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Evaluation of susceptibility to fruit tree canker caused by *Neonectria ditissima*

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ii. Abstract

Fruit tree canker caused by *Neonectria ditissima* is a major bark canker disease on apple especially in temperate regions with high humidity and precipitation. The fungus can infect plant material through natural or artificial wounds. It can lead to loss of yield with possible high impact on economy for the apple producer. It has been reported that there is more fruit tree canker attack on apple in recent years.

To evaluate susceptibility of genotypes to fruit tree canker, one year old potted tree and detached shoots from top of main stem of one year old trees were tested in the greenhouse and growth chamber. All experiments were inoculated with macroconidia suspension after artificial wounding. The inoculum used in these trials was kept in deep freezer and activated on rootstock before inoculation on wounded areas. In general, infection percent and area under the disease progress curve (AUDPC) are commonly calculated to examine resistance of apple genotypes to fruit tree canker. This study showed that infection percent or AUDPC values alone were not enough to conclude about resistance level to fruit tree canker in apple genotypes. It may be beneficial to determine the resistance level by using several parameters, as evaluation of several parameters together seem to ensure more reliable results. In this study, a range of parameters were applied for recording symptom development. External lesion size was measured at fixed points during experiment period, also giving number of days until the first symptoms were visible. Several extra parameters were recorded at the last observation date, such as internal lesion size, wilting, girdling, swelling, flakes, bubbles, ring pattern, color and sporulation.

In general, the developing of lesion surface size increased rapidly 4 weeks after first symptoms was visible in potted tree trial and in detached shoot trial symptoms increased rapidly already one week after first symptoms were visible. We investigated susceptibility of apple cultivars/genotypes by analysis of datasets including or not including inoculation points with no infection symptoms. It was a clear pattern that when including only inoculation points with infection in data analysis, susceptibility of cultivars with low infection percentage can be overestimated.

The number of days until the first symptoms were visible after inoculation seem to be a good parameter to investigate susceptibility to fruit tree canker, together with other parameters such as AUDPC level and lesion size at the end of experiment. Wilting and girdling are important parameters to include when examining resistance of apple genotypes, both parameters presented a good association with other parameters such as AUDPC level and external lesion size at last

recording date. Swelling is a parameter that may discriminate significant difference among apple cultivars. ‘Aroma’ is known to have low to medium susceptibility to *N. ditissima*, and on swelling character ‘Aroma Fagravoll’ differed from the susceptible cultivars ‘Elise’ and ‘Discovery’. PCA plot showed a relationship between the characters swelling and bubbles. These two characters might be related to resistance, while flakes seem to be related to susceptibility.

The tested apple genotypes differ in level of susceptibility to fruit tree canker. MA042 10041 and ‘Bramley’s Seedling’ showed stronger resistance against the canker than ‘Aroma’ in this study.

The study shows that recording of several symptoms can give a clearer picture of susceptibility to canker in apple genotypes. Phenotyping with several characters is necessary to get reliable results on resistance in breeding material for use as parents and for introduction of new resistant cultivars. Such phenotyping is also necessary when searching for resistance genes or markers for resistance genes.

iii. Sammendrag

Frukttrekreft forårsaket av soppen *Neonectria ditissima* er en viktig plantesykdom på eple både på treet og fruktene, spesielt i områder med mildt og fuktig klima. Soppen kan infisere plantemateriale gjennom naturlige sår som for eksempel frostskade, sårdannelse etter bladfall (bladarr) eller beskjæring. Angrep av frukttrekreft kan medføre økonomisk tap for epleprodusenter. Det er rapportert at det er mer frukttrekreft nå enn det har vært før.

For å evaluere motstandsevne mot angrep av frukttrekreft hos nummersorter og sorter ble det gjennomført forsøk på ettårige tre (pisker) og avskårne toppskudd av ettårige tre i veksthus og vekstrom. Plantemateriale i alle forsøk ble inokulert med makrokonidia etter såring, der inokulum ble lagret i fryseboks og aktivert på grunnstammer før det ble brukt i forsøkene. Vanligvis blir infeksjonsprosent og «area under the disease progress curve» (AUDPC) beregnet for å kartlegge motstandsevne mot angrep av fruktrekreft hos eplesorter. I denne studien fant vi at infeksjonsprosent og AUDPC-verdier alene ikke var nok til å konkludere om motstandsevne. Evaluering av flere parameter sammen ser ut til å sikre mer pålitelige resultater. I denne studien ble et vidt spekter av parametre brukt til å registrere ulike symptomer i forsøkene. Lengde av ytre skade ble registrert med faste intervall undervegs i løpet av forsøksperioden, og dette ga samtidig antall dager til første symptomer ble synlig etter inokulering. Sluttregistrering omfattet registrering av både ytre symptomer og indre symptomer, som for eksempel visning, ringing (sår vokser rundt tre), svelling, flassing, bobling, ringmønster, farge og sporulering.

