes in root fresh weight from January through May for 'Miyabi Fuji' in 2020, on semi vigorous Ma (Marubakaido) and dwarfing M.9. Means ± standard error; different letters indicate statistically significant differences among the rootstocks according to a T-test $P \leq 0.05$, ns-no significance (n=3).

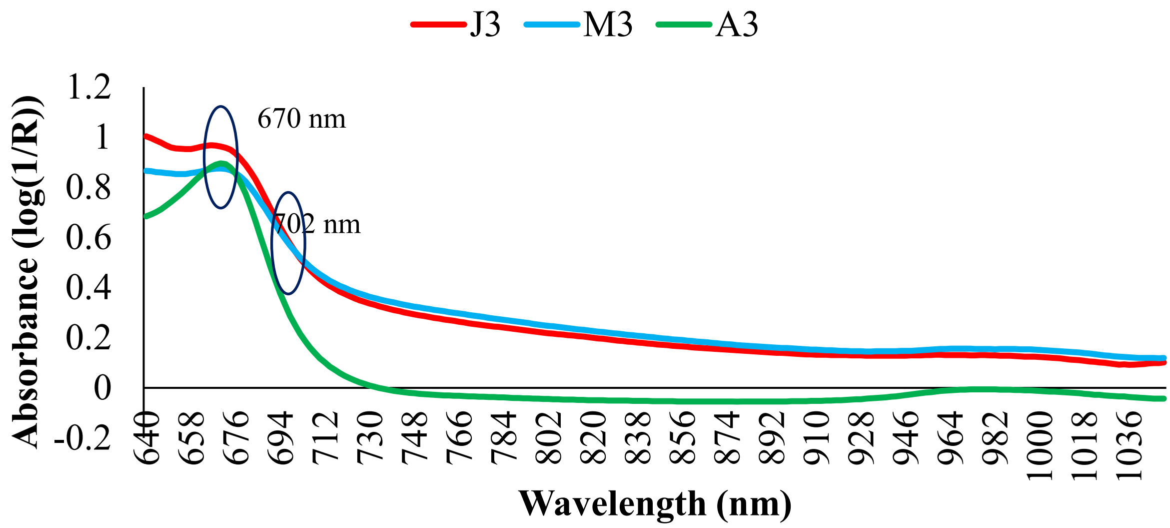


Figure 3.8 Changes in bud light absorbance on a visible/near-infrared spectrometer from January through March and April for 'Miyabi Fuji' on Ma in 2020, J3-January, M3-March, and A3-April from the buds on the top third of the trunk.

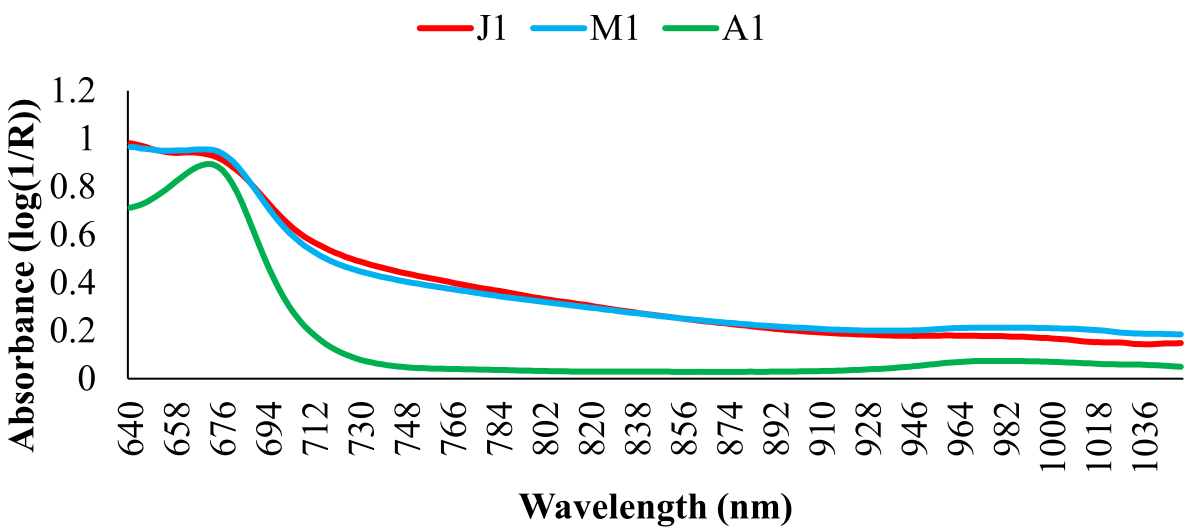


Figure 3.9 Changes in bud light absorbance, measured by the visible/near-infrared spectrometer, from January through March and April for 'Miyabi Fuji' on M.9 rootstock on 2020, J1-January, M1-March, A1-April (1-from the first bud at the top of the trunk).

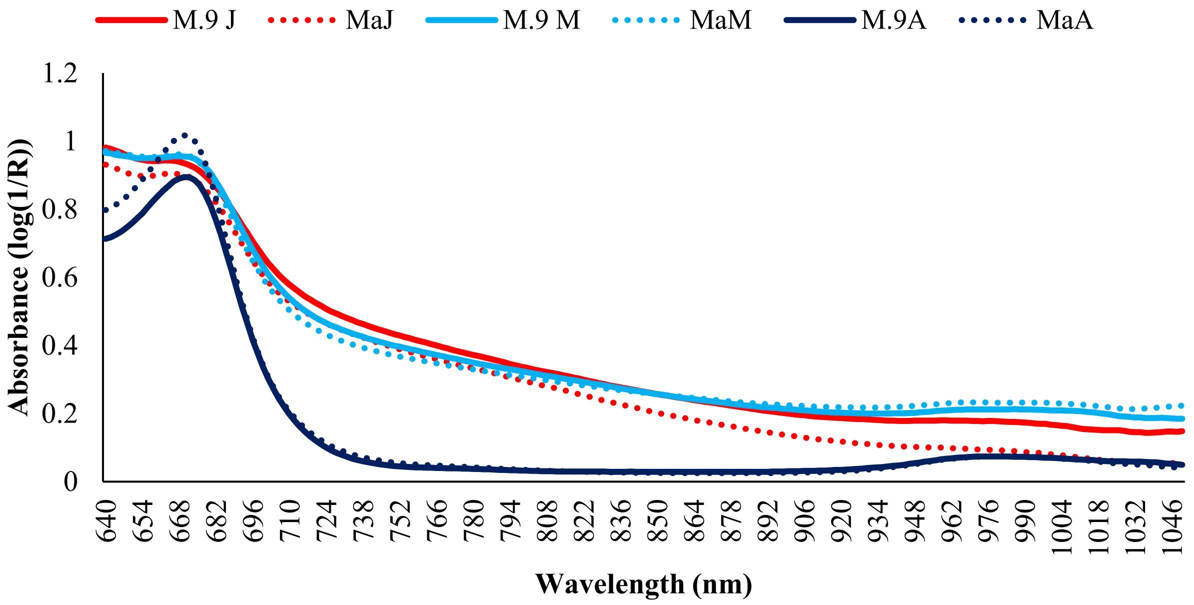
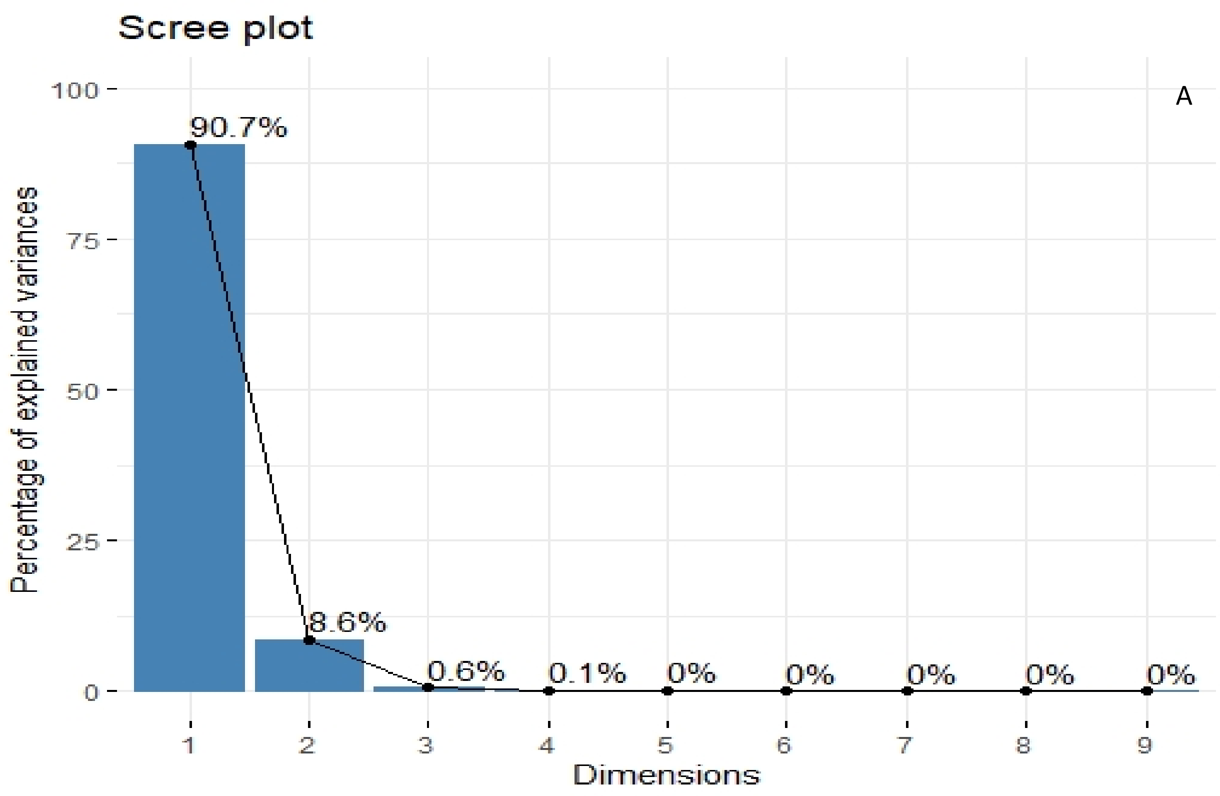


Figure 3.10 Light absorbance of the buds at the top of the trunk as measured by the visible/near-infrared spectrometer for 'Miyabi Fuji' grafted onto the semi-vigorous Ma (Marubakaido) and dwarfing M.9 rootstocks, January through April 2020. J=January, M=March, A=April.



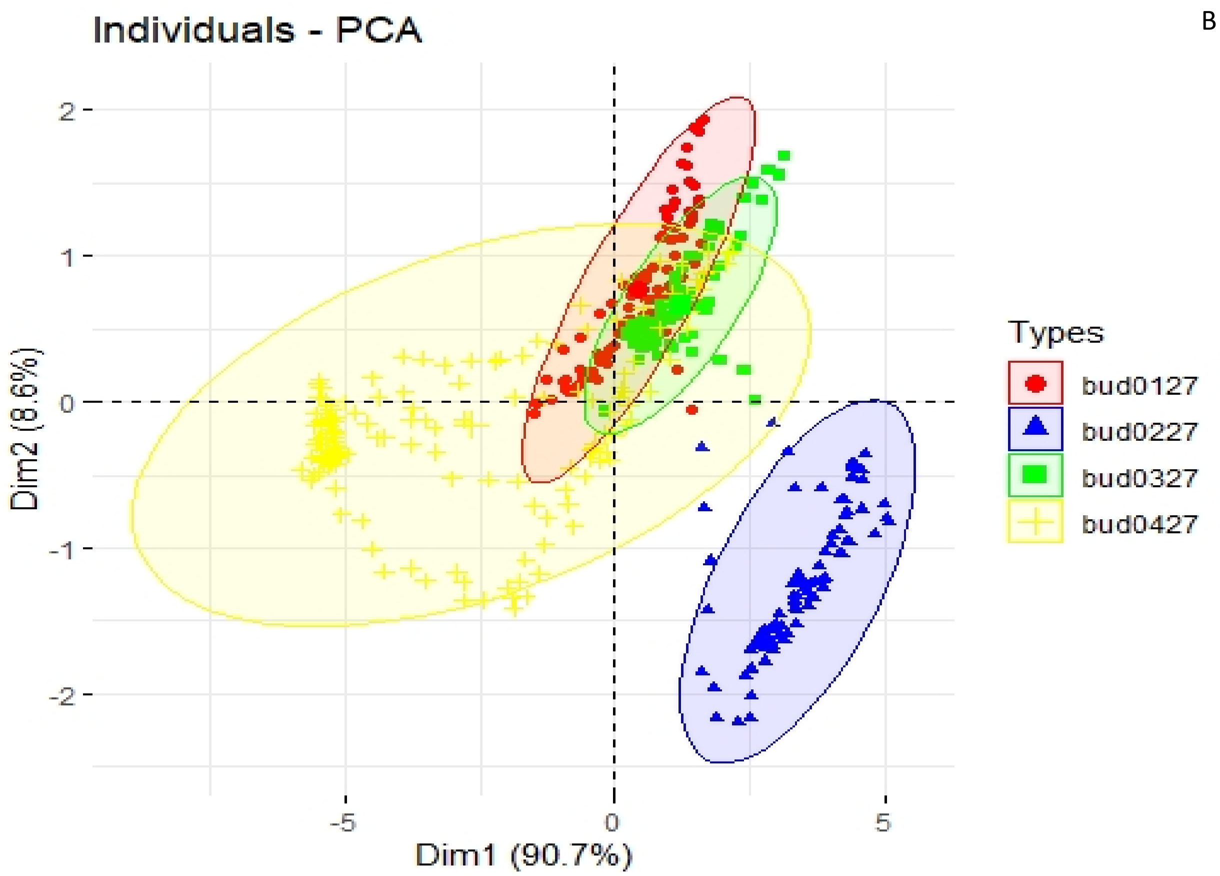


Figure 3.11 PCA of buds through January-April for 'Miyabi Fuji' on M.9 rootstocks, 2020; A=percentage of variance, B=Individual plot of PCA.

