Title:	The effect of pre-harvest shielding and spraying on yield, scab incidence, phenolic compounds and fruit quality in organic apple production
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Master's programme:	Agrobiology – Plant Nutrition and Health Aarhus University
Credits of the thesis:	45 ECTS
Date of submission:	1st of June 2017
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Cover page picture by Anna Borum, 2011

Preface

Today, sustainability and food production has gained a lot of attention and these key aspects are an important part of my personal interest on production. I have been happy to write about apples in this master thesis in Agrobiology, Plant Nutrition and Health at Aarhus University. Apples have a good storability and are fascinating in their application and opportunities, i.e., juice, cake, food and decorations. Furthermore, apples are a representative species for other fruit productions hence the knowledge gained on this matter can be transferred to other subjects, hereby making the research of high interest. It is a 45 ECTS thesis conducted in collaboration with the Research Centre Aarslev, Department of Food Science. The centre has given me raw data sets originating from 2014 and I have been using these throughout the thesis.

Thanks to my family and friends for supporting me all the way through this project and my years at the University. Thanks to my main supervisor, Merete Edelenbos for asking me if I wanted to be a part of this project on organic apples. I also want to thank my co-supervisors Katrine H. Kjær and Marianne G. Bertelsen. Thanks to Birgitte Foged and Elisabeth Kjemstrup for doing measurements in the laboratory and for introducing me to the instruments and methods. I send a gratitude to my friend, Julie Christensen, for competent feedback and to Martin Henriksen and Kent Grigo for proofreading of this thesis. Last but not least, I want to thank my friends, office mates and fellow students for being a great support.

Aarhus, June 2017

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CONTENT

ABBREVIATIONS

DW	Dry weight
FW	Fresh weight
HPLC	High Pressure Liquid Chromatography
Rt	Retention time
SE	Standard error
TSS	Total soluble solids

Abstract

Demand of organic apples is increasing but the yield is low due to apple scab disease. The aim of the present study was to investigate the effect of exposing three apple (*Malus domestica* Borkh.*)* cultivars 'Red Elstar', 'Rubens' and 'Santana' to different pre-harvest treatments in the orchard during the growing season 2014. Treatments were (i) untreated (control), (ii) sprayed with sulphur and potassium bicarbonate (sprayed), (iii) sprayed with sulphur and potassium bicarbonate and from fruit set supplemented with an extract of Acadian algae (sprayed + Acadian), and (iv) trees covered with a rain shield (shielded). The yield was recorded for every treatment. The incidence and severity of apple scab in the harvested fruits was evaluated. In addition, the content of 7 different phenols in apple peel was determined using HPLC. Further, the quality of apple fruits in relation to physio-chemical parameters was investigated. Results showed that all pre-harvest treatments reduced scab disease where shielding provided the best control: The rain shield reduced scab severity by 85-100 % in the three cultivars compared to the control and meanwhile increased the marketable yield of apple fruits. Control apples contained a significantly higher concentration of total phenols compared to apples receiving a treatment. Pre-treated apples had lower firmness, contained less total soluble solids and acids and had a higher sugar/acid ratio compared to control. It was concluded that shielding of apples showed to be a promising tool to diminish the presence of apple scab, increase yield and positively affect the eating quality at harvest.

Summary

Apple scab is a major challenge in organic apple production and requires a lot of energy in time, money and effort to control fully. This disease control is done in order to maintain a high yield with apples of high quality meaning scab-free, sweet in taste and with 3-5 months of shelf-life. This master thesis aims to investigate possible treatments for control of apple scab in organic apple production and meanwhile investigate whether these treatments influences the harvested fruit in terms of yield, the incidence and severity of apple scab, fruit quality and the content of phenolic compounds in the apple peel.

The apple cultivars ‘Rubens’, ‘Red Elstar’ and ‘Santana’ were in the year 2014 subjected to four different treatments in an orchard at Research Centre Aarslev, Denmark. The pre-harvest treatments investigated was (i) untreated (control) (ii) spraying with sulphur and potassium bicarbonate (sprayed), which is used in organic production today; (iii) application of the same amount of sulphur and potassium bicarbonate as (ii) but supplemented six times from the time of fruit set until harvest with an Acadian algae extract as biostimulant, (sprayed + Acadian), (iv) placement of a rain shield above the trees during the entire growing season in order to reduce leaf wetness (shielded).

Fruits were harvested from the 10^{th} of September to the 3^{rd} of October depending on cultivar. The severity of scab was visually evaluated according to a scab index where a high value corresponded to a high degree of scab infection. The scab incidence was calculated as the percentage of discarded apples that were useless for sale. Apples are useless for sale when they contain more than $1cm^2$ of scab lesions according to EU standards. The yield was determined according to number of fruits and the weight of all the fruits pr. tree harvested. The yield was calculated both brutto (total yield) and netto (without discarded fruits). In addition, the content of different phenolic compounds in the apple peel of the three cultivars was measured using HPLC. There was searched for 12 phenols and seven identified: catechin, epicatechin, phloridzin dihydrate, chlorogenic acid, rutin, quercetrin hydrate and quercetin. The quality of apples at harvest was evaluated on physio-chemical parameters such as starch content, total soluble solids (TSS), firmness and acid content.

In all cases, compared with the control, treatments significantly increased overall netto yield pr. hectare. This was the case for all the three cultivars investigated. Especially for the scab-susceptible cultivar ‘Rubens’ the yield increased significantly from 0.03±0.01 t/ha for control to 26.2±1.4 t/ha when the trees were covered by a rain shield. Shielding showed to be significantly better than the treatments sprayed and sprayed + Acadian in terms of fruit yield.

Moreover, the study showed a great effect of protecting trees with a rain shield in relation to reducing the scab incidence and scab severity. The percentage of discarded fruits was highest for the control where as much as 98 % of apples of ‘Rubens’ had more than 1 cm^2 apple scab and were therefore useless for sale.

For all cultivars, the highest scab index was found for apples in the control and lowest scab index in apples with a rain shield as pre-harvest treatment. The only exception was ‘Santana’, where no significant differences were found between the sprayed treatment and the shielded treatment. Furthermore, pre-harvest treatments were found to change the phenolic content of apple peel. Total phenols were significantly higher in the control for ‘Santana’ and ‘Red Elstar’ while there was no data for control ‘Rubens’ due to severe scab infections. The higher phenolic content in control apples is in agreement with the significantly higher scab index for these apples. A positive correlation was found between scab index and total phenolic content with correlation coefficients of r=0.82 for pooled and individually r=0.70 for ‘Rubens’, ‘Santana’ r=0.84 and ‘Red Elstar’ r=0.88. The different treatments seem to have an influence on the fruit quality at harvest. Control contained lower starch, had higher firmness, higher sugar and higher acid content compared to all treatments. This indicates a difference in maturity at the point of harvest. Moreover, the shield treatment seemed to significantly change the sugar/acid ratio of ‘Red Elstar’ from a ratio of 15 in the control to a ratio of 21 in the shielded apples. This change has an influence on the taste of the apple as it is perceived sweeter.

1 INTRODUCTION

Over the recent decades, a growing interest has blossomed for organic production. This focus has led to a noticeable increase in the market share for organically produced food in many European countries, especially in Denmark (Wier et al., 2008, Willer and Lernoud, 2016). In 2004, the Danish market for food and beverages amounted for 3 % being organic and this share increased to 8.4 % in 2015 (Statistik, 2016). Regarding the future, the market of organic food is expected to further increase (Willer and Lernoud, 2016). Accompanying the increase in consumption of organic food, an increasing part of consumers is concerned about residues of pesticide in their food and caring about environmental aspects of food production. This is an additional argument that organic production practices, which entail no use of synthetic pesticides, have gained ground. Today, there is a netto import of organic apples to Denmark (Statistik, 2016), but there is a large potential for organic apple production in Denmark since both organic and local food are desired by and increasing part of consumers.

Organic apple production is a challenge since apple fruits are susceptible to a wide range of diseases. They can be attacked by diseases, both during growing and the following post-harvest storage period. One of the main challenges in domesticated, large-scale production is the disease apple scab, which is caused by the fungus *Venturia inaequalis* Cook (Wint.). The disease can easily spread in the orchard and cause both reduced fruit quality and serious losses in production (MacHardy, 1996). Currently, in conventional and integrated production, chemical sprayings are applied frequently during the growing season in order to control the disease as much as possible. For the organic grower, one major challenge is the limited availability of fungicides, since the same compounds are not available for this type of production. Besides choosing scab-resistant cultivars, organic growers depend to a great extent on the use of inorganic fungicides, such as sulphur sprayings, for scab control in apples. This inorganic fungicide is widely used and has been for many years, but several studies have shown that continuously application of sulphur compounds has ecotoxicological and phytotoxic side-effects (Holb et al., 2003, Palmer et al., 2003) and is harmful to beneficial organisms in the orchard (Kreiter et al., 1998). Therefore, alternative methods are needed to be investigated.

One alternative to fungicide application could be rain shielding of trees during the growing season. This has shown great promise in for example cherry production (Borve and Stensvand, 2003) and will be further elaborated on in section 2.2.2. Another alternative to chemical protection is to enhance the plants own defending system, as a kind of induced resistance, hereby making the fruit able to withstand pathogenic infections. Researchers have earlier found that the application of seaweed extract onto leaves, as a so-called ‘biostimulant’, may enhance fungal disease resistance in carrots (Jayaraj et al., 2008) by enhancing defence enzyme activities and accumulation of phenolic compounds in the plant. Phenols are secondary metabolites found in plants and within the framework of plant physiology serving to defend stresses, e.g., a pathogen attack (Slatnar et al., 2016). Therefore, a high content of phenols in the apple is thought to strengthen the apple’s own immune response, hereby rendering the apple able to withstand attacks from pathogens. It is interesting to test whether application of Acadian, a seaweed extract, can also enhance resistance to fungal diseases in apples by an upregulation of phenolic compounds.

On one hand, consumers wish to buy apples that have been grown organically. On the other hand, consumers do not want to compromise with food quality aspects like taste, storability and cosmetic appearance. These criteria put a further demand on the organic sector to meet consumers’ expectations. Therefore, it is important to not only study the effect of spraying and shielding on yield, scab incidence and severity, but also investigate whether these pre-harvest treatments have consequences for the quality of the harvested fruits and its storability. Regarding the scope of this master’s thesis, the fruit quality is restricted to quality determination at harvest and therefore it does not include measurement of quality after storage.

2 THEORETICAL FRAMEWORK

2.1 THE LIFE CYCLE OF AN APPLE

Apple (*Malus domestica* Borkh.) is a deciduous plant in the Rosaceae family that produces pomaceous fruits, suitable and liked for consumption by humans. The plant is cultivated as a fruit tree worldwide in regions of New Zealand, Asia, USA, Europe and southern parts of America and Africa. The tree is suitable for growing in North European countries like Denmark due to coastal climate conditions (Lindhard, 2009) and cold winters, which is required to break bud dormancy. A great portion of apples produced are sold as fresh fruits for human consumption, but can also be used for production of juice, cider and as an ingredient in different meals. In Denmark, the apple growing season starts in spring around April-May, where trees bloom with white-pink flowers. Later, fruits develop during summer and are finally harvested in the autumn. The specific time of harvest depends on cultivar and climate conditions during the growing season. The typical fruit yield is in the range of 20-40 t/ha (Bertelsen, oral communication), depending on cultivar, production practices etc.

After harvest, storage management is pivotal in order to keep the harvest quality until consumption or processing, and to ensure a steady supply of apples during the winter and pre-spring. Apples have a good storage life compared to other fresh products and they can be kept at storage for several months. If the fruit is not treated or conserved in any way, it will consist of living tissue. Apples are therefore, even after harvest, metabolically active. This entails fruit respiration and conversion of carbohydrates to carbon dioxide, water and energy. The quality of the fruit will therefore change after harvest and generally deteriorate so that the fruit becomes softer, more mealy and sweeter in taste with increasing storage time (Harker and Hallett, 1992). The fruit will lose weight during storage due to transpiration of water. Apple fruits will subsequently decompose due to microbial life. Storage conditions with low temperature and low oxygen will lower the rate of deterioration and can therefore prolong storage (Wright et al., 2015). As a food source, apples are regarded as an important part of the human diet because of their role as providers of sugars, acids and biologically active compounds such as vitamins and phenolic compounds (Wu et al., 2007).

2.1.1 The cultivars ‘Red Elstar’, ‘Rubens’ and ‘Santana’

A wide range of cultivars exists, bred for specific purposes and attributes. Although a lot of different apple cultivars exist, relatively few cultivars are commercially cultivated, while many more are to find in private gardens or small-scale hobby orchards. One of the most popular cultivars on the Danish market for fresh apples is ‘Elstar’. This cultivar is a cross-breeding between other two popular cultivars: ‘Golden Delicious’ and ‘Ingrid Marie’ (Nielsen, 2005). ‘Elstar’ is a cultivar that shows great storability and due to its popularity, organic growers would like to cultivate this apple (Bertelsen, oral communication), but the cultivar is susceptible for apple scab, which makes it challenging for organic production. ‘Rubens’ are described as firm and sweet in taste. As the situation stands, ‘Rubens’ are highly susceptible to scab infections (Nielsen, 2005) and are therefore not suitable in organic production. ‘Santana’ is a relatively new cultivar that originates from the Netherlands and a cross between ‘Elstar’ and ‘Priscilla’. ‘Santana’ apple is red in colour and is larger in size than both ‘Rubens’ and ‘Elstar’. Only few years ago, ‘Santana’ was regarded as a scab resistant cultivar and has in research been used as a model of a scab-resistant apple. But this resistance has now been broken, and the cultivar is no longer scab resistant (Bertelsen, oral communication).

2.2 MAIN CHALLENGES IN APPLE PRODUCTION

Intensive production in agriculture and horticulture is a challenge because diseases can easily spread in monocultures. Diseases reduce the yield and quality of a crop if they are not controlled and in the worst case leads to massive losses. Apple production is no exclusion. A wide range of pests can attack the apple fruit during production in the orchard and in a subsequent period of storage. Apples suffering from storage diseases do not have a real value for fresh consumption or processing. Fungal diseases known in apple production are for example powdery mildew (*Podosphaera leucotricha*), Monilia (*Monilia laxa*) and apple stem canker (*Nectria galligena*). One of the most serious diseases in apple production is ‘apple scab’ caused by the fungus *Venturia inaequalis* Cook (Wint.) and losses can be severe in areas with a humid and cool climate (MacHardy, 1996) such as Denmark.

2.2.1 The life cycle of apple scab

Apple scab is caused by the organism *V. inaequalis*, which is a hemibiotrophic fungus (Bowen et al., 2011) because it is parasitic on living tissue and continues to live on dead plant tissue. The lifecycle of *V. inaequalis* is shown in figure 1.

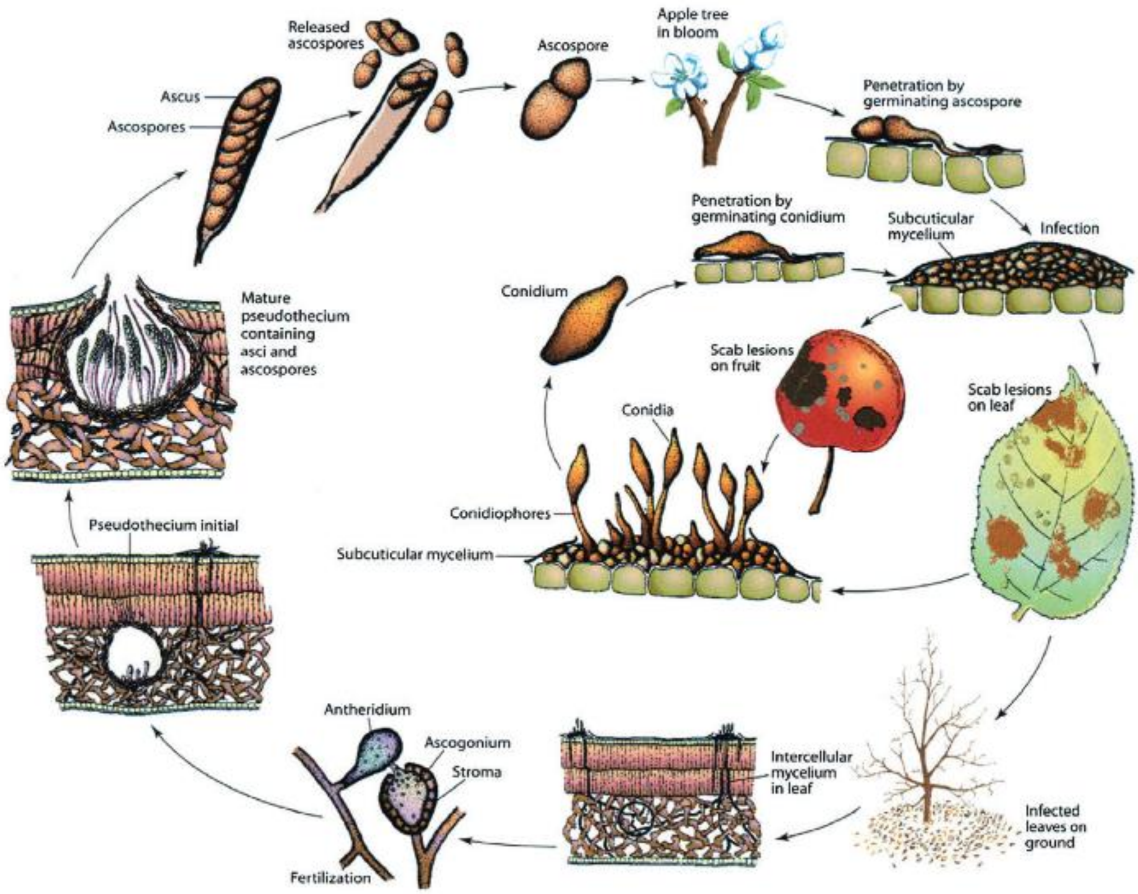

Figure 1. Life cycle of Venturia inaequalis, the causal agent of apple scab disease. Figure (Agrios, 2005).

The primary infection is initiated in spring or early summer by sexual ascospores spreading to the apple trees. The ascospores are released from pseudothecia during rainfall and the risk of infection is greatest for young leaves and fruits (Bowen et al., 2011). A germinating ascospore that lands on a leaf or a fruit can penetrate through the cuticle and create an infection, which will result in a visible lesion. Free moisture on the plant tissue is required for germination (Biggs, 1990). After infection, the fungus starts to create an intercellular mycelium in the infected leaf or fruit, forming asexual conidia spores. This makes apple scab a so-called 'polycyclic disease' because a secondary infection takes place if the conidia spores are disseminated, typically by wind and rain, to other tissue. Secondary infection can take place several times throughout the season, until leaves and fruits fall from the tree. When pseudothecia formation is initiated on infected leaves on the ground, new ascospores are created, ready to be spread and to infect leaves and fruits next spring. *V. inaequalis* thereby overwinters on infected leaves and fruits in the orchard and creates a 'green bridge' between two consecutive seasons.