Observasjon av ytre skade viste at størrelse øker fort 4 uker etter første synlige symptom på hele trær og bare en uke etter første ytre skade var synlig på avskårne skudd. Vi undersøkte motstandsevne hos eplesorter ved analyse av datasett både med og uten inkludering av inokuleringspunkt uten synlig ytre skade. Resultatene viser at vi kan overestimere motstandsevne hos eplesorter med lav infeksjonsprosent når bare inokuleringspunkt med synlig skade er inkludert. Ved å analysere datasett både med og uten punkt uten synlig skade kan vi få et sikrere bilde av mottakelighet for *N. ditissima.*

Antall dager til første symptom er synlige etter inokulering ser ut til å være en god parameter for å undersøke motstandsevne mot frukttrekreft, sammen med AUDPC-verdi og lengde av ytre skade. Visning og ringing er viktige parametere å inkludere for å vurdere motstandevne hos eplesorter, begge parametrene viste god tilknytning til andre parametere som for eksempel AUDPC-nivå og lengde av ytre skade. Svelling er en parameter som kan skille betydelig mellom eplesorter. Sorten 'Aroma' er kjent for å ha lav til middels mottakelighet for *N.*

ditissima, og for karakteren svelling var 'Aroma Fagravoll' signifikant forskjellig fra de mottakelige sortene 'Elise' og 'Discovery'. PCA-plot viste en sammenheng mellom karakterene svelling og bobling. Disse to karakterene kan være knyttet til resistens, mens flassing ser ut til å være knyttet til mottakelighet.

Eplesorter varierer i motstandsevne mot angrep av fruktrekreft. Nummersort MA042 10041 og 'Bramley's Seedling' viste sterkere motstand mot frukttrekreft enn 'Aroma' i denne undersøkelsen.

Denne studien viser at registrering av flere symptomer gir et klarere bilde av motstandevne mot frukttrekreft hos eplesorter. Fenotyping av flere ulike symptomer gir et sikrere bilde av mottakelighet hos sorter for bruk som foreldre i kryssing og for å lansere en ny sort med høy motstandsevne mot frukttrekreft til epleprodusenter. Slik fenotyping er også viktig og nødvendig når det gjelder å finne resistensgener eller markører for resistensgener.

Content

1. Introduction

Releasing new cultivars with high adaptation to local environmental conditions, with good fruit quality, high yield, and resistance against plant diseases is a major goal for apple breeding programs around the world, including in Norway (Press 2014; Roen 1998). Apple genotypes with high resistance against important plant diseases such as apple scab, powdery mildew and fruit tree canker are important for use in fruit production but also as parents for breeding (Roen 1998). Several breeding programs have carried out research to find sources of resistance against plant diseases to develop new improved genotypes (Schoonhoven et al. 2005).

Fruit tree canker caused by *Neonectria ditissima* (Tulasne and C. Tulasne) Samuels and Rossmann (syn. *Nectria galligena* Bresadola syn. *Nectria ditissima* Tulasne and C. Tullasne), imperfect stage *Cylindrocarpon heteronemum* (Berkeley and Broome) Wollenweber (EPPO Global Database 2021), is an important woody tree disease on apple, especially in the regions with high humidity and precipitation (Saville and Olivieri 2019). The fungus can infect the plant material through natural or artificial wounds (Swinburne 1975). It was reported that apple genotypes vary in resistance levels (Van de Weg 1989). *Neonectria ditissima* can colonize the main stem, side branches and fruit (Cooke 1999), and it may lead to losses of yield (McCracken et al. 2003), with possible high impact on economy for the apple producer (Garkava-Gustavsson et al. 2013).