CHAPTER 4

NON-DESTRUCTIVE WAYS OF DETECTING BUD GROWTH ON YOUNG APPLE TREES AFTER PLANTING

4.1 INTRODUCTION

In young apple orchards, the determining factors in their future productivity are the healthy growth of young apple trees, the number of fruit branches, and the accurate construction of the tree to establish where the fruit will appear future fruit location. It is also essential to know the exact physiology of the buds before effective orchard management activities can be carried out. Knowing the bud's physiology and correctly evaluating it could help the grower identify the shoot or leaf that will be formed from this bud at an earlier stage of development and enable him or her to carry out fieldwork in a timely way. It is not easy to distinguish the “growing buds” that continue to develop into fruit from the “non-growing buds” that remain dormant. In addition, physiological changes in the buds also depend on the apple variety and climactic conditions. In this study, the bud light absorbance of ‘Jonagold’, ‘Miyabi Fuji’ and ‘Orin’ varieties were tested and analyzed.

‘Orin’ is a hybrid of the ‘Golden Delicious’ and ‘Indo’ (apple cultivars). It has a yellow-green appearance, a pleasant taste, and a unique aroma.” (Yang et al., 2021). ‘Jonagold’ is a ‘Golden Delicious’ and ‘Jonathan’ cross that was carried out in 1943 at the New York State Agricultural Experiment Field breeding program in Geneva (New York State), and was introduced in 1968 (Ferre, 2003).

Kramer and Kozlowski, (1960) found that, “A bud is an embryonic axis with its appendages. Height growth results from the activity of apical meristems or growing point.” One of the most practical ways to examine bud physiology is to utilize modern technologies that do so in a non-destructive way. Fortunately, intelligent agriculture technologies now make it possible to identify bud characteristics before pruning without destroying the bud in the process. However, no research has been reported on detecting shoot and non-shoot buds using non-destructive measurement methods. A visible/near-infrared spectrometer is one such non-invasive technological tool. It is relatively easy to use in the field and is also very efficient timewise. Therefore, the experiments that follow used a visible/near-infrared spectrometer to identify and enable the distinguish shoot from non-shoot buds.

4.2 MATERIALS AND METHODS

4.2.1 Plant materials

Shoot and leaf buds from three young 'Miyabi Fuji', 'Jonagold' and 'Orin' apple trees, grafted onto semi-vigorous 'Marubakaido' (*Malus prunifolia* 'Ringo') rootstocks, were used in this study. The three trees, one of each cultivar, were planted on April 30, 2021. Before planting, all saplings were scaled to the same size by cutting them to a length of 70 cm; roots were cut back to 10 cm. The young apple trees were then placed in 11 L black plastic nursery pots. All trees were purchased from "HARADA NURSERY" Co, Ltd. The experiments were conducted on the campus of the Hirosaki University, Faculty of Agriculture and Life Science. The 'Jonagold' and "Orin' buds were tested on April 30 through May 6 and the buds on the 'Miyabi Fuji" were tested on April 30 through May 8 (the 'Jonagold' and 'Orin' bud burst was observed May 6, 2021 and for 'Miyabi Fuji' it was May 8 2021). Buds used for testing on the respective testing dates and it was implemented on directly the trunks of each apple trees varieties. On each of these dates, the buds were examined with an ultra-mini visible near-infrared spectrometer.

4.2.2 Non-destructive measurement

The visible near-infrared spectrometer is a device that measures the amount of light that passes through an object without destroying it, where some wavelengths are transmitted and others are absorbed. The OMT-NIR-M1 spectrometer used in this study was manufactured by Optcom Co., Ltd. using SpectralRatio Version 1.1.0.1 software. This spectrometer measures from a range of 640 nm to 1050 nm, with an interval of 2 nm. Measurement parameters were adjusted to amp gain-high, to memory integration-16, and to smoothing points-16 nm. All buds were measured with this spectrometer and the spectral data were collected. The spectral data was then used to identify and distinguish shoot buds from leaf buds before bud burst. Which bud growth from the bud or which bud continued during the dormancy period was fully known 18 days after bud burst.

4.2.3 Statistical analysis

The bud Spectro data were analyzed using the R studio version 1.3.1073 (© 2009-2020 RStudio, PBC). Spectro data were analyzed individually for the dates on which the measurements were taken, and collected every data was analyzed using principal component analysis.

4.3 RESULTS

The light absorbance levels of the leaf buds, as shown on the visible/near-infrared spectrometer, were higher than those of the shoot buds from five days before bud burst until one day before bud burst for all three cultivars tested: ‘Jonagold’ (Fig. 4.1 A), ‘Miyabi Fuji’ (Fig 4.5 B), and ‘Orin’ (Fig. 4.7 C).

The ‘Orin’ leaf and shoot bud light absorbance levels were higher than those of the ‘Jonagold’ and ‘Miyabi Fuji’. The light absorbance of the leaf and shoot buds are shown at five and three days as well as one day before bud burst.

A principal component analysis (PCA) of the leaf and shoot bud light absorbance seen on with the visible/near-infrared spectrometer showed 85.6% of accuracy for Dimension 1 and 12.3% on Dimension 2 for the ‘Miyabi Fuji’ tree five days before bud burst (Fig. 4.4 B, C). Three days before bud burst Dimension 1 was 87.5% and Dimension 2 10.2% (Fig.4.5 B, C). One day before bud burst, Dimension 1 was 86.8% and Dimension 2 was 11.5% (Fig. 4.6 B, C).

The PCA of the leaf and shoot bud light absorbance shown 82.6% of accuracy for Dimension 1 and 14.9% for Dimension 2 for the ‘Jonagold’ tree (Fig. 4.1 B, C) five days before bud burst. Three days before bud burst the PCA of the leaf and shoot bud Dimension 1 seen 83% of accuracy and Dimension 2 accuracy of 14.8% (Fig. 4.2 B, C). One day before bud burst 82.5 % of accuracy for Dimension 1 and 15.3% for Dimension 2 in (Fig. 4.3 B, C).

The PCA of the leaf and shoot bud light absorbance seen 78.3% of accuracy for Dimension 1 and 12.4% for Dimension 2 for the ‘Orin’ apple tree (Fig. 4.7 B and C) in five days before bud burst. Three days before bud burst the PCA of the leaf and shoot bud Dimension 1 shown 83% of accuracy and 11.2% for Dimension 2 (Fig. 4.8 B, C). The leaf and shoot bud PCA seen 80% for Dimension 1 and 11.3% for Dimension 2 in one days before bud burst (Fi. 4.9 B, C).

4. 4 DISCUSSION AND CONCLUSIONS

The precision of observations can be improved, initially, by detailing the internal development of buds before bud burst, and likewise, by considering the entire annual cycle of trees as a single continuous process, where bud formation, stress induction, and bud vigilance spring issues identified to affect the timing (Viherä-Aarnio et al., 2014). In this research study shoot and leaf bud light absorbance were tested using a visible/near-infrared spectrometer. The leaf bud light absorbance was higher than that shoot bud light absorbance for all measured dates and cultivars. Additionally, there was little change in the leaf bud light absorbance, although shoot bud light absorbance decreased near bud burst for both ‘Miyabi Fuji’ and ‘Jonagold,’ although no changes were observed for ‘Orin’. The spectrometric data for the growing buds before bud burst were much lower than the spectrometric data for the non-growing buds. The highest first factor effect (87.5%) was determined by a PCA test that was conducted on growing and non-growing ‘Miyabi Fuji’ buds three days before bud burst, whereas the lowest (78.3%) was observed for 'Orin' buds five days before bud burst. 640, 650, and 700 nm were determined by PCA testing to be significant wavelengths before bud burst for all three-cultivar studied, while 950 and 1050 nm wavelengths were also significant for the ‘Jonagold’ variety (changes in the above-mentioned 950 and 1050 nm of wavelength applied only for ‘Jonagold’ and only three days before bud burst).

In conclusion, it is suggested that a visible/near-infrared spectrometer could be used to distinguish shoot buds from non-shoot buds before bud burst.

4.5 SUMMARY

Being able to ascertain the physiological condition of the buds on a young tree before bud burst could help farmers manage their orchards more efficiently, especially if they could do so without destroying the buds in the process. The experiments carried out in this study were conducted with the aim of distinguishing growing from non-growing buds before bud burst using a visible/near-infrared spectrometer, a device that does not destroy the buds being tested. Tests on spring-planted (April 30, 2021) trees were conducted to check growing and non-growing bud physiology and the winter dormancy of young apple trees (‘Jonagold’, ‘Miyabi Fuji’ and ‘Orin’). The spectrometric data for the growing buds before bud burst were much lower than the spectrometric data for the non-growing buds. The highest first factor effect (87.5%) was determined by a PCA test conducted on growing and non-growing ‘Miyabi Fuji’ buds three days before bud burst, whereas the lowest (78.3%) was observed for 'Orin' buds five days before bud burst. 640, 650, and 700 nm were determined by PCA testing to be significant wavelengths before bud burst for all three varieties studied, while 950 and 1050 nm wavelengths were also significant for the ‘Jonagold’ variety (changes in the above-mentioned 950 and 1050 nm of wavelength applied only for ‘Jonagold’ and only three days before bud burst). These findings suggest that growers can more effectively manage the development of the young trees in their orchards their orchards with a visible/near-infrared spectrometer.

Keywords: Forecasting, **s**hoot bud, leaf bud, visible/near-infrared spectrometer.

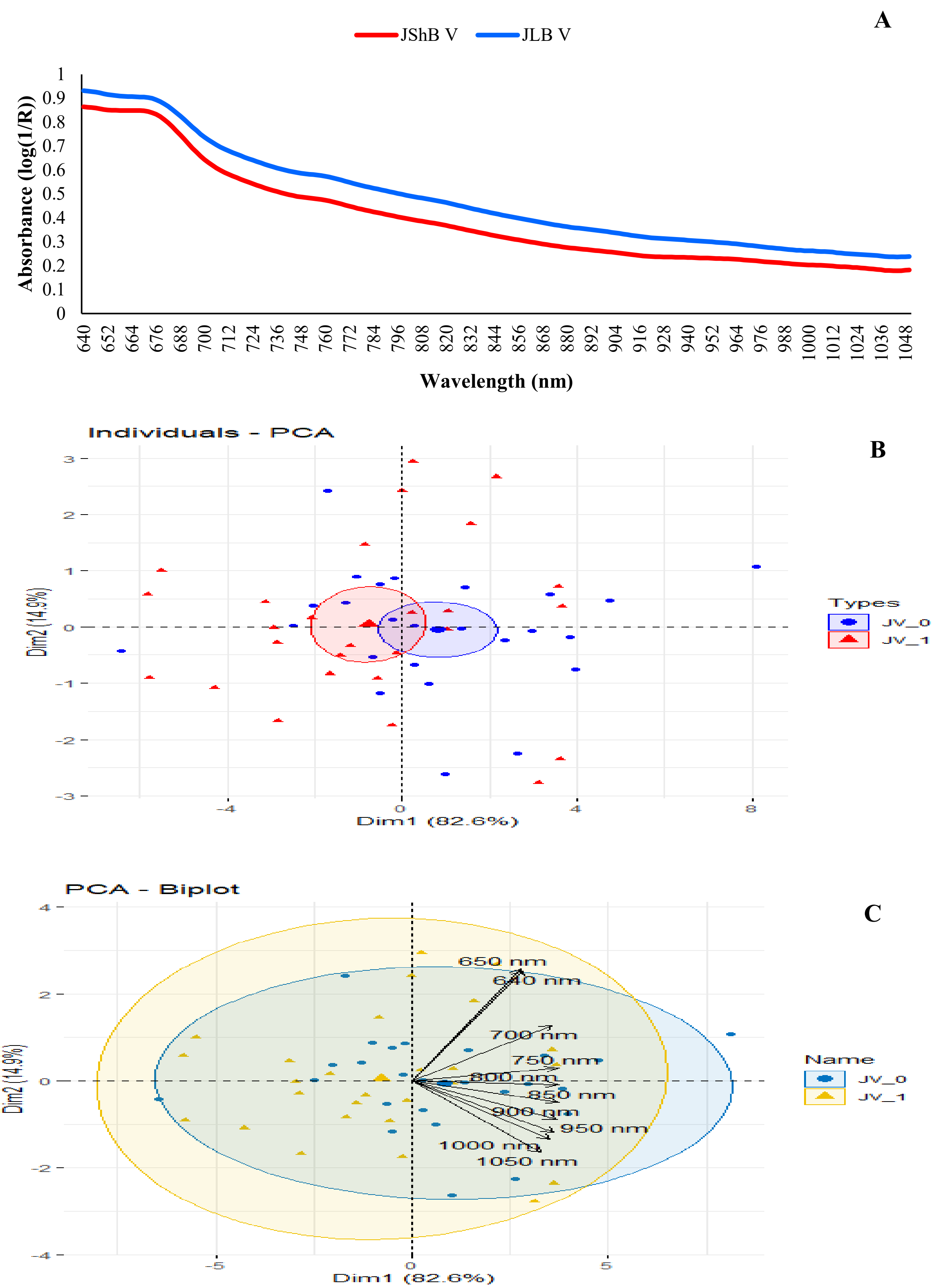


Figure 4.1 Shoot and non-shoot bud light absorbance shown on the visible/near-infrared spectrometer and the PCA analysis for 'Jonagold' (J) five days before bud burst (V), A-raw data; B-Individuals PCA; C-Biplot PCA, JShB-'Jonagold' shoot bud; JLB-'Jonagold' leaf bud.