Apple scab *V. inaequalis* causes dark lesions on the surface of the fruit and in case of severe infection, the fruit can crack, which facilitate invasion by secondary pathogens (figure 2). Besides from causing lesions on fruits, severe scab lesions on foliage decrease the rate of photosynthesis due to the destruction of the leaf tissue and a reduction of leaf surface for photosynthesis (Mikulič Petkovšek et al., 2009). Apple fruit may become infected at any stage of development but is most susceptible when young and then becomes increasingly resistant as it matures (MacHardy, 1996). Especially infections early in the season can be detrimental as the fruit develops unevenly. Infected leaves and fruits can remain on the tree for the rest of the season, but defoliation can occur if severely affected leaves shrivel and fall off. This may negatively affect bud formation and fruit yield the following year.

Figure 2. Apples suffering from severe scab in the orchard of Aarslev, Denmark (photo: Anna Borum).

Mills (1944) were one of the first to investigate the disease and published his infection curves in 1944. Even though researchers since have tried to improve Mills data and adjust it to local conditions, the basic observations are still the same: A minimum hours of leaf and fruit wetness is required to result in a scab infection on leaves and fruits inoculated with ascospores or conidia spores from *V. inaequalis*. An example on the basis of Mills data is shown in table 1 below.

Table 1. The hours of wetting required for a scab infection to be established at a given temperature. From Pometet (2017).

Temp. (°C)	0-4	5	6	7	8	9	10	11	12-13	14-15	16-24	25
Wetting period (h)	48	35	25	20	17	15	14	12	11	10	9	11

Apple scab infection periods are the hours of wetness from the beginning of rain. From table 1 it can be seen that the wetting period is dependent on temperature and is accelerated at temperatures above 10°C.

2.2.2 Present tools to control apple scab

In order to control diseases in a production system, pest-management strategies are important. The foundation for a successful strategy is to gain knowledge about the life cycle of the pest which is causing the problems and additionally the environment in which the disease occurs. In the case of apple production, several chemical fungicides exist to protect or cure against attack from pathogens. In conventional apple production, fungicides are applied several times during the growing season – normally from bud break until the end of July, depending on weather conditions. In this period, the disease pressure is high and the young plant tissue is susceptible to infection (MacHardy, 1996). However, there are other tools available for pest control and these are especially pivotal in organic production, where use of synthetic pesticides is not allowed. Apple scab can be controlled by different strategies, and preferably a combination of strategies.

Preventive actions can hinder scab infections and diminish disease pressure in the orchard. Since scab overwinters on infected leaves on the ground (figure 1), removal of fallen leaves in the autumn is a sanitation practice that can reduce ascospore inoculum and remove the green bridge between seasons. A high turnover of fallen leaves can be enhanced by grinding leaves on the ground and make favourable conditions for earthworms and microbial life on the ground around the tree, which increases decomposition of organic matter. Pruning of trees is recommended to ensure a light and airy environment in the canopy so that fruits and leaves can quickly dry after rainfall to lower the risk of infection by *V. inaequalis* (cf. table 1).

As mentioned, a limited range of fungicides are available in organic production. One commonly used solution is sulphur and potassium bicarbonate, often in combination. Sulphur works as a systemic and protective inorganic fungicide against *V. inaequalis*. The compound is not persistent and therefore spraying with sulphur should take place right before or while it is raining. Unfortunately, sulphur compounds are less effective in controlling scab, compared to synthetic fungicides used in conventional production (Ellis et al., 1998). Potassium bicarbonate is a relative new bio-fungicide authorized for organic production in EU. The compound acts as a contact fungicide and it has a multi-site mode of action (Marku et al., 2014), among others linked to the perturbation of pH and osmotic pressure. Potassium bicarbonate is an unstable compound, it is water-soluble and easily washed off by rain (Jamar et al., 2010).

Another tool in control of apple scab is to choose plant material that has low susceptibility to the disease. Some cultivars are more resistant to apple scab than others, and therefore not all cultivars are suitable for organic growing. Scab resistance is thus an important trait, when choosing a cultivar for commercial organic growing.

Disease-management programs and forecast programs have been developed as a tool to optimize fungicide application, which is a part of the integrated production system. This type of management relies on biological and meteorological data. Scab-warning systems takes basis in Mill's data and his results about scab-infection periods has been used to build a forecasting system to be used in scab management. This warning system can help growers to apply fungicide at the most optimal time and it can help to lower the amount of fungicide and make the application more efficient.

As mentioned, a major limitation in organic production is the available fungicide options for control of apple scab. Because of the negative attributes with the commonly used sulphur spraying program, on for example the population of useful insects, other types of methods approved for organic cultivation is of interest. Not only is this useful in organic practise but can be of interest in both conventional and integrated production practices. Moreover, in many European countries, copper is used as fungicide in organic production but this is supposed to be phased out in EU (EU Council Regulation No. 2092/91) and this further enhances the need for alternatives to control of diseases common in apple production.

With knowledge about the environment favourable for development of apple scab, the idea behind placing a rain shield above the apple trees is to reduce leaf and fruit wetness and thus prevent an infection with *V. inaequalis* to foliage and fruits. The inspiration for installation of a rain shield above the tree canopy comes from cherry production, where plastic rain shields have shown great promise in reducing fruit decay and fruit cracking (Borve and Stensvand, 2003, Thomidis and Exadaktylou, 2013). Fruit-decaying organisms like the fungus *Monilinia laxa* (Aderh. & Ruhl.) need free water or high relative humidity to infect cherries. Therefore shields over the tree canopy should decrease the number of fungal infections by excluding precipitation (Borve and Stensvand, 2003). In addition, cracking of cherries, is a great problem due to rain near harvest because it creates a pathway for pathogen entry to the fruit, predominantly fungi of the genus *Alternaria* (Thomidis and Exadaktylou, 2013). Preventing free water on the fruit can decrease the number of fungal infections so the need for fungicides is diminished (Borve and Stensvand, 2003).

2.2.3 Acadian algae as biostimulant

The search for natural and biological compounds to either replace or enhance the effect of chemical treatments in agriculture is increasing. This is partly because still more agrochemicals are banned in the pesticide legislation because of the negative consequences and unintended effects on ecology (Douglas et al., 2015) and our health (Buratti et al., 2007). Moreover, the development of resistance towards a wide range of previously utilised pesticides makes some agrochemicals less effective as crop-protecting agents. The search for substitutes is therefore important in future agriculture and horticulture. One promising approach has been the use of agricultural biostimulants such as an Acadian algae product (Acadian Seaplants Ltd., Dartmouth, Canada). This fluid is a brown algae extract from the seaweed *Ascophyllum nodosum*. The use of algae preparations are considered safe to use in crop production because of their natural occurrence in the environment and they are therefore easily degradable (Basak, 2008). The algae preparation are applied to the plant leaves or into the growth medium to regulate and enhance the crops physiological processes and make the processes more efficient (Sharma et al., 2014). A biostimulant is not regarded as a pesticide, nor as a fertilizer, but placed in-between. Application of Acadian algae extracts improve resistance to stress and the crops ability to recover from stresses. The effect of applying Acadian algae has been studied in many different crops. In a review on the use of macroalgae for crop management to reduce abiotic and biotic stresses, by Sharma et al. (2014), effects such as increased yield, improved growth and improved crop quality has been reported for crops such as tomato, maize and potato. Furthermore, application of seaweed extracts onto leaves of carrots has showed to enhance disease resistance in carrots, probably through induction of defence genes or proteins (Jayaraj et al., 2008). The purpose of Acadian is to stimulate the plant’s nutrient uptake, hereby improving the cell structure, but the mode of action is not definitely clear. Algae extract is a source of phytohormones such as cytokines and auxins but also phenols, oligosaccharides and other compounds. Currently, Acadian is not approved for use in Denmark.

2.3 APPLE QUALITY

The quality of apples has several aspects depending on the observer's position in the food supply chain. Quality aspects can relate to parameters such as texture, colour, storability and sensory perception. In addition, properties that are not readily perceived such as nutritional profile and chemical constituents are quality attributes which might be of importance for a particular use. For determination of eating quality: firmness, soluble solids content and acidity are the most important parameters (Hoehn et al., 2003). In addition, these parameters are used in evaluation of maturity. Fast and instrumental measurements of these factors are important in determining the optimal harvest time of apples in order to ensure both a good quality for eating and an appropriate quality for storage. The optimal harvest time differs in relation to the purpose of the fruit and in relation to the sales organisation.

The starch index can be used for determining the stage of development of the fruit. In order to evaluate the content of starch, a Lugol's test can be made: A slice of apple is dipped into an iodine-iodine-potassium solution and the iodine will colour the starch grains dark. The slice is given a starch score from 1 to 10 with reference to a standard scale (Appendix 1). Immature fruits contain a lot of starch and the iodine-iodine-potassium solution will colour the fruit slice dark, whereas an apple containing less starch and more sugar will remain lighter after dipping. The starch level declines (the index goes up) as the starch in the fruit is degraded into sugars in the fruits during ripening.

Firmness is used as a measurement of ripeness and it tells about the fruits maturity as fruits become softer and less firm as they ripen. Firmness is measured as the pressure needed to punch a hole with a probe into the apple tissue without the skin. The basic metric unit for fruit firmness is Newton (N), sometimes stated as kg pr. area. The mostly used instrumental texture measurement is the penetrometer test. Firmness is in particular important for the crispiness of an apple and Harker et al. (2002a) found a positive correlation between penetrometer value and the sensory attributes crispiness and juiciness. Firmness of fruits decreases during storage of apple fruits, and is further accelerated by increasing temperatures (Bourne, 1979). Low temperatures are an important factor for long-term storage management of apples. A Swiss study by Hoehn et al. (2003) which investigated consumers acceptance of some apple varieties reported that for 'Elstar' apples, firmness should exceed 46 N (corresponding to 4.7 kg cm^{-2}), soluble solids above 12 % and acidity not be less than 4.0 g L^{-1} or higher than 6.5 g L^{-1} for high consumer acceptability.

Apple fruits contain different types of sugars, where the mainly present sugars are fructose, sucrose and glucose (Ackermann et al., 1992). What is regarded as a nice tasting apple is among others a balance between the content of organic acids and sugars (Petkovsek et al., 2007) and therefore sugar and acid content have an influence on the sensory quality of the fruits. Consumers often prefer a sugar/acid ratio of 15-20 (Bertelsen, oral communication). As the level of organic acids in an apple decreases, the perception of sweetness increases. Consumers select fruits on the basis of appearance since it is not possible to know the taste of an apple during purchase. Apple cultivars with sugar/acid ratio lower than 20 are appropriate for processing and cider production because of the acidity, while cultivars with higher sugar/acid ratio are sweet and good for direct consumption (Vieira et al., 2009). The classification of cultivars between sweet and acidic taste based upon sugar/acid ratio, might differ between studies. For example, Kühn (2010) classifies apples with sugar/acid less than 14 as an acid cultivar, while apples with sugar/acid of 20 would fall into the category of a harmonised (sugar/acid ratio of 14-21) cultivar by Kühn (2010) and as a sweet apple by Vieira et al. (2009).

Total Soluble Solids (TSS) can be measured by refractometry. Sugars are the major soluble solid in fruit juice and therefore TSS is often used as an estimate of sugar content. Main organic acids in apples are citric, malic and lactic acid, where malic acid accounts for around 90 % of the organic acids (Ackermann et al., 1992). Titratable acids are the best predictor of an acid taste, while soluble solid content is the best predictor of a sweet taste (Harker et al., 2002b). One thing is what can be measured by instrumental, objective methods; another is the sensory measurements of apple taste and flavour by consumers.

2.4 PHENOLIC COMPOUNDS

Secondary metabolites and are a group of naturally occurring chemical compounds, which has different functions in plants e.g. as pigments, hormones or antioxidants. In contrary to primary metabolites, which are necessary for plant growth, secondary metabolites are present in the plant tissue in very low concentrations. One major class of secondary metabolites are phenolic compounds, which can be further subdivided into chemical families such as flavonoids, polyphenols and hydroxycinnamates, depending on their chemical structure (Agrios, 2005). It is widely accepted that phenols are involved in immune responses in plants (Mikulič Petkovšek et al., 2009, Singh et al., 2015). When a plant is attacked by a pathogen or undergoes chemical, physical or biological stress, several biochemical changes occur. These changes could be an up- or downregulation of specific phenolic compounds, which therefore plays an essential role in resistance/susceptibility of the plants (Singh et al., 2015). The phenolic compounds, implicated in disease resistance, are found also in healthy plant tissue as a kind of basis resistance, but phenolic compound synthesis and accumulation seems to be accelerated after infection and can therefore be seen as a post-infection response. This accumulation of polyphenols at the infection site slow down the pathogen growth (Mikulic Petkovsek et al., 2011). At the molecular level, fungal invasion in plant tissue triggers the transcription of messenger-RNA for certain enzymes that stimulates the synthesis of phenolic compounds in plant tissue (Slatnar et al., 2010). In relation to apple fruit and leaves, cultivars show varying degrees of resistance to apple scab. Singh et al. (2015) found a higher accumulation of total phenols in resistant genotypes as compared to susceptible ones. In addition, Mayr et al. (1997) demonstrated that phenol biosynthesis had a pronounced role in the expression of resistance in apple trees. Petkovsek et al. (2007) observed that concentration of some phenolics from the flavonoid family e.g. chlorogenic acid, epicatechin and catechin were higher in scab resistant cultivars compared to susceptible cultivars. However, the content of phenolic compounds in apple fruits is not only under endogenous control but is also influenced by environmental conditions (Treutter, 2001). Parameters such as pesticide treatment and stress situations, e.g., wounding and pathogen attack, influence the phenolic content and biosynthesis (Treutter, 2001). For example, Slatnar et al. (2010) found that infection of apples with the fungus causing apple scab, *V. inaequalis*, enhanced the metabolism of phenolic compounds at both the scab spot, in the tissue around the spot and in the healthy peel. Owing to biotic and abiotic stressors peel shows greater content of phenols in comparison to flesh (Awad et al., 2000, Veberic et al., 2005). In addition to having a role in the immune response of plants, phenolic compounds are beneficial for humans because they may have anticarcinogenic effects through their antioxidative activity (Treutter, 2001).

3 AIMS, HYPOTHESES AND EXPERIMENTAL DESIGN

In order to limit scab disease and the use of fungicides in organic apple production, methods for disease management are necessary. The overall objective of this master's thesis was to investigate some possible alternatives to control of scab disease to increase the percentage of marketable yield of organic apples. On background of the theoretical framework, the overall expectation is that pre-harvest shielding of apple trees and use of sulphur, potassium bicarbonate and Acadian spraying will reduce the number of fruits with scab and increase the marketable yield of organic apple fruits, compared to no pre-treatment (control) without changing the fruit quality. Following hypotheses are stated:

1. Shielding of the apple trees in the orchard, during the growing season will perform protection against the disease apple scab. This rain-shield protection is expected to result in apples with less scab infection and therefore lower scab incidence and severity at harvest compared to control apples (unsprayed and non-rain shielded). Because of a lower infection pressure, these apples are expected to have a lower concentration of phenolic compounds than control apples.
2. Spraying with sulphur and potassium bicarbonate will result in apples with lower scab incidence and severity than control apples. This is expected to increase the yield compared to control apples. The phenolic content in apple peel will be lower than control apples because of the protection of fruits with inorganic fungicide.
3. Apples sprayed with sulphur and potassium bicarbonate and from fruit set with a supplement of Acadian algae extract will result in apples with lower scab incidence and severity than apples in the control. Moreover, spraying with Acadian algae after bloom will decrease the incidence of apple scab compared to control because the algae extract enhance the concentration of phenolic compounds in the apple peel and thus boosts the immune system of the fruit.
4. A higher scab index will result in higher total phenolic content in the peel of apple, because phenols are involved in defense against invading pathogens.

The aim of this master's thesis was to investigate the effect of exposing three apple (*Malus domestica* Borkh.) cultivars 'Rubens', 'Red Elstar', and 'Santana' to different pre-harvest treatments in the orchard. The study looked for possible effects of the pre-harvest treatments in relation to the incidence and severity of the disease apple scab. Further, the different treatments effects on yield were studied and it was investigated how the pre-harvest treatments influenced the content of 7 phenols in the apple peel at the time of harvest. Besides, the project also searched to evaluate the quality of the harvested fruits on factors such as fruit weight, total soluble solids, firmness, acid content, starch content and the sugar/acid ratio. The process of measuring scab incidence, scab severity, yield, apple quality and phenolic compounds in apple peel are visualised in figure 3.

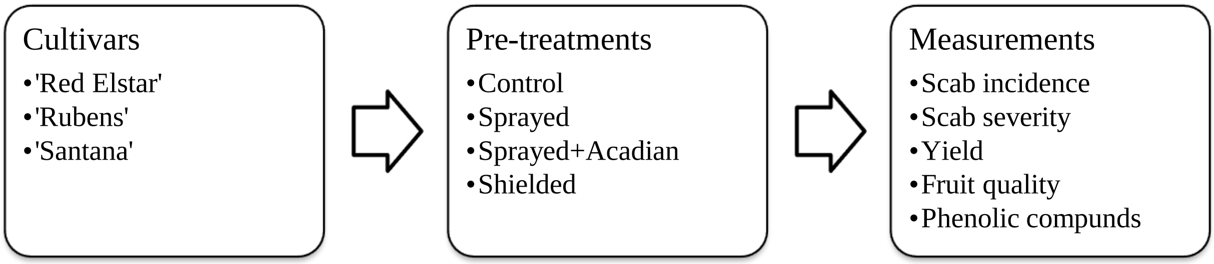

Figure 3. An overview over the apple experiment.

The previously stated hypotheses were tested in an orchard during the growing season 2014 at Aarhus University, Aarslev, Denmark. The field had previously been managed according to standard organic practices. The study design consisted of three blocks (block 1-3) placed in the same field with trees planted in three rows with one row per cultivar (figure 3).