To better understand the epidemiology of the fungus, a public - private partnership project (PPP) in pre-breeding was initiated in Norway, Sweden, and Finland, aiming to build up knowledge about resistance levels against fruit tree canker and storage diseases in apple. It aims to improve genotypes with high resistance, having potential for use in breeding in the Nordic countries, by finding sources for resistance against fruit tree canker (Nybom et al. 2016a).

Several methods can be used to discriminate susceptibility level to fruit tree canker caused by *N. ditissima* on apple (Gomez-Cortecero et al. 2016; Van de Weg 1989), including inoculating on whole tree and the detached shoot method, inoculation with *N. ditissima* conidia or ascospore solutions (Wenneker et al. 2017) or by natural or artificial wounding. It is reported that the detached shoot method did not perform optimally and gave unreliable results compared to the whole tree method. The results from detached shoot method differed from observations under field conditions and the test cultivars did not give the similar results between two treatments (detached shoot trial and potted tree trial). It was suggested not to replace the whole

tree method with the detached shoot method as it seems that the whole tree method is the best method to determine the level of resistance against fruit tree canker (Scheper et al. 2018). Inoculation on whole trees can be with *N. ditissima* conidia (Van de Weg 1989; Wenneker et al. 2017), ascospores or mycelia (Borecki and Czynczyk 1985), wounded with scalpel (Garkava-Gustavsson et al. 2013; Garkava-Gustavsson et al. 2016; Nybom et al. 2016a), rasp (Amponsah et al. 2017) and map pins (Talgø and Stensvand 2013) or unwounded treatment on leaf scars after leaf fall (Crowdy 1952; Lichtfouse 2009; Wenneker et al. 2017).

2. Literature review

2.1. Apple (*Malus* × *domestica*)

The *Malus* genus belongs to the Rosaceae family. The domesticated apple (*Malus* × *domestica*) is a hybrid originating mainly from *Malus sieversii* M.Roem in Central Asia (Janick & Moore 1996). *M.* × *domestica* is a diploid (2n = 2x =34 chromosomes); however, some cultivars are triploids (3x = 51 chromosomes) (Janick and Moore 1996).

The genetic variation of *Malus* has been used in breeding to make new genotypes adapted to local climate, with higher quality, promising yield, and disease resistance. Apples are the fourth most important fruit crop worldwide (Janick and Moore 1996), and the apple production was around 870 million tons in 2019 (FAO 2019).

Apple is a temperate fruit that grows mainly in temperate regions in Asia, North and South America, South Africa, New Zealand, Australia, and Europe (Janick and Moore 1996). In Norway, apple production is principally in areas around the Oslo Fjord, in Telemark and along the fjords of Western Norway, and in 2019 the apple production was about 15593 tons (SSB 2019), with approximately 50 percent used for processing. The main cultivars grown for domestic commercial market are ‘Aroma’, ‘Summered’, ‘Discovery’, ‘Gravenstein’ and ‘Rubinstep’ (GPS 2018). Apple bloom normally begins in mid-May and depending on cultivars harvest is from mid-August to mid-October. By selection of cultivars for commercial production, it is important to select cultivars which can grow and reach maturity under climatic conditions in Norwegian production areas.

Environmental conditions such as temperature and photoperiod influence and stimulate signal processes for vegetative and generative growth phases for perennial crops (Funt and Hall 2013; Heide et al. 2020). Apple is a perennial crop and requires optimal environment such as

temperature and photoperiod especially autumn and winter the year before. In addition, favorable conditions during growing season are significant importance factors to get good fruit quality and yield. A high yield potential is depending on the climate the year before (Heide et al. 2020; Roen and Sønsteby 2019).