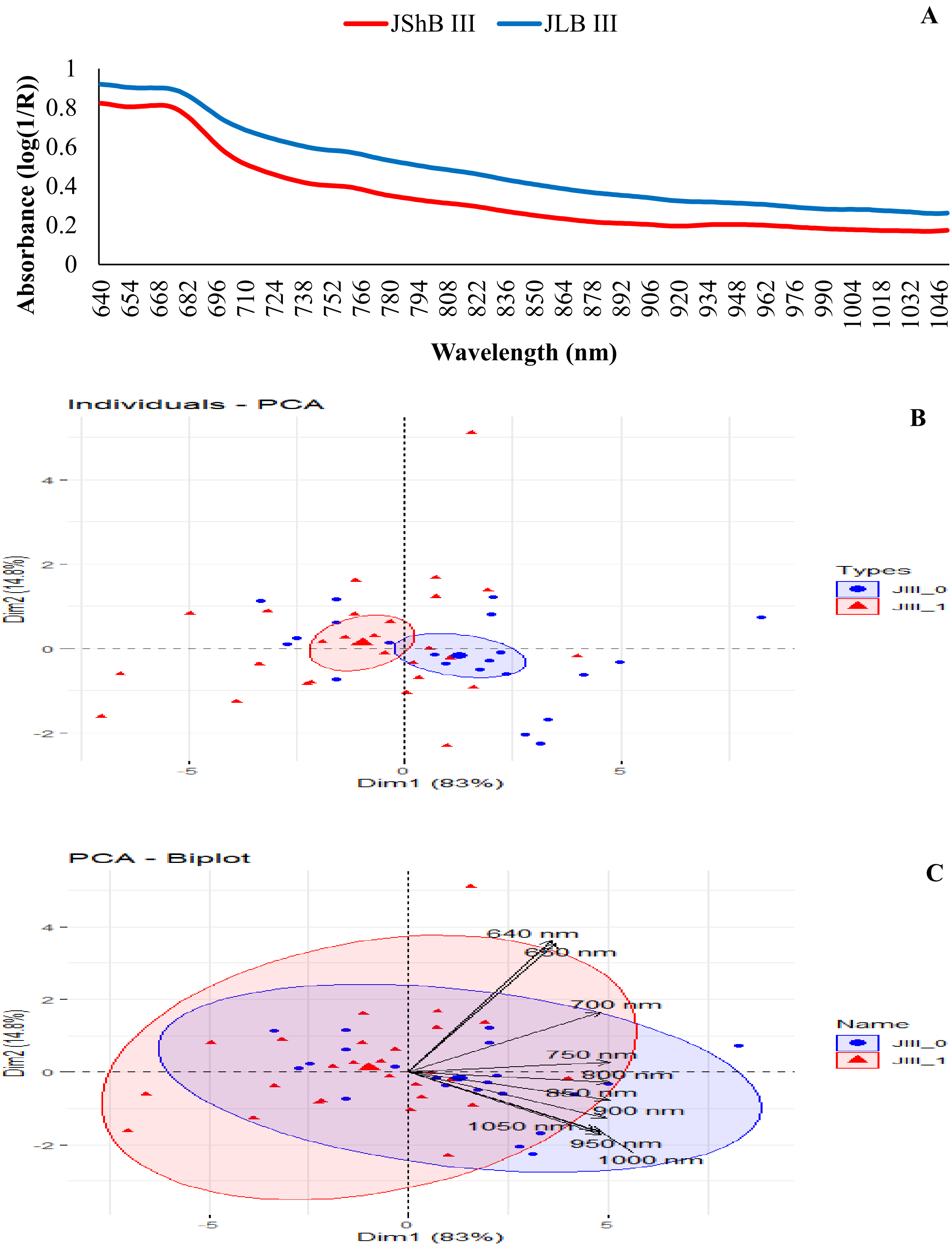


Figure 4.2 Shoot and non-shoot bud light absorbance seen on the visible/near-infrared spectrometer and PCA analyze for 'Jonagold' (J) in three days before bud burst (III), A-raw data; B-Individuals PCA; C-Biplot PCA, JShB-'Jonagold' shoot bud; JLB-'Jonagold' leaf bud.

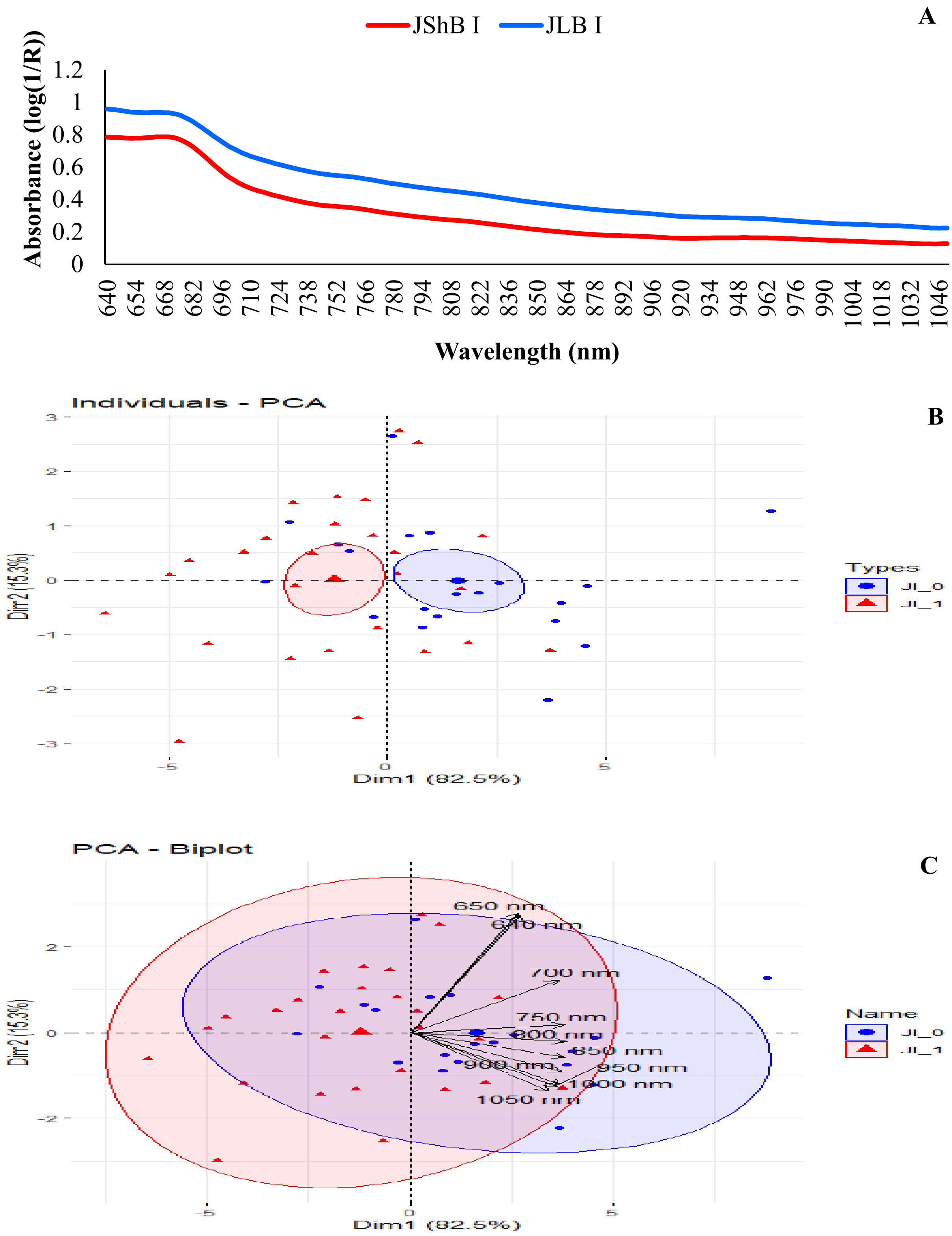


Figure 4.3 Shoot and non-shoot bud light absorbance shown on the visible/near-infrared spectrometer and PCA analyze for 'Jonagold' (J) in one day before bud burst (I), A-raw data; B-Individuals PCA; C-Biplot PCA, JShB-'Jonagold' shoot bud; JLB-'Jonagold' leaf bud.

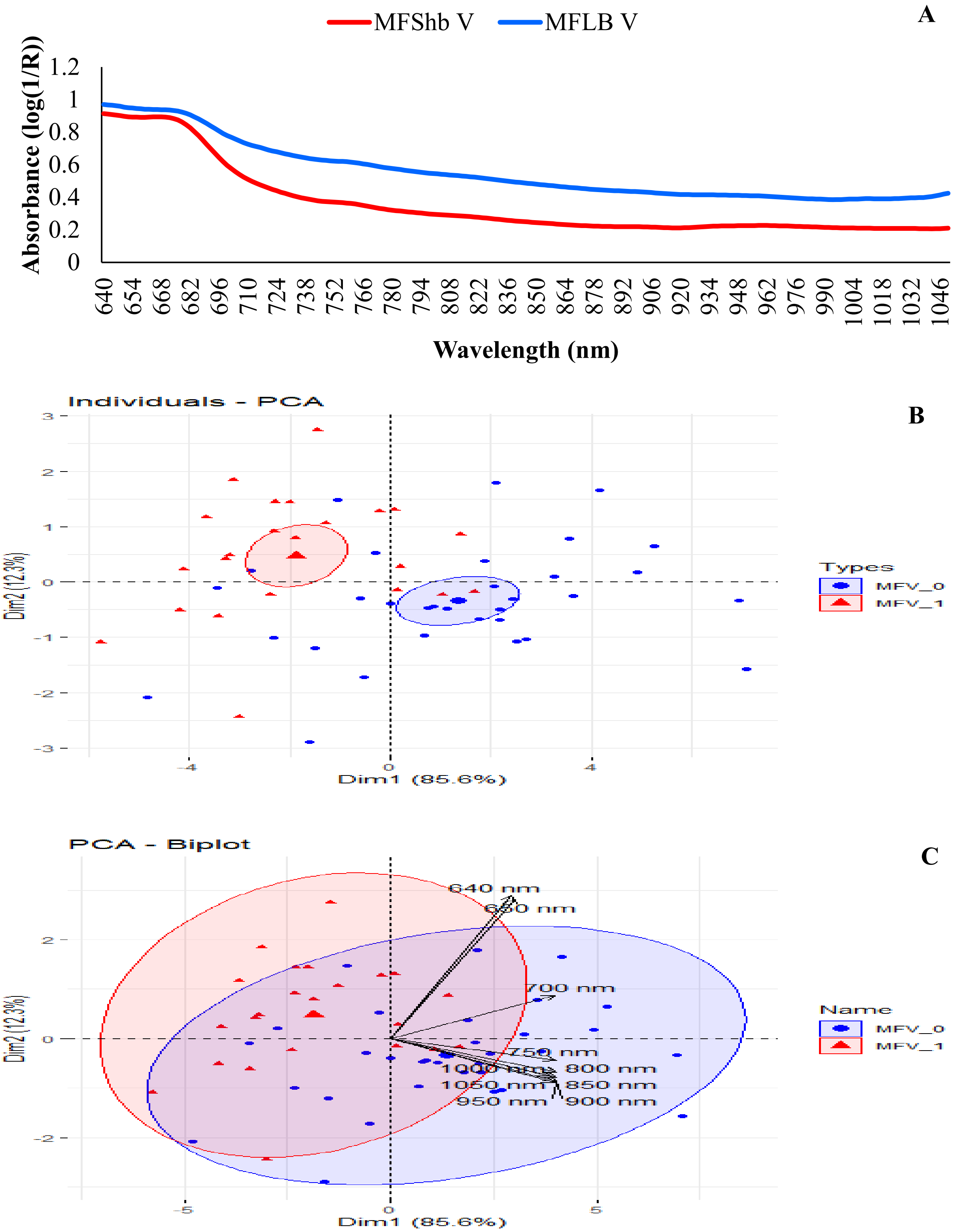


Figure 4.4 Shoot and non-shoot bud light absorbance seen on the visible/near-infrared spectrometer and PCA analyze for 'Miyabi Fuji' (MF) in five days before bud burst (V), A-raw data; B-Individuals PCA; C-Biplot PCA, MFShB-'Miyabi Fuji' shoot bud; MFLB-'Miyabi Fuji' leaf bud.

