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IN NATURAL SCIENCE SPECIALIZING IN BIOLOGY

Preharvest Conditions Affecting Apple Quality, Antioxidant Responses and Susceptibility to the Infection by Grey mould (*Botrytis cinerea*)

Author:
Tuyet T. A. Bui

Main Supervisor:
Assoc. Prof. Mikael Molin

Assistant Supervisor:
Prof. Björn Berg

Examiner:
Prof. Malte Hermansson

UNIVERSITY OF GOTHENBURG

Department of Chemistry and Molecular Biology
University of Gothenburg

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Tuyet Bui

Cover: Apples flowering, photo: Tuyet Bui

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Department of Chemistry and Molecular Biology
University of Gothenburg
SE-405 30, Gothenborg, Sweden

I dedicate this work to my loving parents and my brother

Abstract

Apple fruits are rich in vitamin C and other antioxidants, which are beneficial for human health. During postharvest storage, there are losses as a result of diseases such as grey mould, caused by the fungus *Botrytis cinerea*. The present work has investigated how to obtain a high quality of apples, allowing long-term storage. One focus of this study was to investigate whether preharvest weather conditions affect apple quality, antioxidant responses and the susceptibility to infection by grey mould. We have tested the hypothesis that high levels of sunlight increase the quality of the fruit and its tolerance to grey mould. To this end, we examined the patterns of several antioxidants, including enzymes, in apple fruit in fending off attack from grey mould. The results show that preharvest exposure to high levels of sunlight can reduce the susceptibility of apples to postharvest disease. The susceptibility of apples also depends on the apple cultivar tested. 'Braeburn' was found to be more susceptible than 'Golden Delicious'. Further studies focusing on 'Braeburn' confirmed a strong effect of sunlight on both quality and susceptibility. In addition, high levels of protein and phenolic compounds were positively associated with the tolerance of apple fruits to grey mould infection. A field study in Sweden following eight orchards growing the cultivar 'Ingrid Marie' over three years shows that the quality of apples and the development of disease varied strongly among years of harvest and with the orchard's location. Preharvest weather conditions strongly affected the growth and development of apples as well as their quality, among which high humidity and high rainfall during flowering and fruit set and low temperature during maturity were the most influential on apple quality and the susceptibility of fruit to infection by grey mould. Knowledge of such crucial factors may guide apple growers to interventions aiming at improving apple quality and postharvest storage.

Keywords: *Malus x domestica*, grey mould, preharvest weather, apples' quality, antioxidants, harvest year

Author's address: Tuyet Bui, Department of Chemistry and Molecular Biology, University of Gothenburg (GU), P.O. Box 462, SE-405 30 Gothenburg, Sweden.
E-mail: tuyet.bui@gu.se, tuyetbui005@gmail.com

Sammanfattning Abstract in Swedish

Äpplen är rika på C-vitamin och andra antioxidanter, vilka är gynnsamma för vår hälsa. Under lagring efter skörd sker förluster, som resultat av infektioner exempelvis av gråmögel, vilken orsakas av svampen *Botrytis cinerea*. I det föreliggande arbetet undersöks hur en så hög kvalitet kan erhållas på äpplen att de kan långtidslagras. Ett fokus för detta arbete var att bestämma om en hög exponering för solljus före skörd ökar fruktens kvalitet och dess motståndskraft mot gråmögel. Vi har också undersökt flera antioxidanters, bland annat enzymers roller, vad gäller att avvärja angrepp av patogener efter skörd. Våra resultat visar att hög exponering för solljus minskar äpplens känslighet för angrepp efter skörd. Emellertid berodde känsligheten hos äpplen också på vilken sort, som vi undersökte. Sålunda var ’Braeburn’ mera mottaglig än ’Golden Delicious’. Fortsatta studier, som fokuserade på ’Braeburn’ bekräftade en stark effekt av solljus på både kvalitet och mottaglighet. Vi fann att höga halter av protein och fenoliska ämnen korrelerade positivt med motståndskraften mot gråmögel. I en treårig fältstudie i Sverige där åtta fruktodlingar undersöktes fann vi att både äpplenas kvalitet och utvecklingen av gråmögel varierade starkt mellan skördeår och fruktodlingens läge. Även hos lagrade äpplen varierade kvalitet och mottaglighet för gråmögel mellan skördeår och fruktodling. Vidare påverkade olika väderförhållanden under växtsäsongen, speciellt luftfuktigheten och mängden regn under blomningen och fruktsättningen samt temperaturen och luftfuktigheten under den slutliga mognaden starkt äpplenas kvalitet och deras motståndskraft mot gråmögel vid lagringen. Kunskap om sådana nyckelfaktorer ger äppelodlare möjligheten att förbättra äpplenas kvalitet och hållbarhet vid lagring.

List of Papers

Paper I **B.T.A. Tuyet**, T. Vanwalleghem, B. Vorstemans, P. Creemers, M. Hertog, B. Nicolaï, J. Keulemans and M.W. Davey*. (2012). **"**Cross-Tolerance and Antioxidant Metabolism as Determinants of the Resistance of Apple Fruit to Postharvest *Botrytis* Decay". Proc. XXVIIIth IHC-IS on Postharvest Technology in the Global Market Eds.: M.I. Cantwell and D.P.F. Almeida. *Acta Hort. 934, ISHS 2012, 319-326. DOI:10.17660/ActaHortic.2012.934.40.*

Paper II **Bui, T.A.T.,** Wright*, S.A.I., Falk, A.B., Vanwalleghem, T., Van Hemelrijck, W., Hertog, M.L.A.T.M., Keulemans, J., Davey, M.W. (2019). "*Botrytis cinerea* differentially induces postharvest antioxidant responses in 'Braeburn' and 'Golden Delicious' apple fruit". *J. Sci. Food Agric., Vol. 99(13): 5662-5670. DOI:10.1002/jsfa.9827.*

Paper III **Bui*, T.A.T.,** Lönn, M., Berg, B., Molin, M. 2020. "Higher levels of protein and phenolics in 'Braeburn' apples correlate with fruit tolerance to grey mould". *Revised Manuscript*.

Paper IV **Bui*, T.A.T.,** Lönn, M., Berg, B., Molin, M., Wright, S.A.I. "Varying susceptibility of 'Ingrid Marie' apples to grey mould infection among years and orchards in southern Sweden". *Manuscript.*

Paper V **Bui*, T.A.T.,** Stridh, H., Berg, B., Molin, M. "Influence of weather conditions on the quality of 'Ingrid Marie' apples and their susceptibility to grey mould infection". *Manuscript submitted.*

* Corresponding author.

Author's contribution to the papers

The contribution of Tuyet Bui to the papers included in this thesis is as follows:

Paper I	All the experimental work, collection of data, all the statistical analysis.
Paper II	All the experimental work, collection of data, part of the statistical analysis, interpretation of data as well as writing a draft manuscript.
Paper III	All the experimental work, collection of data, part of the statistical analysis, interpretation of data as well as writing a draft manuscript.
Paper IV	Conceptualisation and design of the study, all the experimental work, collection and interpretation of data, as well as writing a draft manuscript.
Paper V	Conceptualisation and design of the study, all the experimental work, collection and interpretation of data, as well as writing a draft manuscript.

Contents

List of abbreviations and terms

- APX: Ascorbate peroxidase
- AsA: Ascorbic acid - Vitamin C (L-ascorbic acid)
- *B. cinerea*: *Botrytis cinerea*
- 'Br': 'Braeburn'
- CA: Controlled atmosphere
- CAT: Catalase
- cm: Centimetre
- CO_2: Carbon dioxide
- °C: Degree Celsius
- DHA: Dehydroascorbate
- DHAR: Dehydroascorbate reductase
- DPI: Day post inoculation
- 'GD': 'Golden Delicious'
- GSH: Glutathione
- GSSG: Oxidized glutathione
- GR: Glutathione reductase
- H_2O_2: Hydrogen peroxide
- MDHA: Monodehydroascorbate
- MDHAR: Monodehydroascorbate reductase
- MH: Mean humidity
- mL: Milliliter
- µL: Microliter
- mm: Millimetre
- MT: Mean temperature
- POX: Flavonoid peroxidase
- $O_2^{\cdot -}$: Superoxide anion
- ORAC: Oxygen radical absorbance capacity
- SOD: Superoxide dismutase
- SS: Sum of sunlight
- RH: Relative humidity
- ROS: Reactive oxygen species
- RS: Sum of rainfall

Aims

Main aims

1/ To determine and understand mechanisms involved in the susceptibility of apple fruit to grey mould (caused by *Botrytis cinerea)* by studying the antioxidant responses.

2/ To test the hypothesis that pre-harvest weather conditions can influence both the quality and the resistance of apples to grey mould infection during postharvest storage.

Specific goals

1/ To find and determine a test system for investigating the influence of antioxidant responses on apple postharvest resistance to pathogen attack by comparing two apple cultivars ('Braeburn' and 'Golden Delicious') infected by grey mould. **(Papers I and II).**

2/ To determine in detail how different antioxidants in 'Braeburn' apples increase fruit tolerance to infection by grey mould. **(Paper III).**

3/ To determine the quality of 'Ingrid Marie' apples and their susceptibility to grey mould infection in 8 different Swedish orchards and among 3 years of harvest. **(Paper IV).**

4/ To determine the effects of pre-harvest weather conditions on the quality of apple fruits and their susceptibility to grey mould infection. **(Paper V).**

Research questions

- How do apple antioxidants respond to infection by grey mould?
- How does variation in weather conditions influence the quality of apple fruits and their susceptibility to grey mould infection?

1. Introduction

1.1. Apple Production, Quality and Harvest

1.1.1. Apple Production

Apples (*Malus x domestica* Borkh.) is one of the most popular fruits and is consumed around the world as an excellent source of vitamins and minerals. Apples are the fourth most important fruit worldwide after citrus fruits, grapes and bananas with global production reaching 84.6 million tonnes in 2014 (Musacchi and Serra, 2018). Apples have a high content of water (>80%) but also constitute a rich nutritional food, with high levels of e.g. vitamin C (2.3-31.1 mg/100 g dry mass (DM), organic acids (0.2-0.8%), minerals (= ash 0.34%-1.23%) as well as dietary fibres (≈2-3% and pectin < 50% of apple fibres). Apples have high contents of antioxidants (≈20-25%) as well as of fiber and potassium (≈10-30%) (Musacchi and Serra, 2018). 'An apple a day keeps the doctor away' is a well-known statement about the health benefits of apples. Apples help people become resistant to many diseases such as Parkinson's, cataracts, Alzheimer's, gallstones, and even certain cancers (https://www.worldatlas.com/articles/top-apple-producing-countries-in-the-world.html).

Apples grow in temperate, subtropical and tropical regions wordwide. The original apple fruit came from Central Asia and is now cultived all over the world. The global apple production reached 83.1 million tonnes in 2017, of which the highest share came from China with 41.4 million tonnes. The European Union contributed with 9.6 million tonnes and the USA with 5.2 million tonnes (https://en.wikipedia.org/wiki/List_of_countries_by_apple_production). Apple production in Sweden covers about 1660 hectares and the total annual yield was 27 000 - 28 000 tonnes in 2017 of which almost 90% were cultivated in the province Scania, southernmost Sweden (the Swedish Agricultural Agency, October 24, 2018).

The traditional way to produce apples still continues in some areas, but in many countries commercial growers are adopting the best international practices. Globally, growers continue to produce their region's traditional cultivars while at the same time new cultivars are being introduced. For example, in the 1950s and 1960s 'Red Delicious' and 'Golden Delicious' became popular in the USA and in the 1970s and 1980s 'Granny Smith' became popular in the Northern hemisphere and in the 1980s and 1990s 'Jonagold', 'Gala', 'Fuji' and 'Braeburn' arrived. These apple cultivars were selected for long-term storage, since apples are transported over long distances. Nowadays, more than 10 thousand apples cultivars are recorded in the European Apple Inventory (Musacchi and Serra, 2018). Today, in 2020 'Ingrid Marie' and 'Aroma', 'Jonagold',

‘Gloster’, ‘Elstar’, ‘Alice’ and ‘Katja’ are the most common cultivars in Sweden (Ferree and Warrington, 2003; Tahir, 2006).

1.1.2. Apple Quality

Apples attract consumers by their appearance (colour, size and shape) and by taste, texture, aroma, nutritional value, sweetness and acidity (Vanoli and Buccheri, 2012). Firmness level, sugar content, starch index and weight of fruit are the most important quality properties that are directly connected with the consumer’s decisions on buying and eating fresh fruit (Lu, 2004; Mussachi and Serra, 2018). Firmness, sugar content and starch index are three parameters monitored (Kingston, 1992) to assess apple maturity and to determine the optimal harvest dates (Qing et al., 2007). Firmness is made as a practical test and as an indicator of apple quality, which is determined at harvest (Ornelas-Paz et al., 2018). Sweetness is an internal fruit quality trait, that is crucial for consumer acceptance, and is determined chemically by measuring the total sugar content. Sugar content for each apple cultivar may fluctuate among years, among orchards and between picking dates (Iwanami, 2011). Starch starts to be degraded and converted into sugar during the ripening process (Musacchi and Serra, 2018; Doerflinger et al., 2015; Mesa et al., 2016; Tromp, 2005). Numerous environmental factors and growing conditions may influence starch accumulation and degradation; there is a tendency for higher starch index at harvest with increased latitude of the orchard (Watkins et al., 1982; Smith et al., 2012).

Environmental factors such as temperature, rainfall, sunlight, humidity, nutrition, conditions in the orchards, and orchard management, all may have effects on growth, development and quality of apples (Musacchi and Serra, 2018; Tromp, 2005; Tahir, 2006), as well as on susceptibility of fruit to infection and the development of disease (Dutot et al., 2013). Preharvest conditions during the cultivation of apples have an impact on the levels of phytochemicals and these can relate to the postharvest resistance of apple fruit to pathogens (Davey et al., 2007; Bui et al., 2019).

1.1.3. Apple Harvest

Most commercially grown apples are harvested before they are ripe and stored at low temperatures for several months in a controlled atmosphere (Davey et al., 2007; Nilsson and Gustavsson, 2007). Since apples have a high market value, the ability to maintain their long-term quality after harvest is of economic importance to growers (Tahir, 2006; Tahir et al., 2009).

After harvest, the fruit continues to remain a living organism with uptake of oxygen and release of carbon dioxide. Apples no longer receive nutrients from the tree, but are still respiring, using their nutrients during storage, thereby changing their sugar, starch and acid content (Paliyathe et

al., 2008). Eventually, the tissue breaks down, the fruit becomes mealy, develops an 'off' flavour, and the loss of water makes the fruit soft. Good postharvest storage conditions preserve the quality of the fruit by allowing it to ripen slowly and by reducing water loss.

During long-term storage of apples in cold rooms, postharvest diseases may appear, and losses by the decay of fruit may amount to about 50-60% in storage bins prior to packing (Alkan and Fortes, 2015). There are more than 90 different phytopathogens infecting apples and the most important are *Botrytis*, *Penicillium*, *Gloesporium* and *Phytophthora spp*. (Davey et al., 2007; Dutot et al., 2013; Tahir, 2006; Tahir et al., 2009). The infections may originate from the field or from the storage area, which depending on both preharvest and postharvest factors (Davey et al., 2007).

Botrytis cinerea is a necrotrophic fungus responsible for both pre- and postharvest diseases, collectively known as grey mould, in over 1400 plant species, including cultivated apples (reviewed by Fillinger and Elad, 2016; Elad et al., 2016; Romanazzi and Feliziani, 2014). The annual global economic losses due to *B. cinerea* are estimated at US$10-100 billion (Romanazzi and Feliziani, 2014). The interaction between *B. cinerea* and the host plant it infects has given rise to intense research worldwide (Fillinger and Elad, 2016; Elad et al., 2016). *B. cinerea* often causes latent infections of immature apple fruits that are still attached to the tree, with grey mould becoming apparent as postharvest decay (Elad et al., 2016; Siegmund and Viefhues, 2016; van Kan, 2006). During harvest and cold storage, *B. cinerea* may infect mature apples and spread among stored fruits (Sholberg and Conway, 2004).

Most postharvest pathogens attack fruit through wounds during harvest, shipping or handling. Therefore, it is very important to manage and minimize the postharvest disease by very careful handling of fruit during harvest and transport and the provision of adequate tree nutrition (Grove et al., 2003). Worldwide, apple producers are requested to produce high-quality apples for consumption as fresh fruit. Further, apples should be tolerant to pathogen attack both at harvest and after long-term storage and postharvest transport (McCluskey et al., 2007; Greene et al., 2014; Juhnevica-Radenkova et al., 2018). Postharvest storage is becoming increasingly important and storage procedures as well as of treatment of fruit before harvest require improved understanding and development (Paliyath et al., 2008). Reseach on pre- and postharvest factors are very important to increase fruit quality and improve fruit storage (Tahir, 2006; Tahir et al., 2009).

1.2. Apple Growth and Development

1.2.1. Apple Physiological Growth Stages (BBCH-scale)

The BBCH-scale is used to identify the phenological development stages of plants (Meier et al., 1994). BBCH-scales have been developed for a range of crop species where similar growth stages of each plant are given the same code.

There are 8 main growth stages in pome fruit including apple

Growth stage 0: Sprouting/Bud development

Growth stage 1: Leaf development

Growth stage 3: Shoot development

Growth stage 5: Inflorescence emergence

Growth stage 6: Flowering

Growth stage 7: Development of fruit

Growth stage 8: Maturity of fruit and seed

Growth stage 9: Senescence, beginning of dormancy

1.2.2. Phenological Stages of Apple Flower Buds

There are 9 main stages of apple flower buds.

a) **Dormant -** Code 00: Winter - Leaf buds and the thicker inflorescence buds are closed and covered by dark brown scales.

b) Silver tip - Code 01'/51: March - Beginning of leaf bud swelling: buds visibly swollen, bud scales elongated, with light coloured patches.

- Code 07'/53: March - Beginning of bud break: first green leaf tips just visible.

c) Green tip - Code 10'/5: April - Mouse-ear stage: Green leaf tips 10 mm above the bud scales; first leaves separating.

d) Half-ich green - Code 31: End of April - Beginning of shoot growth: axes of developing shoots visible.

e) Tight Cluster - Code 55: End of April - Flower buds visible (still closed).

g) Pink - Code 57: Beginning of May - Pink bud stage: flower petals elongating, sepals slightly open and petal just visible.

h) Bloom - Code 59: 5-10 May - Most flowers with petals forming a hollow ball (Bloom).

- Code 61: 5-20 May: Beginning of flowering, about 10% flowers open (Bloom).

i) Petal fall - Code 71: First half of June - Fruit size up 10 mm, fruit fall after flowering

k) Fruit set - Code 75: First half of August - Fruit about half final size - Harvest starts around the 10th of August, depending on varieties and the latitude of the orchard.