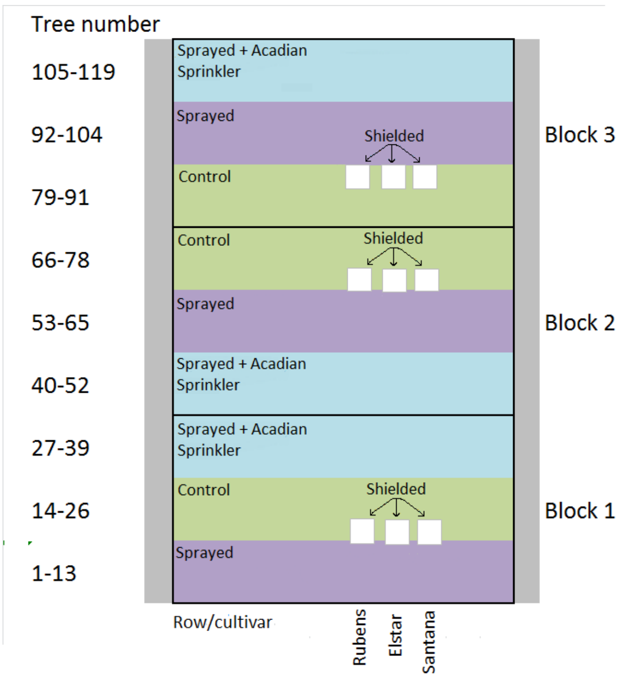

Figure 4. Experimental design for the treatment of the apples in the orchard. The design consisted of three blocks with four treatments: control (unsprayed, non-covered), sprayed (sulphur and potassium bicarbonate spraying during production), sprayed + Acadian (sulphur and potassium bicarbonate spraying until blooming, then only Acadian algae) and shielded (covering of the trees with rain shield). White boxes indicate where the rain shields were placed. Trees were planted in three rows.

Within each block, there were four treatments as follows:

(i) Control (untreated and under open sky).

(ii) Treatment with an organic spraying schedule with sulphur and potassium bicarbonate.

(iii) Treatment with the same organic spraying schedule as for treatment (ii) but after bloom additionally sprayed with extracts of Acadian algae.

(iv) Trees covered with a plastic rain shield during the growing season.

For each cultivar ‘Rubens’, ‘Red Elstar’ and ‘Santana’, the four treatments have been applied to three trees in each of the tree blocks (nine trees in total), resulting in three true replicates. From each tree in the experiment, five apples were harvested for quality analysis and sampling for phenol analysis. Scab evaluations were made on approximately 20 apples pr. tree. Since quality analyses and sampling for phenol analysis are destructive methods, the same apples have not been evaluated for scab.

4 MATERIALS AND METHODS

Apple fruits were obtained from Aarhus University, Aarslev, Denmark (55°18’ N, 20°26’ E, altitude 47 m). The apple (*Malus* x *domestica* Borkh.) cultivars ‘Santana’, ‘Rubens’ and ‘Red Elstar’ were planted on M9 rootstocks with 1.0 m between trees x 3.3 m between rows in a north-south orientation. The trees were established in spring 2009. The rain shields above ‘Rubens’ and ‘Red Elstar’ were installed in March 2012 and for ‘Santana’ in 2014. The rain shield itself was constructed of polyethylene plastic (having no penetration of UV-B light). The rain shields were installed before bud break and remained for the entire season. All trees were managed according to normal cultivation practices with mechanical weed control and pruning of trees. The only difference between the trees was the pre-harvest treatments during the growing season.

The four treatments applied to apple trees were:

- **Control.** Untreated and under open sky.
- **Sprayed.** Sulphur and potassium bicarbonate sprayed according to the spraying schedule in table 2 and applied with a standard sprayer.
- **Sprayed + Acadian.** Sulphur and potassium bicarbonate sprayed according to the spraying schedule in table 2 applied with a sprinkler sprayer. Trees were from fruit set supplemented with an extract of Acadian seaweed algae extract.
- **Shielded.** Covered with a rain shield.

Between each treatment in one row, there were five guardian trees. Spray timing were determined by the RIMpro software warning system (BioFruitAdvices, Zoelmond, Holland), which is based on Mills’ findings. In 2014, a total of 18 sprayings with sulphur and potassium bicarbonate were given to the trees in the sprayed treatment and in the sprayed + Acadian treatment (table 2). Sulphur and potassium bicarbonate in the sprayed treatment were applied with a standard sprayer and in the treatment sprayed + Acadian with a sprinkler sprayer (NaanDan Jain Irrigation Ltd., Jalgaon, India). The droplets were set to have equal size in both spraying techniques. In addition to sulphur and potassium bicarbonate application, two sprayings with insecticides were given to the trees: 29th of April with 2 kg ha^{-1} of DiPel (Valent BioSciences, Libertyville, USA) and 17th of May with 3 L ha^{-1} of NeemAzal (Trifolium-M, Lahnau, Germany).

Table 2. Fungicide application for apples receiving sprayed and sprayed + Acadian pre-harvest treatments in the orchard in the season 2014.

Date (d/m)	Fully sprayed	Amount pr. ha
19.03	sulphur	4 kg
03.04	sulphur	4 kg
07.04	sulphur	4 kg
10.04	sulphur	4 kg
17.04	sulphur	4 kg
22.04	sulphur	4 kg
23.04	potassium bicarbonate	5 kg
04.05	sulphur	4 kg
07.05	sulphur + potassium bicarbonate	4 + 5 kg
11.05	sulphur + potassium bicarbonate	4 + 5 kg
18.05	sulphur	4 kg
23.05	sulphur	4 kg
05.06	sulphur	4 kg
11.06	sulphur + potassium bicarbonate	4 + 5 kg
26.06	sulphur	4 kg
02.07	sulphur	4 kg
07.07	potassium bicarbonate	5 kg
14.07	sulphur + potassium bicarbonate	4 + 5 kg

Apples receiving the sprayed + Acadian treatment were treated with an Acadian algae extract (0.2 %) (containing protein/amino acids 3–5 %, lipid 1 %, alginic acid 12–18 %, fucose-containing polymers 12–15 %, mannitol 5–6 %, other carbohydrates 10–15 %), (Acadian Seaplants Ltd, Dartmouth, Canada). These apples received the algae extract six times during the season, beginning just after flowering with the time of fruit set and until harvest of fruits. Application amount was 1 L ha^{-1} every time, recommended with 14-days interval by manufactures. Dates of application were: 11.06, 24.06, 03.07, 17.07, 04.08 and 20.09.

The apples were harvested at commercial maturity. The exact harvest dates can be seen in table 3. Commercial maturity was defined as fruits having sugar content at about 12 %, firmness above 6 to 7 kg cm^{-2} in combination with a red colour, which was visually evaluated. At harvest, five apples pr. tree were collected for quality analysis. The quality of apples was analysed within a few days after harvest in September and October 2014. The remaining apples were stored for 125-132 days depending on the cultivar and its storability, in darkness at 2°C and 98 % relative humidity. After storage, scab incidence and scab severity was evaluated as described in section 4.1. Date of harvest, date of quality analysis and date of scab evaluation after storage are summarised in table 3.

Table 3. Harvest date, date for determination of quality and date for evaluation of incidence and severity. The days of storage are days between analysis and scab evaluation.

Cultivar	Harvest date	Quality analysis date	Scab evaluation date	Days of storage
’Red Elstar’	22.-23. Sept.	29. Sept.	3. Feb. 2015	125
’Rubens’	2.-3. Oct.	6. Oct.	18. Feb. 2015	135
’Santana’	10. Sept.	10.-12. Sept.	20. Jan. 2015	130-132

4.1 Yield, Scab Incidence and Severity

From each tree, all apple fruits were harvested, which included apples with scab lesions and other damages. They were counted and weighed as a total, to be able to calculate the yield from each treatment within each of the three cultivars investigated. In calculation of yield pr. hectare, 3.300 trees pr. hectare was assumed as an estimate. The severity of fruit scab was visually evaluated by one expert and rated, corresponding to a scab index (table 4). The rating system and index categorization was developed from MacHardy (1996) with modification.

Table 4. Scab index category and rating description used in this experiment, inspired by MacHardy (1996).

Scab index category	Rating
0	Fruit uninfected – No visible lesions
1	1 lesion
2	2-3 lesions
3	4-10 lesions
4	11-50 lesions
5	> 50 lesions

The scab index for each treatment in each block was computed using the formula modified from MacHardy (1996), where percent fruit infected (scabbed) can be described with following formula

$$P = \frac{Sum\ of\ (n \cdot V)}{Z \cdot N} \cdot 100\ \%$$

P=scab severity (% infection), n=number of apples in each category, V= numerical values of categories (scab index category), Z=numerical value of highest category in total, N=total number of apples. Another aspect of scab evaluation is to identify the degree of commercial damage and whether the fruit is useless for sale due to visible scab infection, which is of high interest to growers. This is called scab incidence. The fruit was categorised as “discarded” if the total area with visible scab was bigger than 1 cm^2. This threshold is in accordance with

EU standards for marketing of class II fruits, which is allowed to sell for fresh consumption (Council Regulation (EEC) No 1234/2007 and specified in 543/2011). The yield has been calculated as both brutto yield (total) and netto yield (without discarded fruits). Netto yield is therefore regarded as the marketable yield. Assessment of fruit scab severity and incidence was made after storage due to practical reasons. Nevertheless, this should be representative for the scab incidence and severity at harvest since scab obtained in the orchard will not change during storage (MacHardy, 1996).

4.2 Quality Determination

Quality measurements were done in the same order as described below.

4.2.1 Fruit weight and firmness

Apples were taken out of storage the day before analysis to ensure that the fruits had room temperature. Before destruction, the apple was weighed at ambient temperature on a scale (±0.5 g), which was connected to the Fruit Texture Analyser. Weight was determined for all apples, both at harvest and after storage, with one repetition.

Figure 5. Apple on a scale.

Firmness was measured with a Fruit Texture Analyser (FTA) (Güss Ltd. GS-2005, Strand, South Africa) with an 11 mm probe, connected to a PC. Two spots on the apple was selected – one spot on the most red side and one on the opposite side, where the peel was most yellow. At these two spots, a slice of 1 cm in diameter of the apple skin was removed with at fruit peeler from Fruit Pressure Tester (Wagner FT 327, Greenwich, USA) and firmness was measured. Firmness was measured on all apples at harvest with one repetition on each selected spot. Firmness was determined as the average of the two measurements.

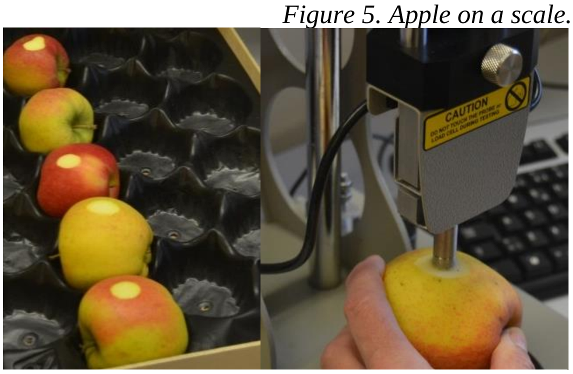

Figure 6. Five apples lined up and prepared for measurement of firmness (left) and analysis in progress (right).

4.2.2 Sampling for phenols

Figure 7. Apple peels for phenol analysis.

Samples for polyphenol analysis were taken from apparently healthy peel around the stem and around the flower. A minimum of 15 gram fresh sample was collected. The collected apple peel was frozen in liquid nitrogen and stored in a freezing box at -24 °C until samples were freeze-dried. The weight of the peel was measured both as fresh sample (fresh weight (FW)) and after freeze-drying (dry weight (DW)), which made it possible to calculate phenol-concentrations on basis of FW. After freeze drying, the samples were grinded to powder before HPLC analysis for selected polyphenols. The HPLC procedure is described in the section “Phenolic Compounds from HPLC Analysis of Apple Peel” in chapter 4.3. The freeze-dried samples that originated from the same tree were pooled to one sample.

4.2.3 Starch index

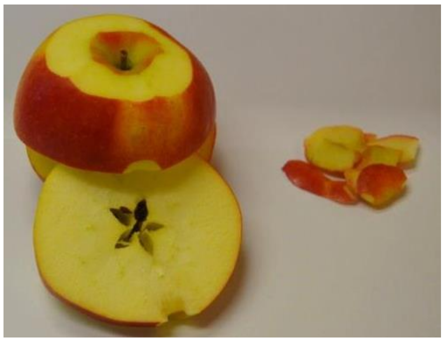

Figure 8. Apple cut for sample to starch.

A slice of 1.0 cm in height was cut transversally from the center of the fruit. This ensured that the kernel house would be present on the cut slice, which was analyzed for starch content: An iodine dissolution was made of 10 g potassium iodine (Merck, art. 105043, Germany) in a 1 L bottle and dissolved in 10 mL demineralised water. As potassium iodine was dissolved, 3 g iodine (Merck, art. 4761, Germany) was added and afterwards filled with demineralised water up until the 1 L mark. The apple slice was submerged in the iodine dissolution so the surface area was fully wetted and was immediately after removed to a paper towel with the dipped site upwards. After 10 minutes, a visual evaluation of the slice was compared to a standard. The standard is given in the “Starch conversion chart for apples” from EUR FRU, which can be found in the Appendix 1. Score 1 indicates a high starch content and score 10 indicates low starch content. Measurement of starch content was measured on all apples at harvest.

4.2.4 Sugars content

Two samples were prepared from the same spots used for firmness determination and were cut in a 45° angle towards the kernel house, resulting in two boat-shaped samples. Pure juice from the two cut samples was used for determination of sugar content by refractive index with the instrument RFM 712 from Bellingham + Stanley Ltd. and calibrated with demineralised water. Sugar content for one apple was calculated as an average of the two samples originating from the same apple and measured with one repetition.

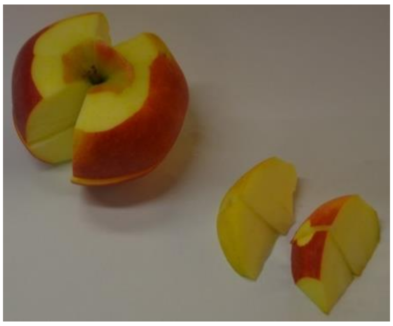

Figure 9. Boat-shaped apple samples for sugar analysis.

4.2.5 Organic acids

Acid content was determined with the instrument 719 SET Titrino (Metrohm, Herisau, Switzerland). A buffer solution with pH 4.00±0.01 and a buffer solution with pH 7±0.01 (Reagecon, Co. Clare, Ireland) were used to calibrate the instrument before sample analysis. All apples from one tree (five apples) were cut into smaller pieces, pooled and weighed. An amount of water was added to the pooled apple pieces corresponding to 1.5 times the weight of the collected apple pieces from five apples originating from the same tree. Water and apples were blended for 2 minutes to a smooth mixture. 6-10 g of the apple mixture was used for titration analysis with addition of 0.1 M sodium hydroxide (Titrisol Sodium hydroxide solution, Merck KGaA, Germany) as alkaline solution. The instrument was set to stop adding alkaline solution as pH reached 8.1.

Figure 10. Apple cut into pieces for acid analysis.

4.3 PHENOLIC COMPOUNDS FROM HPLC ANALYSIS OF APPLE PEEL

Apple peel was collected as described in section 4.2.2 “Sampling for phenols”. Phenols were extracted according to Escarpa and González (2000) with some modification. In this experiment 0.15 g DW apple peel sample were extracted, whereas they used samples of 5 g FW. Further, a small modification in the HPLC elution gradient was made.

4.3.1 Extraction

Phenols were extracted from 150 mg dried apple peel in a 2 mL Eppendorf tube with safe-lock. 1.5 mL methanol was added to the tube and further was added 1 % 2,6-di-tert.-butyl-4-methylphenol (BHT) as an antioxidant agent. Samples were placed in sonification with ice for 60 minutes and afterwards centrifuged (3000g, 5°C, 10 min.). The supernatant was transferred to a weighted 8 mL glass tube and same extraction procedure was done with 1.5 mL for 30 min. and with 0.9 mL for 30 min. The three extracts were then combined in the same 8 mL glass and weighted. The solution was filtered into a HPLC-glass through a 0.45 µm pore-size membrane filter prior to injection.

HPLC of phenols. The analyses were conducted on an UltiMate 3000 LC System (Dionex GmbH, Germering, Germany) consisting of a pump, autosampler, column compartment and a Diode Array Detector. The separation of compounds were carried out with a reverse phase C18 column LiChroCart 250-4,6 (Merck, Damstadt, Germany) at a temperature of 25°C. The HPLC was operated at a flow rate of 1 mL/min, interval detection 200-400 nm and injection volume was 10 µL. Eluent A as mobile phase was 0.01 M aqueous phosphoric acid (Sigma Aldrich, Steinheim, Germany) and eluent B was 100 % methanol (Sigma Aldrich, Steinheim, Germany). The elution was started with 5 % methanol, reached 50 % at 10 min. and stayed with 50 % until 13 min. in order to reach 55 % at 16 min, 70 % at 17 min., 80 % at 22 min., 100 % from 24-29 min., down to 5 % at 31 min. and ended with 5 % at 36 min. hereafter the elution was stopped.

4.3.2 Identification

The phenolic compounds in the apple peel were identified by comparison of retention time and UV spectra against external standards. Six standards were acquired from Sigma-Aldrich, Steinheim, Germany (catechin hydrate, chlorogenic acid, epicatechin, phloridzin dihydrate, quercitrin hydrate and quercetin) and one standard from Fluka BioChemika, Buchs, Switzerland (rutin trihydrate).

4.3.3 Quantification

Quantitative analysis was done by external standards of each phenol. Standard curves were made for each of the phenols of interest by diluting the stock sample (1 mg mL^{-1} methanol) with dilution factors ranging from 50 to 250, with an interval of 50, resulting in 7 standard dilutions of each phenol. A linear regression was made from the curve with concentration as a function of the response (area). Generally, there was a good correlation between the dilution of stock sample and its response, see an example of standard curve for chlorogenic acid in Appendix 4. Quantification was performed by normalisation of data against the external standard at two wavelengths using the software program Chromeleon™ version 6.80. Calculation of concentration on basis of FW was done by multiplication with dry weight of the sample. The content of total phenols has been found as a sum of the concentrations of the 7 different phenols.

4.4 Statistical Analysis

ANOVAs and Tukey tests were performed with R version 3.3.1 (Appendix 6-7). Data was checked for variance homogeneity by Bartlett's test and a histogram was made to check if data was normally distributed. One-way analysis of variance (ANOVA) were conducted to test for the impact of treatment on the measured parameters and a Tukey's test were used to determine significant differences at significance level $p=0.05$.