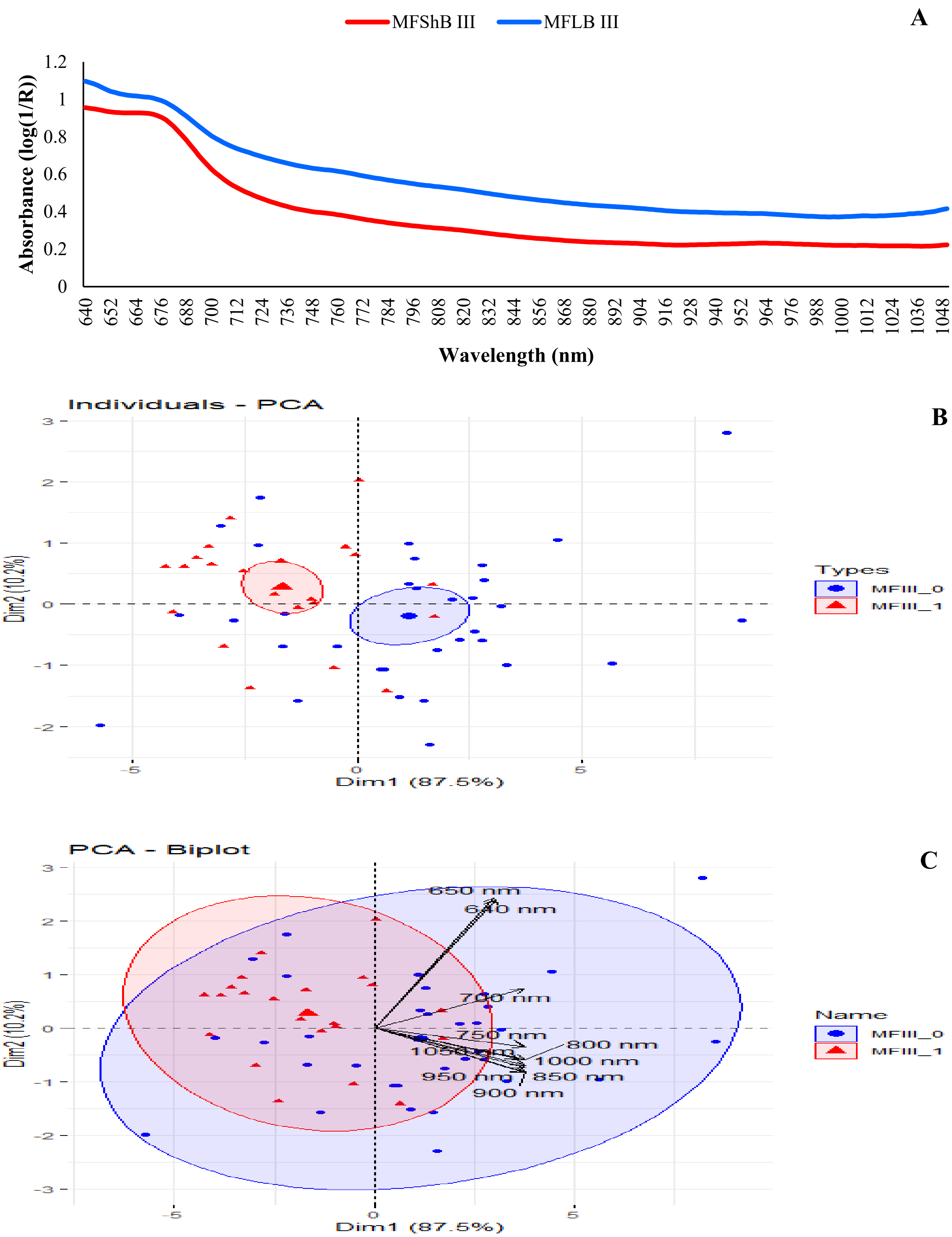


Figure 4.5 Shoot and non-shoot bud light absorbance seen on visible/near-infrared spectrometer and PCA analyze for 'Miyabi Fuji' (MF) in three days before bud burst (III), A-raw data; B-Individuals PCA; C-Biplot PCA, MFShB-'Miyabi Fuji' shoot bud; MFLB-'Miyabi Fuji' leaf bud.

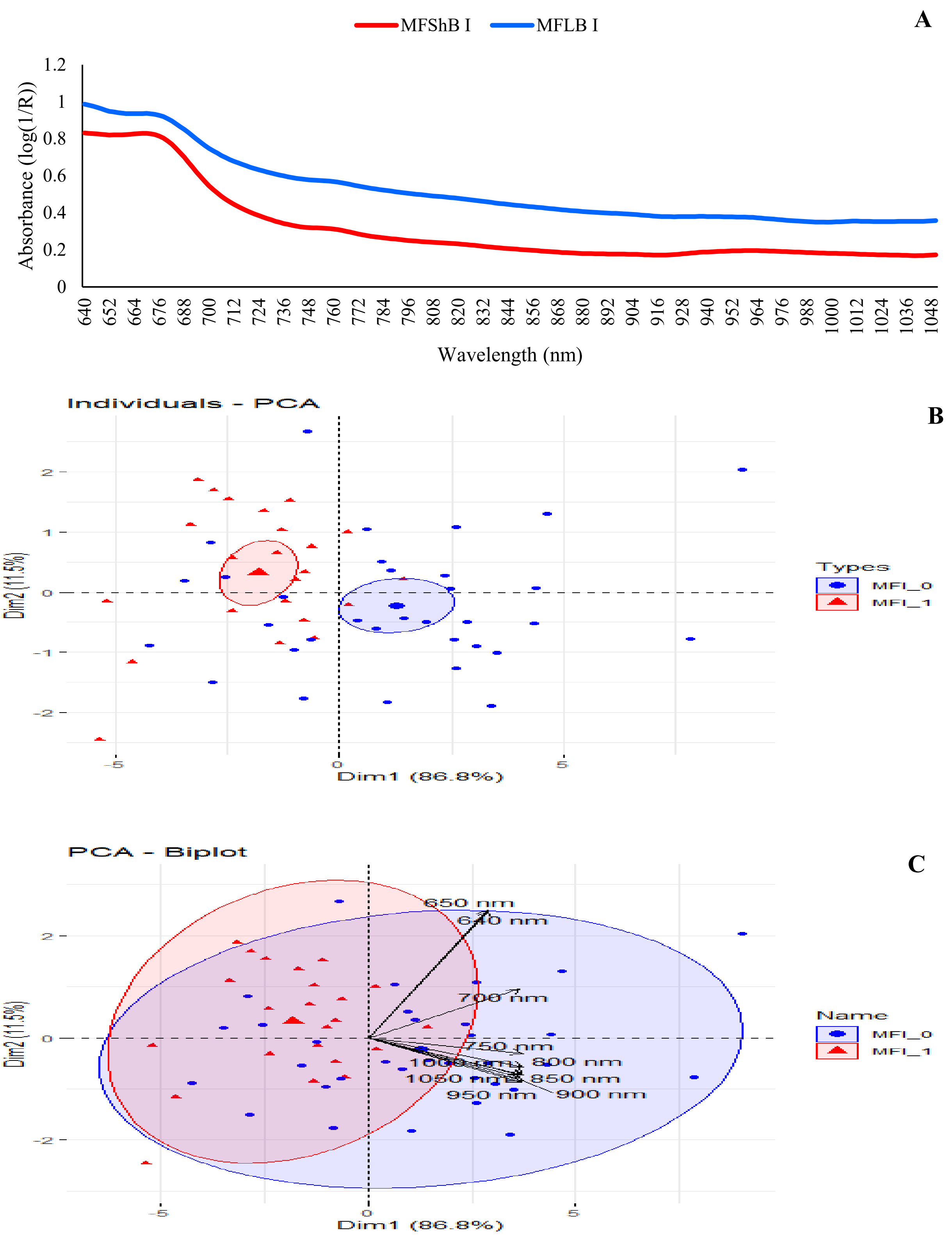


Figure 4.6 Shoot and non-shoot bud light absorbance seen on visible/near-infrared spectrometer and PCA analyze for 'Miyabi Fuji' (MF) in one days before bud burst (I), A-raw data; B-Individuals PCA; C-Biplot PCA, MFShB-'Miyabi Fuji' shoot bud; MFLB-'Miyabi Fuji' leaf bud.

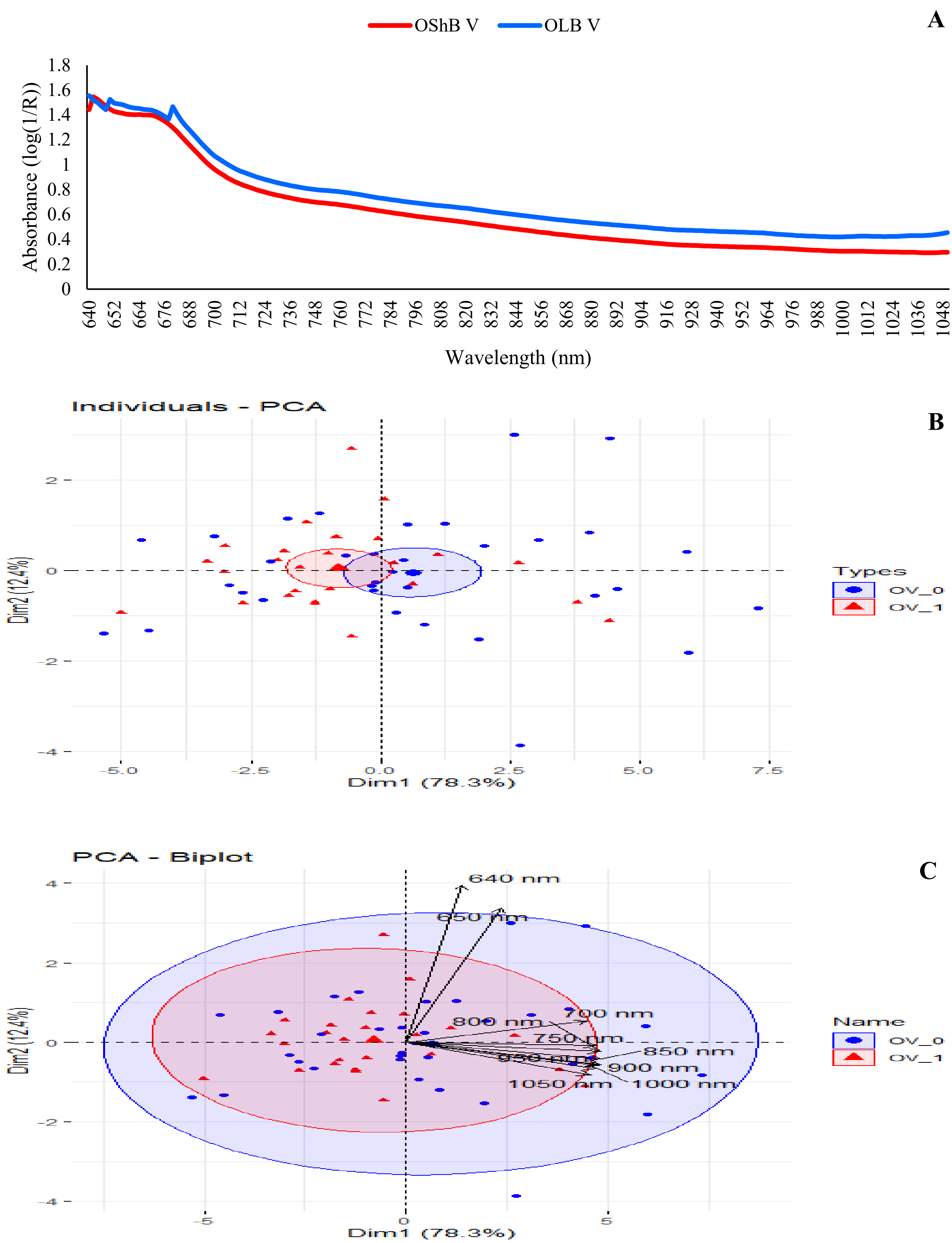


Figure 4.7 Shoot and non-shoot bud light absorbance seen on the visible/near-infrared spectrometer and PCA analyze for 'Orin' (O) in five days before bud burst (V), A-raw data; B-Individuals PCA; C-Biplot PCA, OShB-'Orin' shoot bud; OLB-'Orin' leaf bud.