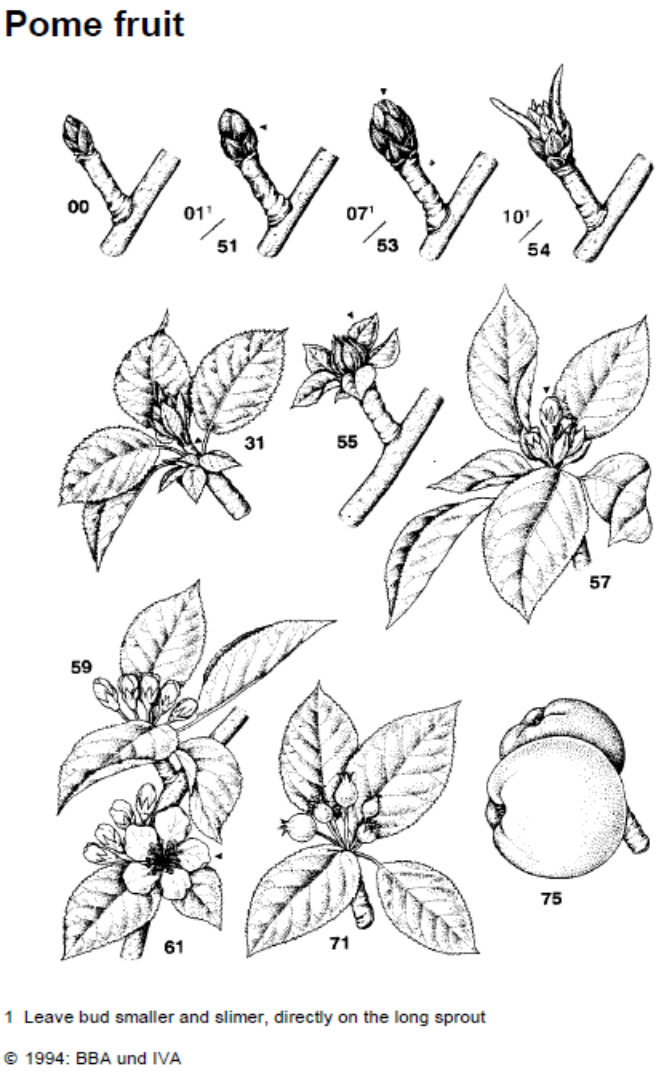

Figure 1. Phenological stages of apples' flower buds

1.2.3. Flower-Bud Formation

Flower-bud development occurs during the period of the winter dormancy until the bloom time (May-June). The flower formation process starts in early spring, February or March. In apple, flower buds are on spurs or short shoots, which may be damaged by spring frost. Buds increase in size by 20-25% during December and January, 120-150% between mid-February and mid-March. At the end of April, a bud contains 6 leaves and flower formation will not occur before the first half of August. 30% of full sunlight is necessary for flower-bud formation in apple. A heavy fruit load and a strongly reduced flower-bud production in one year has an influence on flower-bud formation in the following year (Tromp, 2005).

1.2.4. Flowering, Pollination and Fruit Set

Flowering starts in the meristem to lay down flowers, which occurs in May or June (Tromp, 2005). During early spring when the air and soil temperatures both increase to around 5^{o}C, the development of flower-buds is finished by cell division and expansion in apples. During flowering, temperature has an important role for the germination rate of pollen, while pollen vectors (such as insects or wind) are needed for a good pollination of the orchard's trees. The honeybee is a main pollination insect for apple trees in the orchard during bloom. However, honeybees do not fly at low temperatures. After flowering, the sexual part of flowers, the pollen grains and the egg apparatus are formed (Wertheim and Schmidt, 2005).

In apple, late flowering occurs under conditions of higher chilling and post-dormant heat. In winter, cold causes a delay of foliation, a reduced, prolonged flowering and reduced fruit production, size and quality. In early spring temperature has a strong influence on flowering. In addition, weather conditions during the previous season affect the time of bloom. Warm weather early in the summer and autumn delays bud burst and blossoming the following spring, while warm weather with high temperature later in the summer has a positive effect on bud burst. Flowering time is an important stage and is strongly influenced by weather; warm weather may shorten the length of the flowering period and cold weather may prolong the length of the flowering. Late flowering increases the risk of fire-blight attacks at high temperatures because infection via the flowers is stimulated in warm weather. The flowering period begins with the date of the first open flowers and ends when 90% or 100% of the flowers are worn. Full bloom occurs with 80% open flowers. Fruit set occurs in May-June after fertilization and the fruitlets remain on the tree may be loss after June drop (Wertheim and Schmidt, 2005).

1.2.5. Fruit Growth and Development

Petals fall and undeveloped fruitlets are shed within a few days after bloom is over. A first wave of fruit drop and abscission occurs a few days later by growth of the pistils and the vacuoles in the remaining flowers increase strongly in volume. A second wave of fruit shedding occurs 4 to 6 weeks later, namely in the June drop. If the temperature drops during this period fruit size and quality at harvest are influenced. There are two main phases of cell fruit development, namely cell division and cell enlargement. During flowering, cell-division activity in the ovary is very low and there is no cell expansion. A period of rapid cell division starts after fertilization and continues for 3 to 6 weeks. Cell enlargement has already commenced at around the time of fertilization and starts after cell division has stopped. An important point is that the first few weeks after full bloom (approximately during the period of cell division) are of paramount significance for fruit maturation

at the end of the season. Enhanced cell division increases cell numbers. Cell number and cell size (cell volume) determine fruit size, fruit quality and storage behaviour.

Apple Fruit Development

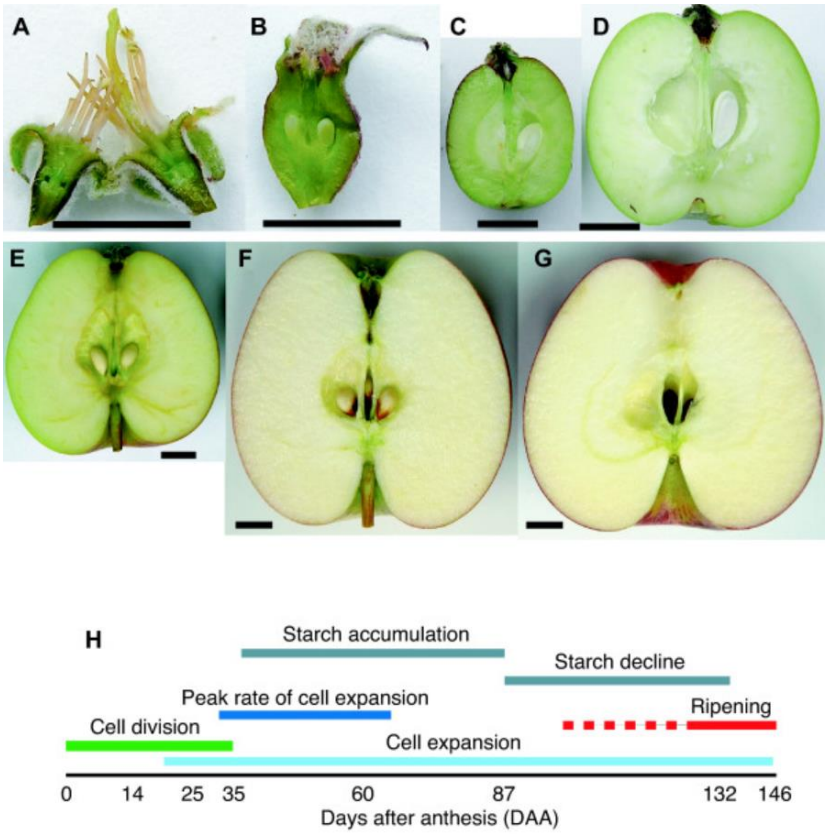

Figure 2. Apple fruit development (http://www.geochembio.com/IMG/apple-fruit-development.jpg)

A: Petal fall: after bloom finished
B-C: Fruit and seed set: after fertilization/ full bloom - June drop - cell division phase
D-E: Fruit growth: after anthesis - cell expansion phase
F: Fruit development: cell expansion phase
G: Fruit maturity and ripening: end of cell expansion phase

Apple fruits grow in two phases; an early exponential cell division phase that occurs 1 to 4 weeks after full bloom is followed by a cell expansion phase during the remaining part of the season (Bollard, 1970; Lakso et al., 1995). The cell division phase is a period when low temperatures occur in the apple-growing areas of the world (Corelli-Grappadelli and Lakso, 2004). Smaller fruit are produced from trees growing under low temperature conditions, especially during the first 40 days after full bloom (Warrington et al., 1999).

1.3. Effect of Preharvest Weather Conditions

During the growth season, environmental and agronomic factors (such as temperature, rainfall, sunlight, humidity, orchard design, training system and pruning) strongly affect the development and quality of apple fruits (Sharma et al., 2008; Musacchi and Serra, 2018). Differences in growing seasons and locations result in a high variability in fruit quality. Even different locations of trees in the same orchard and different positions of fruit within the same tree influence their quality (Minas et al., 2018; Bui et al., Paper IV). Preharvest weather conditions influence fruit in each of the different periods of growth and development. Preharvest exposure to high light intensity and high temperatures can have an influence on many fruit qualities (Lee and Kader, 2000; Cisneror-Zevallos, 2003; Woolf and Ferguson, 2000). It is generally accepted that preharvest exposure of apple to stress, for instance from intense sunlight or high temperatures, increases the levels of antioxidants in fruit (Solovchenko and Schmitz-Eiberger, 2003; Zupan et al., 2014). Indeed, repeated exposure of fruits to intensive sunlight and high temperatures results in adaptive mechanisms and the acquisition of tolerance to subsequent elevated and decreased ambient temperatures (Bramlage and Watkins, 1994; Woolf et al., 1999; Woolf et al., 2003). Such heat stress treatments can also be used to reduce postharvest disease incidence via cross-tolerance (Terry and Joyce, 2004). Since fruits have a high market value, the quality after harvest is an important matter to growers. Environmental conditions, cultivars, cultural practices, susceptibility to pests and diseases, time of harvest, and postharvest conditions all determine the quality of fruits and vegetables (Sharma et al., 2008).

1.3.1. Temperature

Temperature is an important local climatic factor that may influence fruit growth and development. Temperature determines the formation of flower buds, fruit set, fruit drop and final fruit at harvest. Temperature has effects on flower development. High temperatures in February, March and April have a negative effect for several apple cultivars (Tromp and Werthiem, 2005). During May to June temperatures are around 14 ^{o}C to 23 ^{o}C and have a strong influence on flower-bud formation, and the date of flowering. Low temperatures decrease the number of flowers the following year, while high temperatures increase the number of flowers.

The temperature in the first week after bloom has the strongest influence on the degree of flower-bud formation. Fruit set is determined by post-bloom temperature and is better at high than at low temperatures. High temperature during late June to early July doubled the number of fruits.

Fruit set decreased when temperatures during bloom were around 11 ^{o}C to 19 ^{o}C and when pollination was delayed (Tromp, 2005).

High temperature stimulates the metabolism of fruit at harvest and maturity. However, a short time before maturity fruits request low temperatures. Temperatures at harvest and maturity influence fruit quality attributes, such as size, firmness and colour. The temperature in the first part of season, ca 36 days after bloom (during period of cell division) is more important and has stronger influence on fruit size and maturity than the temperature in the later part of the season, namely between 60 and 90 days after bloom (during the period of cell expansion, Tromp and Wertheim, 2005).

1.3.2. Water Supply

Water relates to fruit growth and flowering by its effect on the hormonal balance within the tree. Low relative humidity increases flower-bud formation and reduces shoot growth (Tromp and Wertheim, 2005). Apple quality (such as firmness, soluble sugars and red colour) increases under water-stress conditions. Water has a higher influence on fruit size and on the quality of apples in the second part of the season (the period of cell expansion) than in the first half of season (the cell division phase). The trees' access to water affects the weight of the fruit at harvest (Tromp and Wertheim, 2005). Water is also important in russet formation, as this brown scarring tends to be produced under conditions of high humidity, frequent rain or dew. These conditions lead to apples developing a thin cuticle that is prone to cracking under high turgor conditions also known as russeting (Musacchi and Serra, 2018).

1.3.3. Sunlight

The intensity of solar radiation is important during flowering, while the photoperiod plays little or no role. Flowering progresses very well in trees in areas with high solar radiation and in the sun-exposed sections of the tree. Flowering is reduced when the light level is reduced. Cultivar crop load and light exposure are major factors determining the ultimate size of apple fruits. Solar radiation and temperature are also the most important factors affecting the time to maturation of fruit. Exposure to sunlight also increases red coloration and fruit sugar content because of its stimulatory effects on photosynthesis in the adjacent leaves. Fruits in the shaded part of the tree are generally smaller, greener and less mature (Dennis, 2003). The duration of light influences the partitioning of carbon between carbohydrate fractions, including sorbitol, sucrose, glucose, fructose and starch (Wang et al., 1997). Visible light, i.e. radiation in the 400 - 700 nm wave bands,

provides the driving force behind tree biomass production and the partitioning of resources such as nutrients, carbon and water into fruits.

Davey et al. (2007) and Bui et al. (2019, Paper II) found that apple tissues that have been exposed to high intensity sunlight accumulated higher levels of antioxidants and had an improved ability to resist pathogens. Sunlight stimulates the production of vitamin C, phenolics and antioxidants in apple peel, which makes the fruit less susceptible to infection by pathogens (Davey et al., 2007; Bui et al., 2019). In addition, light has been reported to prevent the infection of grey mould in plants (Jakopic et al., 2009; Canessa et al., 2013; Schumacher et al., 2017).

1.4. Postharvest Pathogen - Grey Mould (*Botrytis Cinerea*)

Botrytis cinerea, causing grey mould, is a necrotrophic pathogen that attacks more than 200 agricultural crop hosts globally. *B. cinerea* causes soft rotting of all aerial plant parts of vegetables, fruits and flowers after harvest to produce prolific grey conidiophores and conidia, which are typical of the disease grey mould. The fungus enters plant tissues at early stages of development and causes symptoms only after the fruits have ripened. *B. cinerea* causes a wide range of symptoms across plant organs and tissues that are difficult to generalize. Soft rots are accompanied by collapse and water soaking of parenchyma tissues, then by a rapid appearance of grey masses of conidia, which are the most typical symptoms, on leaves and on soft fruits. Grey mould is one of the major diseases of apple fruit and causes severe economic postharvest losses (Sholberf and Conway, 2004).

The infection process of grey mould is divided into the following stages: penetration of the host surface, killing of host tissue/primary lesion formation, lesion expansion/tissue maceration and sporulation (van Kan, 2006). *B. cinerea* is difficult to control and it attacks host plants via several modes. The fungus survives as mycelia and conidia and for long periods as sclerotia in crop debris. The mycelium survives in infected dead host tissues left by crop debris and inside some seeds as a primary inoculum. The dead tissues of plant hosts contain masses of mycelium that can produce conidia and initiate infections in crop canopy. Sclerotia develop in dying host tissues and represent an important survival mechanism for *B. cinerea* and are not readily apparent in all susceptible plant hosts. Sclerotia grow in early spring in temperate regions to produce conidiophores and multinucleate conidia, which are primary sources of inocula in crop hosts (Agrios, 2005).

1.5. Response of Host Plant Antioxidants to *B. cinerea*

The host plant's response to infection has been described in detail by Elad (2007). The collapse of epidermal cells is an early reaction in the successful penetration (Clark and Lorbeer, 1976). When a plant recognizes an attacking pathogen, one of the first reactions induced is an 'oxidative burst', during which rapid production of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) takes place. These highly reactive molecules, also known as Reactive Oxygen Species (ROS), prevent the spread of the pathogen to other parts of the plant by restricting fungal movement and reproduction (Torres et al., 2006). ROS play a central role in redox-dependent signalling, which regulates several processes of importance in plant-pathogen interactions, e.g. cell death (Dickman and Fluhr, 2013). H_2O_2 was detected at the interface of *B. cinerea* and host cells and was present in the plasmin space both in the host cell wall and on the outer surface of the host cell, and on outside of the fungal cell wall. H_2O_2 is produced in the host cell at the plasma membrane and diffuses through the host cell wall into the intercellular space (Prins et al., 2000b; Schouten et al., 2002). However, these defence reactions do not block *B. cinerea* infection, they only cause damage its hyphal structure (El-Ghaouth et al., 2004).

During host infection, *B. cinerea* contributes to programmed cell death as a part of its infection strategy (Shlezinger et al., 2011). The fungus, together with the host plant, produces ROS at the infection site (Tudzynski and Kokkelink, 2009). However, both fungus and plant produce antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), flavonoid peroxidase (POX) and ascorbate peroxidase (APX), during disease development (Heller and Tudzynski, 2011) that counter the effect of ROS. Antioxidant enzymes remove ROS by catalyzing antioxidant reactions. In the plant cells, SOD is in the first line of defence enzymes converting the superoxide radical ($O_2^{-\cdot}$) into H_2O_2. An excessive amount of H_2O_2 is toxic to cells and this compound is decomposed by CAT, POX and APX, of which CAT is able to convert H_2O_2 into water and oxygen, but neither APX nor POX (Larrigaudière et al., 2004). CAT metabolises H_2O_2 via iron-heme groups that are attached to the enzyme. Furthermore, POX detoxifies H_2O_2 by using flavonoids as a substrate whereas APX uses vitamin C as a reducing agent (Foyer and Noctor, 2011).

2. Methods

2. 1. Fruit Materials and Orchards Studied

Fruit Materials

Papers I, II and III: 'Braeburn' and 'Golden delicious' apples (*Malus x domestica* Borkh.) were harvested in the period of October 22 to 29 in 2008 (papers I and II), and in 2010, 'Braeburn' apples were harvested between October 22 and 29 at the experimental station Fruitteelt, Experimental Garden for Pome and Stone fruit, Sint-Truiden, Belgium (paper III), which is the optimal harvest period for long-term storage of 'Braeburn' apples as recommended by the Flanders Centre of Postharvest Technology (VCBT), Belgium. After picking, the healthy apple fruits were immediately transported and stored at VCBT under controlled atmospheric conditions; for 'Braeburn' that is 0.5 °C, 1-2 % O_2, 2 - 2.5 % CO_2, and 95 % relative humidity. Apples were stored until July next year, when inoculation was carried out at the Laboratory of Fruit Breeding and Biotechnology, Catholic University of Leuven (K. U. Leuven), Belgium.

Papers IV and V: In each of the years 2015, 2016 and 2017, 150-180 fruits (cv. Ingrid Marie) were harvested from each of the eight orchards in the province Scania, in southernmost Sweden. Sampling dates were those of the regular time for harvest, ranging from end of September to mid-October, and dependent on the maturity of the fruits in each year and orchard as judged by Äppelriket Österlen (an apple production company in south of Sweden). The apples sampled in 2015 were immediately transported to the pcfruit, a research institute in Belgium, and those collected in 2016 and 2017 to the University of Gävle, Sweden. The experiments were carried out using the same design in both laboratories. The harvested apples were stored in a cold room (~ 2ºC) until the measurements started.

Studied Orchards *(only for Paper IV and V)*

We collected apple fruit and weather data from eight orchards located in Sweden's southernmost province, Scania. General information about their locations is given below and in Figure 3.

Orchard No.1 (55.43ºN;13.07ºE), Orchard No.2 (55.48ºN; 13.99ºE), Orchard No.3 (55.66ºN; 14.21ºE), Orchard No.4 (55.71ºN; 14.19ºE), Orchard No.5 (55.72ºN; 14.10ºE), Orchard No.7 (56.16ºN; 14.46ºE), Orchard No.8 (55.72ºN; 13.10ºE) and Orchard No.9 (56.05ºN; 12.75ºE).

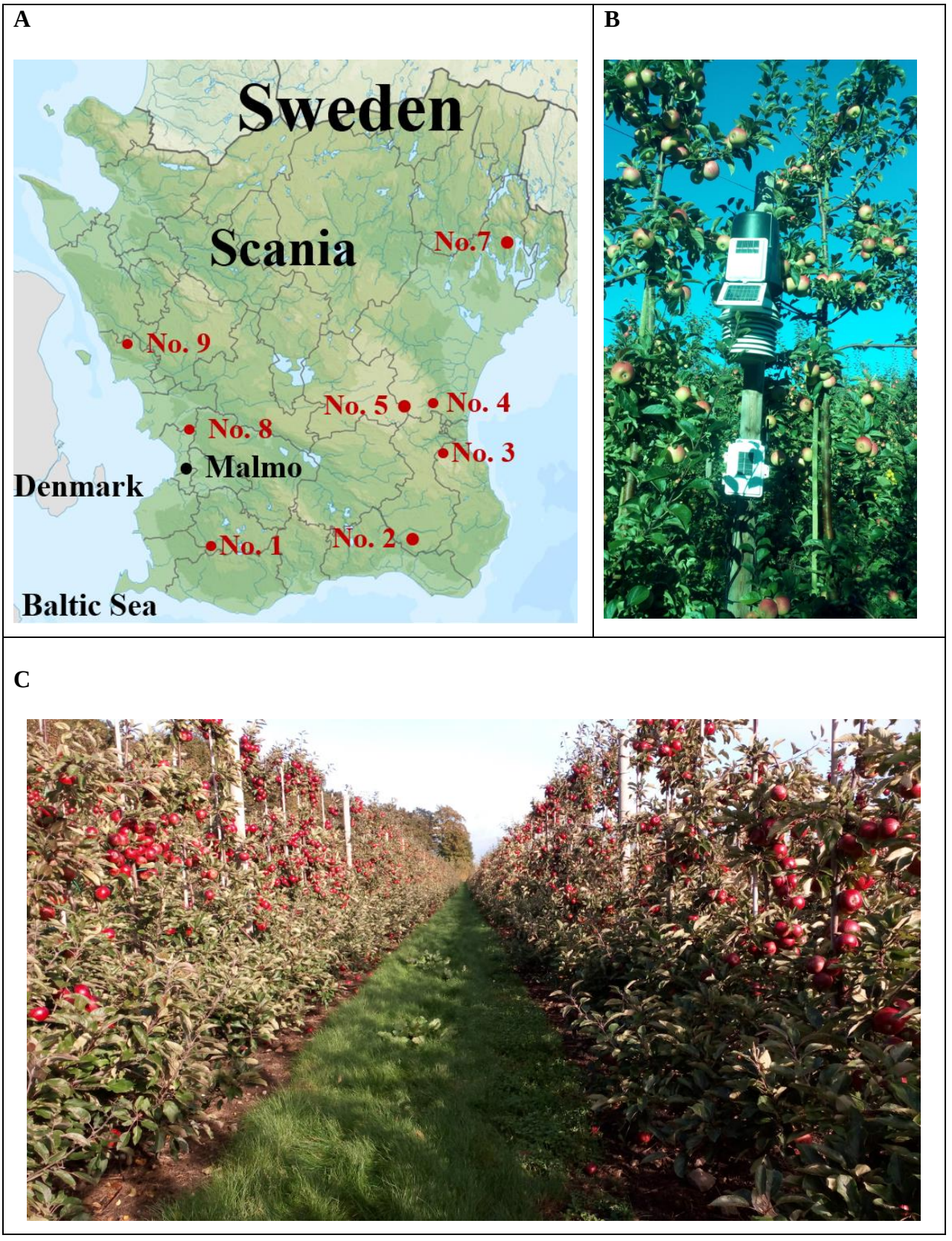

Figure 3. The location of eight orchards in the province Scania in southernmost Sweden (A). A David weather station in the orchard (B). Apple trees and fruits in the orchard (C).