5 RESULTS

5.1 SCAB INCIDENCE AND SEVERITY

All pre-harvest treatments significantly reduced the apple scab index compared to the control (table 5). This shows that scab severity was lower for treated apples. This was observed for all three cultivars investigated. Even though the sprayed treatment reduced the scab index by 78 %, 44 % and 97 % for ‘Red Elstar’, ‘Rubens’ and ‘Santana’ respectively, in relation to the control treatment, an even better scab control was observed for the shielded fruits. Here the lowest scab index was observed for all cultivars and for ‘Red Elstar’ and ‘Rubens’ it was the significantly lowest scab index by far. The sprayed + Acadian treatment performed less good in comparison to both the sprayed treatment and the shielded treatment in controlling scab, expressed by a higher scab index and this was significant for ‘Red Elstar’ and ‘Rubens’. Sprayed + Acadian was still significantly better than control in reducing scab severity. Furthermore, a higher percentage of the apples ‘Red Elstar’ and ‘Rubens’ from the sprayed + Acadian treatment were discarded compared to the sprayed treatment. This was not observed for ‘Santana’ though. For all three cultivars, the highest percentage of discarded apples were in the control, where as many as 98 % of ‘Rubens’ were discarded. This is in contrary to between 0-3 % discarded fruits for the shield treatment, depending on cultivar.

Table 5. %-apples discarded (apples with more than 1 cm^2 scab) and scab index calculated as described by MacHardy (1996) in the three apple cultivars, exposed to different pre-harvest treatments and one control (untreated). Values for Scab index are means ± SE for the three field replicates. Means followed by different letters within one cultivar are significant different according to Tukey’s test at p=0.05.

Cultivar and treatment	% discarded (more than 1 cm^2 scab)	Scab index
‘Red Elstar’		
Control	81	84.4±2.2^a
Sprayed	1	18.3±6.1^b
Sprayed+Acadian	8	41.7±3.0^c
Shielded	0	0.45±0.2^d
‘Rubens’		
Control	98	98.3±1.0^a
Sprayed	24	54.9±6.7^b
Sprayed+Acadian	52	74.0±1.1^c
Shielded	3	14.3±2.0^d
‘Santana’		
Control	97	84.5±0.6^a
Sprayed	1	2.8±0.9^b
Sprayed+Acadian	0	11.9±2.6^c
Shielded	0	0.00±0.0^b

5.2 FRUIT YIELD

Fruit yield varied a lot between cultivars and treatments, but generally, for all of the three cultivars investigated both brutto and netto, the yield was significantly lower for apples in the control (table 6). The lowest yield was found for 'Rubens' control with a netto yield of 0.03±0.01 t/ha. The highest netto yield among the three cultivars was rain shielded 'Red Elstar' with 44.60 t/ha, although this was not significantly different from the sprayed or sprayed + Acadian treated trees of same cultivar. Between sprayed, sprayed + Acadian and shielded trees, there were no significant differences in the number of fruits pr. tree and brutto yield for 'Red Elstar'. Additionally, this was seen for all the cultivars investigated. However, there was a significant difference between the control apples and all treated apples for both fruits pr. tree, yield pr. tree and average fruit weight, where it was lowest in control. This was the case for 'Red Elstar' and 'Rubens', while 'Santana' showed no significant differences in fruit weight between controls and treated, mainly because of the high SE for control. High SE was observed for all control apples. For 'Santana' and 'Red Elstar' the shielding resulted in higher yield than sprayed and sprayed + Acadian, although the tendency was not significant. Brutto yield for sprayed, sprayed + Acadian and shielded was not significantly different for 'Rubens', but if the percentage of discarded apples were taken into account, the yield was significantly higher than for all the other treatments in 'Rubens'.

Table 6. Fruits pr. tree, yield pr. tree, fruit weight, yield in t/ha for apples 'Red Elstar', 'Rubens' and 'Santana', receiving different pre-harvest treatments. Values are means ± SE for the three field replicates. Means followed by different letters within one cultivar are significant different according to Tukey's test at p=0.05.

Cultivar and treatment	Fruits pr. tree	Yield pr. tree (kg)	Fruit weight (g)	Brutto yield (t/ha)	Netto yield* (t/ha)
'Red Elstar'					
Control	$44±9^{b}$	$5.48±1.11^{b}$	$100.4±16.9^{b}$	$18.1±3.7^{b}$	$3.44±0.7^{b}$
Sprayed	$77±10^{a}$	$11.7±1.28^{a}$	$152.8±5.4^{a}$	$38.5±4.2^{a}$	$38.1±4.2^{a}$
Sprayed+Acadian	$85±6^{a}$	$13.1±0.61^{a}$	$155.2±4.7^{a}$	$43.1±2.0^{a}$	$39.6±1.9^{a}$
Shielded	$90±6^{a}$	$13.5±0.79^{a}$	$150.9±2.1^{a}$	$44.6±2.6^{a}$	$44.6±2.6^{a}$
'Rubens'					
Control	$3±1^{b}$	$0.40±0.19^{b}$	$95.4±21.0^{b}$	$1.32±0.6^{b}$	$0.03±0.01^{d}$
Sprayed	$56±5^{a}$	$8.39±0.60^{a}$	$149.9±3.8^{a}$	$27.7±2.0^{a}$	$21.0±1.5^{b}$
Sprayed+Acadian	$52±2^{a}$	$7.96±0.42^{a}$	$152.8±2.6^{a}$	$26.3±1.4^{a}$	$12.6±0.7^{c}$
Shielded	$55±3^{a}$	$8.17±0.44^{a}$	$150.0±4.9^{a}$	$27.0±1.5^{a}$	$26.2±1.4^{a}$
'Santana'					
Control	$13±3b^{b}$	$1.83±0.36^{b}$	$175.0±43.4^{a}$	$6.04±1.2^{b}$	$0.18±0.03^{b}$
Sprayed	$40±6^{a}$	$7.36±0.94^{a}$	$185.1±3.3^{a}$	$24.3±3.1^{a}$	$24.1±3.1^{a}$
Sprayed+Acadian	$46±6^{a}$	$8.04±0.93^{a}$	$177.5±5.2^{a}$	$26.5±3.1^{a}$	$26.5±3.1^{a}$
Shielded	$48±5^{a}$	$9.21±0.89^{a}$	$193.7±3.9^{a}$	$30.4±2.9^{a}$	$30.4±2.9^{a}$

*netto yield calculated as brutto yield minus % discarded apples (see table 5)

5.3 Quality of Apples

5.3.1 'Red Elstar'

Generally, the sugar/acid ratio for this cultivar was in the range of 15-21 and therefore suitable for fresh consumption. Control 'Red Elstar' apples had significantly lower starch index (meaning high starch content), sugar/acid ratio and fruit weight, compared to the treated apples (figure 11). On the other hand, the refractive index, acid content and firmness were significantly higher for control apples in comparison to treated apples. Shielded apples had the lowest refractive index of 12.8 % and lowest acid content of 6.3 mg/g apple and it was significantly lower than both the control, sprayed and sprayed + Acadian treatments. This influenced the sugar/acid ratio, where shielded apples have the highest ratio of 21, although not significantly different from sprayed + Acadian.

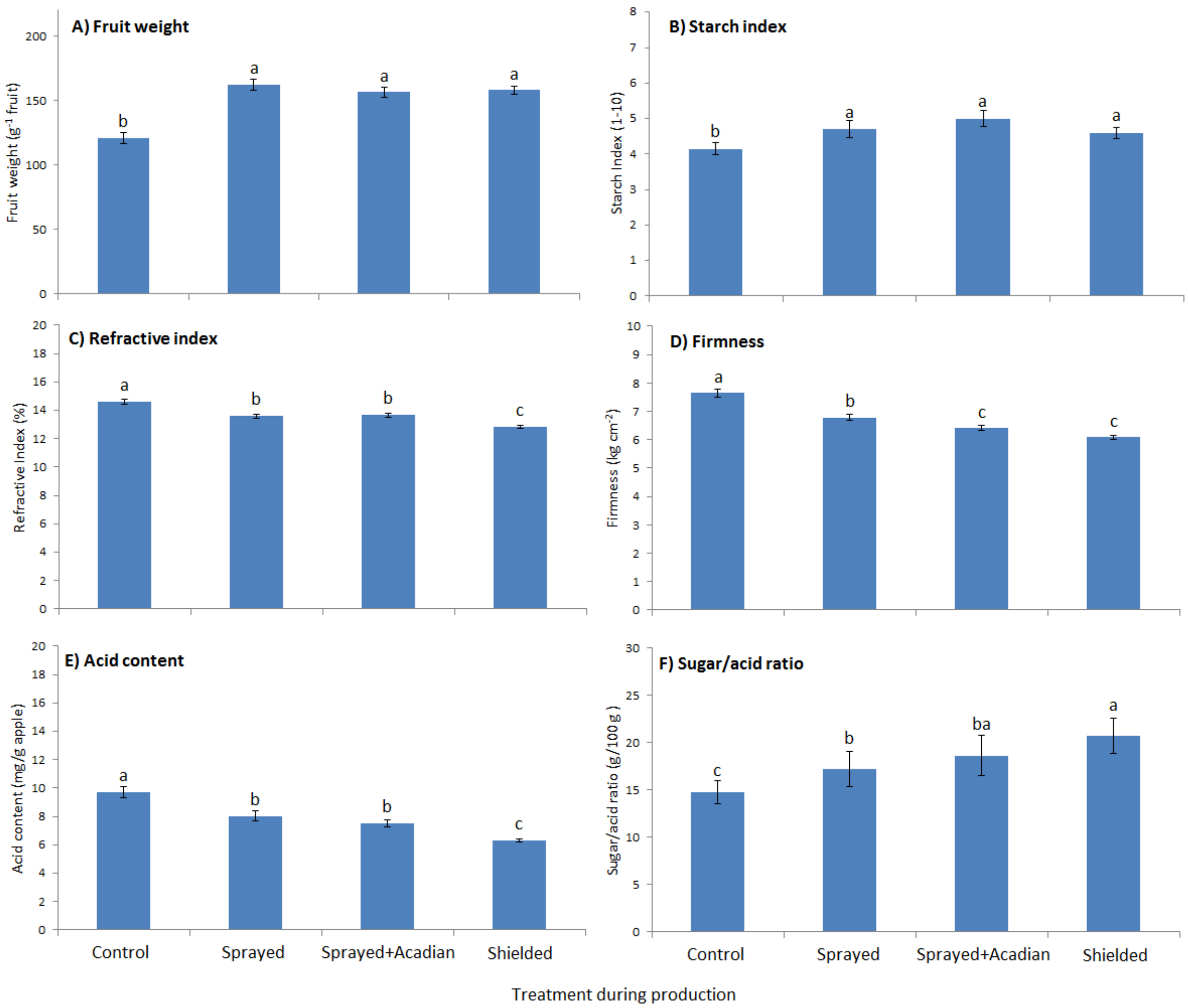

Figure 11. Quality of 'Red Elstar' grown under different production practices (control, sprayed, sprayed + Acadian and shielded) in 2014. Values are means ± SE for the three field replicates. Means followed by different letters within evaluation time are significant different according to Tukey's test at p=0.05.

5.3.2 'Rubens'

Apples of 'Rubens' in the control treatment were severely infected by scab and no apples could be harvested for fruit quality analysis, therefore no results were available for this group. Fruit weight did not differ by treatment (figure 12). There was a significantly lower refractive index of 12.8 % and firmness of 6.9 kg cm^{-2} for shielded apples, compared to sprayed and sprayed + Acadian treatment. The acid content for rain shielded apples was only significantly different from the sprayed apples, but not from sprayed + Acadian. The sugar/acid ratio for this cultivar was in the range of 23-24 and thus regarded as a sweet apple and there was no significant difference between treatments.

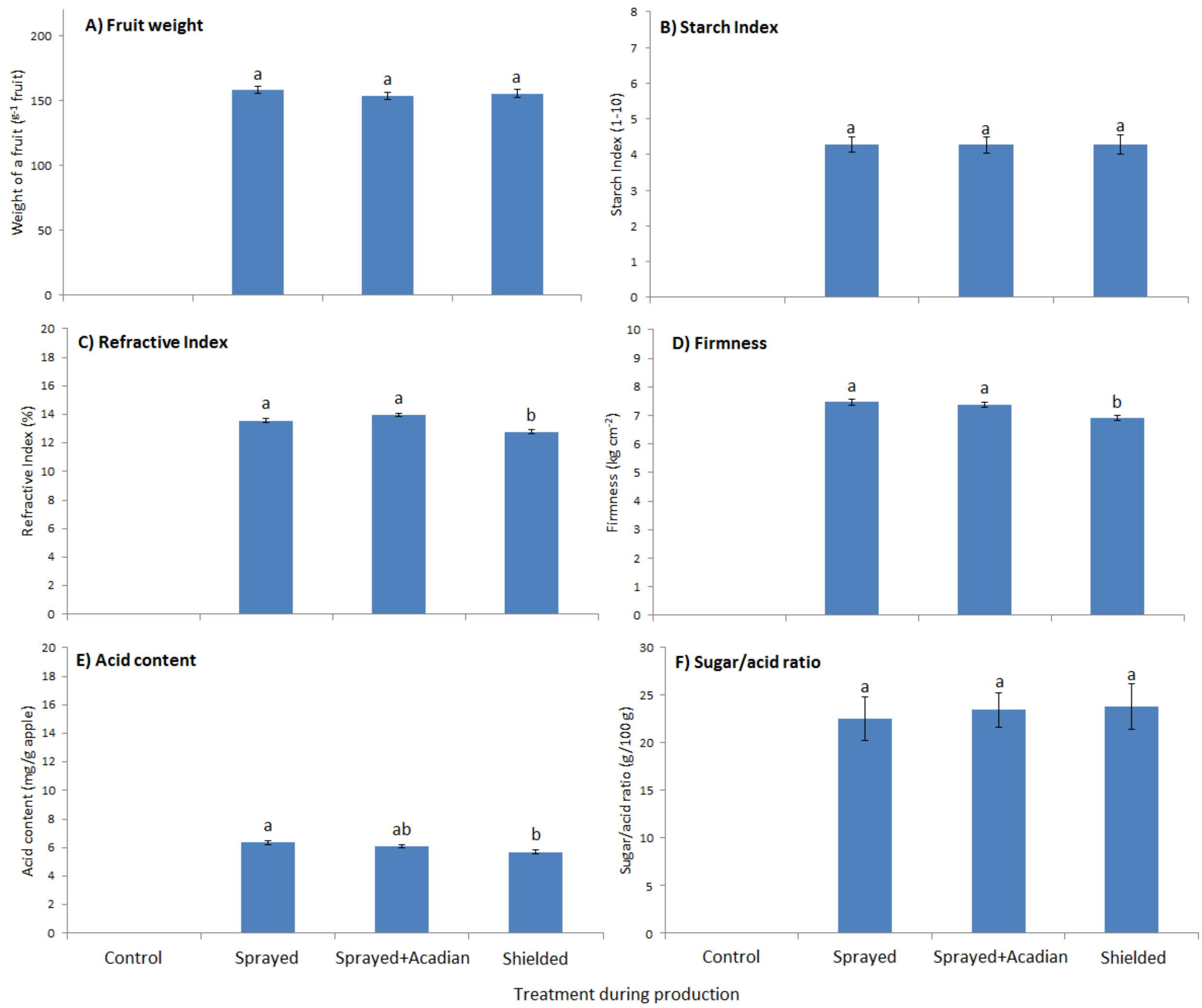

Figure 12. Quality of 'Rubens'grown under different production practices (control, sprayed, sprayed + Acadian and shielded) in 2014. Values are means ± SE for the three field replicates. Means followed by different letters within evaluation time are significant different according to Tukey's test at p=0.05. No apples harvested in control due to severe scab infection.

5.3.3 'Santana'

Significant differences in fruit quality were found only between treated and control apples (figure 13). There was no difference between sprayed, sprayed + Acadian and shielded apples on parameters fruit weight, starch index, refractive index, firmness, acid content and sugar/acid ratio. The starch index was around 5 for the treated apples and 3.5 for the control apples, which indicates that the control apples had higher starch content. Meanwhile, the refractive index was highest for control apples with a value of 15 % and significantly lower for treated apples with refractive index of 12 %. The sugar/acid ratio for this cultivar was in the range of 10-13 and therefore perceived as acid in taste. Control apples had the significantly lowest sugar/acid ratio.

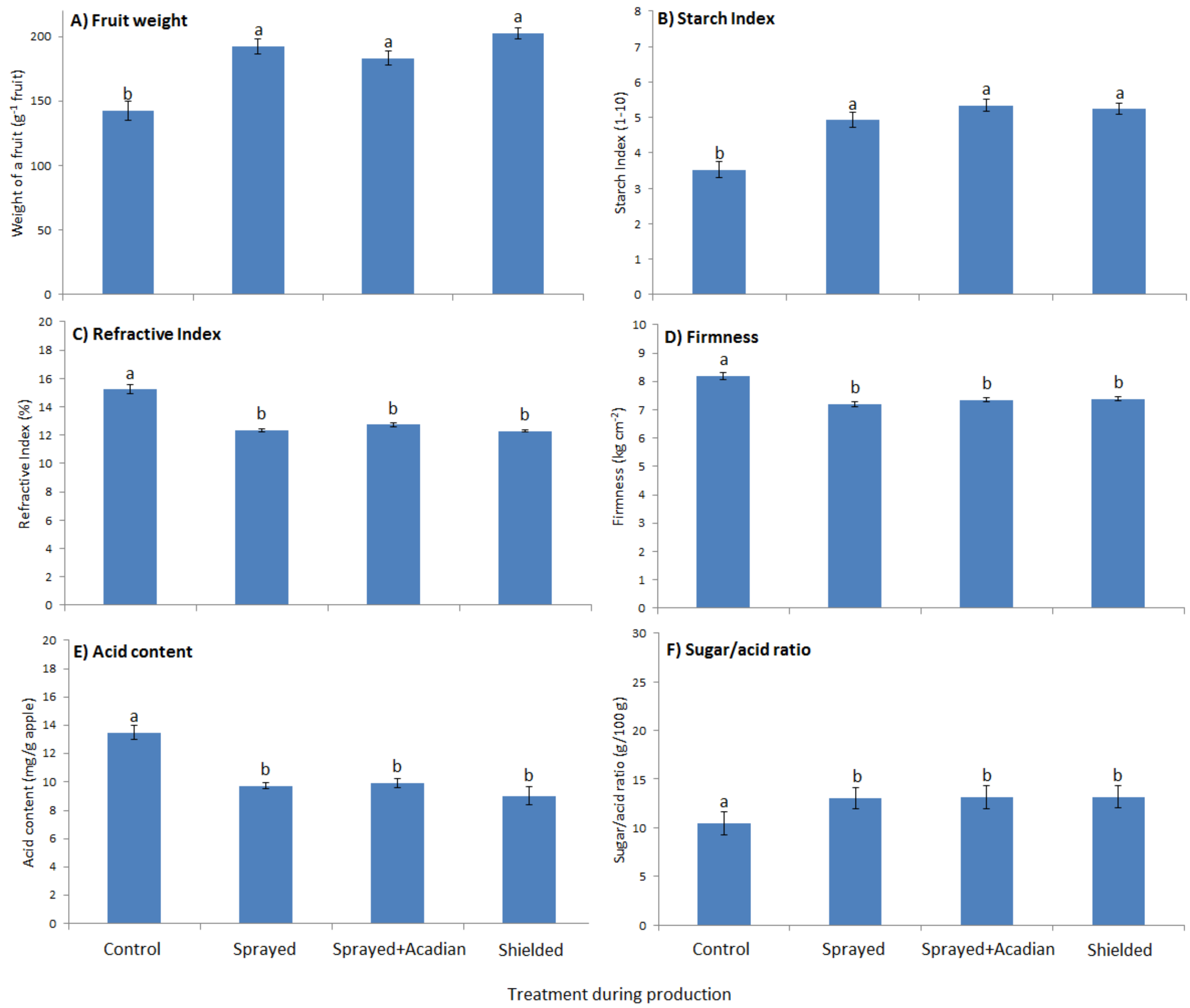

Figure 13. Quality of 'Santana' grown under different production practices (control, sprayed, sprayed + Acadian and shielded) in 2014. Values are means ± SE for the three field replicates. Means followed by different letters within evaluation time are significant different according to Tukey's test at p=0.05.