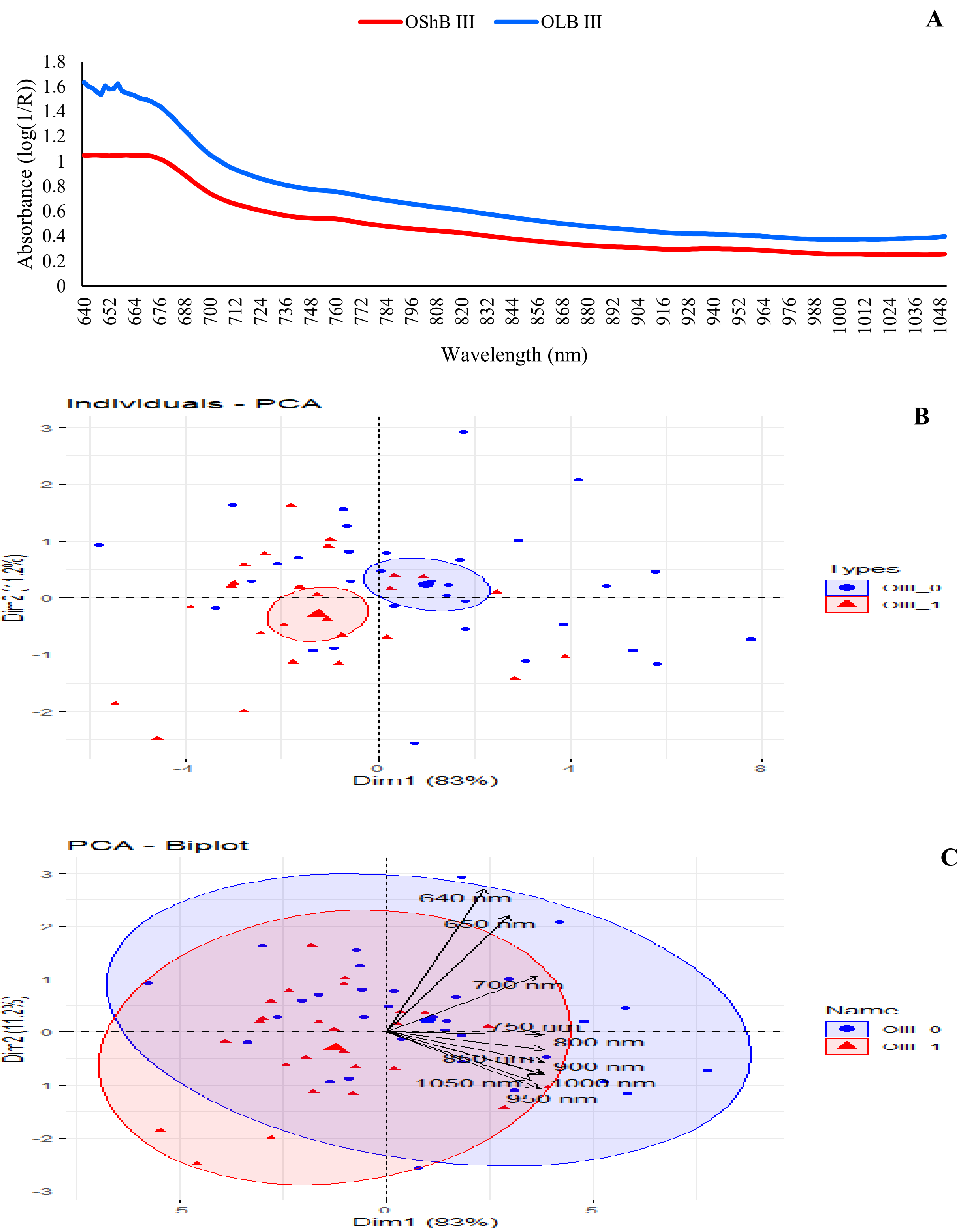


Figure 4.8 Shoot and non-shoot bud light absorbance seen on visible/near-infrared spectrometer and PCA analyze for 'Orin' (O) in three days before bud burst (III), A-raw data; B-Individuals PCA; C-Biplot PCA, OShB-'Orin' shoot bud; OLB-'Orin' leaf bud.

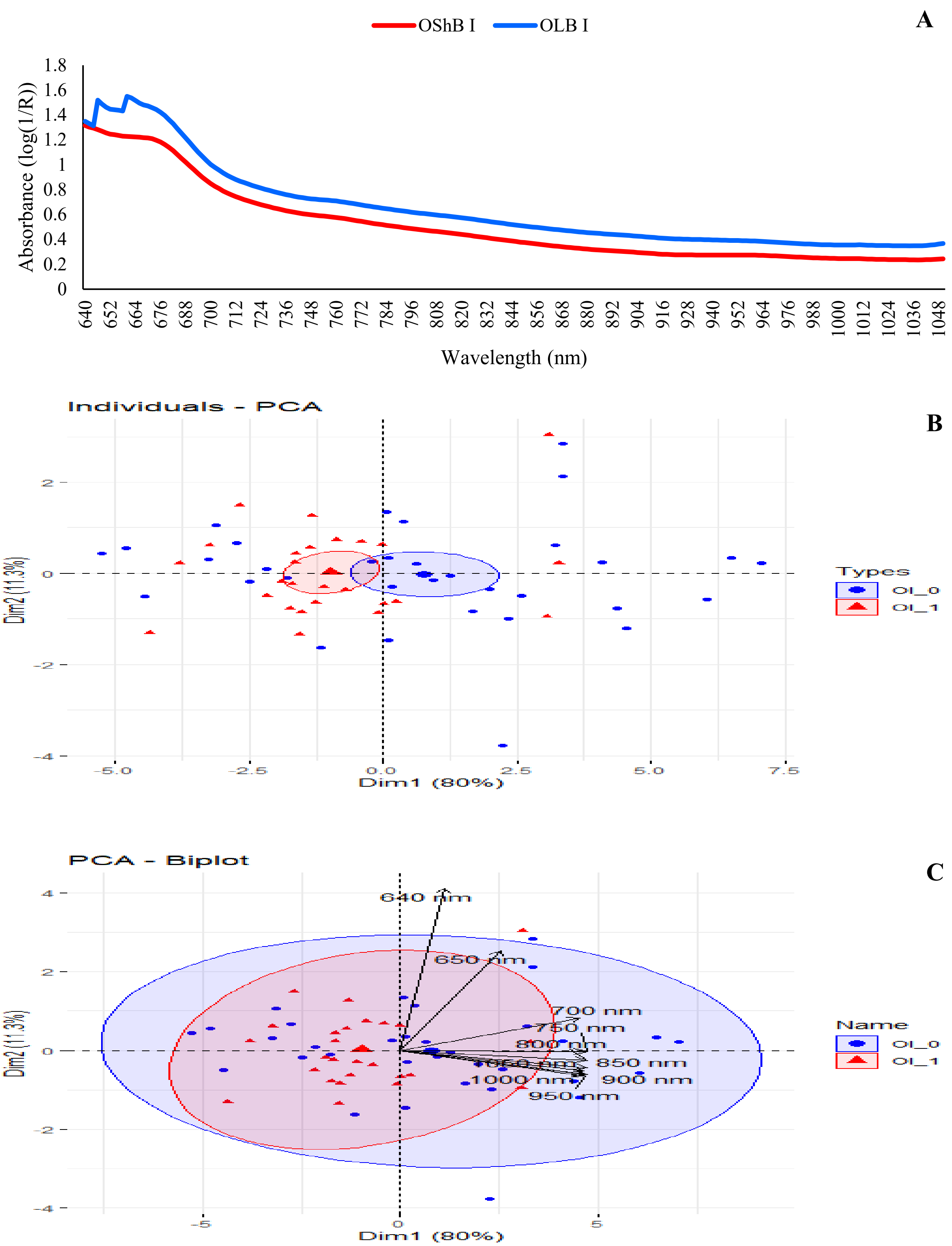


Figure 4.9 Shoot and non-shoot bud light absorbance seen on visible/near-infrared spectrometer and PCA analyze for 'Orin' (O) in one days before bud burst (I), A-raw data; B-Individuals PCA; C-Biplot PCA, OShB-'Orin' shoot bud; OLB-'Orin' leaf bud.

CHAPTER 5
APPLICATION OF A VISIBLE/NEAR-INFRARED SPECTROMETER IN IDENTIFYING FLOWER AND NON-FLOWER BUDS ON 'FUJI' APPLE TREES

5.1 INTRODUCTION

The 'Fuji' apple (*Malus domestica* Borkh.), a cross between 'Ralls Janet' and 'Delicious', was introduced in Japan in 1962 (Soejima et al., 1998). This fine-grained apple, with its high sugar and low acid content, is juicy, firm and crisp and has a sweet, spicy flavor (Rojas-Grau et, al., 2006). Today, it is one of the world's most widely consumed apples and is cultivated in apple-producing regions across the globe.

On the other hand, the 'Fuji' poses a number of problems for growers. Due to its vigorous growth, it is necessary to prune aggressively in order to open up the canopy to control this growth. In the case of the 'Fuji,' it is crucial to distinguish between flower and non-flower buds when pruning, because if these buds are not identified and flower buds thinned, they will cause over-vigorous growth and lower productivity.

Chlorophyll is the pigment that gives a plant its green color and is a crucial component of a plant's physiology (Palta, 1990, Gitelson et al., 2003). Until now, the bud chlorophyll content of 'Fuji' apple buds has not been used to identify flower and non-flower buds. Moreover, no research has been reported on the detection of flower and non-flower buds using non-destructive measurement methods. Therefore, it was decided to check bud chlorophyll content and changes in chlorophyll levels, and to use a visible near-infrared spectrometer to identify and enable the separation of flower from non-flower buds.

In this study, the classification of flower and non-flower buds was determined by a visible and near-infrared of spectrometer and their related intervals that identifies chlorophyll content. However, although to identify changing levels of chlorophyll in the days leading up to bud burst can help determine which buds are flower buds and which are non-flower buds, and it also involves the destruction of the bud itself. On the other hand, it offers verification of and insights into non-destructive approaches.

Buban and Faust (1982) have reported that determining whether a bud is a flower bud or non-flower bud is crucial for ‘applied horticulture’ and the future productivity of a young orchard. Flower bud formation is a complicated process because it is affected by the tree’s spurs and long shoots, the character of the cultivar, as well as the age and strength of the tree (Charlotte, 1998). Moreover, apple tree flower bud growth emerges on different parts of the tree. However, smart agriculture technologies now make it possible to discern bud characteristics before pruning, without destroying the bud in the process. The device that can be used to determine hidden parts of examined objects is the visible near-infrared spectrometer. This spectrometer is easy to use and produces results quickly.

Non-destructive testing includes a broad range of techniques that are used in science and technology industries to evaluate a material, component, or a system's properties without causing damage. According to Crowley (2020), the visible region of the spectrum of electromagnetic radiation identified by a visible near-infrared spectrometer is typically considered to be made up of wavelengths ranging from 400 nm (violet light) to between 700 and 800 nm (red light). Manley and Baeten (2018) have noted that, “the essential origins of NIR spectroscopy include the production, reporting, and understanding of spectra resulting from the interaction of electromagnetic radiation with an object.” Osborne (2000) reported that “the infrared (IR) region comprises that part of the electromagnetic spectrum in the wavelength range between 780 and 100,000 nm and is divided into near-IR, mid-IR, and far-IR subregions; the NIR region covers the wavelength range from 780 to 2500 nm.” Therefore, in this study are used a visible near-infrared spectrometer to identify and enable the separation of flower from non-flower buds.

The aim of this research project was to detect flower and non-flower buds on 'Fuji' apple trees before bud burst without destroying the buds. To do so, in this Chapter 1) was analyzed buds before bud burst using an ultra-mini visible near-infrared spectrometer and 2) was measured the chlorophyll content before bud burst to explain what was visible on the spectrometer. Results showed that the most reliable spectrometer readings of flower and non-flower buds occurred three days before bud burst.

5.2 MATERIALS AND METHODS

5.2.1 Plant materials

Flower and non-flower buds from a 'Fuji' apple tree were used in this study. The 'Fuji' tree studied was in one of the orchards located on the grounds of the "Hirosaki University Fujisaki Research Station" (Fujisaki, Aomori Prefecture, Japan). The tree was ten years old and had been grafted onto semi-vigorous Marubakaido rootstock. The dates selected to test the buds were January 29, February 15, March 1, March 15, and March 31 (64, 47, 33, 19, and 3 days before bud burst), the latter being three days before the tree's 2021 bud burst (Table 5.1). Buds used for testing on the respective testing dates were taken from different branches on the same tree. On each of these dates a branch was cut off and brought to a laboratory in the Hirosaki University Faculty of Agriculture and Life Science. There, the buds were separated from the branch and examined with an ultra-mini visible near-infrared spectrometer and tested for chlorophyll content.