2.2. Pathogen and Inoculation of Apples

2.2.1. Pathogen

Botrytis cinerea strain B05.10, a standard reference strain, was obtained from the Centre of Microbial and Plant Genetics (CMPG), K. U. Leuven. Cultivation and harvesting of *B. cinerea* spores was performed as described previously (Broekaert et al., 1990). Spore suspensions of a frozen stock of *B. cinerea* were transferred to a fungal culture medium, i.e., potato dextrose agar. After two weeks, at the time for inoculation, *B. cinerea* spores were collected from the agar plates and a spore suspension of 1.5 x 10^5 spores per mL distilled water was prepared. One day before the experiments, apples were rinsed in water with 10% of Chlorine for 5 minutes, washed with tap water 2 more times and dried overnight.

2.2.2. Fruit Inoculation

Apples were inoculated with *B. cinerea* by making 0.3 cm wide and 0.6 cm deep wounds on opposite sides of each individual fruit using a pipette tip of 10µL. Each apple was wounded midway between the calyx and the stem on both the sun exposed ('red') and the shaded ('green') sides of the fruit. Inoculation took place immediately after wounding. Of the 20 µL of *Botrytis* solution used on each side, 10 µL solution infected the peel tissue (0.3 cm deep) and 10 µL infected the flesh tissue (0.3 cm deep). After inoculation, the fruits were stored at 4 ^oC and 100 % relative humidity for 24 h by sealing them in plastic bags, before they were transferred to a constant-climate room (4 ^oC and 80% relative humidity) for the remaining time of the experiment (Fig. 4).

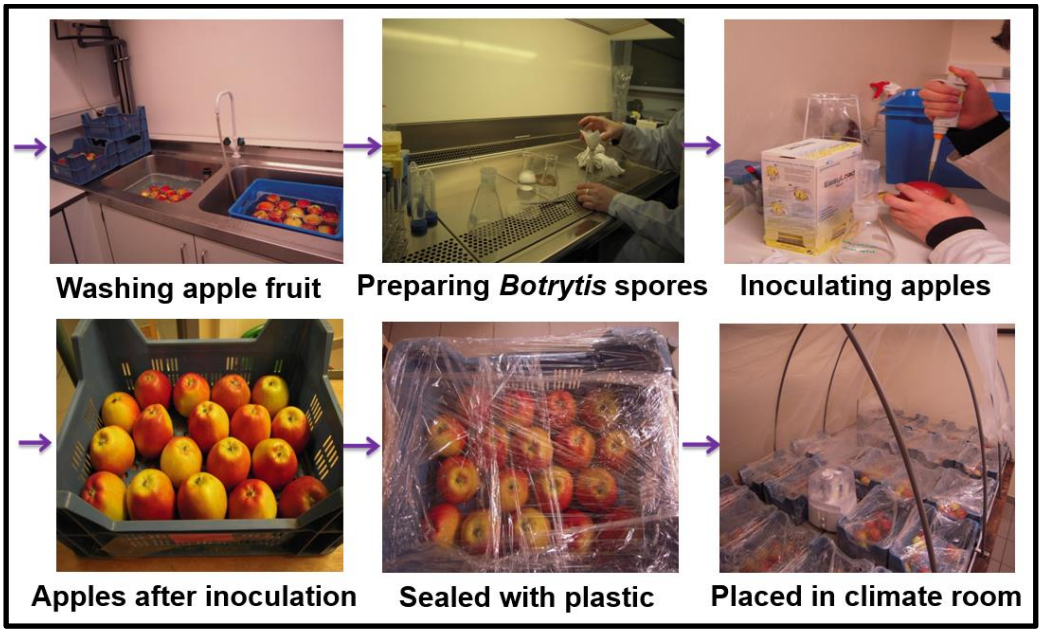

Figure 4. An outline of the steps in the procedure of inoculating apple fruit with a spore suspension of *B. cinerea* and studying the progress of grey mould disease over time.

Papers I, II and III: Three treatments were carried out: Control (neither wounded nor inoculated), Mock-inoculated (wounded and inoculated with 20 µL sterile water), and *B. cinerea*-inoculated (wounded and inoculated with 20 µL of a suspension containing of 1.5 x 10^5 spores/mL). We used 6 replicate fruits for each of the three treatments and sampling, with the exception that there were 10 fruits for the control treatment day 0.

Papers IV and V: Two treatments were carried out; Control (neither wounded nor inoculated) and *B. cinerea*-inoculated (wounded and inoculated with 20 µL of a suspension containing of 1.5 x 10^5 spores/mL). We used 5 or 10 replicate fruits for control treatment and 15 or 30 replicate fruits for *B. cinerea*-inoculated treatment.

2.3. Experimental Setups

2.3.1. Experimental Setup for Papers I, II and II

An overview to sampling and analytical procedures is given in Fig. 5.

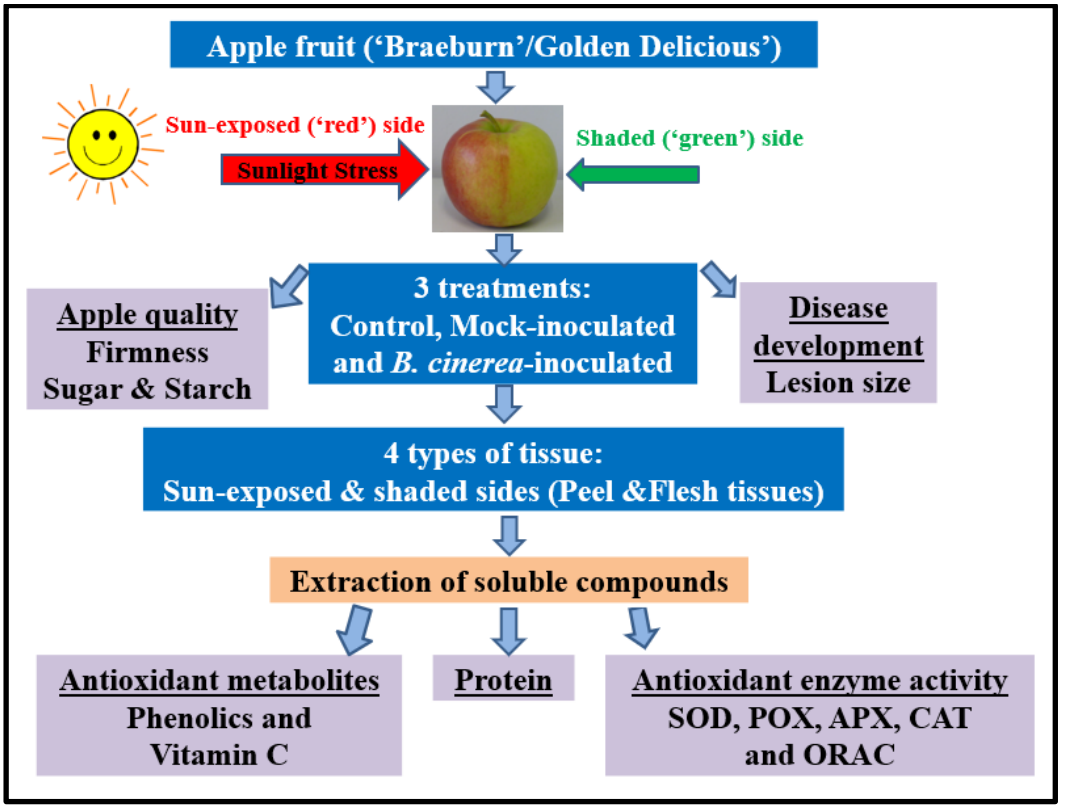

Figure 5. Experimental setup for project 1 (papers I, II and III). Flow chart and overview of sampling and analytical procedures. We extracted and analysed protein for the determination of antioxidant enzyme activities as well as antioxidant metabolites. We also determined apple quality by analyzing for firmness, sugar and starch index. Lesion size was measured on 5 and 14 days after inoculation with *B. cinerea* in papers I and II and on 1, 3, 6, 8 and 10 days after inoculation with *B. cinerea* for paper III.

Around 50 individual healthy apples of each cultivar viz. 'Braeburn' and 'Golden Delicious' were harvested in October 2008 and 100 healthy apples of 'Braeburn' were harvested in October 2010 from one orchard at Fruitteelt, Experimental Garden for Pome and Stone fruit, Sint-Truiden, Belgium. We inoculated *B. cinerea* in two apple cultivars 'Braeburn' and 'Golden Delicious' in year 2009 (papers I and II) and in 'Braeburn' in year 2011 (paper III).

Three treatments were carried out: Control, Mock-inoculated, and *B. cinerea*-inoculated. In papers I and II, we sampled, measured lesions size and antioxidant metabolism at three time points (0, 5 and 14 days after inoculated with *B. cinerea*) in both cultivars 'Braeburn' and 'Golden Delicious'. In paper III, we sampled, measured lesions size and antioxidant metabolism at six time points (0, 1, 3, 6, 8 and 10 days after inoculation with *B. cinerea*) in the cultivar 'Braeburn'. Tolerant and susceptible, refer to the responses of apple tissues to inoculation with *B. cinerea* without and with disease symptoms, respectively (Table 1, Figures 5 and 9).

2.3.2. Experimental Setup for Papers IV and V

The experimental setup for papers IV and V is outlined in Fig. 6. Eight orchards in the province of Scania provided 'Ingrid Marie' apples in the years of 2015, 2016 and 2017. Fruit in 2015 was collected without separating apples from different positions in the tree, while those picked in 2016 and 2017 were collected from two different positions (high and low) in the trees, thus obtaining an extra explanatory variable. For each of the three harvests we investigated apples immediately after harvest (0 month) and after storage for 1 and 3 months. At these time points ten apples were used for the determination of firmness and sugar content in the sun exposed and shaded sides; starch index was determined for the whole fruit. These values were used as untreated control values for the apples to be inoculated and incubated. Incubations were made using 40 apples, of which 30 were inoculated with *B. cinerea*, and ten were control apples. Disease development was measured on the sun exposed and shaded sides of the apples on days 3, 6 and 9 after inoculation.

Data for the two years 2016 and 2017 was analysed separately when exploring effects of position in tree. We investigated the effect of the position of fruits in the tree and related it to the quality of apples. We separated apples from two positions in the trees, namely 'high' (> 1.5 m above the ground) and 'low' (≤ 1.5 m above ground). The fruits were the same as those in the first data set with the exception that the replicate apples were divided as coming from the two positions. Thus, for each of the two positions, five apples were used to determine firmness, sugar content, and starch, while fifteen apples were used for inoculation with *B. cinerea* and five apples were used as controls.

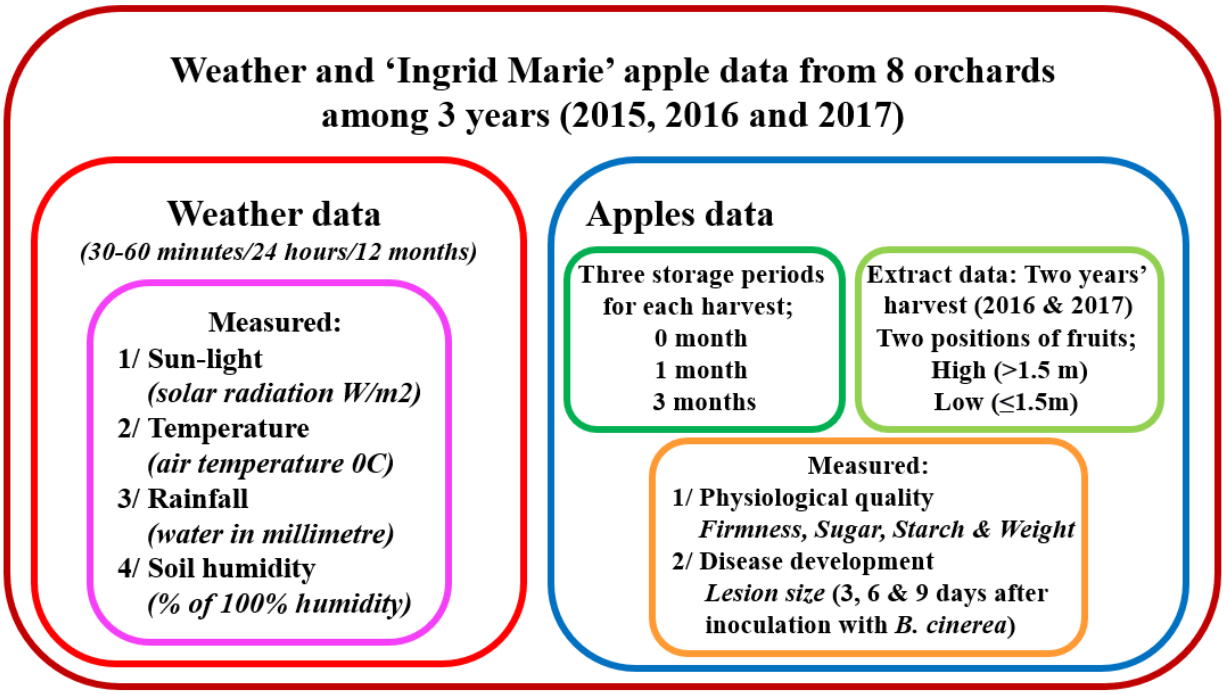

Figure 6. Overview of the experimental setup. Apples from eight orchards were harvested in the years 2015, 2016 and 2017. In addition, apples in 2016 and 2017 were harvested at two different positions in the trees. Apples from each harvest were investigated directly after harvest and after storage for 1 and 3 months. Firmness, sugar content and starch index were determined. The development of grey mould was measured 3, 6 and 9 days after inoculation. Paper IV analysed only apple datain each of the three periods of storage, while paper V analysed the correlation between weather data and apple data combined at all three periods of storage.

2.4. Quality Analysis of Apples

2.4.1. Evaluation of the Development of Disease

The disease symptoms were quantified as the diameter of the lesions around the inoculation points of the apples on both the sun-exposed and shaded sides at 5 and 14 days after inoculation with *B. cinerea* for papers I and II, at 1, 3, 6, 8, and 10 days after inoculation with *B. cinerea* for paper III, and at 3, 6 and 9 days after inoculation with *B. cinerea* for papers IV and V. Lesion size was measured (in cm) using a plastic ruler.

2.4.2. Fruit Sampling

Papers I, II and III: Apple tissue 'Braeburn' (and 'Golden Delicious') was excised from around the point of inoculation using a 0.5 cm diameter cork-borer. The uppermost 0.3 cm from the surface was considered peel tissue (hypanthium - fruit cortex), and the part taken out from 0.3 cm to 2.0

cm of the fruit plug was considered flesh tissue. Samples were ground to a fine powder in liquid nitrogen and stored at - 80 °C until analyzed. Samples were later analyzed for antioxidant metabolites (protein, vitamin C, phenolics) and antioxidant enzyme activities (ORAC, SOD, POX, APX and CAT).

Papers IV and V: On 'Ingrid Marie' apples we measured the lesion size, firmness level, sugar content, starch index and weight of fruit.

2.4.3. Physiological Measurements

Firmness level was estimated using a penetrometer (Bishop, fruit pressure tester, model FT327 - Italy) on the sun-exposed and the shaded sides. The determination was made at the middle point of each side, after removing a 0.5-0.7 cm diameter disc of peel, using a penetrometer (approximate depth 8 mm) (Tahir, 2006). Firmness was determined as kilogram per square centimeter (kg/cm^2).

Sugar content was determined using a rugged portable digital refractometer for sucrose measurements (model HI 96801 - Hanna Instruments Inc. Company, USA). We used juice obtained from the two holes in the apple made by the penetrometer (Davey and Keulemans, 2004). Sugar content was determined as gram sucrose per kg fresh weight (g/kg fw).

Starch test. The starch-iodine test was used to estimate starch index according to Tahir (2006) with slight modifications. The starch-iodine solution contains 40 g potassium iodide and 10 g iodine are dissolved in 1 L demineralized water. The solution is stirred for 3 h and then stored in the dark in the refrigerator until use. After dipping the surface of the slice of an apple into the iodine solution for 10 minutes, blue to black coloration shows the concentration of starch. For cv. 'Ingrid Marie' we used the European scale for starch index, which ranges from 1 to 10, where 1 = completely black and indicates maximum starch whereas 10 = completely white and indicates no remaining starch.

Weight of fruit has been given in grams and measured as the average of the weights of 10 apples by using a balance showing two decimals.

2.4.4. Biochemical Measurements (only for papers I, II and III)

Extraction of Protein

In a first step we extracted and separated protein from the powdered tissue. In a second step, we analyzed the extract for protein content, antioxidant metabolites and antioxidant enzyme activity (Fig. 5). Extraction and separation were carried out according to Ahn et al. (2007) with minor modifications. For the extraction, 0.1 g of tissue sample was homogenized with 1.0 mL of the

extraction buffer (50 mM potassium phosphate buffer, pH 7.8), 1 mM ethylene-diamine-tetra-acetate (EDTA), 1 % polyvinyl-polypyrrolidone (PVPP), 0.3 % Triton X - 100, 10 % glycerol, 0.1 mM dithiothreitol (DTT), and 50 µM vitamin C. The samples were then centrifuged for 15 min at 14000 rpm at 10°C (centrifuge Hettich 220R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The supernatant (1.0 mL) was transferred to a sterile Eppendorf micro centrifuge tube and stored at - 80 ^{o}C. In a next step the high-molecular components were separated from the low-molecular ones, using a Sephadex column and washing three times with column buffer. Column buffer consisted of 50 mM potassium phosphate buffer (pH 7.8), 1 mM EDTA, 1 % PVPP, and 10 % glycerol. The supernatant (1.0 mL) followed by 1.5 mL of column buffer was added and allowed to pass through the column to separate high molecular weight compounds. From the eluate, 1.5 mL aliquots were collected and used for determination of protein, antioxidant metabolites and antioxidant enzyme activities.

Analyses

Protein content (g/kg fw): Protein content was determined using the Bio-Rad Protein Assay, based on the method of Bradford (1976). This method uses bovine serum albumin (BSA) as a standard and added acidic dye to compare the solution analyzed to a standard curve. We used 20 mL of ¼ diluted Bio-Rad solution (protein assay dye reagent concentrates - catalog number 500-0006, Bio-Rad, USA) and 80 mL of methanol. The 96-well plate contained 20 µL of supernatants and 180 µL of reaction mixture in each well. We used 20 µL of 5 different concentrations (0 mg/mL, 0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL and 0.8 mg/mL) of BSA standard substrate added in triplicate for each concentration. The plate was agitated briefly and incubated in the microplate spectrophotometer at room temperature for at least 5 minutes before absorbance was recorded at 595 nm, with triplicates for each treatment. The protein content was expressed as gram per kilogram fresh weight (g/kg fw).