5.4 POLYPHENOL CONTENT IN APPLE PEEL

5.4.1 HPLC chromatograms for samples

Running a sample with 'Santana' gave the following chromatogram at 280 nm and 350 nm (figure 14).

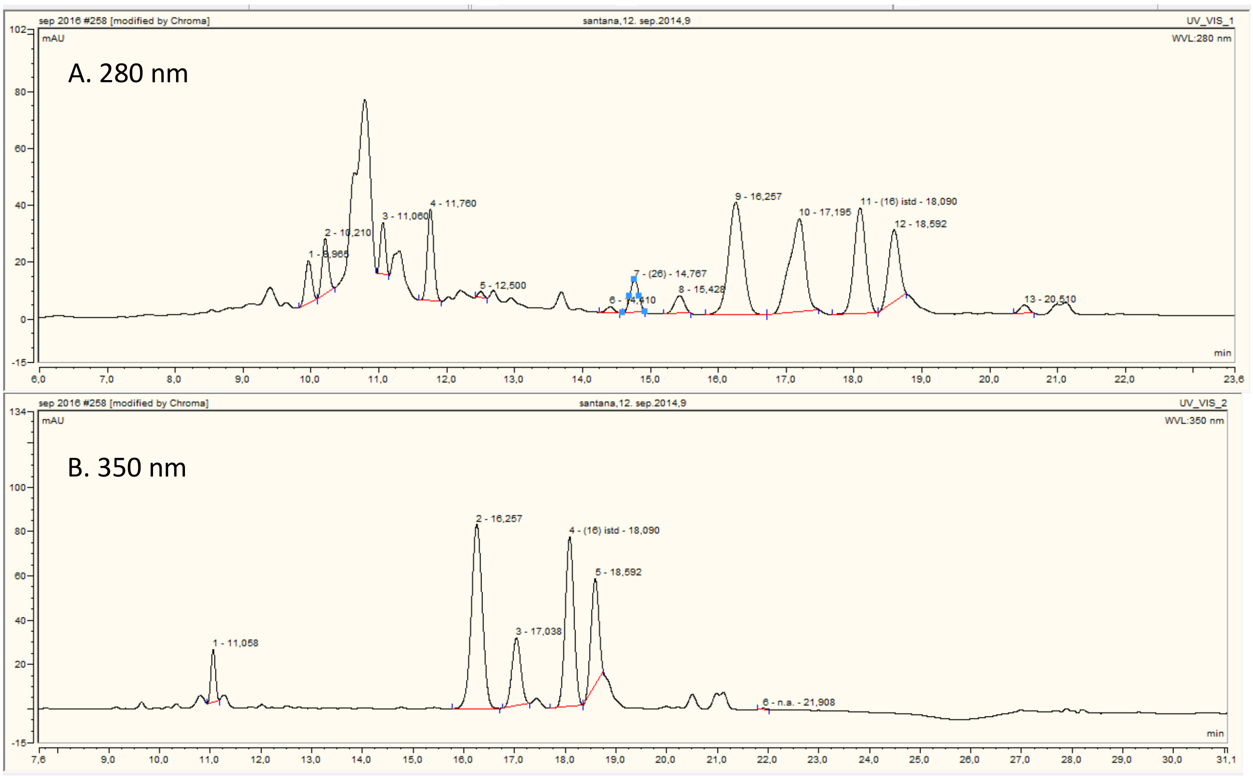

Figure 14. Example of a chromatogram for HPLC analysis of apple peel from 'Santana' at (A) 280 nm and (B) 350 nm.

Seven different phenols out of twelve were identified in the apples peel by standard addition, comparison of retention time and UV spectrum for a single compound.

Table 7. Peak number on the chromatogram for 'Santana' at 280 nm and 350 nm. The seven phenols which could be identified in the samples of apple peel where the compound has been identified and quantified.

Peak #	Compound name	Rt (min.)
280 nm		
2	(+)-Catechin hydrate	10.21
3	Chlorogenic acid	11.06
4	(-)-Epicatechin	11.76
10	Phloridzin dihydrate	17.19
350 nm		
2	Rutin trihydrate	16.25
5	Quercitrin hydrate	18.59
6	Quercetin	21.90

5.4.2 Dry matter in apple peel

Dry matter content of apple peel from the different cultivars subjected to either control, sprayed, sprayed + Acadian or shielded treatment is shown in table 8. Results showed that dry matter for apple peel in the control was significantly higher than in all of the other treatments. This was the case for both 'Red Elstar', 'Rubens' and 'Santana'. Additionally, in 'Red Elstar' there was a difference in dry matter between the lowest dry matter on 20.76 % for shielded apples, which was significantly lower than for both the apples receiving the sprayed treatment and for those treated with sprayed + Acadian. Dry matter of the apple peel was used to calculate phenol concentration in units of mg/kg FW. The values for dry matter in table 8 were used for these calculations. Since there was a higher dry matter in the peel of control apples, the phenolic content pr. gram DW would be lower than for the other treatments, even though this was not the case on FW basis. Calculations of phenolic content in units of mg/g DW are to find in Appendix 5.

Table 8. Dry matter content in the apple peel of different cultivars subjected to different pre-harvest treatments in 2014. Means ± SE followed by different letters are significant different according to Tukey's test at p=0.05, (n=3). No data available for 'Rubens' control.

Cultivar and treatment	% dry matter
'Red Elstar'	
Control	24.7 ± 0.4^{a}
Sprayed	22.6 ± 0.3^{b}
Sprayed+Acadian	22.3 ± 0.3^{b}
Shielded	20.8 ± 0.2^{c}
'Rubens'	
Control	-
Sprayed	23.3 ± 0.2^{a}
Sprayed+Acadian	23.1 ± 0.3^{a}
Shielded	21.6 ± 0.1^{b}
'Santana'	
Control	25.6 ± 0.8^{a}
Sprayed	20.4 ± 0.4^{b}
Sprayed+Acadian	20.5 ± 0.3^{b}
Shielded	20.3 ± 0.3^{b}

5.4.3 Content of phenols in 'Red Elstar'

The content of catechin, epicatechin, phloridzin, rutin, chlorogenic acid, quercetrin and total phenols in the apple peel was significantly higher for control apples than for shielded apples. There was no significant difference between 'Red Elstar' receiving the sprayed treatment and apples receiving the sprayed + Acadian treatment. In some situations, control apples contained higher amounts of phenols than the sprayed and sprayed + Acadian apples. This was the case for phloridzin, rutin, chlorogenic acid, quercetrin and total phenols.

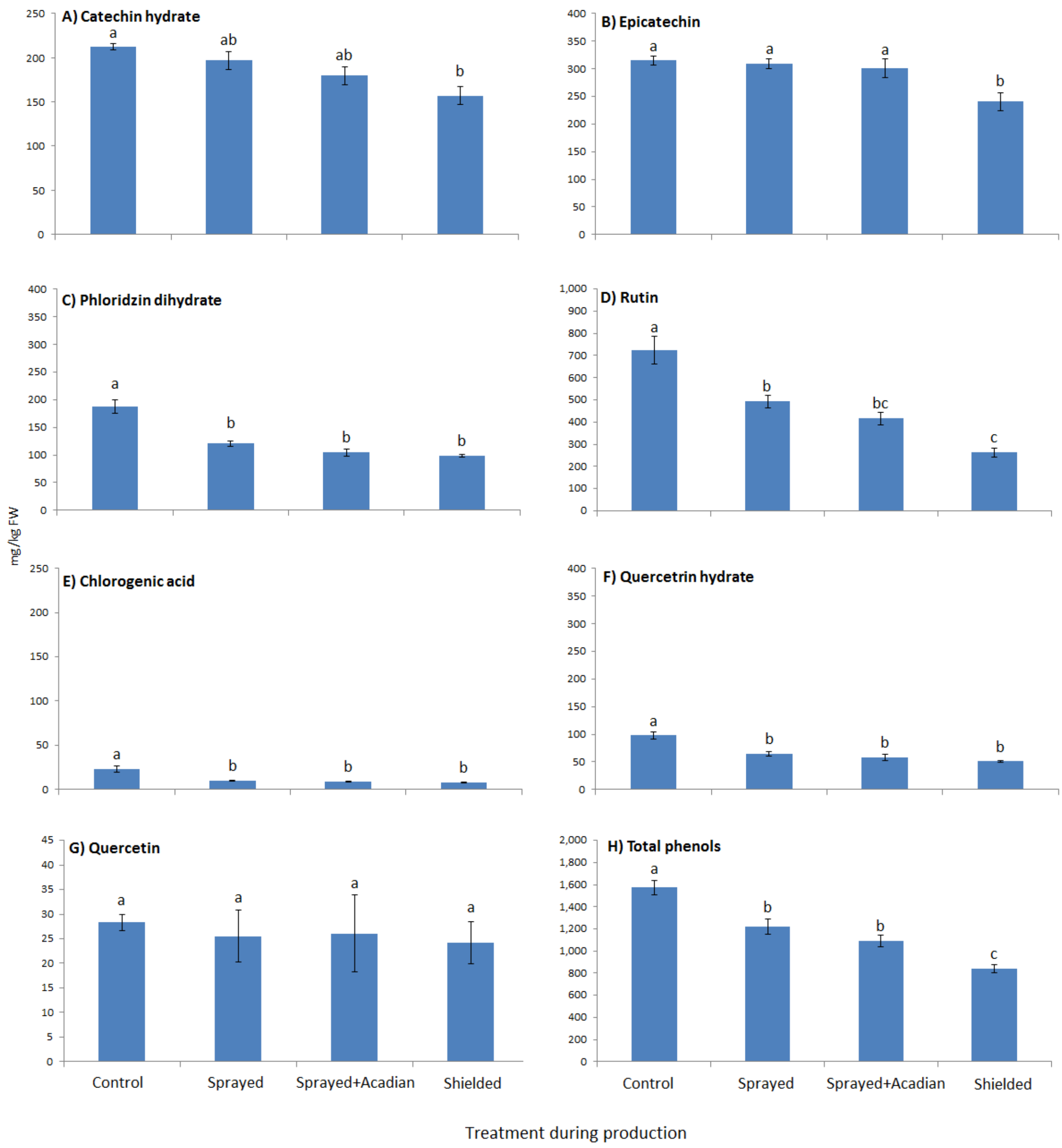

Figure 15. Content of individual phenols and total phenols in apple peel cultivar 'Red Elstar' grown under different practices in 2014. Values are means ± SE for the three field replicates. Means followed by different letters are significant different according to Tukey's test at p=0.05. Total phenols are the sum of the 7 phenols.

5.4.4 Content of phenols in 'Rubens'

Apples could not be harvested for the control. There was no significant difference in total phenolic content between sprayed, sprayed + Acadian treated or shielded apples. The only significant difference between treatments was seen for chlorogenic acid, where the lowest concentration was measured in shielded apples. This concentration was not significantly different from what was measured in apples treated with spraying, but higher for apples receiving spraying + Acadian treatment during production.

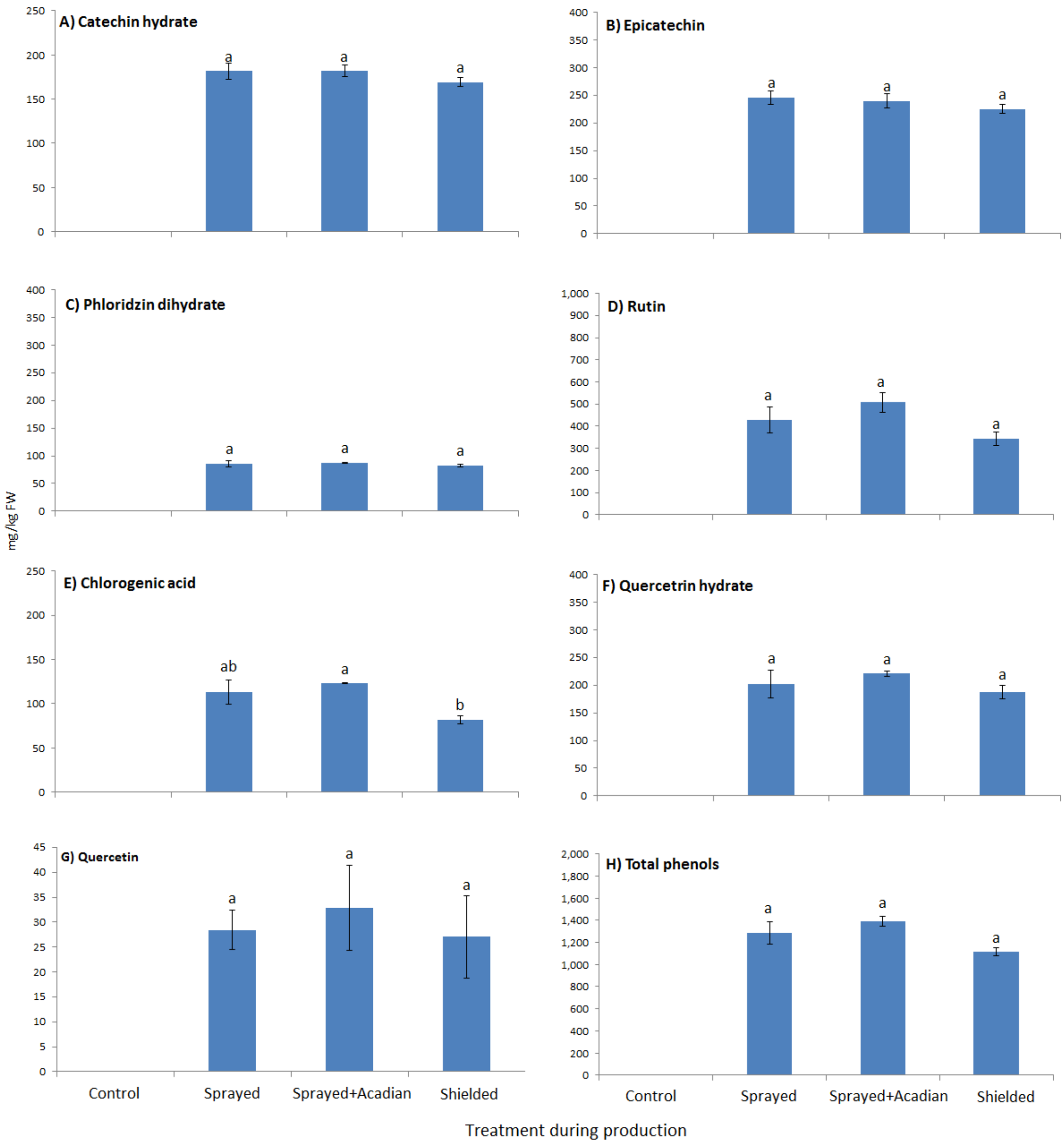

Figure 16. Content of individual phenols and total phenols in apple peel cultivar 'Rubens' grown under different practices in 2014. Values are means ± SE for the three field replicates. Means followed by different letters are significant different according to Tukey's test at p=0.05. No apples harvested in control due to severe scab infection. Total phenols are the sum of the 7 phenols.

5.4.5 Content of phenols in 'Santana'

The content of phloridzin, rutin, chlorogenic acid, quercetrin and total phenols was significantly higher in control apples compared to the sprayed, the sprayed + Acadian and the shielded apples. There were no significant differences between sprayed and sprayed + Acadian apples. Moreover, there was no difference in total phenols between sprayed, sprayed + Acadian and shielded apples.

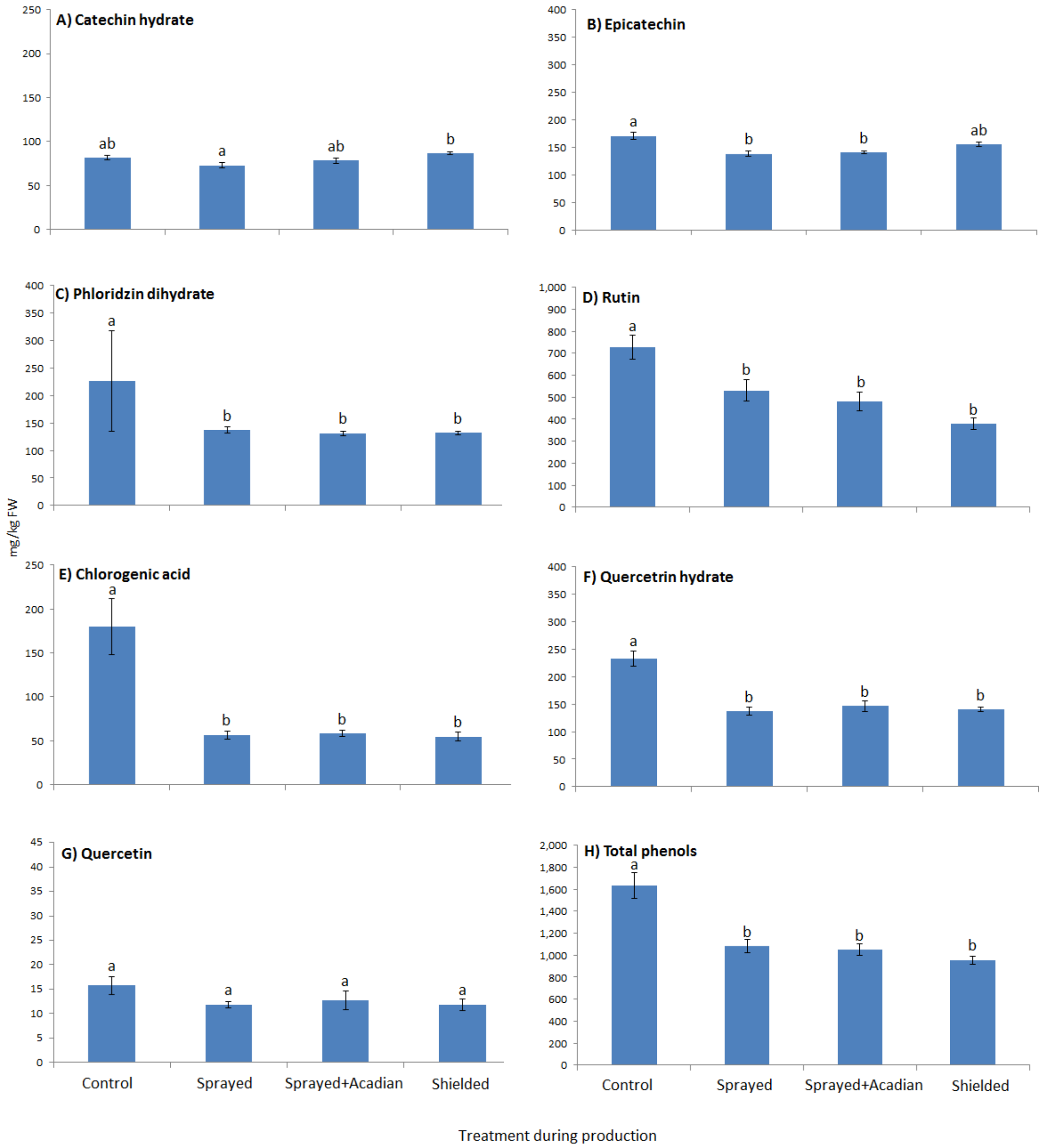

Figure 17. Content of individual phenols and total phenols in apple peel cultivar 'Santana' grown under different practices in 2014. Values are means ± SE for the three field replicates. Means followed by different letters are significant different according to Tukey's test at p=0.05. Total phenols are the sum of the 7 phenols.