5.2.2 Non-destructive measurement

The visible near-infrared spectrometer is a device that measures the amount of light that passes through an object without destroying it. The OMT-NIR-M1 spectrometer used in this study was manufactured by Optcom Co., Ltd.. The software used in this study was the SpectralRatio Version 1.1.0.1 software. This spectrometer measures from a range of 640 nm to 1050 nm, with an interval of 2 nm. Measurement parameters were adjusted to amp gain-high, to memory integration-16, and to smoothing points-16 nm. All buds were measured with the spectrometer and the spectral data were collected. The buds were then examined under a microscope and the spectral data were used to determine whether a bud was a flower or a non-flower bud.

5.2.3 Grouping into flower and non-flower buds

The buds were weighed and sliced in two with a razor blade from the middle of the top through to the bottom of the bud and then checked with (Olympus) a microscope (Olympus Corporation Tokyo, Japan, made in the Philippines). In the upper part of the flower buds was a yellowish-green oval stamen, whereas in the upper part of the non-flower buds there was no such oval, though there was some light green matter (Fig. 5.1 A and B). The flower buds were then separated from the non-flower buds and their chlorophyll content was measured.

5.2.4 Measurement by destructive means

The flower and non-flower buds were classified by shape, weight, and also by chlorophyll content. Bud chlorophyll content is related to bud physiology and differs depending upon the type of bud. The separated buds were carefully placed inside 2 ml tubes and pulverized in a Homogenizer ShakeMan6 (model PS-SMNO6), after which the buds were infused for 10 minutes in a 1.5 ml 80% acetone solution inside 2 ml tubes. The mixtures were then moved to different 1.5 tubes, which were then put into a high-speed Micro Centrifuge and set at a speed of 140,000 (rpm) for 10 minutes. The liquid rose to the top of the tubes and debris from the buds settled at the bottom. The liquid solution at the top of the tubes was then moved into a quartz glass tube in order to measure the chlorophyll content with a UV spectrophotometer (Shimadzu Access Corporation). The UV spectrophotometer was set at three measuring ranges (645, 663, and 750 nm), and chlorophyll content was checked within these Spectral ranges and calculated using the following equation:

$$C = 7.22(A663\ \mathrm{nm} - \mathrm{A}750\ \mathrm{nm}) + 20.30(A645\ nm - A750\ nm)$$

(5.1)

5.2.5 Statistical analysis

Chlorophyll levels were analyzed using a one-way ANOVA (comparing the difference between dates) and a Tukey test. Flower and non-flower bud chlorophyll content differences were analyzed using the Student's t-test. All of the above analyses were conducted by applying the R studio version 1.3.1073 (© 2009-2020 RStudio, PBC). Different software was used to obtain Spectro data.

The bud Spectro data were analyzed using MATLAB R2018b version 9.5.0.1298439 (©1984-2018 the MathWorks, Inc), with the Classification Learner App tool. Spectro data were analyzed individually for the dates on which the measurements were taken, and each new session was set as cross-validation folds:

10 without PCA. Although all 22 machine-learning algorithms in the Classification Learner App were chosen, the results of only 9 of these are included in the table (Table 5.2). Accuracy, sensitivity, and specificity were verified by a confusion matrix plot.

5.3 RESULTS

5.3.1 Changes in bud chlorophyll content

Flower bud and non-flower bud chlorophyll levels exhibited significant differences three days before bud burst. Chlorophyll levels in the flower buds were significantly lower than those in the non-flower buds (Fig. 5.2). Flower bud chlorophyll levels showed dramatic differences depending upon the date. These levels increased significantly with the approach of bud burst.

5.3.2 640-798 nm range

The amount of light absorbed by the non-flower buds, seen on the visible spectrometer three days before bud burst, was higher than that of the flower buds (Fig. 5.3 A). Bud Spectro absorption dropped sharply from 670 nm to 720 nm.

5.3.3 800-1050 nm range

The absorption in the flower bud was lower than the Spectro absorption in the non-flower bud on the as shown on the near-infrared spectrometer three days before bud burst (Fig. 5.4). Flower and non-flower bud Spectro light highly absorbed on the 930 nm and lower absorbed on 1016 nm.

5.3.4 Spectro variation of buds on different dates

The flower and non-flower bud absorption seen on the visible spectrometer decreased near the approach of bud burst (Fig. 5.5). Non-flower bud absorption observed on the visible spectrometer 33 days before bud burst was lower than the flower bud absorption.

Absorption in the flower bud shown on the near-infrared spectrometer absorption three days before bud burst was lower than it was 33 days before bud burst (Fig. 5.6). On the other hand, absorption in the non-flower buds seen on the near-infrared spectrometer three days before bud burst was greater than it was 33 days before bud burst. Near-infrared spectrometer light absorption readings showed that that light absorption of the non-flower buds was greater than that of the flower buds, for both 33 and three days before bud burst.

5.3.5 Classification analyses of Spectro data

The Classification Learner App of the MATLAB was used as a classification model, and the 10-fold of cross-validation was used to set the training data for the model (Table 5.2). The classification results were obtained using the 22 machine learning algorithms; 9 of which are also shown in Table 5.2 The highest classification accuracy was 75.9%, performed by cubic k-nearest neighbor (KNN), accompanied by medium KNN with 72.4% accuracy, cosine KNN (72.4% accuracy), and weighted KNN (72.4% accuracy). The highest sensitivity was found by cubic KNN (86%), then medium KNN with 80% accuracy, cosine KNN 80% (accuracy) and weighted KNN (80% accuracy).

5.4 DISCUSSION AND CONCLUSIONS

In this study, the identification of flower and non-flower buds was determined by a visible near-infrared border that can identify their chlorophyll content. Until now, the bud chlorophyll content of 'Fuji' apple buds has been measured by examining the leaves and not the buds themselves. Our analysis of the buds showed that, three days before bud burst, the chlorophyll level of flower buds was significantly higher than that of non-flower buds However, no significant differences in chlorophyll levels were found between flower and non-flower buds when measured 33 days before bud burst.

This study introduced a non-destructive method of identifying the flower and non-flower buds of 'Fuji' apple trees. The results show that this method accurately classifies and distinguishes flower buds from non-flower buds near bud burst. This non-destructive method is an important way to ascertain chlorophyll content and the water index (Agati et al., 1995, Penuelas, et al., 1997). However, this non-destructive way of differentiating the flower from the non-flower buds of 'Fuji' apple trees has once not been examined. The results of this study showed that the absorption of light in the flower buds shown on the visible/near-infrared spectrometer just before bud burst had diminished, whereas that of the non-flower buds had risen. Classification Learner App testing methods confirmed that the classification and differentiation of flower from non-flower buds was 75.9% accurate when tested with Cubic k-nearest neighbor (k-NN). Vitola (2017) has reported that Cubic KNN is the simplest way to separate various data and obtain accurate results (Vitola, et al., 2017). According to Buban and Faust (1995), bud growth and development depend indirectly on the availability of "free water amounts of buds" (Bubán & Faust, 1995). Penuelas et al. (1997) reported that 680, 900, and 970 nm reflectance provide the best estimation of plant water content (Penuelas et al., 1997). Fruitlet drop measured by Vis-Nir in situ is beneficial in terms of time efficiency and its high level of accuracy (Orlova et al., 2020).

A deficiency of this study is that measurements were made only within a restricted wavelength range (640-1050 nm). Further research should be done using a more comprehensive range on the near-infrared spectrometer (above 1050 nm).

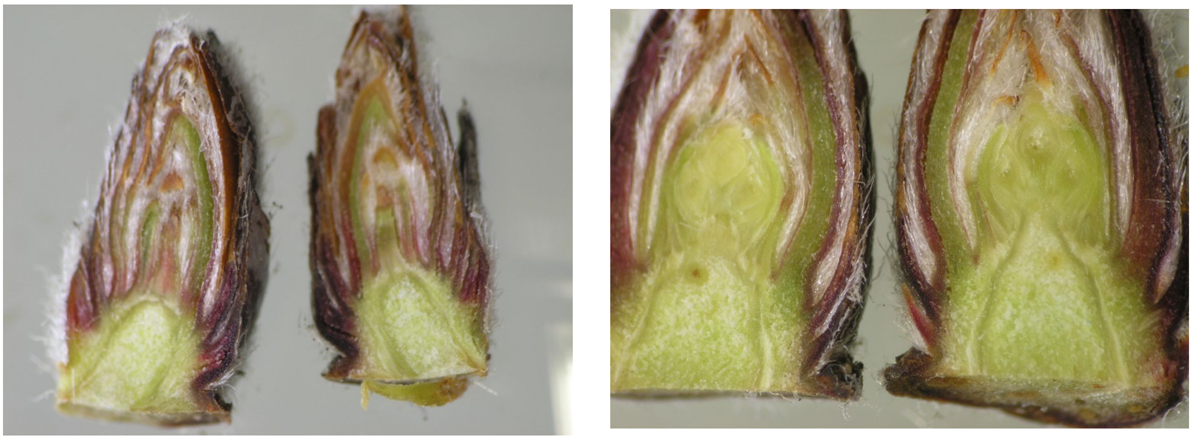
In this Chapter, an investigation of the non-destructive detection of flower and non-flower buds before bud burst on a ’Fuji’ apple tree was undertaken. Data analysis showed that the best time to detect and differentiate between flower and non-flower buds, utilizing a visible near-infrared spectrometer, is three days before bud burst. In addition, significant changes in bud chlorophyll in the flower and non-flower buds were observed. This suggests that deeper non-destructive measurements especially adapted for chlorophyl might be distinguish flower from non-flower buds before bud burst.

Hence, the use of a device that does not destroy the bud can be beneficial for detecting flower and non-flower buds before bud burst and can help growers in their management of ‘Fuji’ apple orchards. In addition, the ultra-mini visible near-infrared spectrometer could offer apple growers a tool that would enable them to distinguish flower from non-flower buds, which could then help them fine tune their pruning practices to better manage their orchards and forecast future harvest yields. Moreover, researchers working on smart agriculture technologies could use this data to develop pruning robots that can identify and separate flower from non-flower buds.

5.5 SUMMARY

Forecasting bud physiologic conditions can help 'Fuji' apple farmers manage their orchards more efficiently. Being able to determine the nature of a bud before bud burst is one such forecast that could be of use to these 'Fuji' growers. The aim of this research project was to determine if a device, a visible near-infrared spectrometer, could be employed to determine whether a bud is a flower or non-flower bud without destroying the bud. Experiments were conducted on buds taken from a 'Fuji' apple tree, beginning on January 29 through to March 31, 2021, three days before bud burst. The data from the NIR spectrometer clarified whether a bud was a flower or a non-flower bud. Three days before bud burst, the chlorophyll content levels of the non-flower buds were markedly higher ($P \leq 0.05$) than those of the flower bud, which explains why the visible border of the near-infrared spectrometer might have been changed by the chlorophyll content of the buds. The visible and near-infrared bands of the buds showed that the Spectro data of the non-flower buds were higher than those of the flower buds when measurements were made three days before bud burst. The Spectro data Classification Learner App proved to be an accurate classification method to analyze flower and non-flower bud Spectro data. Three days before bud burst Cubic KNN of KNN classifier analyzed flower and non-flower buds smoothly. Spectro data (accuracy 75.9%, sensitivity 86% and specificity 67%). The results that were obtained suggest that farmers could use a visible near-infrared spectrometer to identify flower and non-flower buds in their orchards, without damaging the buds, three days before bud burst.

Key words: Chlorophyl content, classifier learner app, flower and non-flower buds, visible near-infrared spectrometer.



(a) (b)

Figure 5.1 Difference between flower and non-flower buds, (a) 'Fuji' flower bud, (b) 'Fuji'- non-flower bud, January 29, 2021.

Table 5.1 Flower and non-flower buds: destructive and non-destructive testing and their distribution before bud burst in 2021.

Name	**Number of buds**			
	29-Jan	**1-Mar**	**15-Mar**	**31-Mar**
Flower	17	23	20	17
Non-flower	7	10	9	12
Total bud	**24**	**33**	**29**	**29**

Note: Bud burst occurred on April 2, 2021.