Antioxidant Defense Metabolites

Phenolic compounds (mg GAE/kg fw): Total contents of phenolics was estimated using a photometric method with the Folin-Ciocalteu reagent (Singleton et al., 1999). Approximately 5 mg powder of apple tissue was extracted with 500 µl of a solvent consisting of methanol and water (80:20, % v/v) containing 0.1% 1 mM EDTA. For each sample, triplicate analyses were carried out using absorption at 280 nm. We used a standard curve based on gallic acid at 6 different concentrations (0, 25, 50, 100, 150, 200, and 250 µg/mL) and the concentration of phenolics was

expressed as milligrams of gallic acid equivalents (GAE) per kilogram fresh weight (mg GAE/kg fw).

Vitamin C (g/kg fw): Vitamin C was determined by high-performance liquid chromatography (HPLC) analysis, essentially as described by Franck et al. (2003). Approximately 0.2 g of tissue sample was homogenized with 1.0 mL of extraction buffer (3% metaphosphoric acid and 1 mmol L^{-1} EDTA). The samples were then centrifuged for 15 min at 14000 rpm at 10^{o}C (centrifuge Hettich 220R, Andreas Hettich GmbH & Co. KG, Tuttlingen, Germany). The supernatant (1.0 mL) was filtered through a PVPP filter with 0.45µm pore size (Millipore, Brussels, Belgium) and analyzed immediately with HPLC. Vitamin C content was analyzed as absorption at 242 nm and expressed as gram per kilogram fresh weight (g/ kg fw).

Antioxidant-Enzyme Activities

The Oxygen Radical Absorbance Capacity (ORAC) (µmol TE/g fw): The ORAC assay is based on the measurement of the antioxidant scavenging activity against peroxyl radicals induced by 2,2$^{'}$-azobis (2-methylpropionamidine) dihydrochloride (AAPH, Ou et al., 2001). All reagents were prepared using a 75 mM phosphate buffer (pH 7.4). In the final assay mixture (0.4 mL total volume), $6.3*10^{-8}$ M fluorescein (FL) was used as a target of free radical attack and AAPH ($1.28*10^{-2}$ M) was used as a peroxyl radical generator. Samples of apple tissue were compared to 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox) standards, made in 7% Random Methylated β Cyclodextrin (RMCD) solvent (50:50% acetone-water mixture). Seven percent RMCD solvent was used as blank, and Trolox (12.5, 25, 50, and 100 µM) was used as control standard. The analyzer was programmed to record the fluorescence of FL every minute after the addition of AAPH. All measurements were expressed relative to the initial reading. Final results were calculated using the differences in areas under the FL decay curves between the blank and the sample. The results were expressed as µmol Trolox equivalent (TE) per gram fresh weight (µmol TE/g fw).

For the activities of selected antioxidant enzyme activities (SOD, POX, APX, and CAT) in triplicate we used the supernatants from extracts (see extraction of protein), assayed as modified from Ahn et al. (2007). All antioxidant enzyme activities were measured using 96-well microtiter quartz plates, containing 20 µL of enzyme extract and 180 µL of reaction mixture in each well in triplicate, and incubated at 18-20 ^{o}C. Readings were recorded every 30 seconds using a Multiscan Spectrum-Microplate Spectrophotometer (Thermo Lab systems, Helsinki, Finland) and expressed in units per gram of fresh weight for each enzyme (units/g fw).

SOD activity (units/g fw): SOD activity was determined by measuring the ability of SOD to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) at 450 nm (Alscher et al., 2002). The assay mixture contained 50 mM PIPES buffer pH 7.5, supplemented with 0.4 mM *o*-dianisidine, 0.5 mM diethylene triamine pentaacetic acid (DTPA) and 26 µM riboflavin. SOD activity was measured at 450 nm at time = 0 min. To start the reaction the plate was placed 12-20 cm below two 15 W fluorescent lamps and the light switched on. The reaction was stopped by switching off the light after which the plate was covered with aluminum foil until absorbance was measured at 450 nm. Eight different concentrations of SOD, each added in triplicate served as a standard. The SOD substrate stock solution had 2 mg protein/mL in water, equivalent to 3277 units/mg protein - Sigma-Aldrich, Saint Louis, Mo. USA, and was diluted to the eight concentrations with 1, 10, 20, 30, 40, 50, 80, and 100 units/mL.

POX activity (units/g fw): POX activity was determined as the rate of guaiacol oxidation in the presence of H_2O_2 (extinction coefficient, 26.6 mM^{-1} cm^{-1}) at 470 nm (Rao et al., 1996). The reaction mixture, containing 50 mM potassium phosphate buffer at pH 7.0, 0.01 M EDTA, 0.02 M pyrogallol, and 1.47 mM H_2O_2, was added to the wells of a 96-well microtiter plate. The plate, which had 20 µL of enzyme extract and 180 µL of reaction mixture in each well, was agitated briefly and incubated in the microplate spectrophotometer for 4 minutes before absorbance was recorded.

APX activity (units/g fw): APX activity was determined by monitoring the decomposition of H_2O_2, based on the method described in Nakano and Asada (1981) with a slight modification. The reaction mixture, containing 50 mM potassium phosphate buffer of pH 7.0, 0.1 mM EDTA, 0.88 mM vitamin C, and 0.1 mM H_2O_2, was added to the wells of a 96-well plate. The plate containing 20 µL of enzyme extract and 180 µL of reaction mixture in each well was agitated slightly and incubated in the microplate spectrophotometer for 30 seconds. Absorbance was measured at 290 nm (extinction coefficient of 2.8 mM^{-1} cm^{-1}).

CAT activity (units/g fw): CAT activity was determined using the method of Du and Bramlage (1995), with slight modifications. The reaction mixture contained 30 mM H_2O_2 in 50 mM potassium phosphate buffer of pH 7.0. The 96-well plate, which contained 20 µL of enzyme extract and 180 µL of reaction mixture in each well, was agitated briefly and incubated in the microplate spectrophotometer for 30 seconds before absorbance was recorded at 240 nm, with triplicates for each treatment. We also used 8 different concentrations of the CAT standard substrate (25 mg protein/mL, 423000 units/mg protein - Sigma, C-100), namely 20 µL of 0, 20, 50, 80, 100, 500, 1.000 and 5.000 units/mL added in triplicate for each concentration.

2.4.5. Weather Data Collection *(only for paper V)*

In each of the years 2015, 2016 and 2017, we collected weather data (temperature, rainfall, humidity and sun-light) from the weather stations close to or in the 8 orchards, from the website of Fruit Web, Lammed, Sweden and the website of the Swedish Meteorological and Hydrological Institute (SMHI, Fig. 6). Data for apples namely weight of fruit, firmness level, starch index and lesion size of the fruits were collected at harvest and after storage for 1 and 3 months. For all three years we collected weather data from 1st of January until 31st of December for each of the orchards. Because of the large data set we analyzed data only from 1st April to 30th September for each of the three years, which is the period of time for flowering, fruit growth and development (Tromp and Wertheim, 2005).

Temperature (^{o} C), rainfall (mm) and humidity (%) were recorded automatically every 30 minutes using Davis weather stations (Davis Vantage pro2 - wireless 6152, produced by Cole-Parmer, 625 East Bunker Ct Vernon Hills, IL 60061 USA) and stored in the website of Fruit Web, Lammed, Sweden. Data for sunlight measured every 60 minutes was collected from the website of SMHI (W/m^2).

2.5. Terminology

We have used the term ‘tolerant’ for apples/tissues that were inoculated with *B. cinerea* without showing disease symptoms. The term ‘susceptible’ means that apples/tissues inoculated with *B. cinerea* showed disease symptoms (lesions). We used the term ‘temperature’ for weekly mean temperature (MT), and ‘humidity’ for weekly mean humidity (MH), ‘rainfall’ for sum of rainfall per week (RS) and ‘sunlight’ for sum of sunlight per week (SS).

2.6. Statistical Analysis

Paper I: Basic data analyses were performed in the Excel program. We presented data as mean values and error bars representing 95% confidence intervals based on the standard deviations.

Paper II: Statistical analyses were carried out in R (version 3.5.1, R development core team, Vienna Austria, 2018). Overall statistical analysis was by MANOVA using the manova function in R. Pairwise tests for mean differences were carried out with the emmeans function of the emmeans (previously lsmeans) package in R, followed by Tukey's test to control the family-wise error rate at a predetermined $\alpha = 0.05$.

Paper III: Statistical analyses were performed in R 3.4.3 (R Core Team, 2017). An over-all analysis of the data was made using a Principal Component Analysis (PCA). The input to the ordination was measured for activities of enzymes and concentrations of metabolites in all samples. All analyses of variance are of the ANOVA type 2 (package car, Fox and Weisberg, 2011), which means that each term is evaluated when the effects of all other terms are taken into account.

Paper IV. Statistical analyses were performed in R 3.4.3 (R Core Team, 2017). We used linear mixed models with apple identity as a random factor since 2 samples were taken per apple and lesion size was measured at three occasions. Mixed models used the lmer procedure in lme4 (Bates et al., 2015). Predictions from models were made using the package effects (Fox, 2003) and used for figure construction, differences between factor levels and between interaction terms were tested using the package phia (De Rosario-Martinez, 2015).

Paper V. Statistical analyses were performed in R 3.5.3 (R Core Team, 2019). An overall analysis of the data was made using Principal Component Analysis (PCA). The input to the ordination was the weather in the eight orchards the three years. On this ordination lesion size, firmness, weight of fruit and starch index in apples was tested using the procedure *envfit* in the vegan package (Oksanen et al., 2019). We used random forest as implemented in the wrapper algorithm in package Boruta (Kursa and Rudnicki, 2010). To explore possible interaction effects, we used the selected weather variables in regression trees using the package rpart (Therneau and Atkinson, 2018).

3. Results and Discussion

3.1. Paper I - "Cross-Tolerance and Antioxidant Metabolism as Determinants of the Resistance of Apple Fruit to Postharvest Botrytis Decay".

Susceptibility to Botrytis

My results show that 'Braeburn' is clearly more susceptible to *B. cinerea* infection than 'Golden Delicious'. In 'Braeburn' disease symptoms were evident 5 days post infection (DPI), whereas in 'Golden Delicious' by contrast, disease symptoms only appeared 14 DPI, and only on the shaded (green) side of the fruit (data not shown).

Protein Content

At 14 DPI however, it is clear that in all samples the mean protein contents of the green side of the fruit are substantially lower. While wounding (mock infection) had no impact on protein content at any time (Fig. 7D), infection resulted in substantial decrease in its concentration. Interestingly, infected (but still healthy) sites on the red side of the apple maintained higher protein contents than healthy green sites, indicating a *Botrytis*-specific loss of proteins only in green tissues, occurring before disease symptoms were evident.

Enzyme Activities

Cuticle penetration and primary lesion formation by *B. cinerea* triggers an oxidative burst and the production of H_2O_2, both in the plant plasma membrane and in the extracellular sheath on the surface of fungal hyphae (Schouten et al., 2002). For these reasons we followed changes in the activities of four enzymes involved in plant H_2O_2 metabolism. Ascorbate peroxidase (APX) utilises ascorbic acid (AsA) as a substrate to detoxify H_2O_2. Initial APX activities were similar in both cultivars and in both types of tissue. However, in 'Braeburn' flesh, infection lead to a large increase in APX by 14 DPI, Additionally, this increase was higher on the red side of the fruit, and much higher in infected and diseased tissues than in infected and healthy tissues, suggesting that the fruit is actively trying to compensate for the stress of disease development (Fig. 7A). APX levels were too low in 'Golden Delicious' at 14 DPI to have much effect. CAT directly catalyses the disproportionation of H_2O_2. Both 'Braeburn' and 'Golden Delicious' initially had similar CAT activities, and in both, activities were higher in the flesh than in the peel. CAT activities also increased in both cultivars during storage. In 'Braeburn' flesh samples, CAT activities were noticeably higher on the green side compared to the red by 14 DPI, but levels were reduced in

infected sites (Fig. 7B). SOD catalyses the dismutation of superoxide anion, to form H_2O_2. Initial SOD activities in 'Braeburn' were considerably higher than in 'Golden Delicious' in both flesh and peel samples. In flesh samples, SOD decreased rapidly and by 14 DPI was insignificant in both cultivars.

In peel samples by comparison, activities increased to similar levels in both cultivars. Finally, initial guaiacol POX activities in 'Braeburn' were substantially higher than those found in 'Golden Delicious' in both tissue types. In both cultivars POX flesh activities decreased over the course of the experiment while in peel samples there was a slight increase.

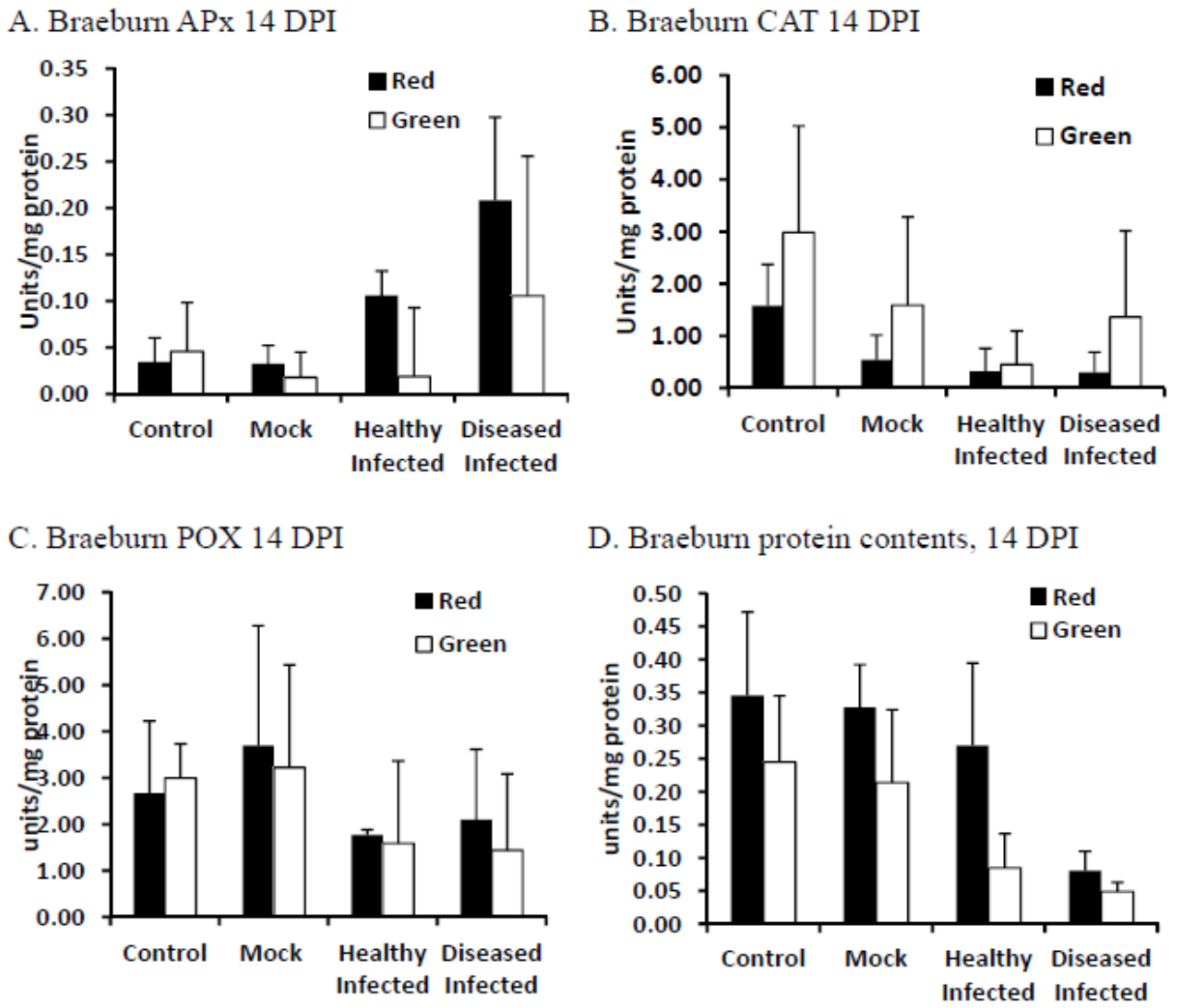

Figure 7. Changes in fruit flesh APX, CAT and POX activities in units/mg protein and the mean total soluble protein contents in apple cultivar 'Braeburn' 14 days post infection (DPI). Samples were either untreated (control), wounded (mock) or wounded and infected with *Botrytis cinerea* (infection) as described. Infected samples were separated into those displaying disease symptoms and those that were apparently still healthy. Error bars represent the 95% confidence intervals of the standard deviations.

Antioxidant Metabolites

Average AsA contents were around 5-fold higher in 'Braeburn' peel compared to 'Golden Delicious' and flesh levels were about 2-fold higher (Fig. 8A, B). However, in 'Braeburn' there was an increase in control and mock AsA contents, but this increase occurred exclusively on the red side of the fruit and AsA contents of green tissues had collapsed by 5 DPI. Mean total phenolic contents were also higher in 'Braeburn' compared to 'Golden Delicious' flesh samples, and decreased over the course of the experiment. Interestingly infection lead to a collapse in total phenols by 5 DPI in both cultivars, but by 14 DPI there was a recovery or even increase in levels in both cultivars (Fig. 8C, D). No significant difference in the response of the red and green side of the tissue were observed.

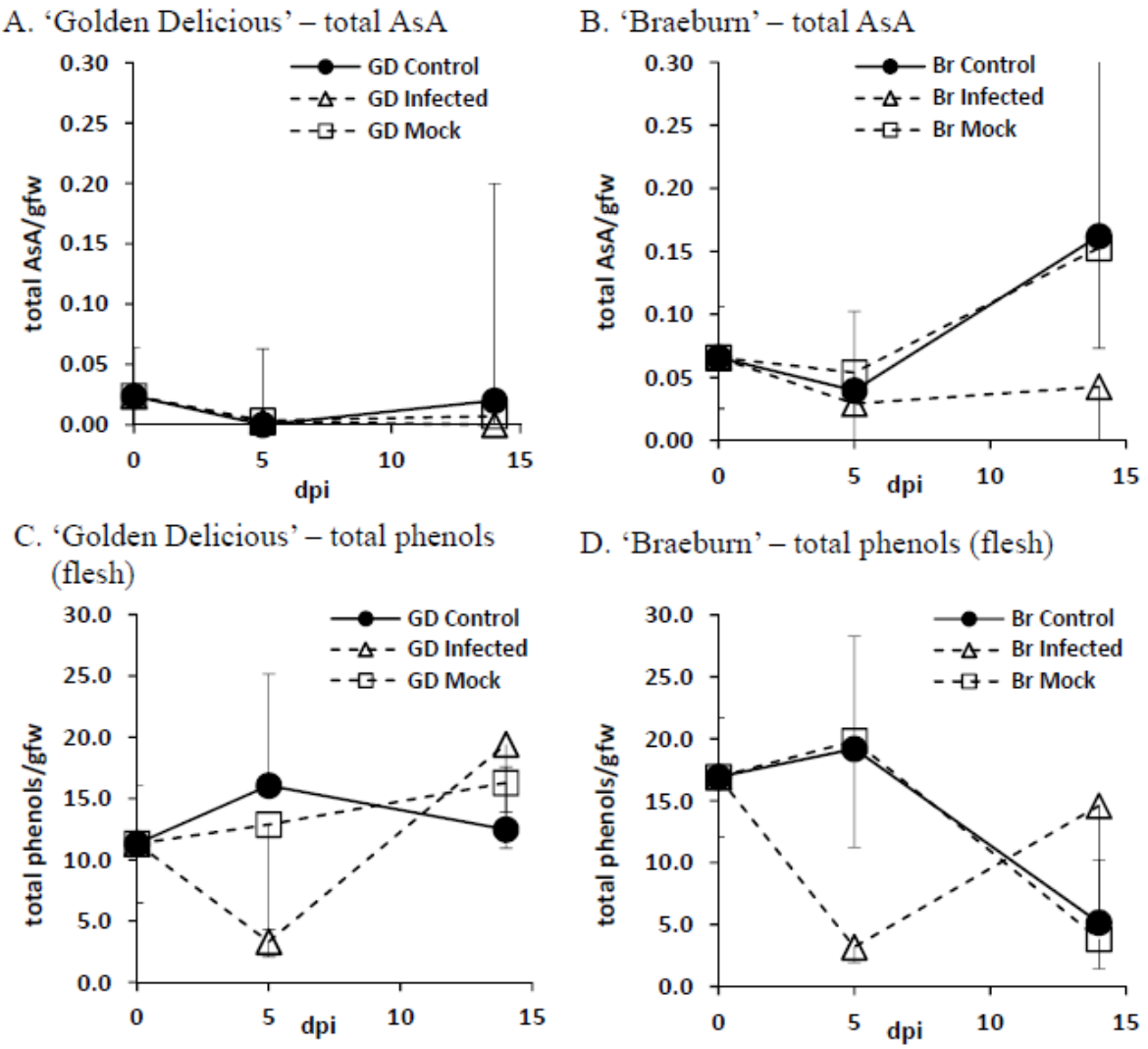

Figure 8. Changes in mean contents of total ascorbic acid (AsA, vitamin C), and total soluble phenols in flesh samples of the apple cultivars 'Braeburn' ('Br') and 'Golden Delicious' ('GD'). All results are expressed per gram fresh weight. Error bars represent the 95% confidence intervals of the standard deviations.