5.4.6 Correlation between phenolic content and scab index

In a correlation analysis between the total phenolic content and the scab index a linear relation was seen for each of the three cultivars (figure 18). This entails that a higher degree of scab infection results in a higher total phenolic content. The correlation coefficients were ‘Rubens’ r=0.70 and ‘Santana’ r=0.84 while ‘Red Elstar’ r= 0.88. The linear correlation coefficient for all cultivars pooled was r=0.82 (data not shown).

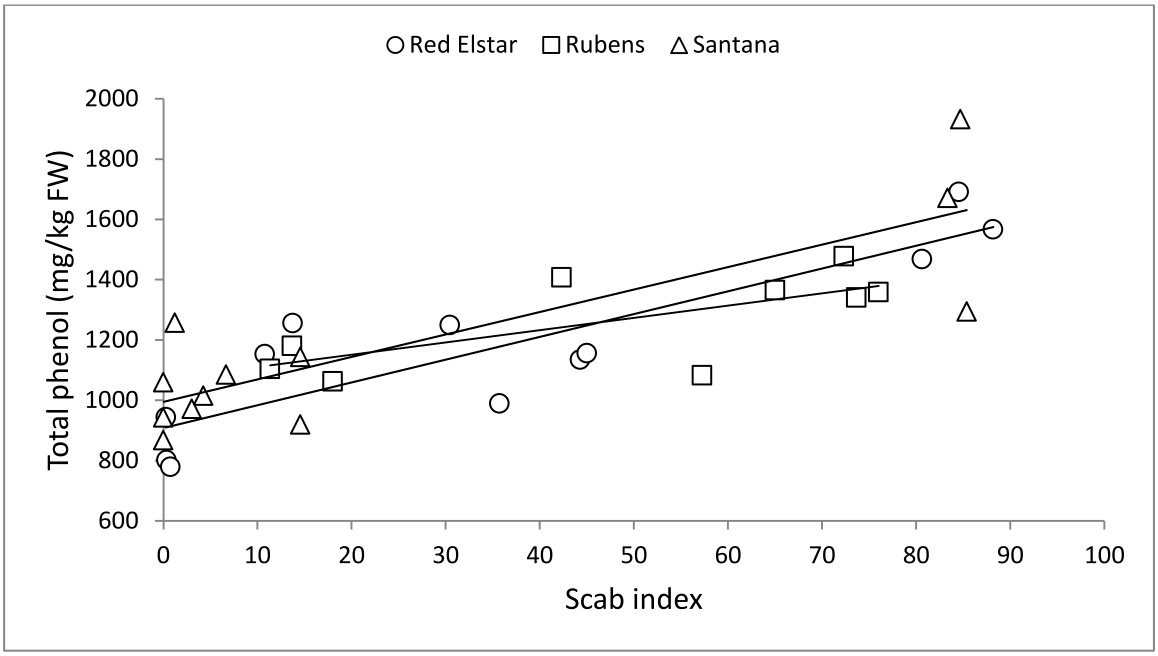

Figure 18. Linear correlation between total phenolic content and scab index for apples ‘Red Elstar’, ‘Rubens’ and ‘Santana’. Values for both total phenols and scab index are means from each of the four pre-harvest treatments (control, sprayed, sprayed + Acadian and shielded apples).

6 DISCUSSION

6.1 RAIN-SHIELDS REDUCED SCAB INCIDENCE AND SEVERITY IN ALL CULTIVARS

Generally, all treatments in this experiment can be used as scab controlling agents in all the cultivars investigated, as they all significantly reduced the scab index compared to control apples. This is in agreement with the overall expectation stated. Especially, the shielded treatment with installation of a rain-shield above the tree canopy reduced scab severity for all the cultivars investigated (table 5). Not only was the scab index highest for control apples, the amount of discarded apples with more than 1 cm^2 scab were also highest in the control for all the three cultivars. This indicates that it is necessary to protect fruits from scab infection if less fruits have to be discarded due to scab disease. Shielded apples resulted in the lowest scab index meaning that this pre-harvest treatment gave the lowest severity of scab compared to both control and the other pre-harvest treatments. In agreement with the first hypothesis, the shield functioned as a physical barrier for the rain to reach leaves and fruits of the apple tree. Therefore, infection by the causal agent of this disease, the fungus *V. inaequalis*, would be diminished since there was no free moisture on the plant long enough for an infection to take place, cf. Mills data. The protection might also lead to fewer ascospores spread when there is a shield above the tree, because the underground is not made wet.

Data in this single-year study was obtained from 2014, and this was a record warm weather, compared to the yearly middle temperature since 2001 (Appendix 2). In addition to this, in 2014, the yearly precipitation was greater than the average for 2001-2010 (Appendix 2). Therefore, there were optimal conditions for fungal growth and spore release and hereby spread of the disease in this particular year. It is known that severity of plant diseases differs from season to season; hence long-term trials should be performed to strengthen the evidence of the results obtained as rain-shields being a promising tool for scab control.

6.2 Sulphur and Potassium Bicarbonate Spraying is Better than Acadian in Scab Control

The third hypothesis was that sprayed + Acadian would give apples with lower scab incidence and severity than control apples. Results showed that the sprayed + Acadian treatment resulted in lower scab index compared to the control. Therefore the hypothesis could be verified since sprayed + Acadian provided control against scab disease. However, scab indexes for sprayed + Acadian were still significantly higher than both the sprayed and the shielded treatment (table 5). This pattern is equal for all the cultivars investigated. It was hypothesised that the spraying with Acadian could stimulate the apples own defending system against infections in the orchard by an upregulation of the phenolic content in the fruits. The phenolic content in sprayed + Acadian treated 'Red Elstar' and 'Santana' were significantly lower than control (table 15 and 17). Due to the higher scab index for control compared to sprayed + Acadian, a higher phenolic content in control was expected, cf. hypothesis 4. There were no significant difference between sprayed and sprayed + Acadian in relation to phenolic content in any of the cultivars investigated; therefore it doesn't seem that Acadian adds extra value to the scab control compared to spraying with sulphur and potassium bicarbonate alone. The sprayed treatment gave significantly better scab control than sprayed + Acadian, seen as a lower scab index and fewer discarded apples for 'Red Elstar' and 'Rubens'. Based on these results, it is hypothesised that the carbohydrates content in the Acadian formulation maybe even ends up stimulating the fungal growth.

Even though the scab index was relatively high for sprayed + Acadian treated apples, this does not seem to significantly influence the brutto and netto yield for 'Santana' and 'Red Elstar' compared to sprayed. This can be partly explained by a higher, although not significant, number of both fruits pr. tree and yield pr. tree for sprayed + Acadian. In the case of 'Rubens' there was a significantly lower netto yield for apples treated with sprayed + Acadian algae compared to the sprayed and the shielded treatment.

In explaining the vague results of Acadian as a plant protecting agent, the dose of applied biostimulant is an important consideration. The amount applied in this experiment was decided on basis of what has been recommended in strawberry production (Bertelsen, oral communication), and is not directly comparable with what is optimal in apple production. Moreover, the stability of the Acadian product is not sure. Often, natural products are unstable and might easily wash off or break down due to, e.g., light and hereby lose its intended functionality.

The method of application is another parameter that might influence the compounds functioning in that it has to be applied close to the plant tissue to diminish the risk of getting carried away by wind or rain. Another important aspect is that the study set-up was not perfect. Although the same amount of sulphur and potassium bicarbonate were applied in the treatments sprayed and sprayed + Acadian, two different spraying techniques were used. Therefore, it is not sure to say whether the higher scab severity for sprayed + Acadian in relation to sprayed is caused by the spraying technique or that Acadian do not have a positive, significant effect. The application amount, method and time have to be investigated in order to know the effect of Acadian and optimise it for apple production.

6.3 DISEASE CONTROL INCREASED YIELD IN ORGANIC APPLE PRODUCTION TREMENDOUSLY

Overall, pre-harvest treatments increased the netto yield (marketable yield) in this organic apple orchard. This has been concluded on the background that yield was significantly higher in the treated apples of all cultivars in comparison to the control (table 6). The netto yield in t/ha was highest in the shielded 'Red Elstar', primarily because of the low scab index and 0 % discarded fruits. This could probably be ascribed to the genotype of this cultivar.

The number of fruits pr. tree was the significantly lowest in the control of all cultivars investigated but there were no significant difference between the treated apples. The low fruit set for control, in combination with a low fruit weight and a high percentage of discarded fruits, gave a low marketable yield for control. This can be explained by the increased amount of stress in control apples because the trees have to cope with a high infection pressure in the orchard during the growing season. When a plant has to cope with stress, it will cost energy on behalf of crop yield.

Additionally and as previously described, there were no results from the control 'Rubens' for quality and phenol analysis, because these fruits suffered severe scab infections. This made it impossible to collect samples with the criteria "apparently healthy". Even though no data exist for these trees, this is in itself a result: It shows that 'Rubens' can hardly be cultivated without protecting it from apple scab and therefore it is not feasible under the present conditions to produce this cultivar without some kind of disease control. Not only did the control 'Rubens' give a low netto yield on 0.03 t/ha, they were also notable smaller than all other trees, had less green area and were generally less vigour (Bertelsen, oral communication). These trees suffered from a high disease pressure.

By placing a rain-shield above the ‘Rubens’ trees, the yield increased to a netto of 26.2 t/ha. This indicates that if one wishes to cultivate ‘Rubens’ apples organically and with a satisfying yield, this is made possible by the establishment of a rain-shield above the trees. It underlines the observation that disease control and management is necessary in organic apple production to meet the wish for a high yield. An important finding is that a rain-shield gives a higher yield (only significant for ‘Red Elstar’) than sprayed, which is the currently most used method of scab control in the Danish organic apple production. Additionally, the shield blocks for some of the light and therefore less light-induced stress to these trees. In connection to this, it could be interesting to investigate whether the shielded apples would also be less red in colour, since the development of pigmentation is dependent on UV-B light (Treutter, 2001).

6.4 Positive Correlation Between Scab Severity and Phenolic Content

There was a positive correlation between phenolic content and scab index (figure 18) seen for both pooled and individual cultivars, which was in agreement with hypothesis number four. For all the three cultivars, a significantly higher concentration of phenolic compounds was found in control apples. The same pattern was observed on DW basis (Appendix 5). This finding is presumably an expression of the stress situation in these fruits, mainly caused by *V. inaequalis*. A critique towards the model for ‘Santana’ is that it is not robust, because there are either high or low values for scab index with no observations in between. Whereas for ‘Red Elstar’, the observations are more evenly distributed in the range from the highest to the lowest scab index observed. Notice that no data on phenol content exist for ‘Rubens’ control treatment (figure 16) and therefore the model for this cultivar could be stronger with more observations.

Vieira et al. (2009) compared six apple cultivars in Brazil and concluded that genotype was the main factor determining the composition of bioactive compounds in apples. Pre-harvest treatments also seem to have an influence, which is supported by the findings in the present study. Overall, the concentrations of total phenols in the peel of control were significantly higher than the sprayed, sprayed + Acadian and shielded apples, although there was no data for ‘Rubens’. Trees were exposed to different pre-harvest treatments for protection against scab and therefore had to cope with different amounts of stress. Higher infection pressure resulted in accumulation and biosynthesis of phenolic compounds. There was no significant difference between ‘Rubens’ and ‘Santana’ receiving sprayed, sprayed + Acadian or shielded treatment, whereas ‘Red Elstar’ showed significant lower phenolic content with shielding compared to sprayed and sprayed + Acadian. There are cultivar differences and this is supported by Singh et al. (2015) who investigated the phenolic acid content of some apple cultivars and found that the phenolic compounds varied widely from cultivar to cultivar. In table 9 below, results from other studies on the phenolic content in peel of ‘Red Elstar’ and ‘Santana’ are given.

Table 9. Results from other studies that investigated phenolic compounds in peel of ‘Red Elstar’ and ‘Santana’. Results given in mg/kg FW.

	Phloridzin	**Chlorogenic acid**	**Catechin**	**Epicatechin**	**Rutin**
‘Red Elstar’					
Petkovsek (2007)	84.0±9.14	67.79±4.15	327.5±22.7	353.4±23.0	741.1±34.8
Veberic (2005)	166±80	165±13	Not quantified*	42±0.7	1232±163
‘Santana’					
Vanzo (2013)	12.356	59.252	0.471	5.597	4.286

*co-eluted

Petkovsek et al. (2007) concluded that concentrations of some phenolic compounds were higher in scab-resistant cultivars hold against scab-susceptible cultivars. They found ‘Red Elstar’ to contain 84.0±9.14 mg/kg FW of the compound phloridzin, whereas Veberic et al. (2005) found concentrations of 16.6 mg/100 g FW for the same compound. Apples in the mentioned study by Petkovsek et al. (2007) gained apples from an orchard in Slovenia in 2005 and ‘Red Elstar’ was treated with fungicides during production. Veberic et al. (2005) cultivated ‘Elstar’-apples in Austria and Slovenia according to integrated pest management-practices. Their results are not directly comparable with results obtained in this study since geography, exact production practices and production year differed. However, it gives an indication that results on phenolic content of apples peel achieved in this study are in the same range as findings from the other authors.

Shielded apples have significantly lower dry weight (cf. dry matter content, table 8) than control apples; this is observed for both ‘Santana’ and ‘Red Elstar’. The significantly higher dry weight in the peel of control apples is ascribed to the plants distribution of dry matter. It can be seen as a plant defense mechanism, where an upregulation of a structural barrier against pathogens is necessary for untreated apples compared to the treated apples. Hence, a thicker peel in control apples is expected, but the thickness of the apple peel has not been measured to support this statement.

Lancaster et al. (2000) found that chlorogenic acid increased markedly with UV-B radiation but meanwhile concluded that the effect of UV-B irradiation was variable, depending on cultivar, previous light exposure, temperature and type of phenol examined. Genes controlling the synthesis of different phenolic compounds might have a different sensitivity to light (Ju 1998) and, furthermore, many of the enzymes involved in phenol synthesis are induced by light (Lancaster et al., 2000). Phloridzin is thought to be connected with resistance to apple scab and therefore the stressed fruits produce greater amounts of especially this compound in order to withstand apple-scab infection, which is also the case for this study, since both 'Red Elstar' and 'Santana' control had higher phloridzin content than treated apples. The polyethylene used was without UV-B light penetration. This type of plastic is the cheapest and the most common plastic to find. Plastic that allows the sun's UV-B light to penetrate is not easy to find.

Since phenols act as an important part of the defense mechanism against stress, it could be interesting to investigate how the apples perform during storage. It might be the case that a higher phenolic content will perform protection against various types of storage diseases.

6.5 SHIELDING RESULTED IN LOWER SUGAR AND ACID CONTENT AT HARVEST

The overall expectation was that pre-harvest shielding and spraying with sulphur, potassium bicarbonate and Acadian would perform protection against apple scab without changing fruit quality. As previously discussed, these pre-harvest treatments showed to significantly reduce scab disease on fruits and increase the marketable yield compared to control apples. Nevertheless, results on quality parameters shows that this protection against disease cannot be made without a change in fruit quality at the time of harvest. For 'Red Elstar' and 'Santana' there was a significant difference in the refractive index between the control and the shielded apples. One possible explanation is that there was also significantly less fruits pr. tree in control than on shielded trees. The higher crop load pr. tree and a consequently lower TSS content can be expected in each apple, since there is a limited amount of photoassimilate available pr. tree. If these assimilate resources have to be allocated between more fruits, each fruit will consequently contain less TSS. Another explanation is that control apples were not mature at the time of harvest. This statement is supported by significantly higher starch content (low starch index) in control apples. Moreover, the content of acid was lower for treated apples in comparison to control. As described in section 2.3 on “Apple Quality”, consumer acceptance for ‘Elstar’ in relation to acidity was in the range of 4.0 g L^{-1} to 6.5 g L^{-1} (corresponding to 4.0 mg/g and 6.5 mg/g). With this as a guide line, control apples containing about 10 mg/g for ‘Red Elstar’ and 13 mg/g for ‘Santana’, these apples are not within the limit of consumers’ acceptance. Pre-treated cultivars are closer to consumers’ acceptance regarding acidity. Since apples of the same cultivar have been harvested at the same time, it could indicate that in relation to control, a pre-harvest treatment results in apples that are more mature at this point. In addition, for ‘Red Elstar’ and ‘Rubens’, there is a significant difference in acid content and refractive index between on one hand sprayed and sprayed + Acadian and on the other hand shielded apples. This might indicate that shielded apples mature even earlier than sprayed and sprayed + Acadian, due to less stress and/or better environment for fruits to develop and ripen. The same significance was not found for ‘Santana’ though.

Control ‘Red Elstar’ in this experiment have sugar/acid ratio of 14.73±1.26, which is similar to results obtained by Petkovsek et al. (2007) who found the sugar/acid ratio in ‘Red Elstar’ to be 12.31±0.61 and therefore these apples were regarded as one of the most acidic cultivars in the mentioned study. The shielded apples in the present study showed significantly higher sugar/acid ratio of 20.74±1.87 compared to control. Therefor the shielded apples will probably appear sweeter in taste than control apples.