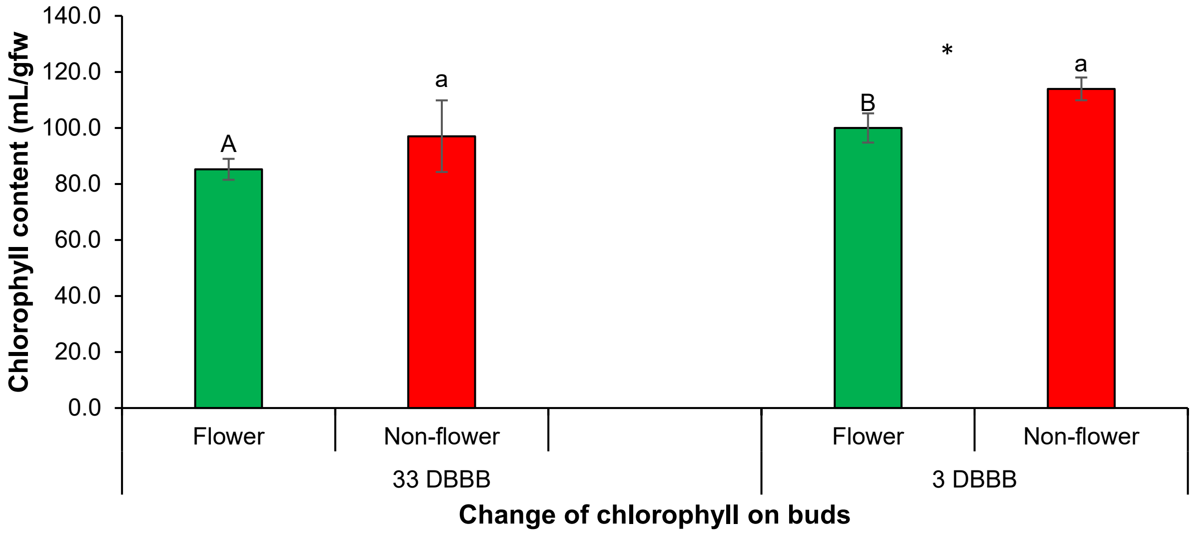


Figure 5.2 Changes in chlorophyll content: a comparison of flower and non-flower buds tested 33 and 3 days before bud burst (DBBB). Means ± standard error and different letters indicate statistically significant differences between the dates according to a T-test $P \leq 0.05$.

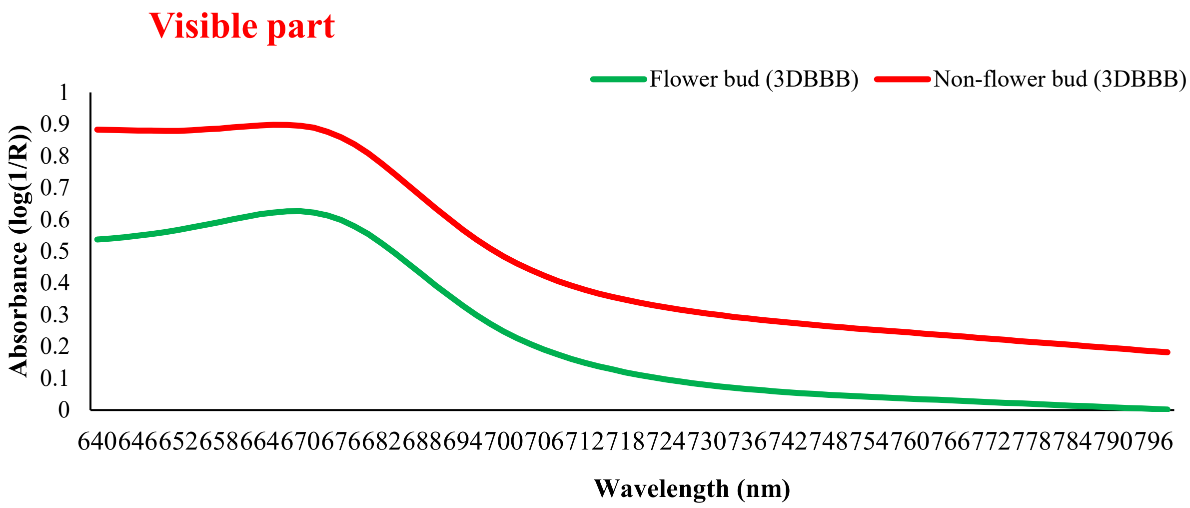


Figure 5.3 Flower and non-flower bud absorbance of the visible spectrometer for 'Fuji' on March 31, 2021; DBBB-days before bud burst g**reen line** = flower bud, **red line** = non-flower bud.

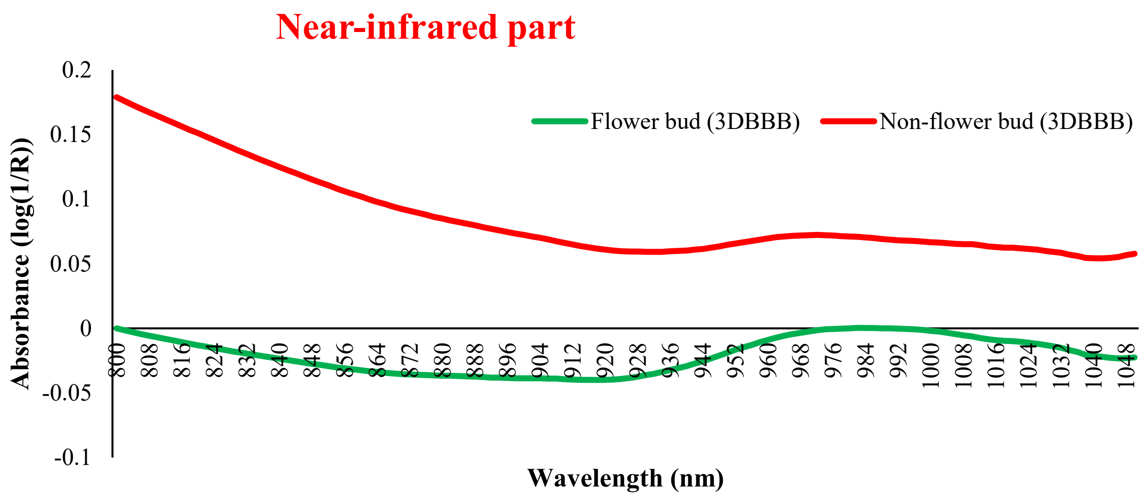


Figure 5.4 Flower and non-flower bud absorbance of the near-infrared spectrometer for 'Fuji' on March 31, 2021; DBBB-days before bud burst: **green line** = flower bud, **red line** = non-flower bud.

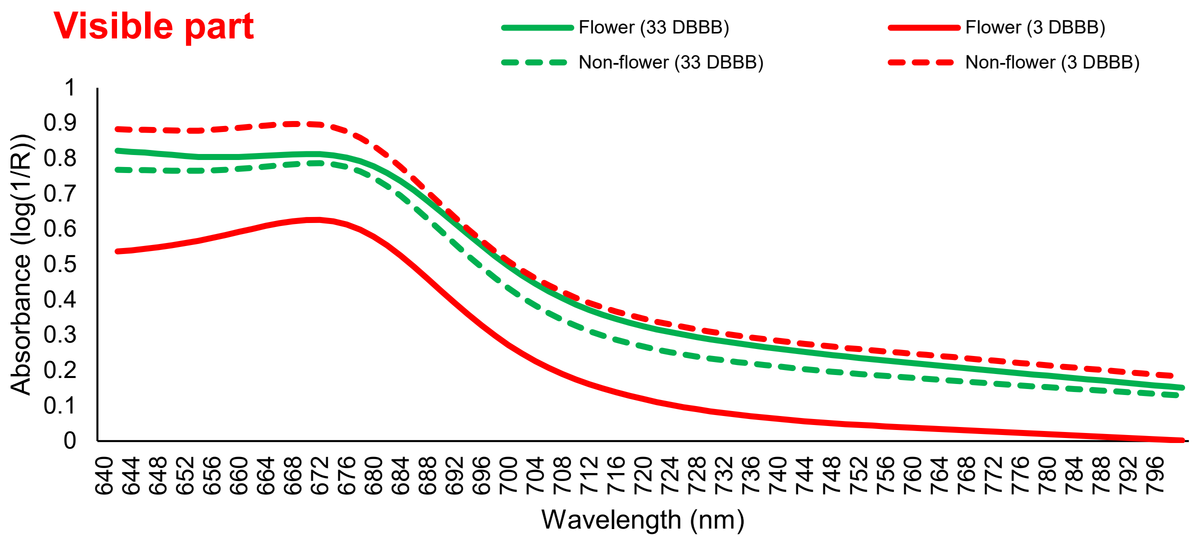


Figure 5.5 Flower and non-flower bud absorbance seen on the visible spectrometer for 'Fuji' on March 15 and March 31, 2021; 33 and 3 days before bud burst (DBBB), **Green and red lines** = flower buds, **green** and **red** ring lines = non-flower buds.

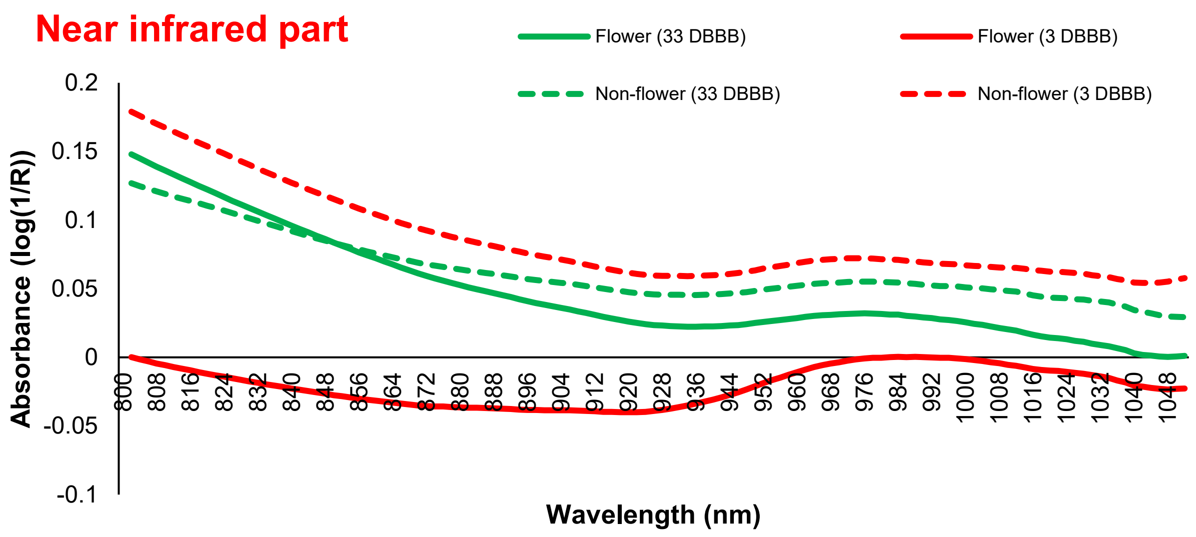


Figure 5.6 Flower and non-flower bud absorbance seen on the near-infrared spectrometer for 'Fuji' on March 15 and March 31, 2021; 33 and 3 days before bud burst (DBBB): **green and red lines** = flower buds, **green** and **red** ring lines = non-flower buds.

Table 5.2 Buds Spectro data classification results using the classification learner app without using the PCA three days before bud burst (DBBB) for the 'Fuji' flower and non-flower buds on the visible/near-infrared spectrometer on 2021.

Classifier	Classifier type	Classification accuracy (%) 2 DBBB		
		Accuracy	Sensitivity	Specificity
Discriminant Analysis	Linear Discriminant	62.1	65	56
	Quadratic Discriminant	F	F	F
Logistic Regression Classifier	Logistic Regression	58.6	65	50
KNN	Fine KNN	62.1	67	55
	Medium KNN	72.4	80	64
	Coarse KNN	58.6	59	0
	Cosine KNN	72.4	80	64
	Cubic KNN	75.9	86	67
	Weighted KNN	72.4	80	64

CHAPTER 6

GENERAL DISCUSSION

Promoting young apple tree growth in water-limited areas is crucial, and several factors were examined in this study, and those are 1) the role of rootstocks, soil water levels, and soil water retention substances; 2) the effects of winter planting and the efficient retention of winter soil moisture; 3) the identifying of bud growth before bud burst in a non-destructive way.

The main objective of this study was to study how rootstock differences, soil water level, and water retention substances in the soil, together or separately will help ensure the healthy growth of young apple trees in areas with limited water resources.

The several results were obtained: Young 'Miyabi Fuji' apple trees planted in the spring took root ten days after planting on both Ma and JM7, whereas the root growth of winter-planted trees started from the end of March (in Chapter 3). Trees on Ma with 70% water content combined with Hydretain ES Plus showed good root growth. Nevertheless, our conclusions do not recommend findings of Greenwell et al. (2017) on the impact of humectants on plant root parameters. In other research, Roberts and Linder (2010) said Hydretain ES was affected for longer days to wilt for forest trees and our findings verify that root fresh weight and root volume changes occurred in trees on Ma with 70% water content in Hydretain ES Plus treated soil, resulting in increased root biomass and root volume.