3.2. Paper II - "*Botrytis cinerea* Differentially Induces Postharvest Antioxidant Responses in 'Braeburn' and 'Golden Delicious' Apple Fruit".

Disease Development and Vitamin C Content

Lesion sizes were smaller on the sun-exposed sides than on the shaded sides (Fig. 8). The shaded sides of 'Braeburn' ('Br') fruit were almost all infected at 5 d, whereas a similar situation did not appear until 14 d for the sun-exposed sides. At 5 d post inoculation, disease symptoms were observed on both sides of 'Braeburn' ('Br') apple fruit inoculated with *B. cinerea* (Fig. 9A, B). For 'Golden Delicious' ('GD') apples, disease symptoms were observed only on the shaded sides of a few 'Golden Delicious' apple fruit (Fig. 9D). The sun-exposed sides of 'Golden Delicious' apples did not develop disease (Fig. 9C). Thus, 'Braeburn' was more susceptible to grey mould than 'Golden Delicious'.

Sun-exposed peel of 'Braeburn' had significantly higher initial total vitamin C content than any other tissue examined (Fig. 10). The total vitamin C content was 5 to 6 folds higher than in sun-exposed peel tissue of 'Golden Delicious'. Over the course of the experiment, the total vitamin C content in the peel tissues of *B. cinerea*-inoculated 'Braeburn' apples was reduced (Fig. 10). At 14 d post inoculation, when rot of grey mould was extensive on the shaded side of inoculated 'Braeburn' fruit, the total vitamin C content in peel was no longer detectable, whereas it was significantly reduced in sun-exposed apple peel (Fig. 10).

Figure 9. Symptoms of *B. cinerea* at 5 d post inoculation; representative fruit of 'Braeburn' sun-exposed (A), and shaded sides (B), and fruit of 'Golden Delicious' showing the absence of symptoms on the sun-exposed sides (C), and occasional symptoms on shaded sides (D).

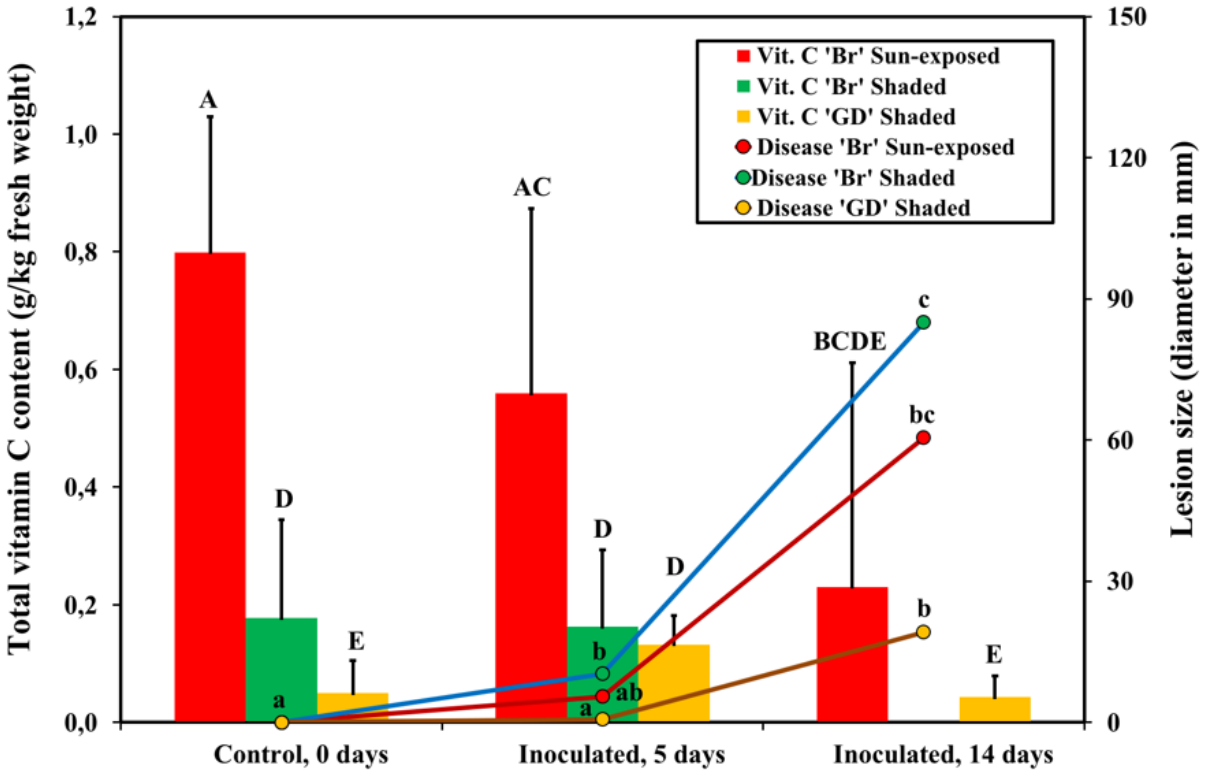

Figure 10. The change over time of total vitamin C content in peel (bars) and in lesion sizes (lines) of apples of the cultivars 'Braeburn' ('Br') and 'Golden Delicious' ('GD'), at 5 and 14 d post inoculation with *B. cinerea*. The inoculated, sun-exposed sides of 'Golden Delicious' were without symptoms at all time points; thus, the data is not presented. The treatments were: control (neither wounded, nor inoculated) at 0 d post inoculation, and *B. cinerea*-inoculated (wounded and inoculated with 10 µL of a 1.5 x 10^5 mL^{-1} *B. cinerea* spore suspension). The results from t-tests without pooled standard deviations are shown as capital letters for total vitamin C content and as lower-case letters for lesion size. Measurements with the same letter were not significantly different at $p \leq 0.05$.

The relationship between total vitamin C content and lesion size over time is presented in Fig. 10. Overall, the total vitamin C content in the apples decreased as the lesion size increased. However, the total vitamin C content at 0 d was significantly different among the tissues that were inoculated (Fig. 10). 'Br' was susceptible to *B. cinerea* whereas 'Golden Delicious' was essentially tolerant or even resistant, which has been observed previously (Davey et al., 2000; 2007).

Overall Antioxidant Enzyme Activity (SOD, APX, POX and CAT)

Significant changes in enzyme activity and antioxidant content were found in 'Br' over time (Fig. 11). In contrast, there was no antioxidant enzyme induction in ''Golden Delicious' and no change

in total vitamin C (AsA + DHA) or phenolic content as a result of inoculation. Nonetheless, 'Br' was more susceptible. Similarly, 'Braeburn' displayed higher initial total AsA content in peel and higher SOD and POX activities in flesh tissues as compared to 'Golden Delicious'. Obviously, the resistance of 'Golden Delicious' must depend on other factors than those investigated here. It was the susceptible 'Braeburn' apples that displayed the observed increase in SOD and APX activity and the decrease in POX activity that had been observed in inoculated apples (Fig. 11).

Since SOD is an important part of plant detoxification of ROS, the increase in SOD activity observed is an attempted plant defense strategy. SOD activity could possibly also originate from *B. cinerea*, since *BCSOD1* was found to be a virulence factor for *B. cinerea* in *Phaseolus vulgaris* (Rolke et al., 2004), in *Arabidopsis thaliana* and in tomato plants (López-Cruz et al., 2017). Inoculation with *B. cinerea* led to a strong increase in 'Braeburn' APX activity, particularly in the flesh tissue of the shaded side.

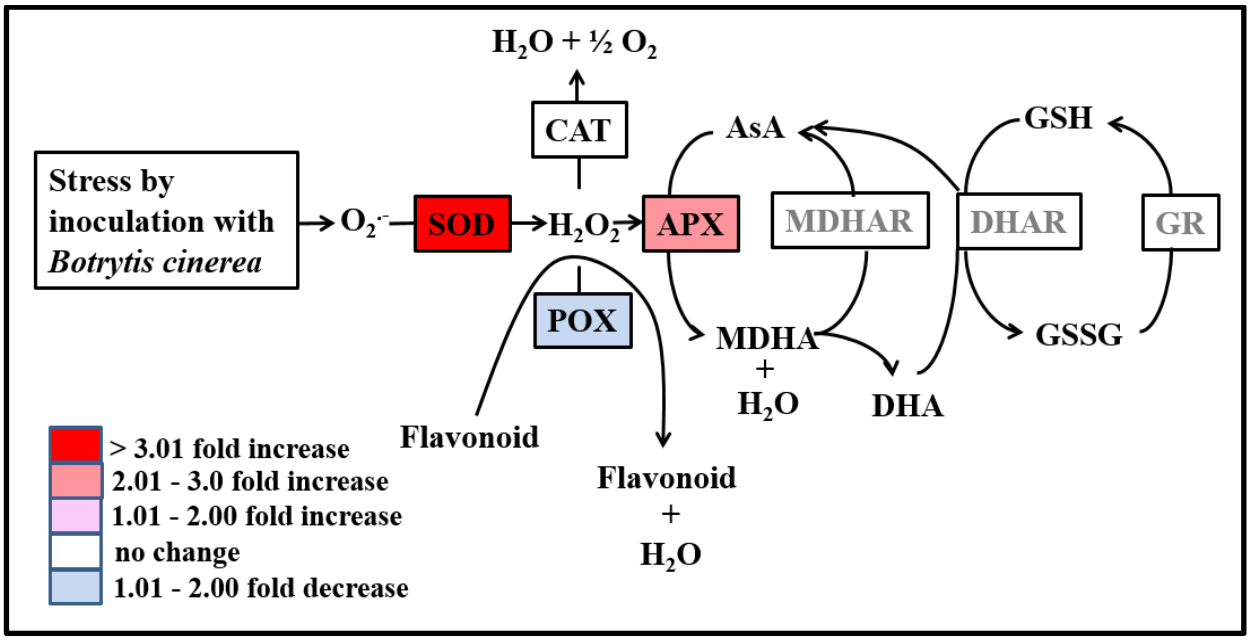

Figure 11. Summary of the antioxidant enzyme responses in 'Braeburn' ('Br') apple fruit as a consequence of infection by *B. cinerea*. The antioxidant enzyme activity is collectively analyzed for shaded and sun-exposed tissue, as for peel and flesh tissue. The colours represent the changes in antioxidant enzyme activity of susceptible fruit at 14 days post inoculation, compared to noninoculated control fruit at the same point in time. The changes in enzyme activity were analyzed by ANOVA, followed by pairwise t-tests. Abbreviations: APX, ascorbate peroxidase; AsA, vitamin C (L-ascorbic acid); CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GR, glutathione reductase; GSH, glutathione; GSSG, oxidized glutathione; H_2O_2, hydrogenperoxide; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbatereductase; O_2.-,superoxideanion; POX, flavonoidperoxidase; SOD, superoxidedismutase.

The increase in APX activity may suggest an attempt by the host to metabolize the H_2O_2 generated from other ROS during infection. The increase in APX activity was accompanied by decreasing vitamin C levels both in flesh and peel tissues over the course of the experiment. This presumably occurred since vitamin C was consumed as a result of APX activity.

POX activity was higher in 'Braeburn' than in ‘Golden Delicious’ tissues, in accordance with previous results (Zupan et al., 2014). POX activity in the shaded side of ‘Braeburn’ at 14 d was lower in the infected flesh tissue than in control and mock-inoculated fruit. The low POX expression correlated well with the low amount of phenolics and progression of disease that was found at 14 d in the susceptible ‘Braeburn’. This could possibly indicate that the availability of the enzyme is regulated by, or dependent on the availability of its substrate. Alternatively, the POX pathway may have been downregulated by *B. cinerea*, as was previously suggested from inoculation experiments with *B. cinerea* on bean (*Phaseolus vulgaris*) leaf discs (von Tiedemann, 1997).

Plant CAT activity is essential to plant health, particularly during grey mould infection (Govrin and Levine, 2000), but also when not infected. CAT activity remained unchanged in ‘Braeburn’ apples after inoculation and during the course of disease development. Some of the measured activity may have been of *B. cinerea* origin, as seven CAT genes are present in its genome and CAT activity has been reported from growth on culture media (Gilad et al., 2000). However, deletion of one of the catalase genes did not affect virulence (Schouten et al., 2002).

3.3. Paper III - "Higher Levels of Protein and Phenolics in 'Braeburn' Apples Correlate with Fruit Tolerance to Grey Mould".

Influence of Sunlight on the Antioxidant Metabolism of Apple Fruits and their Tolerance to Infection with Grey Mould.

The effect of sunlight is shown within individual apples with the sun-exposed side of the fruit being more resistant to infection than the shaded side (Paper III, Table 1: Tolerance). Our results agree with those of Zupan et al. (2014), Davey et al. (2007), Bui et al. (2019) and Ballaré (2014), who reported that the sun-exposed side of the apples accumulated higher levels of antioxidants and thus had an improved capacity to resist pathogens. Inoculated peel tissue developed smaller lesions than the flesh (Fig. 11, Paper III, Table 1: Susceptibility), demonstrating that disease developed faster in the flesh than in the peel. Our result is in the agreement with previous reports, which indicate that the peel had higher contents of antioxidants and a better capacity to scavenger ROS than the flesh (Davey et al., 2007; Bui et al., 2019; Chinnici et al., 2004; Drogoudi et al., 2008; Carbone et al., 2011). The sun-exposed peel tissue had higher levels of antioxidants (protein, phenolics, ORAC, vitamin C and POX activity), and was less susceptible to *B. cinerea* infection. These results are similar to those of previous studies (Solovchenko and Schmitz-Eiberger, 2003; Zupan et al., 2014, Dayvey et al., 2007; Bui et al., 2019), which reported that preharvest exposure to sunlight increased the antioxidant capacity in apples, making the fruit more tolerant to pathogen attack.

Responses in Apple Antioxidant Metabolism to the Development of Grey Mould

Post-harvest storage is becoming increasingly important and requires more understanding and development of procedures and treatment of fruit before harvest (Sholberg and Cinway, 2004; Kevers et al., 2011). Recent work shows that the interaction of the pathogen *B. cinerea* with plant tissue is complex (Heller and Tudzynski, 2011; González et al., 2016; Gill and Tuteja, 2010). During infection, oxidative bursts are generated both by *B. cinerea* and the host as ways to facilitate infection and to defend itself against pathogenic attack, respectively (Tudzynski and Kokkelink, 2009). Two clear phases are distinguished: an early stage characterized by local necrosis, and a later one, characterized by the development of a spreading lesion (van Kan, 2006). Accordingly, a current model predicts that *B. cinerea* uses different types of virulence/effector molecules at each stage. A complex signaling network regulates the secretion of a large set of proteins and phytotoxic secondary metabolites, which are necessary for the progression of the *B. cinerea* infection from the early to the late stages (González et al., 2016). From a fruit-grower's perspective, there is a need to develop knowledge of factors which influence postharvest disease by e.g. *B. cinerea* on the

molecular level, in order to develop practices and/or infection markers that may improve postharvest storage. We defined *tolerance* of an apple as the property of not developing disease symptoms despite having been inoculated with *B. cinerea*. We defined *susceptibility* of an apple as the lesion size after being inoculated with *B. cinerea*. Tolerance to grey mould was predicted by high levels of protein and phenolics (Figure 12, Paper III, Table 1: Tolerance).

Similarly, susceptibility was predicted by low concentrations of protein, phenolics, vitamin C, and ORAC, as well as by low activities of POX and APX in the host tissue (Figure 12, Paper III, Table 1: Susceptibility). This means that the development of disease (lesion size) was negatively associated with high levels of protein and phenolics.

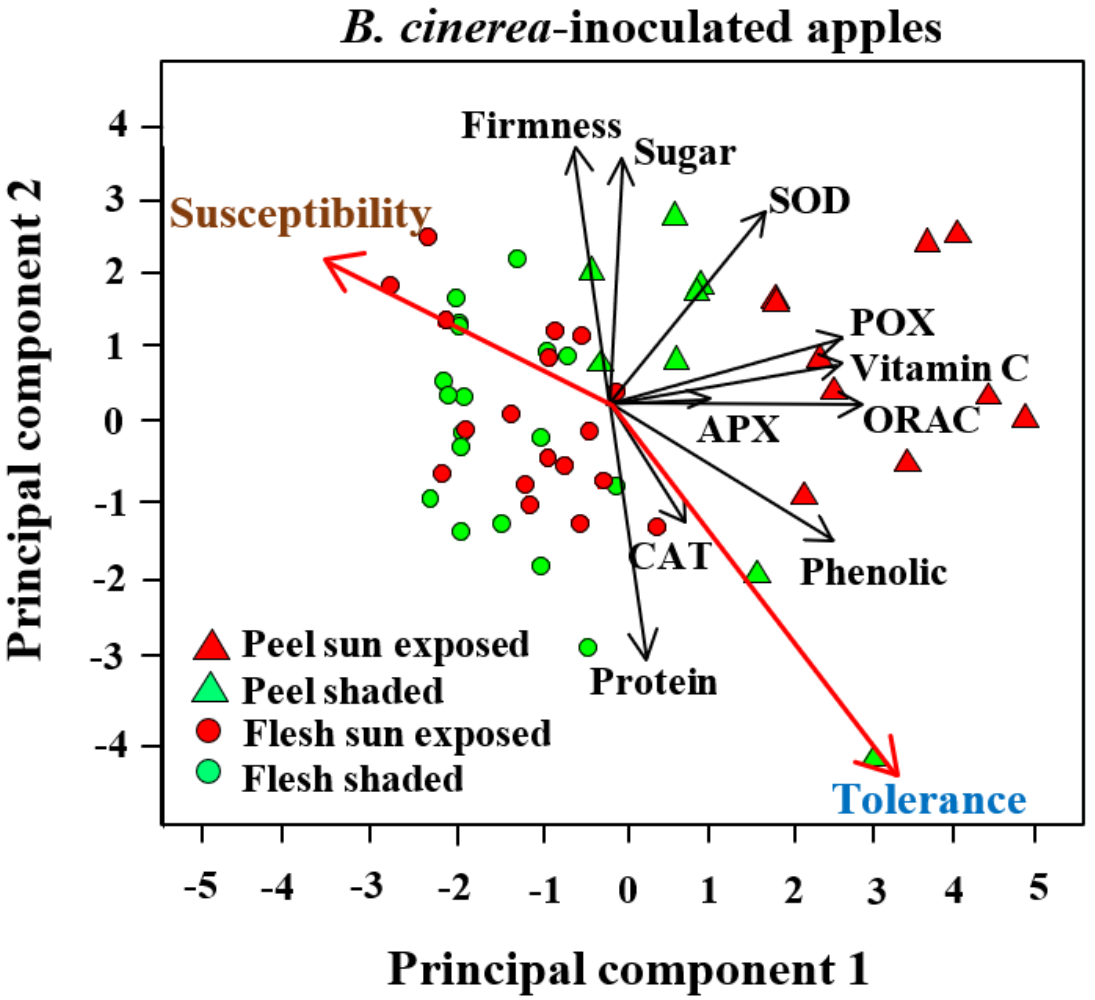

Figure 12. A Principal Component Analysis (PCA) based on the antioxidant (AOX) properties as well as sugar (Brix) and firmness. Samples from four kinds of tissue, namely sun-exposed and shaded peel, as well as flesh from the sun-exposed and the shaded sides are marked with different symbols. Values were taken with start on day 3 after inoculation. Regression of Susceptibility on the ordination gave R^2=0.46, p=0.001 and of Tolerance R^2=0.14, p=0.023.