Vanzo et al. (2013) investigated apple quality in ‘Santana’ using an organic production system. This system was in accordance with the system under EU regulation no. 2092/91, which entails that copper fungicides were used, which is not allowed in Denmark. This is important to notice, when comparing Vanzo’s findings to results obtained in this experiment. In the organic system, they found ‘Santana’ to have a fruit weight of 197 g, a starch index of 6.3 (similar scale as used in present study), TSS content of 13.4 % and fruit firmness 77 N (corresponding to 7.8 kg·cm^{-2}). Fruit weight, starch index, TSS and firmness are similar to what have been measured for treated apples in the present study. It has not been possible to find references in literature on sugar/acid ratio for ‘Santana’ apples, but the same observation is made as for ‘Red Elstar’ that the sugar/acid ratio in shielded apples is significantly higher than control apples. As ‘Santana’ is one of the more acidic apples, the change towards a higher sugar/acid ratio will approach the ratio that is preferred by consumers. Generally, the difference in sugar/acid ratio will change the taste and flavour of harvested apples being shielded during production. As the sugar content increases during storage, the fruit will presumably become even sweeter after storage for several months. Whether this is noticeable and liked by consumers has to be supported by a sensory test.

There was a significant difference in fruit size between control and apples receiving a pre-harvest treatment (figure 19). The size of the fruit is not significantly affected by shielding compared to sprayed and sprayed + Acadian apples. Additionally, control apples have significantly higher firmness than the other groups of treatments. The difference in firmness can be explained by the difference in dry weight between a cultivar receiving different pre-harvest treatments. A higher dry matter content will result in a higher force required to penetrate the fruit tissue. The higher fruit weight of treated apples compared to control in combination with a lower dry weight indicates that treated apples have higher water content. Hereby it becomes easier to penetrate the apples and the firmness is lowered.

6.6 Fruit Quality was Affected by Treatment but the Cultivars Responded Similarly

Figure 19 shows a picture of apples receiving either a control, shielded or sprayed treatment. It can be seen from this picture that control is smaller in size and has several, visible scab lesions. The shielded and the sprayed apple look similar in size and have no visible lesions. In the three cultivars investigated, the same pattern is observed in that the control contains significantly higher total phenolic content than treated apples. The low content of phenols in apples fits the market trend towards selection of cultivars with a less astringent taste as pointed by Veberic et al. (2005). Hence, the pre-harvest treatment fits the quality parameters set for a modern production. Furthermore, the sugar/acid ratio was changed by treating apples during production.

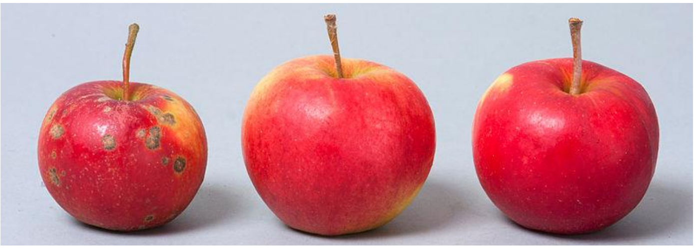

Figure 19. From left: an untreated (control) apple, a shielded apple and a sprayed apple of 'Red Elstar'. Photo: Connie Damgaard.

7 CONCLUSION

The disease apple scab caused by the fungus *V. inaequalis* is a great problem in organic apple production because it reduces yield. The results of this study showed that pre-harvest shielding and spraying with sulphur, potassium bicarbonate and a seaweed extract (Acadian) had a positive effect on apple yield compared to untreated apples, while it reduced scab incidence and severity. On background of the results, it is concluded that shielding of apple trees provided the best scab control of the treatments investigated in that it reduced scab index by 85-100 % compared to control. Hence, shielding can be effectively used to lower scab severity in apple fruits. It was expected that by shielding the apple trees, the time at which leaves and fruits were wet, could be reduced and thereby the infection by *V. inaequalis* diminished. Meanwhile, shielded apples seemed to have a lower content of phenolic compounds in comparison to an untreated control. The lowered content of phenols in shielded apples indicates a less stressed environment for the growing of apples, but it is uncertain how it affects storage life. Further, it was hypothesised that application of Acadian could enhance the apples own immune response by an upregulation of phenols in the apple peel, which may help to reduce scab incidence and severity. This hypothesis could not be fully confirmed due to implications in the study design. An important observation was that all cultivars generally responded in the same manor to all pre-harvest treatments.

Surely, shielding performed significantly better as a scab-controlling agent, but not without changing important taste-related parameters where the treated apples' sugar/acid ratio changed, herby it is expected to be perceived as sweeter. Further, firmness of the apple fruits is lowered because of higher water content in pre-harvest treated fruits and the progress in maturity. The quality obtained in treated fruits is within the limit of consumer acceptance.

7.1 PERSPECTIVES

There is high interest in finding alternatives to pesticide use in agricultural production. Up till now, organic growers have relied on the use of resistant cultivars and sulphur sprayings in organic production. Knowledge in this area is useful for not only organic producers, but also conventional growers, wishing to reduce pesticide input. As can be deduced from the findings of this master thesis, organic cultivation of the very scab-susceptible 'Rubens' has been made possible with a rain-shield treatment, which made the netto yield increase from 0.03 t/ha in untreated apples (control) to 26.17 t/ha for shielded apples. Therefore, shielding of trees during the growing season seems to be a promising alternative to chemical fungicides in production. Furthermore, knowledge on alternatives to chemical fungicides is not restricted to apple production, but can be beneficial for other types of horticultural production as well. In Aarslev, the establishment of rain-shields above trees of pear are currently being investigated.

The present study demonstrated that pre-harvest shielding makes it possible to produce apples that are not sprayed. Except from the benefits on reduced scab incidence and scab severity, by rain-shielding apples during their growing season, establishment of a rain-shield above the apple tree canopy can exempt the grower from always being available and ready to apply sulphur or other chemical fungicides, when the disease pressure in the orchard is high. Moreover, the time and labour costs for spraying are lowered by installation of a rain-shield above the trees. Unfortunately, as the situation stands, the solution is often not economically feasible. The establishment of rain-shields in the orchard is costly, and decisions to establish it have to be made on background of a cost-benefit analysis. The estimated price of a rain-shield establishment is 300.000 DKK/ha and this cost has to be taken into account. The shield is an investment and can last for a couple of years, it has to be renewed due to weather destruction. Presumably, the rain-shield treatment will result in a higher cost of apples produced in this way, which will require consumers that are willing to pay for a product with premium quality. From time of planting of trees until payback of expenses by orchard establishment, the resistance against apple scab can be broken. In these situations, a rain-shield establishment is especially suitable in order to diminish economical losses. Moreover, for the aesthesia, it can be discussed what an installation of rain-shields do for the landscape and whether it fits with the idea of an organic orchard.

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9 APPENDIX OVERVIEW

Appendix 1: Starch index table from EUR FRU

Appendix 2: Climate data 2014 from DMI and local climate station, Aarslev

Appendix 3: HPLC chromatograms for standard solution

Appendix 4: Standard curve example

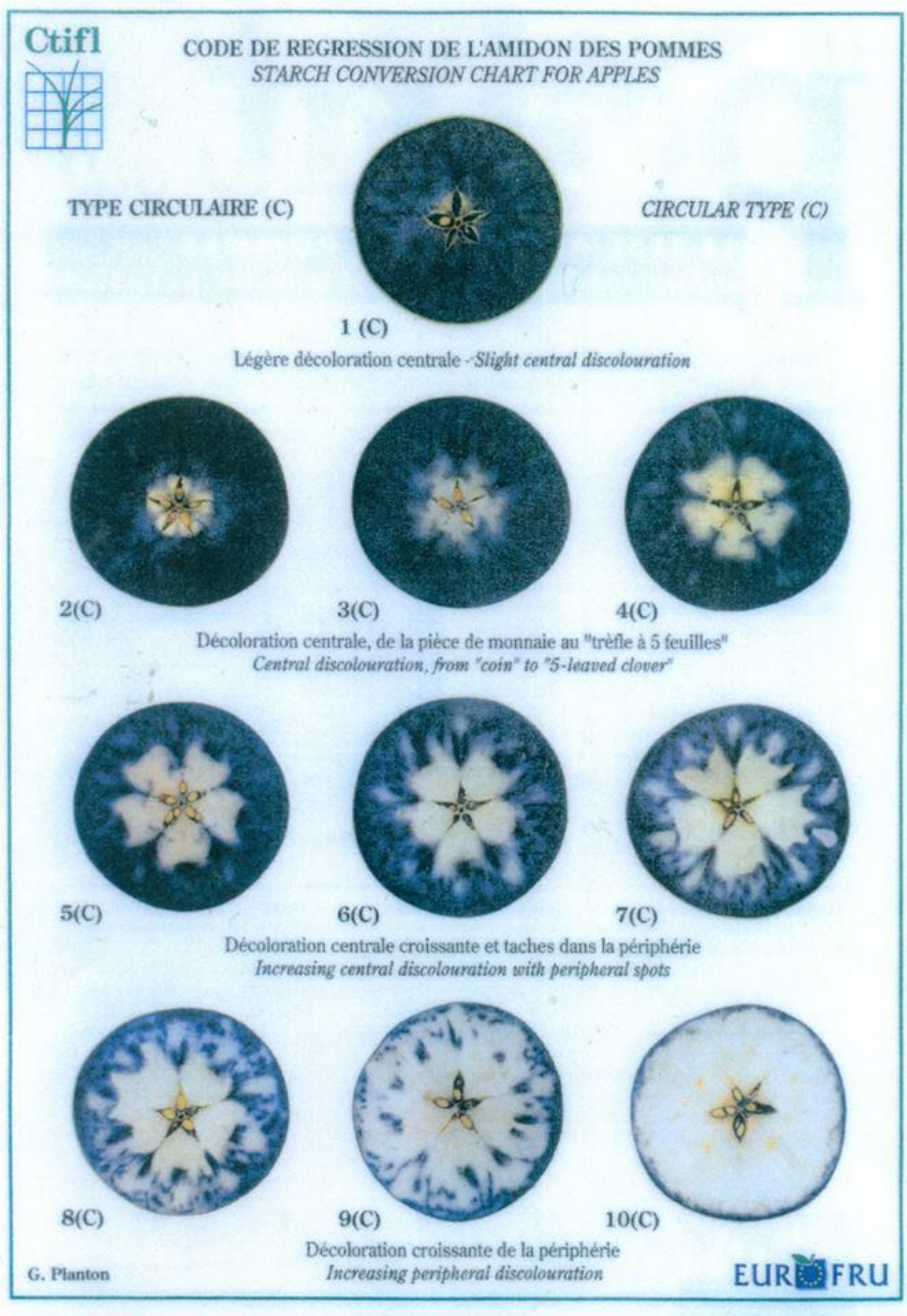
Appendix 5: Phenolic content of apple peel on dry weight basis

Appendix 6: R-codes used

Appendix 7: Example of R-outputs

Appendix 1

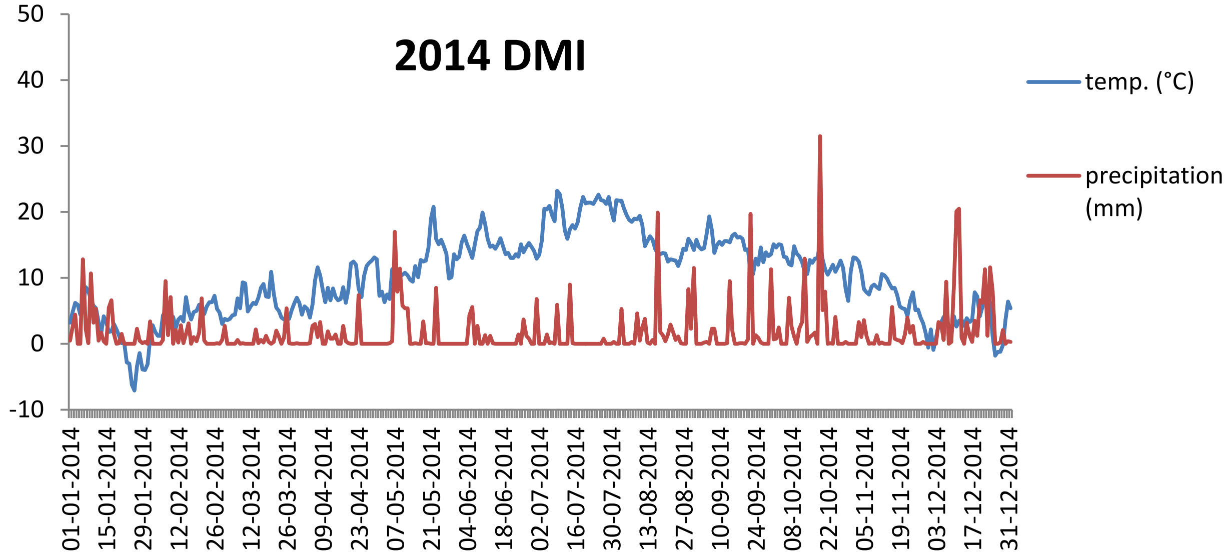
Starch index table from EUR FRU

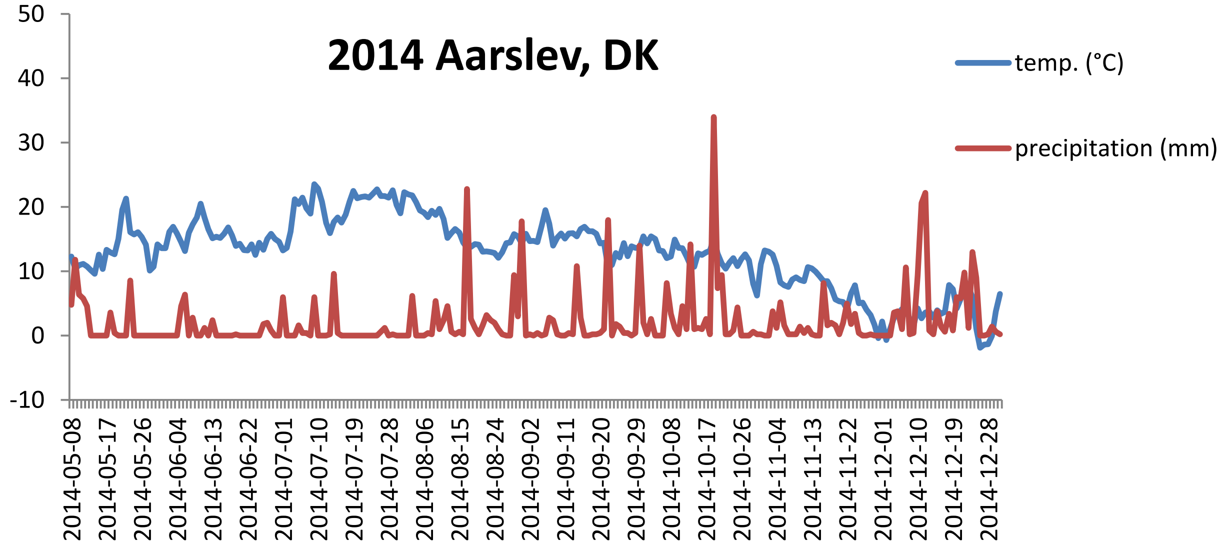


Immature fruit (1) containing a lot of starch. Over mature fruits (8-10) containing low starch but high sugar content. Preferably, category 5-7 for harvest.

Appendix 2

Climate data 2014 from DMI and local climate station, Aarslev.
- NB. Different x-axes on the graphs below.





Since 2001, the middle temperature (°C) in Denmark on a yearly basis:

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
°C	8.2	9.2	8.7	8.7	8.8	9.4	8.5	9.4	8.8	7.0	9.0	8.3	8.4	10.0

Since 2001, the precipitation on a yearly basis:

Year	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
mm	751	864	630	827	647	823	866	779	732	726	779	819	669	818

Data from Danish Meteorological Institute
http://www.dmi.dk/vejr/arkiver/maanedsaesonaar/201402/vejret-i-danmark-aaret-2014/

Appendix 3

HPLC chromatograms for standard solutions

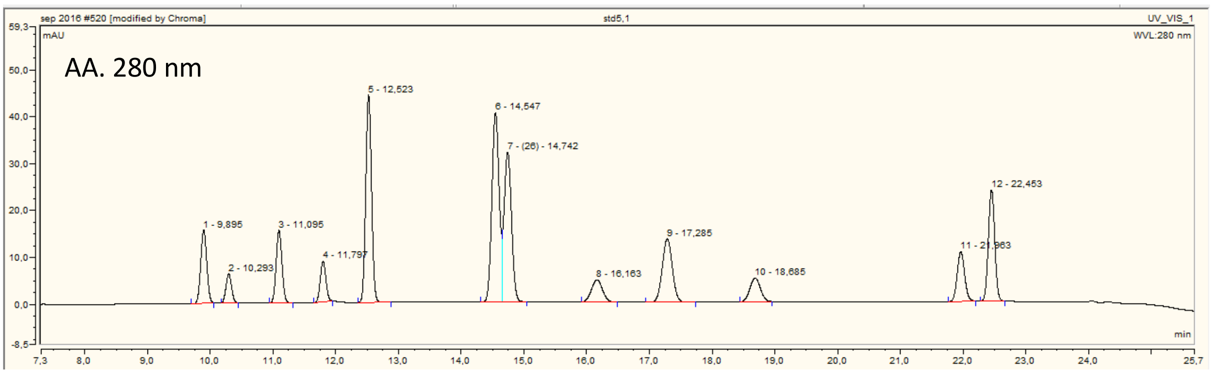


Figure AA. 280 nm: Chromatogram for standards run at 280 nm. Peak 2, 3, 4 and 9 were catechin hydrate, chlorogenic acid, epicatechin and phloridzin dihydrate, respectively.

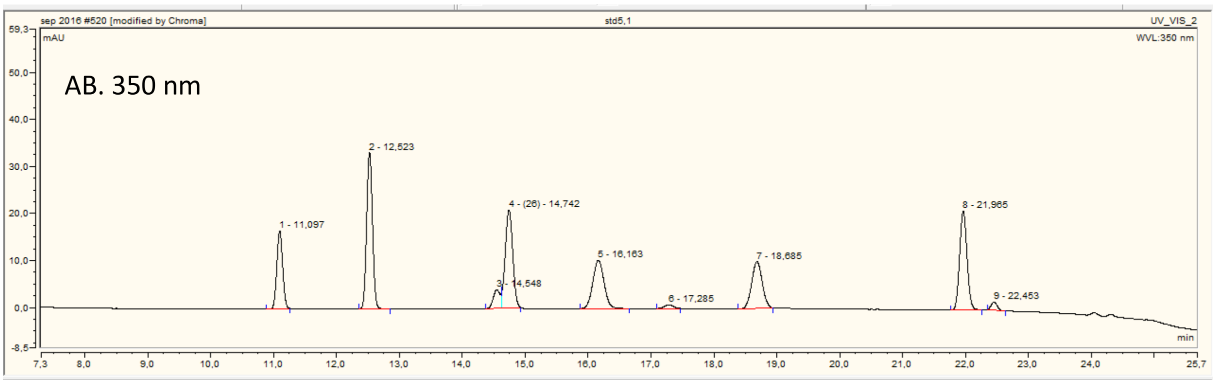


Figure AB. 350 nm: Chromatogram for standards run at 350 nm. Peak 5, 7 and 8 were rutin trihydrate, quercitrin hydrate and quercetin, respectively.