Young apple trees are usually planted as one-year unbranched whips. According to Hull (2018), nursery trees are usually headed 70 to 90 cm above the grafted union before planting to obtain a sufficient number of side branches to promote the growth of new shoots. When this is done, three or four dominant new shoots emerge at the top. It has been observed that when this occurs, only very short shoots grow under these top shoots (Kikuchi et al., 2003). This phenomenon has been understood as a physical characteristic of trees having a top predominance. In the experiment conducted for this study, it found that the upper three to four shoots in spring-planted trees were significantly longer than the lower shoots. Similar results have been reported. Kikuchi et al. (2003) also found that in ‘Fuji,’ top shoot weight was the same for pruned and unpruned shoots. While Kikuchi et al. (2003) only compared pruned and unpruned trees, in my study, it was found that the rootstock impacted top shoot length on pruned trees. Findings in this experiment was expressed that shoot length was greater on Ma with 70% water content than on JM7 with 70% water content (Table 2.8) and that top shoot length differed in soil with a moisture content of 70% depending upon the rootstock. The rootstock's impact on fresh shoot weight was more significant for Ma with 70% water content than for JM7 (for both 50% and 70% water content levels). The fresh trunk weight of the young apple trees was higher on Ma with 50% water content than on JM7 with 70% soil water. These findings extend those of Campbell and Bould (1970), confirming that the number of shoots was closely related to the rootstock.

In areas water resources are scarce and winters are not very cold, the opportune time for planting apple trees is in the early winter months. Apple trees planted in early winter do not branch during the winter months, and root growth usually occurs at five degrees when the soil temperature reaches during temperature become rise. There are many disadvantages as there are advantages to winter planting. For example, some non-wintering animals often feed on these young trees during the winter months when other plant material is scarce (Ferre, 2003). According to several researchers, the best time to plant young apple trees is in spring, continuing with cold weather in winter (Arakawa et al., 2014; Kikuchi et al., 2003).

This research was undertaken because there was no detailed study on the root growth of young apple trees planted in the winter and the root growth of winter-planted apple trees. The findings from this study were to show that no root growth was observed on young apple trees during the winter months. The roots started to grow slowly from March to April, whereas in May vigorous root growth was observed. Van et al. (2011) have reported that root growth for dwarfing M.9

occurred from early spring (December in New Zealand), although they did not check or mention wintertime root growth. Temperature change is also vital for root growth. During the experiment period, the average daily temperature in March was 4.9 °C, which impacted root growth. Lopushinskiy and Max (1990) have found that, for forest trees, root growth occurs when soil temperature is 5° C or above. As a result of the above research and the results of this study were confirmed that the occurrence of root growth in young trees depends not only on temperature changes but also on a specific time because after planting young trees in favorable conditions, reach a growth rate eventually. This may suggest that the root can adapt to the soil conditions before growth and begin to grow after improvements are made in the growing environment.

The MC changes were measured in the lower and upper parts of the trees to determine the relationship between the root condition and the growth of different parts of the tree. The MC of the trunk rose slowly from January to May. Root MC increased from January to April when the new roots appeared. These findings suggest that these MC changes are related to root growth and root activity (water absorption by the roots). An increase in trunk MC and rootstock stem MC may be related to cold-related damage in young trees during the spring, since it has been suggested that the cold hardiness of woody plants is related to water relations parameters (Anisko and Lindstorm, 1996).

In this study, root MC decreased when shoot growth occurred in May on the young apple trees. Diminishing root MC did not affect root growth in May and vigorous root growth continued.

Improved growth of young trees can be achieved through timely planting and through managed watering and other orchard work utilizing modern technologies. Determining the future growth points of a tree can be instrumental in achieving an early and bountiful harvest in a new orchard. To do this, it is crucial to properly assess the nature and content of the shoot buds and flower buds in advance to determine their location, which can be useful for precise and effecting pruning. In this research study, it was investigated shoot and leaf bud light absorbance using a visible/near-infrared spectrometer. The findings revealed that the leaf bud light absorbance shown on the visible/near-infrared spectrometer was higher than that of the shoot bud light absorbance for all the cultivars studied and for all of the dates on which measurement were taken. In addition, although no large changes in bud light absorbance were observed with the visible/near-infrared spectrometer, the visible/near-infrared spectrometer showed that bud light absorbance decreased near bud burst for 'Miyabi Fuji' and 'Jonagold'.

In this study, the classification of flower and non-flower buds was determined by a visible near-infrared border that can identify their chlorophyll content. Until now, the bud chlorophyll content of 'Fuji' apple buds has been measured by examining the leaves and not the buds themselves. An analysis of the buds showed that, three days before bud burst, the chlorophyll level of the flower buds was significantly higher than that of the non-flower buds However, no significant differences in chlorophyll levels were found between flower and non-flower bud when measured 33 days before bud burst. This suggests that neither destructive nor non-destructive measurements of chlorophyll levels are reliable for distinguishing flower from non-flower buds.

This study introduced a non-destructive method of identifying the flower and non-flower buds of 'Miyabi Fuji' apple trees. The results show that this method, using a visible/near-infrared spectrometer, accurately classifies and distinguishes flower buds from non-flower buds near bud burst. This non-destructive method is an important way to ascertain chlorophyll content and the water index (Agati et al., 1995, Penuelas, et al., 1997). However, this non-destructive way of differentiating the flower from the non-flower buds of 'Fuji' apple trees has once not been studied previously. Hence, findings of this study were appeared that the absorption of light in the flower buds, shown on the visible/near-infrared spectrometer just before bud burst, had diminished, whereas non-flower bud absorption had risen. Classification Learner App testing methods confirmed that the classification and differentiation of flower from non-flower buds was 75.9% accurate when tested with Cubic k-nearest neighbor (k-NN).

Vitola (2017) has reported that Cubic KNN is the simplest way to separate various data and obtain accurate results. According to Buban and Faust (1995), bud growth and development depend indirectly on the availability of “free water amounts of buds”. Penuelas et al. (1997) reported that 680, 900, and 970 nm reflectance provide the best estimation of plant water content. Fruitlet drop measured by Vis-Nir in situ has been shown to be beneficial in terms of time efficiency and its high level of accuracy (Orlova et al., 2020). In Chapter 3, was reported on winter planted young ‘Miyabi Fuji’ trunk moisture content changes, finding that changes in bud light absorbance may have brought on physiological changes in the buds before bud burst. Based on the results obtained, can suggest that to know buds physiological condition with visible/near-infrared spectrometer can serve as a valuable guide for growers of young apple trees to ensure the proper growth of young apple trees in areas with limited water resources.

SUMMARY

Young apple trees that are planted in areas with limited water resources face challenges in their early growth stages. Insufficient intake of moisture often stunts the growth of a young tree and impacts its subsequent growth. However, substances that help retain soil moisture have been developed and these can help promote apple tree growth after planting in these water-challenged areas. The following experiments were conducted to explore this issue:

In Chapter 2 the roles that irrigation, water retention materials that are added to the soil, and rootstocks play in the growth of young apple trees were studied to find the best ways to promote young apple tree growth. Specifically, the effects of two rootstocks, 'Marubakaido' and 'JM7', two irrigation levels (normal - 70% soil water level and dry - 50% soil water level) and four soil humectants (Glutain plus Kalpak 66, Hydretain ES Plus, Menedael and Super Sorb C) applied on the upper and lower parts of young apple trees (cv. 'Miyabi Fuji') planted in the spring and harvested in late autumn, were studied.

A greater number of shoots emerged on trees grafted onto Ma than on those on JM7 when both received normal water treatments. The dry water treatment that had the most beneficial impact on the number of shoots for the trees on Ma was Glutain plus Kalpak 66. The length of the very top shoot and of the top three shoots were greater on Ma than on JM7 and the impact of Glutain plus Kalpak 66 was most advantageous under normal water treatment. The top shoot and the top three shoots, under dry water treatment, were longer for Ma than they were for JM7 and the most favorable results for Hydretain ES Plus were observed for the lengths of the top three shoots for Ma.

The substance that had the most beneficial impact on root dry weight was Hydretain ES Plus, while adverse effects from Glutain and Menedael were observed for the 70% soil water level on Ma (2018). The highest coefficient for shoot to root relation was observed for Menedael (0.99), and the lowest was for

Hydretain ES Plus (0.73). In addition, shoot and root dry weights at a 50% soil water level showed positive effects when Super Sorb C was used on JM7. It was also observed that root growth in spring-planted trees occurs 10 days after planting on both Ma and JM7.

The results showed that the interaction of rootstock and water and soil treatments had a significant impact on total shoot length ($p < 0.01$), as did the interaction of rootstock and soil treatments on the length of the top three shoots ($p< 0.05$) and trunk fresh weight ($p < 0.05$) (2020 experiment results). Finally, it was found that the interaction of water and soil treatments impacted shoot fresh weight ($p < 0.05$).

The change in root dry weight ($P<0.01$) was observed in the interaction of water and soil treatments on Ma (2019 experiment results). Rootstock, water, and soil treatments (when only Glutain plus Kalpak 66) was used, significantly impacted total shoot length ($P<0.05$). Additionally, the highest shoot to root relation was observed for Hydretain ES Plus (0.8), while Glutain and Super Sorb C had slightly lower (0.7) coefficients for Ma.

This study revealed that the growth of young apple trees in areas with limited water resources can be aided by providing 70% soil water level with Glutain plus Kalpak 66 or a 50% soil water level with Hydreatain ES Plus for young trees that have been grafted onto Ma and JM7 rootstocks respectively. Growers in these areas should think about which rootstock to use, what soil water retention treatments to introduce into the soil as well the amount of water that should be applied.

In Chapter 3 while the timing of planting is a normal part of any agricultural operation, it plays a significant role in water-challenged areas where soil moisture is an issue. However, during the winter months in these areas, there is usually sufficient precipitation to maintain adequate water content levels. Another aim of this study was to measure root growth and variations in the growth of the upper parts of young apple trees under cold winter conditions. The effects of winter planting (from January through May in the northern hemisphere) on root growth and moisture content on each particular part of young apple trees (cv. 'Miyabi Fuji' grafted onto M.9 and Ma (*Malus prunifolia* 'Ringo') were studied. Additionally, physiological changes that occurred during the winter months were observed using a device, an OMT-NIR-M1 spectrometer, that did not destroy the buds.

The results showed dramatic changes in root growth from March (average root length less than 2 cm) to May (average root length longer than 10 cm) for both rootstocks. Root growth was observed on winter-planted trees two months earlier than in those planted in the spring. Furthermore, trunk moisture content increased over time (51.8 percent in January and 56.1 percent in May on M.9). Having observed the root growth process and tree moisture content changes in the trees under study led to the conclusion that winter planting can be recommended in areas where water resources are limited.

In Chapter 4 being able to forecast bud physiological conditions could help farmers manage their orchards more efficiently, especially since this would enable them to predict the nature of the buds without destroying them in the process. The experiments carried out in this study were conducted with the aim of distinguishing growing from non-growing buds before bud burst using a visible/near-infrared spectrometer, a device that does not destroy the buds being tested. Tests on spring-planted trees were conducted to check growing and non-growing bud physiology and the winter dormancy of young apple trees.

The spectrometric data for the growing buds before bud burst were much lower than the spectrometric data for the non-growing buds when the three varieties were tested: 'Miyabi Fuji', 'Orin' and 'Jonagold'. The highest first factor effect (87.5%) was determined by a PCA test conducted on growing and non-growing 'Miyabi Fuji' buds three days before bud burst, whereas the lowest (78.3%) was observed for 'Orin' buds five days before bud burst. 640, 650, and

700 nm were determined by PCA testing to be significant wavelengths before bud burst for all three varieties studied, while 950 and 1050 nm wavelengths were also significant for the 'Jonagold' variety (changes in the above-mentioned 950 and 1050 nm of wavelength applied only for 'Jonagold' and only three days before bud burst).

In Chapter 5 the experiments were conducted with the aim of distinguishing flower from non-flower buds before bud burst using a visible/near-infrared spectrometer. The near-infrared part of the visible/near-infrared spectrometer data for flower and non-flower buds clarified the difference between flower and non-flower buds. Three days before bud burst, non-flower bud chlorophyll content was markedly higher ($P < 0.05$) than the flower bud chlorophyll content, which explains why the visible and near-infrared border might have been influenced by the bud chlorophyll content. Spectro data Classification Learner App was presented as a useful classification method to analyze flower and non-flower bud Spectro data. Three days before bud burst, the Cubic KNN of the KNN classifier smoothly collated flower and non-flower bud Spectro data (accuracy 75.9%, sensitivity 86% and specificity 67%). The above-obtained results suggest that apple growers can go through their orchards two days before bud burst to identify flower and non-flower buds with a visible/near-infrared spectrometer.

These results suggest that to ensure the adequate growth of young apple trees in orchards in water challenged areas, apple growers can plant in the early winter months and apply Hydretain ES plus a soil water-retaining substance to utilize winter precipitation effectively. Also, for apple growers who want to use an automated system for efficient young apple orchard management, it is suggested that the easy-to-use visible/near-infrared spectrometer be employed.

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