Lesion size was also negatively associated with higher levels of vitamin C, ORAC, and activities of POX and APX. The sun-exposed side of the apples showed higher tolerance than the shaded

side (Paper III, Table 1: Tolerance, Side-Red>Green). The lesions developed much more slowly in the sun-exposed than in the shaded side of apples (Fig. 12, Paper III, Table 1: Susceptibility, Side-Red<Green).

The general trends over time show that inoculation with *B. cinerea* led to that the levels of protein, phenolics, vitamin C, and ORAC decreased strongly during infection, while POX activity decreased and APX activity increased only on day 10 (Fig. 12; Fig. 13, Paper III Tables 1 and 2). In particular, there were significant and large differences in POX and APX activity levels between the control and *B. cinerea*-inoculated apples in the later stage of the infection (day 10) (data not shown; Fig. 13). In general, the levels of protein, phenolics, vitamin C and ORAC decreased strongly as the lesion size increased over time of the experiment (Fig. 12 and Fig. 13, Paper III, Tables 1 and 2).

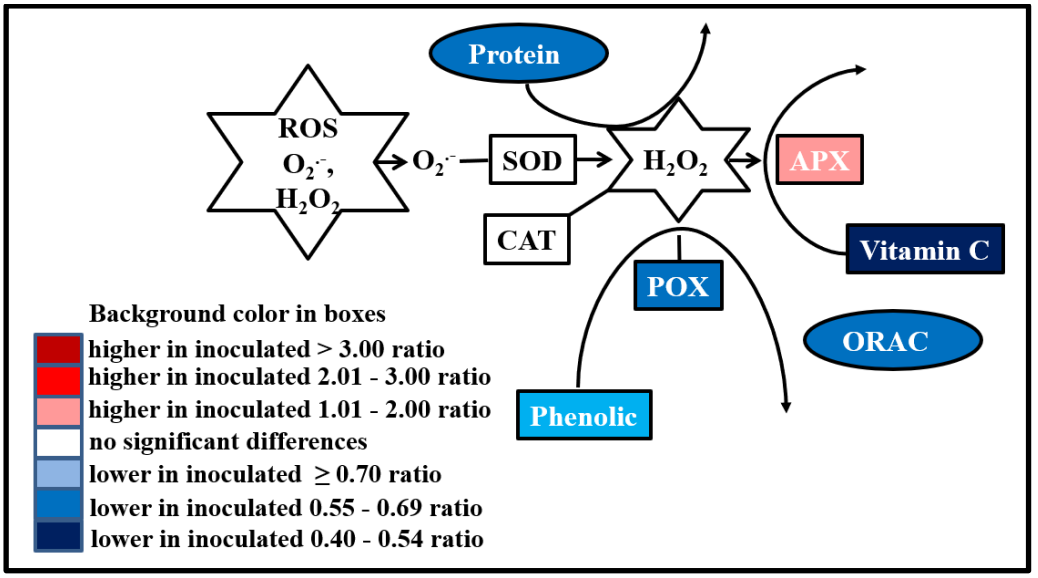

Figure 13. A summary of the response in antioxidant properties in 'Braeburn' apples after infection with *Botrytis cinerea* on day 10 after inoculation. Measurements for all four kinds of tissue were pooled, namely peel and flesh from the sun-exposed and the shaded sides and no separation of tolerant and susceptible fruits. Colours represent relative changes in concentrations of metabolites and enzyme activities of infected apples compared to non-inoculated control apples. White colour indicates no significant change in antioxidant properties in *B. cinerea*-inoculated tissue and control tissue. Pink colour indicates significantly higher values in the inoculated apples at 1.01 to 2.00 ratio, red colour indicates an increase in the ratios (from 2.01 to 3.00) and dark red colour indicates ratios higher than 3.00. Light blue colour indicates a lower value in the inoculated apples with a ratio ≥ 0.70, medium blue colour denotes decreased ratio (between 0.55 to 0.69) and dark blue colour denotes an even stronger decrease (ratio from 0.40 to 0.54) in *B. cinerea*-inoculated tissue and control tissue.

Protein Content

High protein concentration was the strongest predictor of tolerance and was negatively associated with lesion size (Fig. 12, Paper III, Table 1). Protein levels were significantly different among the three treatments (Table 2: Treatment). Using the prediction and interaction tests we found that protein concentration was significantly lower in apples inoculated with *B. cinerea* than in both the control and mock treatments. Protein levels were significantly different in the two-way interaction for Treatment*Side and for Treatment*Tissue (Paper III, Table 2). In general, the levels of protein did not change in the early stages of infection (days 1, 3 and 6) and levels of protein became lower in the later stages of infection (days 8 and 10) (data not shown). Using linear models for combinations of type of tissue, side and day as explanatory factors (data not shown), we found that protein concentration was higher in the sun-exposed peel than in that on the shaded side on day 8 and that disease developed less in the sun-exposed peel than in both the peel and flesh tissues on the shaded side (data not shown). Thus, the higher protein content in the peel on the sun-exposed side reflects apples being more tolerant to infection by *B. cinerea*. The level of protein in all four kinds of tissue was lower in the *B. cinerea*-inoculated apples than in the control on day 10 after inoculation (Fig. 13). Our results agree with those of a previous study (Michielse et al., 2011), reporting that several proteins are consumed by the fungus in the late phase. Proteins have a specific role in the physiology and quality of apple fruits during harvest and postharvest handling as well as during maturation and in different stages of ripening (Shi et al., 2014). The protein content is related to stress response and defense as well as to the energy metabolism of apples (Shi et al., 2014; Qin et al., 2009).

Phenolics

The concentration of phenolics was a strong predictor of tolerance in apples as the level of phenolics was negatively associated with lesion size (Fig. 12; Paper III, Table 1). Levels of phenolics were significantly different among the three treatments (Paper III, Table 2: Treatment). Interaction tests showed that the levels of phenolics were lower in the *B. cinerea*-inoculated apples than in both control and mock-treated apples. Phenolics' levels were significantly different in the two-way interaction for Treatment*Day, for Treatment*Tissue and for Tissue*Side (Paper III, Table 2: Phenolic). In general, the levels of phenolics did not change in the early stages of infection (days 1, 3 and 6), and levels of phenolics became lower in the later stages of infection (days 8 and 10) (Fig. 13). The phenolics' content was higher in sun-exposed peel tissues as compared with that in flesh tissues on both sides of the apples over the duration of the experiment in *B. cinerea*-inoculated apples (data not shown). The level of phenolics decreased as the lesion size increased

strongly on the shaded side of fruit in the inoculated apples. Thus, the higher phenolics content in the peel on the sun-exposed side could contribute to an improved oxidative defense and might also mirror apples being more tolerant to infection by *B. cinerea*. Our results are similar to those of Rysman et al. (2016). Phenolics may be used in defense mechanisms against pathogens through the integration of phenolic esters into cell walls as suggested by Dixon (2001). Phenolic compounds are secondary metabolites with an important role in fruit quality and contribute to taste, colour and nutritional properties (Tomás-Barberán and Espin, 2001). Phenolics in fruits are important for postharvest preservation and human health benefits linked to the phenolic-associated antioxidant activity (Adyanthaya et al., 2009).

Summary

In this study, antioxidant enzyme activity measured in the inoculated apples could either originate in the fruit or be of *B. cinerea* origin. Bui et al. (2019, Paper II) indicated that sun exposure might improve apple fruit tolerance to grey mould. In that study, we evaluated changes in content of antioxidants in apple fruits with time in *B. cinerea*-inoculated apples as compared to non-infected apples at 5 and 14 days after inoculation. We found that antioxidant metabolism in the fruit changed after inoculation with *B. cinerea*, and that vitamin C content and POX activity decreased while SOD and APX activity increased in 'Braeburn' apples on day 14 after inoculation. However, we did not find antioxidant metabolism to be involved in tolerance mechanisms in 'Braeburn' and 'Golden Delicious' apples. In the present study we investigated antioxidant defense mechanisms of 'Braeburn' fruits more in detail using additional time points following inoculation with grey mould and used principal component analysis and linear models by which the relative importance of enzymes and compounds could be evaluated and compared in a more comprehensive manner. Additionally, we found that an antioxidant response in apples upon infection by *B. cinerea* appears as early as 1 day after inoculation.

At the later stage of the infection, there were large and significant differences in levels of antioxidant metabolites between the control and *B. cinerea*-inoculated apples, in which the levels of protein, phenolics, vitamin C, ORAC, and POX activity decreased strongly, while APX activity was low but increased on day 10 after inoculation with *B. cinerea* (data not shown, Fig. 13). Our results show that proteins and phenolics were consumed during the infection process (Fig. 12), in agreement with our finding that high levels of protein and phenolics make apples more tolerant and less susceptible to infection with *B. cinerea* (Fig. 12, Paper III, Table 1). We propose that high levels of protein and phenolics are indicators of an active defense mechanism against pathogens and may serve as predictors of tolerant and less susceptible apples.

The development of disease (lesion size) is negatively correlated to protein, phenolics, vitamin C, POX activity and positively correlated to APX activity (Fig. 12 and Fig. 13, Paper III, Table 1). This is logical in that in samples with large lesions, there is little, or no antioxidant activity left, and proteins are being broken down as the tissue rots. As with SOD activity, early responses suggest that there is a loss of SOD activity, particularly in the red side of the fruit, but these then recover at the later stages. The more resistant red tissues are able to maintain a higher SOD activity. SOD activity and POX activity are down-regulated (or lost), and there is an up-regulation of APX activity, possibly as an attempt to compensate for the loss of SOD activity. Both SOD and CAT are known to be inactivated by singlet oxygen, suggesting an accumulation of ROS in 'Braeburn' tissues. This could reflect an attempt by the tissue to deal with stress, or higher ROS-detoxification activities could be negatively associated with *B. cinerea* resistance.

Ideally, this study should be repeated on a larger scale using entirely natural causes of infection. It would also be of value to follow physiological parameters during infection with increased time resolution, especially at the early stages when oxidative bursts are considered to be most prevalent. Nevertheless, sun-exposed peel tissue showed significantly higher antioxidant content and increased tolerance to infection by *B. cinerea*. High levels of protein and phenolics were positively associated with the tolerance of apple fruits. To our knowledge, such information has not been published before. Our study reinforces and extends previous studies suggesting that preharvest exposure to abiotic stress, such as high light intensity, can modulate post-harvest susceptibility of apples to *B. cinerea* infection after harvest.

3.4. Paper IV - ″Varying Susceptibility of 'Ingrid Marie' Apples to Grey Mould Infection Among Years and Orchards in Southern Sweden″

Year of Harvest

Our first goal was to determine how year of harvest affected the apples' quality and the development of disease. Lesion size was largest in year 2015, somewhat lower in year 2016 and lowest in year 2017 (Fig. 14A). In year 2015, lesion size was smallest in apples infected at harvest, larger after storage for 3 months and largest after storage for 1 month. In year 2016, lesion size was smallest in apples infected after storage for 3 months, larger after storage for 1 month and biggest when infected at harvest. In year 2017, lesion size was largest in apples infected after storage for 3 months (Fig. 14B). Lesions varied significantly for all orchards among storage periods, with the exception of orchard No. 8 (Fig. 14C) and lesions were smaller in apples that had been stored for 3 months than in those inoculated at harvest time or stored for 1 month (Fig. 14C). The differences in lesion size that we found among years may partly be explained by the development of grey mould, which is influenced by the conditions of the year of harvest, such as temperature, rainfall and humidity (Schumacher, 2017). Also, Kader (1999), Lafer (2006), Tahir (2006; 2009) emphasize that year of harvest is very important for the quality of apples and storability.

The results of those studies are similar to ours, namely that the quality of apples (firmness, sugar content and starch index) was clearly different among years of harvest (Paper IV, Table 1). Thus, in the year 2015 apples had a higher firmness (Fig. 15A), sugar content and starch index (figures not shown) than in those harvested in the years 2016 and 2017. Among the three years, the firmness (Fig. 15B) and starch index (figure not shown) were significantly different among the three storage periods. That such quality factors are sensitive to temperature, rainfall and sun light was reported by Mussachi and Serra (2018).

Our results (Fig. 14 and Fig. 15) suggest that in general higher firmness correlated with a larger lesion size. This association within a cultivar is in agreement with Tahir (2006), who reported that during the harvest period, firmness and starch index of fruit decreased and sugar content increased for four apple cultivars in Sweden. Quality properties, such as firmness, starch index, content of sugar, colour and size have been found to vary among growing seasons (Mussichi and Serra, 2018; Minas et al., 2018; Iwanami, 2011). Other studies show that seasonal conditions affect fruit growth which is known to influence the quality of fruit (Lopez and Dejong, 2007; Marra et al., 2002).

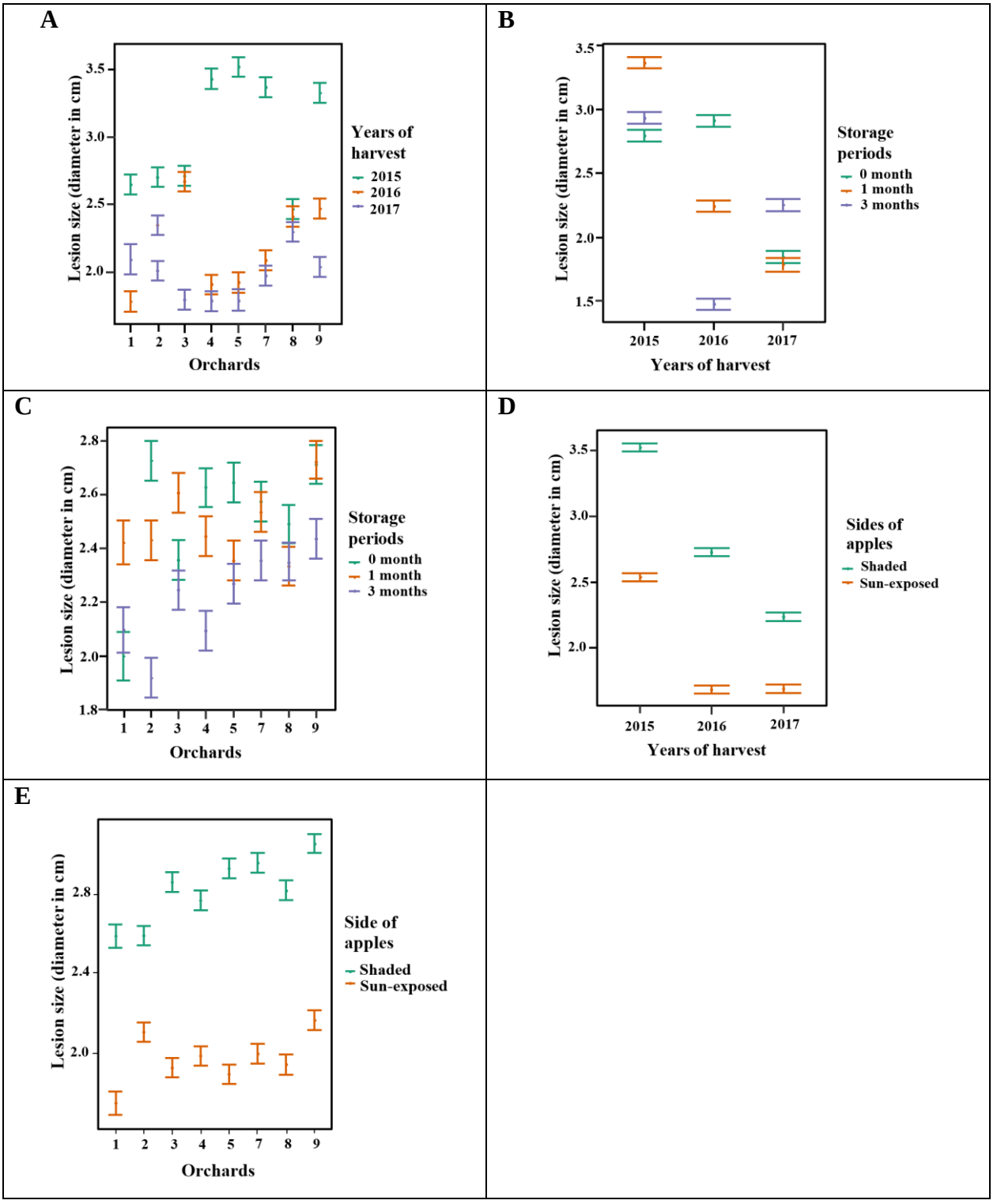

Figure 14. Disease symptoms were measured as lesion size (cm) in apples for the three years of harvest, from eight orchards, numbered 1, 2, 3, 4, 5, 7, 8 and 9, on sun exposed (R) and shaded (G) sides. Effect plots showing predictions from a linear mixed model using Lesion size as response variable. Bars indicate 95 % confidence intervals. (A) Lesion size for three years of harvest vs eight orchards. (B) Lesion size for three years of harvest vs storage periods of 0, 1 and 3 months. (C) Lesion size for eight orchards vs storage periods for 0, 1 and 3 months. (D) Lesion size for two sides of apples vs three years of harvest. (E) Lesion size for two sides of apples vs eight orchards.

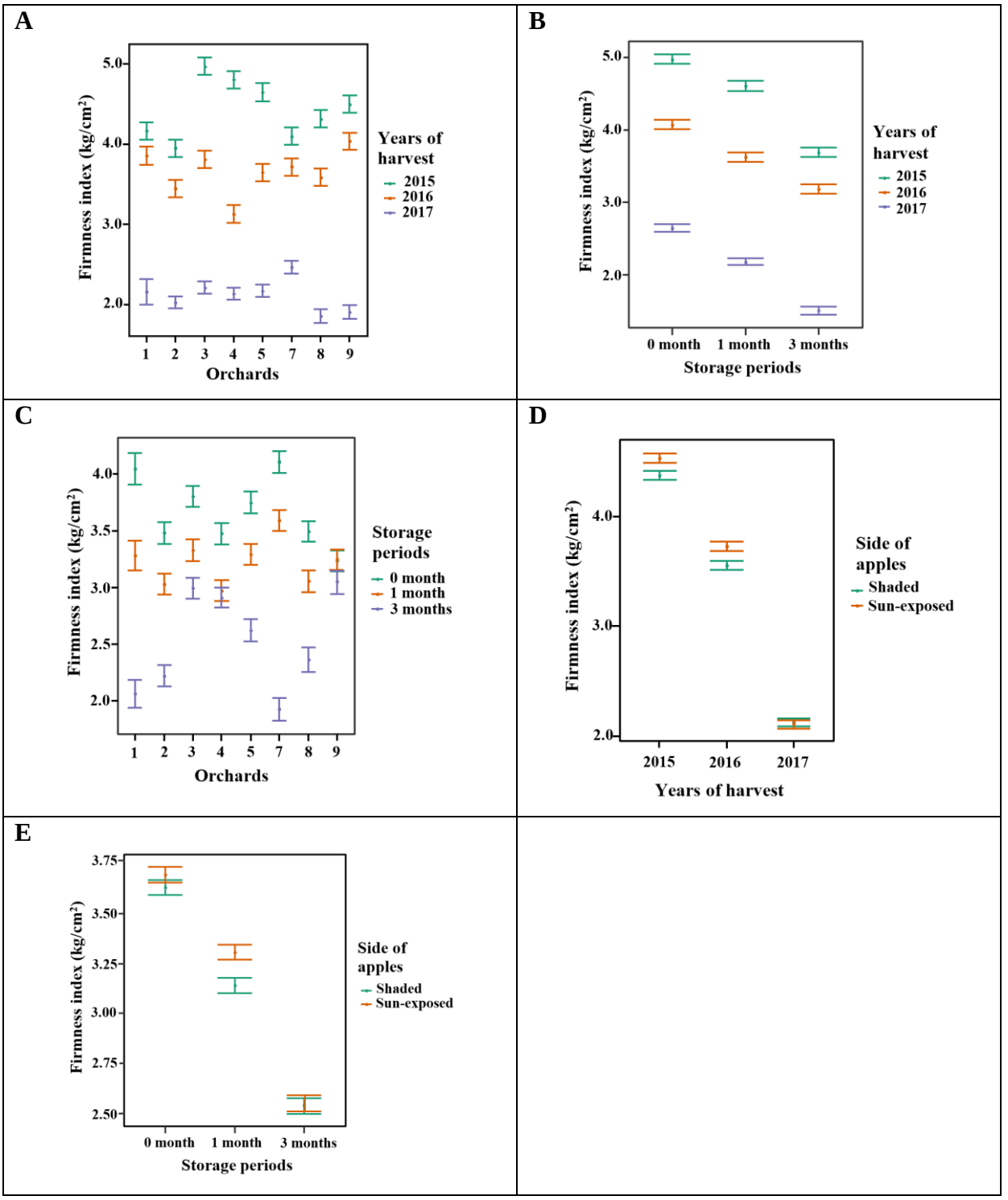

Figure 15. Firmness was determined for the years 2015, 2016 and 2017, for apples from eight orchards, for three storage periods and on sun exposed (R) vs shaded (G) sides of apples. Effect plots showing predictions from a linear mixed model using Firmness as response variable. Bars indicate 95 % confidence intervals. (A) Firmness measured for three years of harvest vs eight orchards. (B) Firmness measured for three harvest years vs three storage periods (0, 1 and 3 months). (C) Firmness for eight orchards vs storage periods for 0, 1 and 3 months. (D) Firmness measured for three harvest years vs two sides of the apples (sun-exposed and shaded sides). (E) Firmness measured for storage for 0, 1 and 3 months vs two sides of the apples, viz. R and G sides.