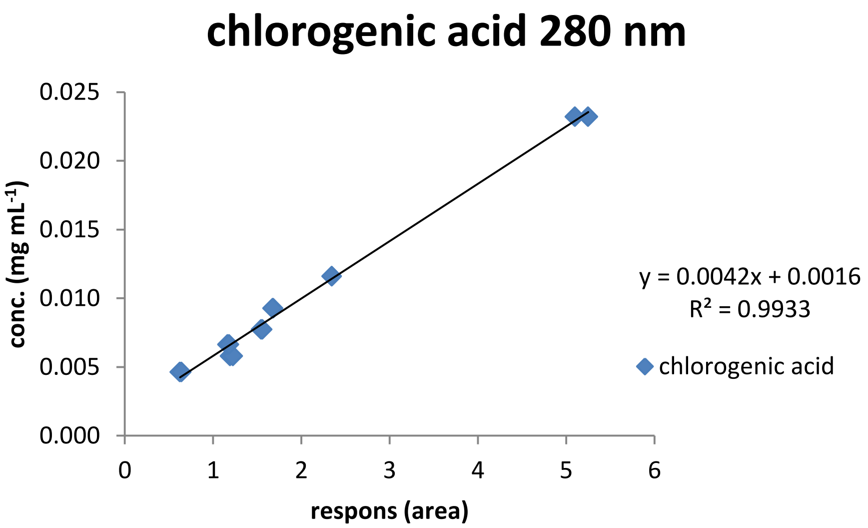
Table Appendix 3. Peak number on the chromatogram at 280 nm and 350 nm, compound name and the compounds retention time (min.).

Peak #	**Compound name**	**Rt (min.)**
280 nm		
1	Protocatechuic acid	9.80
2	(+)-Catechin hydrate	10.20
3	Chlorogenic acid	11.04
4	(-)-Epicatechin	11.72
5	Caffeic acid	14.45
6	Trans-ferulic acid	14.67
7	p-coumaric acid	14.46
8	Rutin trihydrate	-
9	Phloridzin dihydrate	17.16
350 nm		
1	Chlorogenic acid	11.04
2	Caffeic acid	12.45
3	p-coumaric acid	14.46
4	Trans ferulic acid	14.67
5	Rutin trihydrate	16.02
6	Phloridzin dihydrate	17.16
7	Quercitrin hydrate	18.53
8	Quercetin	21.86
9	Phloretin	22.36

Appendix 4
Standard curve example

Example for peak #2 (chlorogenic acid) on standard chromatogram run at 280 nm.

conc. mg mL^{-1}	Respons area
0.0046	0.6261
0.0046	0.6416
0.0058	1.1920
0.0058	1.2263
0.0066	1.1646
0.0066	1.1824
0.0077	1.5452
0.0077	1.5590
0.0093	1.6811
0.0093	1.6771
0.0116	2.3457
0.0116	2.3456
0.0232	5.2510
0.0232	5.0999



Appendix 5

Phenolic content of apple peel on dry weight basic

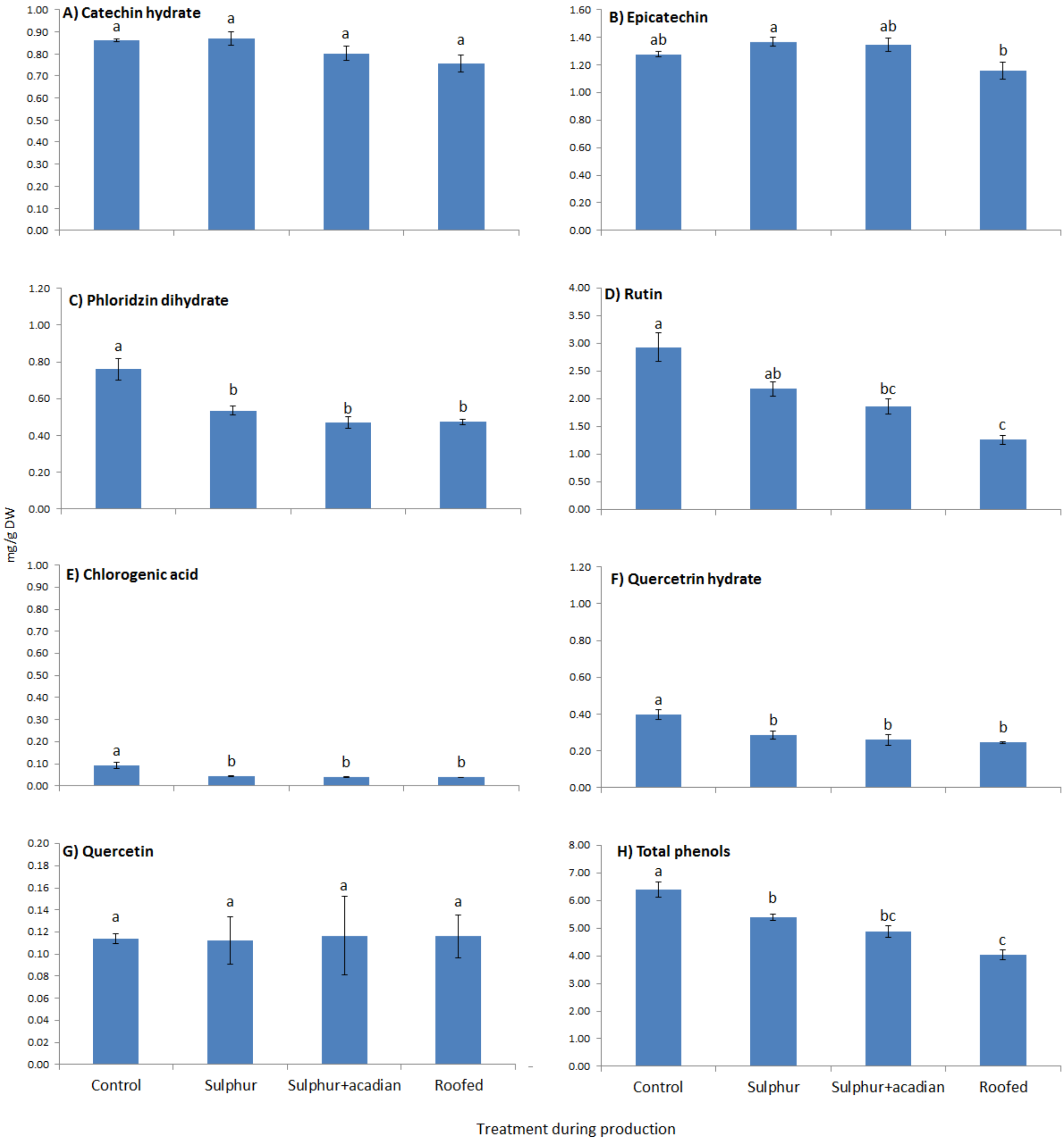


Figure A1. Content of individual phenols and total phenols in apple peel cultivar 'Red Elstar' grown under different practices in 2014. Values are means ± SE for the three field replicates. Means followed by different letters are significant different according to Tukey's test at p=0.05. Total phenols are the sum of the 7 phenols.

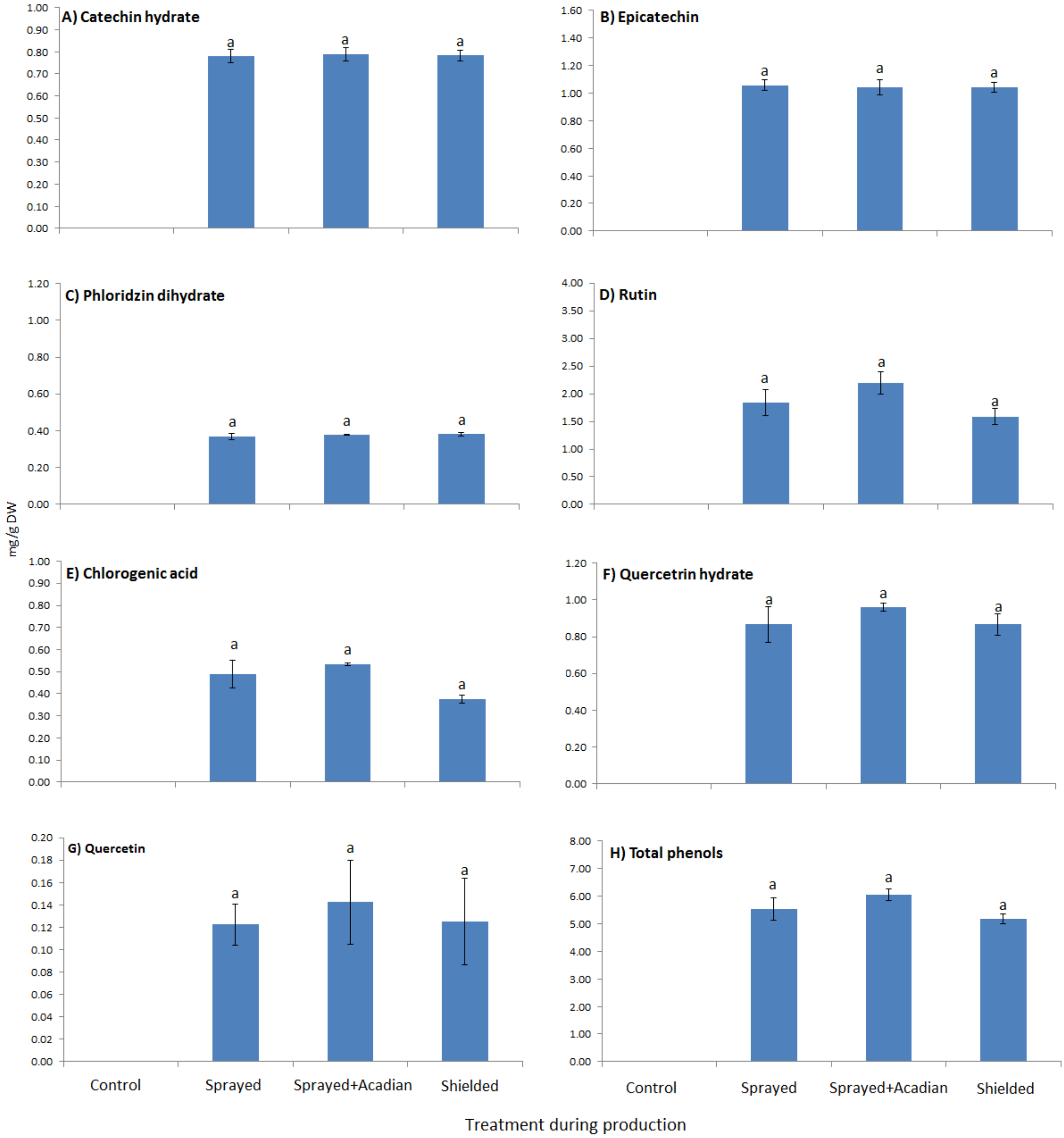


Figure A2. Content of individual phenols and total phenols in apple peel cultivar 'Rubens' grown under different practices in 2014. Values are means ± SE for the three field replicates. Means followed by different letters are significant different according to Tukey's test at p=0.05. Total phenols are the sum of the 7 phenols.

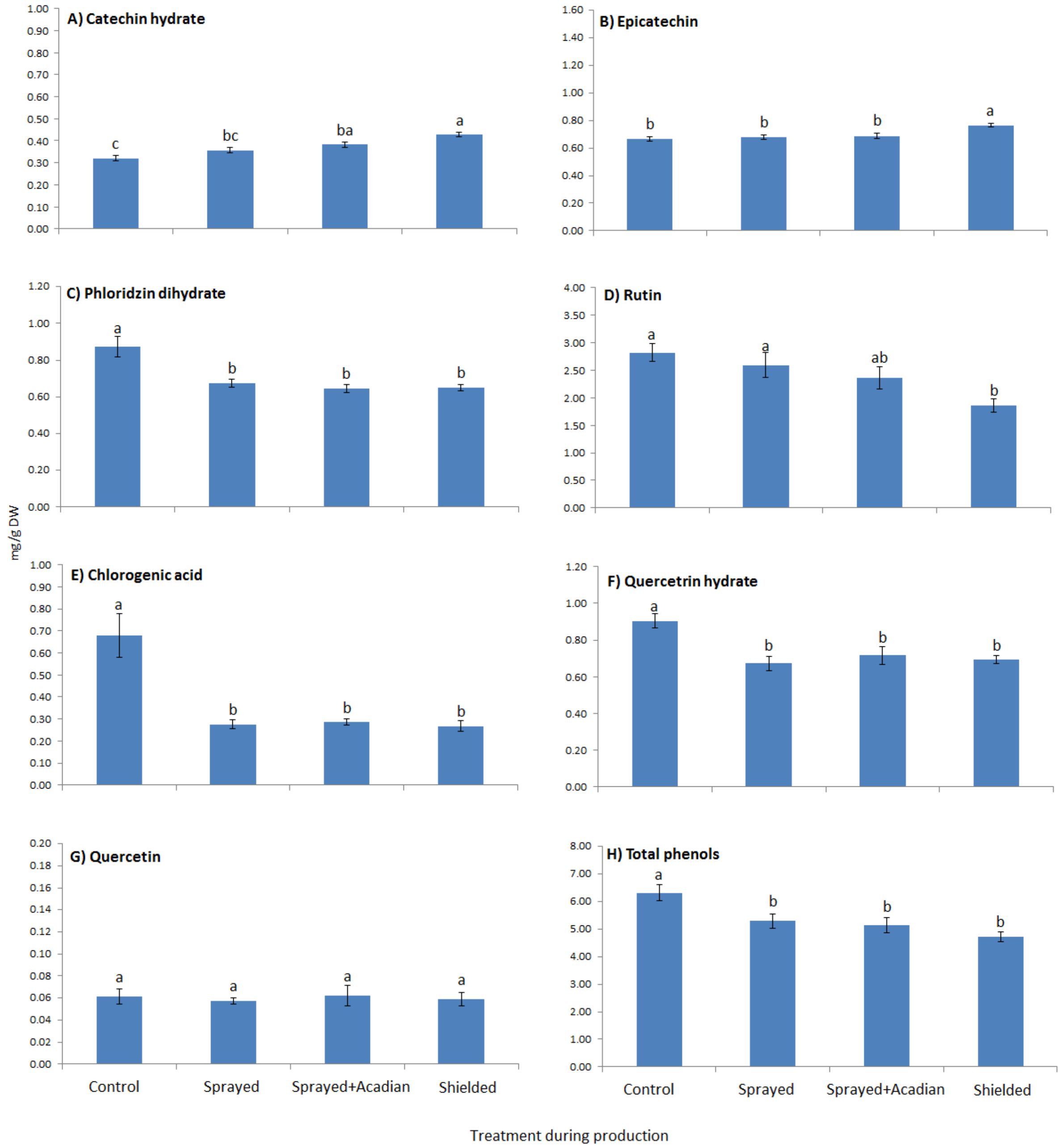


Figure A3. Content of individual phenols and total phenols in apple peel cultivar 'Santana' grown under different practices in 2014. Values are means ± SE for the three field replicates. Means followed by different letters are significant different according to Tukey's test at p=0.05. Total phenols are the sum of the 7 phenols.

Appendix 6

R-codes used

An example is shown for (Red) Elstar dataset on quality

```
#Load dataset into R from csv-file
Elstar <- read.csv("C:/Users/Anna/Desktop/ElstarQuality.csv", sep=";")
#Check for normality for one parameter
(Elstar, hist (weight), probability = TRUE) or (Elstar, hist (log(weight)), probability = TRUE)
#Check for variance homogeneity for the selected parameter
bartlett.test(weight~treatment,Elstar)
#Find means for one selected parameter for each treatment
tapply(Elstar$weight, Elstar$Treatment, mean)
#One-way Anova model
aov.ex1 = aov(weight~treatment)
summary(aov.ex1)
#Post-hoc Tukey-test
TukeyHSD(aov(weight~treatment), conf.level=.95)
plot(TukeyHSD(aov(weight~treatment), conf.level=.95))
```

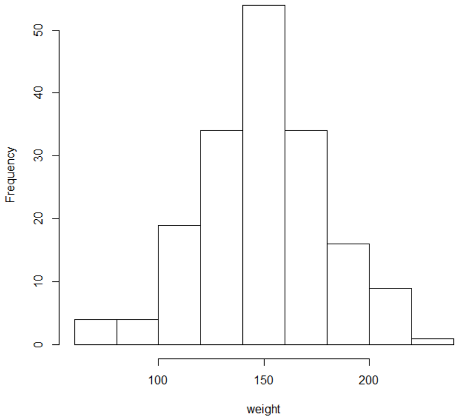
Appendix 7

Example of R-outputs

#Mean for each treatment

```
> tapply(Elstar$weight, Elstar$treatment, mean)
       1        2        3        4 
120.8250 162.3556 156.4000 158.0000 
```

#Check for normality



#Check for variance homogeneity

```
> bartlett.test(weight~treatment,Elstar)

 Bartlett test of homogeneity of variances

data:  weight by treatment
Bartlett's K-squared = 4.3417, df = 3, p-value = 0.2268
```

#Result of Anova analysis

```
> a1 <- aov(Elstar$weight ~ Elstar$treatment)
> summary(a1)
                  Df Sum Sq Mean Sq   F value Pr(>F)    
Elstar$treatment   3  213.4   71.14 3.497e+30 <2e-16 ***
Residuals        171    0.0    0.00                     
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

#Tukey test

```
> TukeyHSD(aov(Elstar$weight~Elstar$treatment), conf.level=.95)
  Tukey multiple comparisons of means
    95% family-wise confidence level

Fit: aov(formula = Elstar$weight ~ Elstar$treatment)

$`Elstar$treatment`
         diff       lwr       upr     p adj
2-1 41.530556  27.04605 56.015063 0.0000000
3-1 35.575000  21.09049 50.059508 0.0000000
4-1 37.175000  22.69049 51.659508 0.0000000
3-2 -5.955556 -20.00759  8.096481 0.6902653
4-2 -4.355556 -18.40759  9.696481 0.8523948
4-3  1.600000 -12.45204 15.652037 0.9910033
```