Orchard's Location

Our second goal was to determine if the orchard's location affects the quality of apples and the development of disease and overall, we were able to group the orchards into two regional groups. Group 1 is located in the southern part and group 2 in the northern part of Scania. We found that apples from group 2 had larger lesion size than apples from group 1 in year 2015 (Fig. 14A). In both groups, firmness was highest in year 2015, lower in year 2016 and lowest in year 2017 (Fig. 15A). It seems that the location of the orchard does not affect the level of firmness whereas year of harvest significantly does. For all 8 orchards, high levels of firmness (Fig. 15A) correlated with larger lesion sizes in the three years of harvest (Fig. 14A). That pattern is in agreement with a finding of Tahir (2006; 2009), who reported that high levels of firmness make apples more susceptible to fungal infection. Our results showed that sugar content and starch index were different among orchards and years (data not shown). However, we did not observe any relationship between the levels of sugar and the starch index with the lesion size among orchards and years, which is a relationship that could have been expected from several previous studies. For example, apples with higher sugar content had higher sensitivity to fungal infection according to Alkan and Fortes (2015).

Storage Periods

Our third goal was to determine the effect of storage on the quality of apples and the development of disease. The firmness of 'Ingrid Marie' apples decreased during storage since the apples continued to ripen after harvest (Fig. 15B, C). Our results agree with those of Felicetti and Mattheis (2010) and Ornelas-Paz et al. (2018), who reported that firmness of 'Golden Delicious' apples continuously decreased during the ripening. There were differences in lesion size among the different years and orchards (Fig. 14B, C). Lesion size decreased strongly over time of storage in year 2016; in contrast, lesion size increased slightly after storage for 3 months in years 2015 and 2017 (Fig. 14B). It seems that the susceptibility to grey mould of apples after storage was strongly influenced by the year of harvest.

After 3 months in storage, the firmness of apples had decreased, which may be the reason why the lesion size decreased because there is a positive relationship between susceptibility to grey mould and the levels of firmness (Fig. 14B). The results of Tahir (2006) and Tahir et al. (2009), who reported that a high level of firmness is related to apples being more susceptible to grey mould, support this explanation. We found such a relationship in year 2016 whereas the effect of storage did not have any clear direction in 2015 and 2017, suggesting that the observation of Tahir (2009) is not a general one.

Our results suggest that higher firmness correlate with a larger lesion size for all the three years and for all orchards. This association within a cultivar is in agreement with Tahir (2006), Tahir et al. (2009) found that several apple cultivars with high firmness were more susceptible to infection by the fungi *Neofabrae spp*. and *Colletotrichim gloesporicides*. Our result, indicating an association of weather conditions with the level of firmness is, furthermore, in agreement with Tromp (2005), de Castro et al. (2007), Harker et al. (2010), Saei et al. (2011) and Jain et al. (2019). Tromp (2005) found that the preharvest factors temperature, soil moisture, nutrition and exposure to sunlight influenced fruit texture including firmness. De Castro et al. (2007) suggested that firmness at harvest time strongly influenced the storability and postharvest quality of apples. Harker et al. (2010) showed that apple fruit firmness is reduced after anthesis and continues to decrease during fruit development and ripening as the intercellular spaces increase in fruit tissue. Larger fruit had softer tissue (lower firmness), larger cells, and more intercellular space than smaller fruit. Saei et al. (2011) demonstrated that firmness is a key factor in determining apple quality, with apples having a better quality when fruits are softer whereas a higher water content. At the same time a higher content of dry matter was associated with higher firmness of apple. Jain et al. (2019) demonstrated that firmness is an important factor in determining the maturity of fruit and is also an important factor in the fruit resistance to pathogen attack.

Sunlight

Our fourth goal was to determine the effect of sun light and position of fruits in the tree on the quality of apples and the development of disease. The sun-exposed side of the apples had much smaller lesion size than the shaded side for all the three years of harvest and for all 8 orchards (Fig. 14D, E). The sun-exposed side had higher sugar content (data not shown), higher firmness for years 2015 and 2016 (Fig. 15D) and after storage for 1 month (Fig. 15E) than the shaded side. Davey et al. (2004), Tuyet et al. (2012, Paper I) and Bui et al. (2019, Paper II) reported that the sun-exposed side of apples was less susceptible to *B. cinerea*. Grappadelli (2003) reported that light affects fruit quality, such as increase in red colour, size, weight, and sugar content of fruit and decrease disease during storage.

Furthermore, Schumacher (2017) found that high sunlight has a positive effect in preventing the fungal infection through an interaction between fungus and fruit. Our results confirm these findings and show that the development of grey mould was larger on the shaded than on the sun exposed side.

Position of Fruit in the Tree

In an earlier study, Nilsson and Gustavsson (2007) found that different positions of the fruit in the tree had no influence on the quality of Swedish apple cv. ‘Aroma’. For the cv. 'Ingrid Marie’ we did not find a major effect of position on quality either (data not shown).

However, interaction terms showed that different positions in combination with orchard and year of harvest gave a significant effect both on quality and development of grey mould (data not shown). For several orchards, apples from a high position displayed higher quality (Fig. 16C, D, E, F) and were less susceptible to infection by *B. cinerea* than those from a low position (Fig. 16A, B). Also, Wagenmakers (1995), Tromp (2005) and Blazek et al. (2007), found that fruits from a higher position in the tree displayed higher quality than those from a lower position.

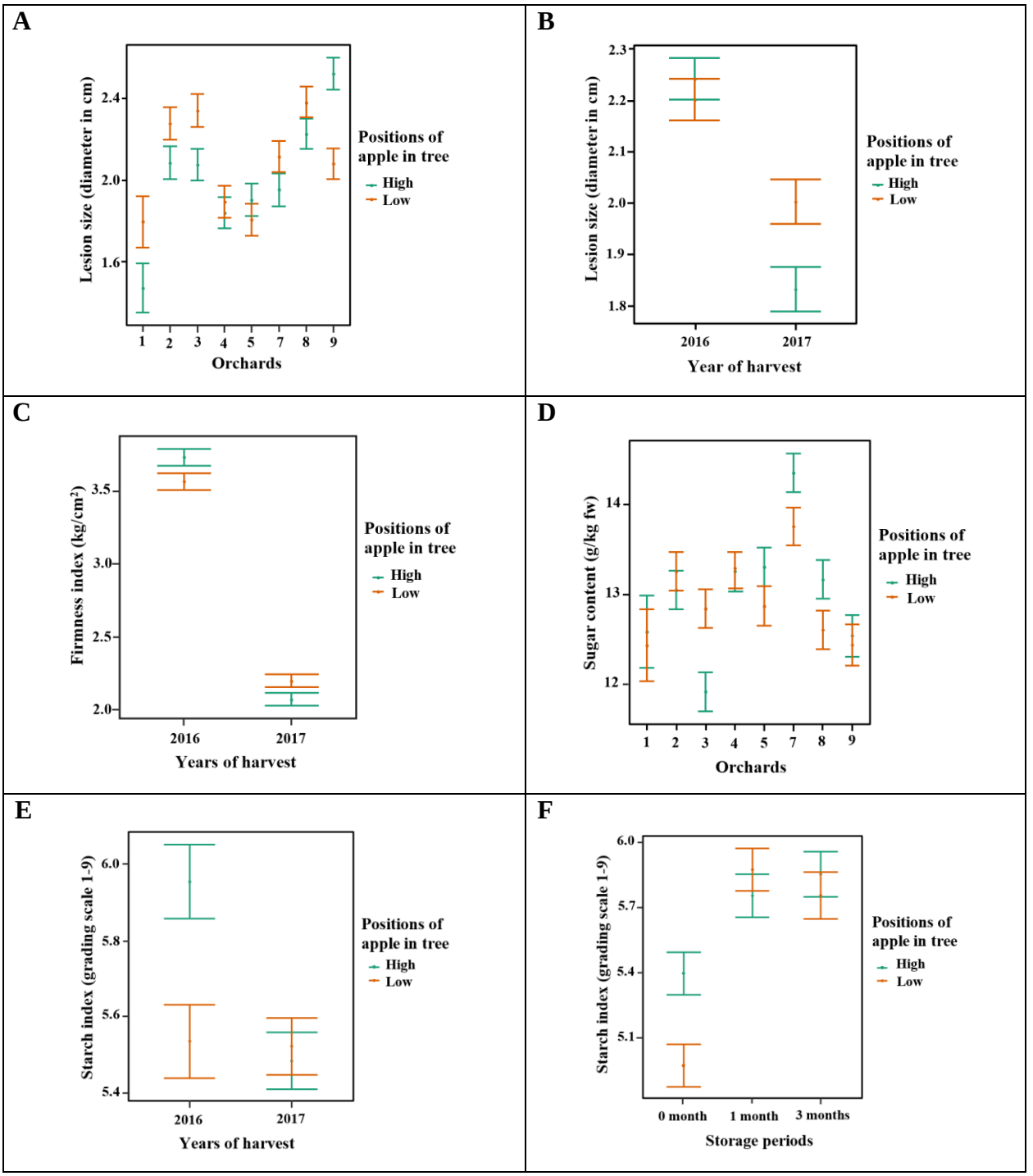

Figure 16. The effect of position of apples in the trees (>1.5 m 'high' and ≤ 1.5 m 'low') on the development of lesion size and quality of apples (firmness and starch indices) for the two years 2016 and 2017, from eight orchards (Nos. 1, 2, 3, 4,5, 7, 8 and 9) and for storage for 0, 1 and 3 months. Effect plots showing predictions from linear mixed models using Lesion size for A and B, Firmness for C, Sugar content for D, and Starch index for E and F as response variable. Bars indicate 95 % confidence intervals. (A) Lesion size for two positions vs orchard. (B) Lesion size for two positions vs years 2016 and 2017. (C) Firmness for two positions vs years 2016 and 2017. (D) Sugar content for two positions vs 8 orchards. (E) Starch index for two positions vs the two years 2016 and 2017. (F) Starch index for two positions vs storage for 0, 1 and 3 months.

3.5. Paper V - "Influence of Weather Conditions on the Quality of 'Ingrid Marie' Apples and their Susceptibility to Grey Mould Infection".

Weather Conditions Among the Three Years of Harvest and Among Eight Orchards

In general, in 2015 the weather was cold, dry, with low sunlight and low rainfall, in 2016 the weather was warm, dry, with high sunlight and heavy rainfall, while in 2017 the weather was cold, wet, with high sunlight and heavy rainfall, and also high humidity (Fig. 17). Temperature in May-June (weeks 21-24) was lowest in 2015, somewhat higher for 2017 and highest for 2016 (Fig. 17A). Rainfall was lowest in April and August (weeks 16, 33) in 2015, somewhat higher later in April and June (weeks 17, 28, 29), whereas in 2016 and in 2017 it was highest in April, June and September (weeks 15, 24, 37) (Fig. 17B). Sunlight was lowest in May, June and July (weeks 18-22, 25 and 30) in 2015, and somewhat higher in 2016 and 2017 (weeks 19-25) (Fig. 17C). In 2015 humidity was lowest in April (week 16), June (week 28-30) and July (week 34), somewhat higher in July for 2016 and highest in June, July and August in 2017. Humidity sank in early May (weeks 18-20) during both 2016 and 2017 (Fig. 17D).

The Influence of Weather Conditions on Apple Quality and Disease Development

An analysis of the data for weather vs apples' quality and susceptibility was made using a Principal Component Analysis (PCA) (Fig. 18). There were major differences in weather conditions and apple quality between the years, and small differences between orchards within a year (Fig. 18).

The harvest in 2017 produced high quality apples with the highest weight and the least susceptibility to *B. cinerea* infection. Year 2016 produced fruit less susceptible to *B. cinerea* infection than in 2017 with a high starch index, while 2015 produced the most susceptible apple fruits with the largest lesion size and the highest firmness (Fig. 18). Our results show that a high level of firmness was positively associated with the development of disease (Fig. 18). In contrast, heavy fruits and higher starch index appeared to make apples more tolerant to infection.

Our results are in agreement with those of several publications, which report that both environment and pre-harvest weather as well as conditions in the orchard affect fruit quality (Saei et al. 2011; Minas et al. 2018; Musacchi and Serra 2018; Marra et al. 2002; Lopez et al. 2007; Wert et al. 2009; Famiani et al. 2012; Pissard et al. 2013; Minas et al. 2018; Musacchi and Serra 2018). The weather during the growing periods affects both fruit growth pattern and size of fruits at harvest (Marra et al. 2002, Loper et al. 2007, Wert et al. 2009). Pissard et al. (2013) and Musacchi and Serra (2018) reported that apple quality depends on a variety of environmental conditions, cultural practices, maturity and storage time. Lee and Kader (2000) and Woolf and Ferguson (2000)

reported that fruit quality is largely affected by pre-harvest conditions such as exposure to high light intensity and high temperatures. Quality properties, such as firmness, starch index, sugar content, colour, weight and size have been found to vary widely among growing seasons (Musacchi and Serra 2018; Minas et al. 2018; Iwanami 2011).

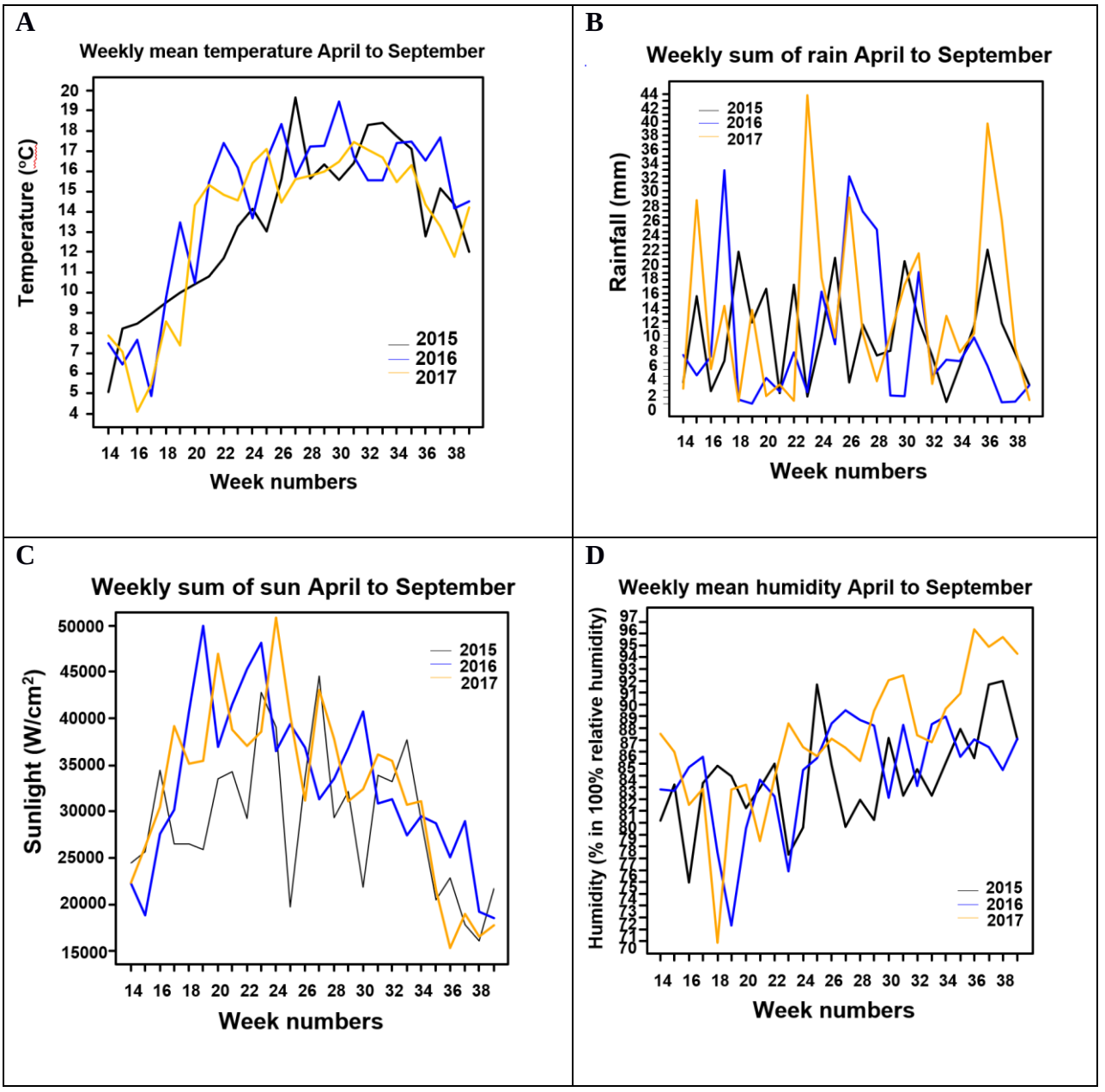

Figure 17. Graphs of weather conditions from April to September (from week 14 in early April until week 39 at the end of September) for each of the three years of harvest (2015, 2016 and 2017). The weekly mean temperature from April to September among the three years (°C) (A). The rainfall from April to September among the three years presented in unit of millimeter (mm) (B). The sum of sun light per week from April to September among 3 years presented in the unit Watts per square centimeter (W/cm^2) (C). The weekly mean humidity from April to September among 3 years presented by as relative humidity (%) (D).

Other studies show that seasonal conditions affect fruit growth, which in turn is known to influence the quality of fruit (Lopez and Dejong, 2007; Marra et al. 2002).

Our results on high starch index and weight of apples showing a negative correlation with the development of disease are in the agreement with Davey et al. (2007) and Doerflinger et al. (2015). Davey et al. (2007) reported that environmental conditions affected apples directly through their impact on plant growth, development and fruit quality at harvest (such as fruit size, weight, vitamin C and antioxidant content).

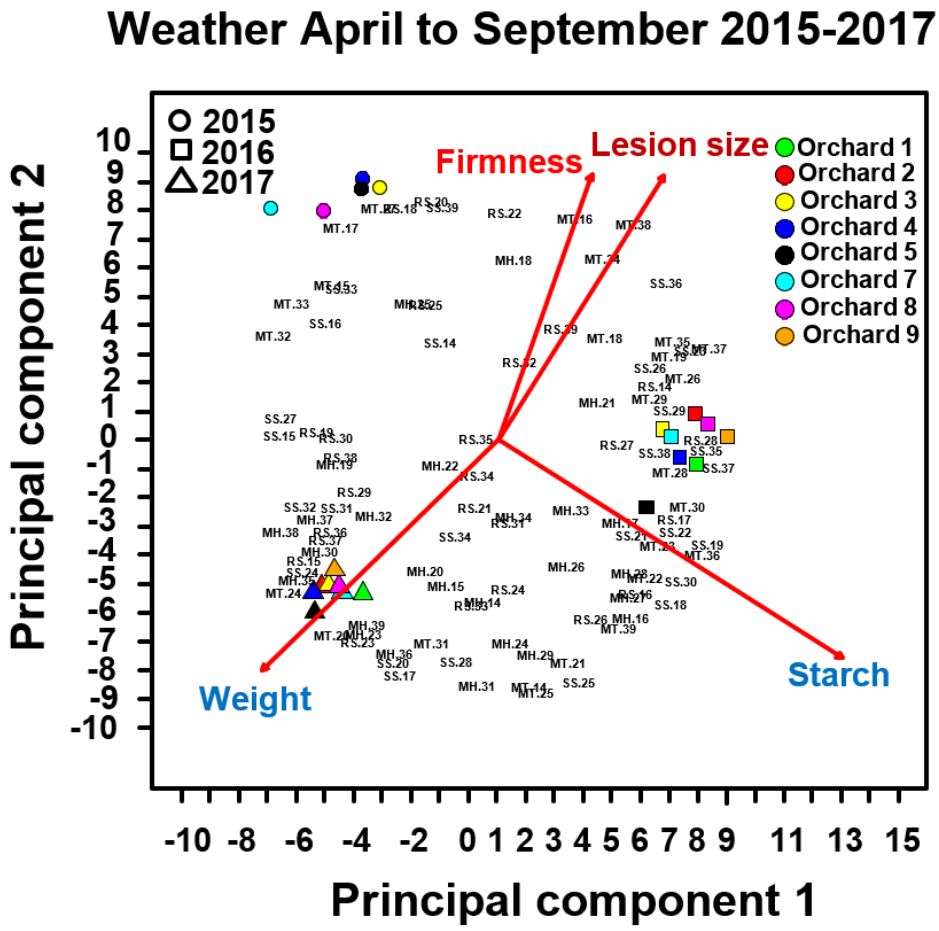

Figure 18. PCA presents the association/correlation between weather conditions and apple quality and the development of disease among the three years of harvest (2015, 2016 and 2017) among eight studied orchards in Scania, the southernmost province of Sweden. The weather conditions are presented as mean temperature-MT, mean humidity-MH, rain sum-RS and sun sum-SS) weekly in April-September. Apple quality is presented as weight of fruit, firmness level and starch index and as the development of disease (Lesion size). The three years of harvest are presented in different forms, circle for year 2015, square for year 2016 and triangle for year 2017. The eight studied orchards are presented using colours. Green, red, yellow, dark blue, black, blue, pink and dark yellow colour represent orchard Nos 1, 2, 3, 4, 5, 7, 8, and 9, respectively.

Apples which at harvest had a higher quality (bigger size, heavier fruit and higher levels of vitamin C and antioxidants) had an increased capacity to resist pathogen attack after harvest. Doerflinger et al. (2015) demonstrated that starch index is one of the simplest and easiest test to determine harvest time of apples and starch index apple is clearly affected by preharvest conditions. Starch hydrolysis begins at the end of the fruit development and the conversion of starch to sugar is one of the processes that most clearly indicate the ripening stage of apple. The maximum starch index in fruit at harvest is affected by cultivar, climate and position in the tree and a light cropping tree have higher starch index in the fruit as seen by darker colour.

Influence of Specific Weather Conditions on Fruit Quality

A novel finding in the study in Paper V is that rainfall, humidity and temperature were the most important weather factors, whereas sunlight was less important as a factor that influenced the quality of apples and their susceptibility to grey mould infection. These results are slightly at odds with the traditional view that rain is an unfavourable weather factor during fruit development and that sunlight is the most important factor. Accordingly, Tromp (2005) reported that sunlight is important for flowering and in determining the size of apple fruits. Tromp and Wertheim (2005) determined that light conditions in the orchard are important for fruit quality and development.

Our results point to that especially high humidity and rainfall in the middle of April and in early June, high temperature and sun-light in in the middle of May, and low temperature at the end of August strongly influenced the quality of apples and their susceptibility to grey mould infection (Fig. 19; Paper V, Table 1). The strong association of humidity with the protection against disease can also be seen in the regression tree for mean lesion size (Fig. 19A). A humidity higher than 77% in early June (week 23) produced tolerant apple fruits with the smallest lesion size (Fig. 19A). When combined with low sunlight week 23 the average lesion size was reduced, whereas, additionally, a humidity higher than 89% this week produced the most tolerant apples. In contrast, a low humidity in early June (week 23) combined with low humidity in the middle of May (week 20), as well as a low rainfall in mid-June (week 24) resulted in the most susceptible apples with the largest lesion size (Fig. 19A).

The mean firmness of apples was strongly associated with rainfall in early June (week 23); at high rainfall apples with low firmness were produced (Fig. 19B). When combined with a high temperature in mid-August (week 34) the highest quality apples with the lowest firmness were produced. However, low rainfall in early June (week 23) resulted in apples of a poor quality with high firmness. When, additionally, the temperature in early May (week 19) was low the apples displaying the highest firmness were produced.

The mean starch index of apples was also associated with rainfall and temperature (Fig. 19C). The apples with the highest starch index (best quality) were produced when the rainfall at the end of April (week 17) was high and that in the end of June (week 26) was low. When, in addition, the temperature in early May (week 18) was low, the highest starch index was recorded. In contrast, a low rainfall at the end of April (week 17) and a low temperature in early June (week 23) produced poor apples with a low starch index.

The mean weight of apple fruits was strongly associated with temperature. A low temperature at the end of August (week 35) produced heavy apples. In addition, when the temperature was high in mid-June (week 24) the heaviest apples were produced. In contrast, with high temperatures at the end of August (week 35) and around 18 ^{o}C in mid-August (week 34), the smallest apples were produced. Moreover, high temperatures in the latter half of August (weeks 34 and 35) also produced smaller fruit (Fig. 19D).

The flowering stages run from dormant buds in early winter to leaf fall in autumn. Apples bloom in April-May, petals fall in early June, fruit sets in July and fruit matures and ripens between 60 days after full bloom up to 180 days before harvest in September and October (Fig. 20). Poor weather conditions at the time of flowering, in particular frost damage, are major causes for poor fruit setting and yields in temperate climates (Tromp and Wertheim 2005).

In agreement with an importance of high temperature during flowering on fruit quality, we observe a negative impact of high sun and high temperature during apple flowering (week 20) on fruit firmness. In contrast, the trend is rather the opposite at earlier time-points, since we can see a strong positive effect of high temperature in April (week 16) on firmness. However, a positive effect of high rainfall during tree flowering (mid-April, week 15) that we observed (Paper V, Table 1) may relate both to the importance of an adequate supply of water in this period as well as to the beneficial effect of rain spreading fertilizer administered. Scudellari (1993) noted that the application of two types of fertilizers prior to tree leafing in two apple cultivars in northern Italy increased fruit weight whereas its quality was only moderately affected.

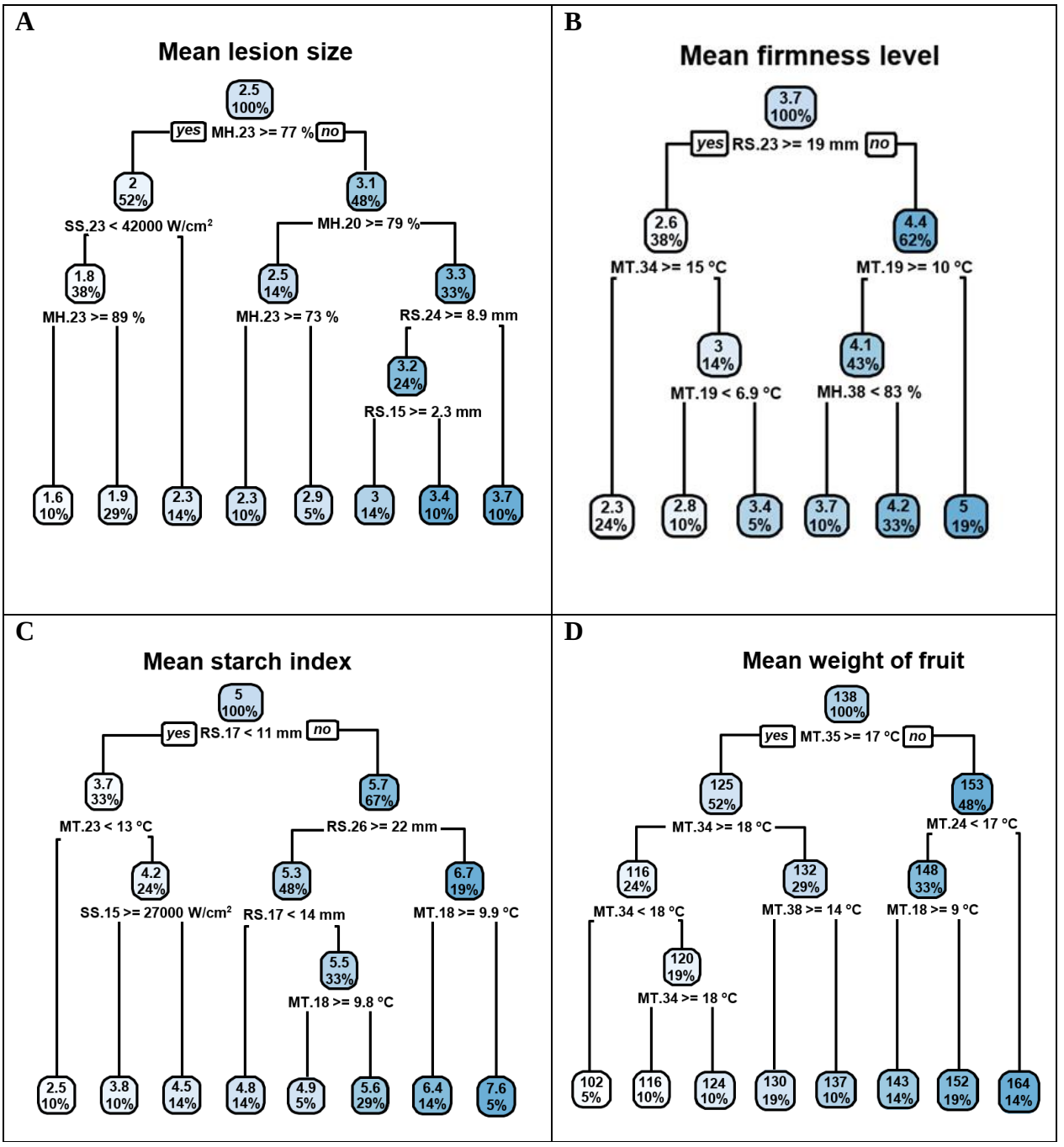

Figure 19. Regression trees of the effect of weather conditions from April to September on specific apples quality parameters for all eight orchards and all three years of harvest. The weather conditions presented are mean temperature - MT, mean humidity - MT, rain sum - RS and sun sum - SS weekly from week 14 in early of April to week 39 at the end of September. Apple quality measurements presented are weight of fruit, firmness level and starch index and the development of disease (lesion size). Weather conditions effected on the mean lesion size (A), on the mean firmness level (B), on the mean starch index (C) and on the mean weight of fruit (D).

Both high rainfall and humidity in early June (week 23) produced tolerant fruit (smallest firmness and lesion size, Fig. 19). At this stage flowering has ceased and early fruit development has started (Fig. 20). During flowering, cell-division activity in the ovary is very low and cell expansion does not occur (Tromp and Wertheim, 2005). After fertilization, a period of rapid cell division starts, which continues for 3 to 6 weeks in apple. Cell enlargement has already commenced at around the time of fertilization but increases when cell division has stopped. Cell number and cell volume together determine ultimate fruit size. In agreement with the importance of weather conditions in mid-May (week 20) and early June (week 23) the first few weeks after full bloom (approximately during the period of cell division) have been pointed out as of paramount significance for fruit maturation at the end of the season (Tromp and Wertheim, 2005). One reason for this is that fruit set and early fruit growth is dependent on the production on hormones, mainly auxin and gibberilins, by the developing seeds, which exert control over the hormone balance within the whole tree.

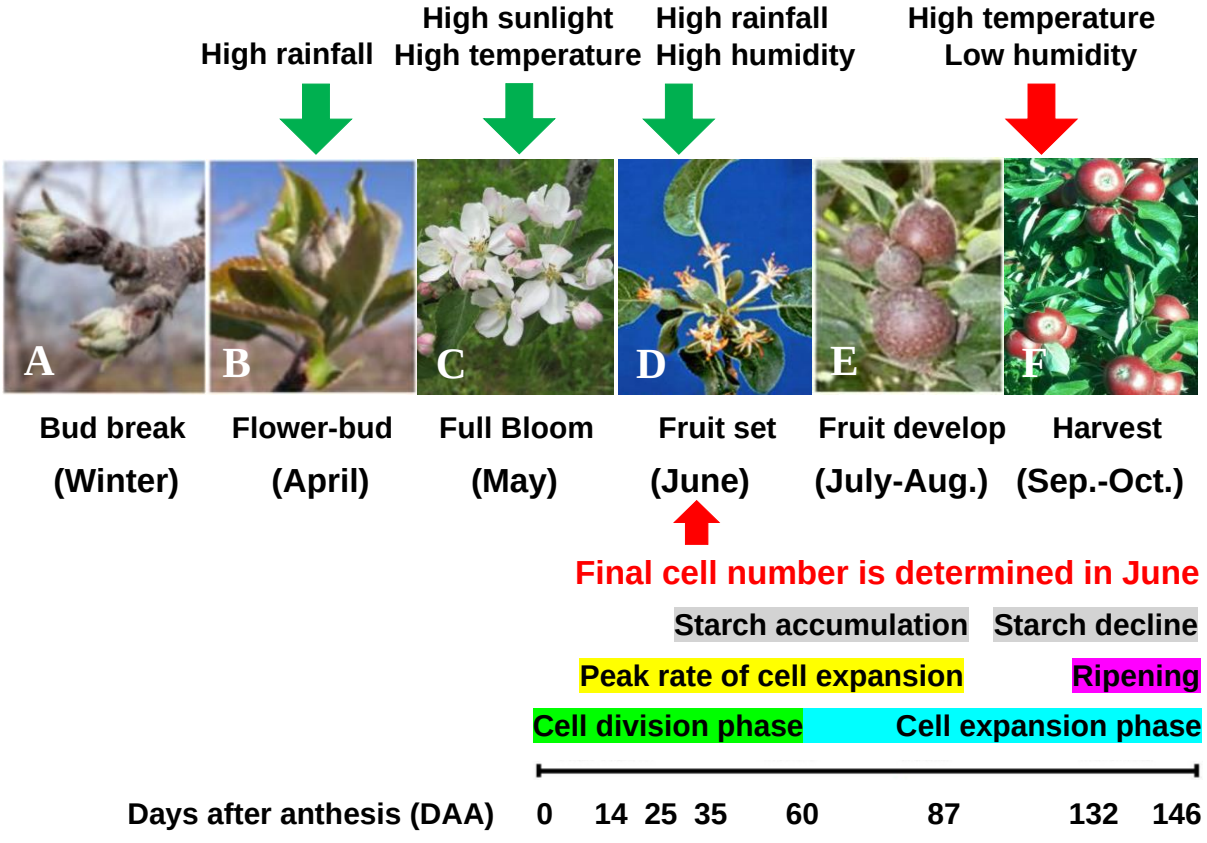

Figure 20. The influence of pre-harvest weather conditions (rainfall, humidity and temperature) on specific stages of growth and fruit development of apples. Bud break occurs during winter (November to March) (A), flower-bud during April (B), full bloom during May (C), fruit set during June (D), fruit development during July-August (E) and harvest during September-October (F). The cell division phase occurs after full bloom (from May to June) and the cell enlargement phase occurs from fruit set to ripening (June-September/October).

Insufficient nutrition during this period may, due to large consequences on hormone balance in the entire tree, stall both seed and embryo development and lead to embryo abortion. In general, shoot growth is more sensitive to water stress than fruit growth but there is a strong dependency on the season (Tromp and Wertheim 2005). Our data also stress the importance of a high-water supply during early fruit development for fruit quality. Our results indicated that low water supply (low humidity and rainfall) in early June (week 23) and low humidity and high temperature in September reduce fruit quality and makes them more susceptibility to grey mould. This finding is opposite to that of Tromp and Wertheim (2005), who reported that low relative humidity increases flower-bud formation and reduces shoot growth and that apple quality increases under water-stress conditions.

Furthermore, the positive effect of high humidity in September (weeks 37-39) on fruit weight and the strongly negative effect of high temperatures in mid-August to mid-September on the same parameter may also relate to water supply (Paper V, Table 1). In pears, the sensitivity of fruit to water stress increases when drought occurs in the later season, in the period of rapid fruit growth (cell expansion), and drought in this period may reduce both fruit size and yield (Tromp and Wertheim 2005). Similarly, in apples water has a higher influence on fruit size and on the quality in the second part of the season (the period of cell expansion). The access of the tree to water affects the weight of the fruit at harvest (Tromp and Wertheim, 2005)

Tromp and Wertheim (2005) investigated the effect of light conditions on the quality of pears from early May until mid-July and found that sunlight in June and July had a positive effect on fruit firmness at harvest. A positive effect of sunlight on weight and starch index of apple fruit as well as on sugar content and a negative effect of sunlight on firmness and acidity was recorded by keeping apple trees at different shade levels in the period from 8 weeks post bloom until harvest (from end of Augus viz. at peak rate of cell expansion phase to September or October at fruit maturity). Similarly, by applying shading to pear trees during two periods after full bloom, namely from 2 to 11 weeks after full bloom and from 11 to 18 weeks after full bloom produced smaller pear fruits (Tromp and Wertheim, 2005). Our results suggest that high sunlight at the end of April (week 17) had a negative influence on firmness, whereas high sunlight in the beginning of May (week 18) positively influenced starch content. In agreement with previous data on the importance of sunlight, we found that high sunlight in mid-June (week 24) stimulated fruit weight (Paper V, Table 1).

4. Conclusions

- Preharvest exposure of apple fruit to high levels of light showed a positive effect of sunlight on the quality of apples as well as on the tolerance of fruits to grey mould infection (pathogen attacks after harvest).
- The results clearly demonstrate that the apple cultivar ‘Braeburn’ was more susceptible to *B. cinerea* infection than ‘Golden Delicious’ and that in both cultivars disease progressed more rapidly through the flesh than the peel as well as it developed more rapidly on the green compared to the red side of the individual fruit.
- Sun-exposed apple peel tissue showed significantly higher levels of antioxidants and increased tolerance to infection by *B. cinerea*.
- Higher levels of protein and phenolics are positively associated with the tolerance of apple fruits to infection by *B. cinerea*.
- The quality of apples and the development of disease varied strongly between year of harvest and among orchards.
- The sun-exposed side of apples and a high position in the tree both lead to higher quality of fruits. Thus, sunlight has a large impact on the quality of apples and their ability to withstand fungal attacks.
- Fruit weight and starch index related negatively, and firmness positively, to disease development.
- High rainfall during tree flowering produced high-quality apples.
- High rainfall and humidity during early fruit development gave more resistant apples of high quality.
- Low temperature and high humidity during fruit-cell enlargement in September produced larger apples.

5. Future Research

I would be grateful if I could continue further research on apples in Sweden and perform further analyses. It would be interesting to investigate also other Swedish apple cultivars such as Aroma, Gloster, Elstar, Alica and Katja. Since differences between individual years turned out to be so marked longer time series (more than 5 years) would be interesting as well as an analysis of soil quality, and more detailed weather and light conditions. Furthermore, modern equipment for molecular analysis, such as the analysis of the antioxidant metabolism (antioxidant metabolites and antioxidant enzyme activities) would be valuable in order to have a more clear picture on antioxidant metabolism in apple tissues at earlier stages of the infection with grey mould (e.g. 8, 12 and 24 hours after inoculation). Predictive instruments may also be further refined by including data on a specific orchard in combination with the weather conditions e.g. temperature and rainfall, during a specific year.

6. Acknowledgements

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