2.2. Apple Breeding

Apple breeding mainly focuses on high yield, good fruit quality, adaptation to local climate, and resistance to diseases. Selection methods have been based primarily on phenotyping traits such as yield, fruit quality and screening for resistance against important plant diseases (Baumgartner et al. 2016). Genotypes with low amounts of allelochemicals have been selected to please consumer with pleasant taste. Breeder selected individuals that produce probably lower content of secondary compounds, such as terpenes, phenolics and nitrogen containing compounds, which can minimize ability to defend the plant against herbivores and microbials (Schoonhoven et al. 2005; Taiz et al. 2018). Level of resistance against natural enemies differ probably due to differences in such allelochemicals (Schoonhoven et al. 2005). Lower levels of allelochemicals can make a new genotype more susceptible to natural enemies (Taiz et al. 2018). For example, 'Nicoter' Kanzi® is a new genotype with high commercial quality (Weber and Hahn 2013), but it is very susceptible to fruit tree canker (Weber and Hahn 2013). It is reported that modern genotypes are generally susceptible to *N. ditissima* but differ in susceptibility levels (Ghasemkhani 2015; Ghasemkhani et al. 2015; Gomez-Cortecero et al. 2016). Another example of a commonly grown cultivar internationally is 'Royal Gala', known to be quite susceptible to *N. ditissima* (Amponsah et al. 2017; Bus et al. 2019; Gomez-Cortecero et al. 2016; Scheper et al. 2018). Other cultivars such as 'Cameo', 'Discovery' 'Fiesta', 'Idared', 'McIntosh', 'Red Delicious' and 'Spartan' are also known to be very susceptible to fruit tree canker (Cross et al. 2013).

Some cultivars, such as 'Golden Delicious', have relatively good resistance to canker (Bus et al. 2019; Garkava-Gustavsson et al. 2013; Ghasemkhani et al. 2015; Gomez-Cortecero et al. 2016; Van de Weg 1989), and can better withstand the pathogen infection, typically repairing wounded areas with a ring of new cortex. Several barriers have been shown beneficial for some cultivars, including mechanisms for protein repair (Willey 2016). It is important to understand plant responses to this pathogen, shown as symptoms of this disease and resistance reactions to handle the damage.

The fruit tree canker have been reported in Europe, North America, Chile, Australia, New Zealand, Japan and South Africa (Kim and Beresford 2012; Weber 2014). Canker is also an important apple disease in the Nordic countries (Nybom et al. 2016a). Important genotypes used in apple production in Norway such as 'Discovery', 'Summered', and 'Gravenstein' are observed to have high susceptibility to canker while 'Aroma' and 'Rubinstep' are relatively resistant (Borve et al. 2019; Garkava-Gustavsson et al. 2013; Kühn 2004).

It is observed that *N. ditissima* isolates seem not to be specific to host plants (Langrell and Barbara 2001; Saville and Olivieri 2019), being a general pathogen to woody plants without specific races (Gomez-Cortero et al. 2016; Saville and Olivieri 2019). As *N. ditissima* does not have different races, a source of resistance can be used to improve cultivars for use in all regions (Saville and Olivieri 2019). It is not like the apple scab pathogen (*Venturia inaequalis* Cooke (Wint.) that have different races (Bus et al. 2011), and some race can attack the cultivar that has a kind of resistance to apple scab. For example, as the Vf (Rvi6) resistance to apple scab is broken down by race 6 (Janick and Moore 1996).

It is desirable for apple producers to eliminate yield losses caused by *N. ditissima* by breeding cultivars with high resistance against the pathogen. The selection method should be precise to ensure a new cultivar with disease resistance. It is needed to implement effective selection methods that are easy to perform or implement molecular techniques to determine individuals with disease resistance (Schoonhoven et al. 2005). Selection methods based on phenotyping is used up to now because genes for resistance to canker was not found (Garkava-Gustavsson et al. 2013). It is important to apply reliable phenotyping methods to evaluate susceptibility to fruit tree canker. Results from evaluations are useful to benefit from by choosing resistant cultivars as parents in crossing (Garkava-Gustavsson et al. 2013; Van de Weg 1989) and for apple growing (Weber 2014). It is a long-term process to develop and improve a new cultivar with disease resistance. Selection methods based on phenotyping in conventional breeding is a time-consuming task, and molecular techniques may be needed to achieve effective and precise breeding (Schoonhoven et al. 2005). Genotype and phenotype must be linked to find which genes gives the effect seen on phenotype, and for fruit canker resistance there seem to be only QTLs (Quantitative trait locus) involved (Bus et al. 2019), QTL mapping is to find areas/positions on chromosomal regions that affects a quantitative character. It is important to collect (big) data both for genotypes (gene maps) and visual characters/phenotyping to analysis QTL. Characters such as the ring of new cortex around wounded area or swelling that may involve resistance mechanism to fruit tree canker, cannot be linked to positions on the

chromosome without good observations in the test experiments and under field conditions. It is promising that the first QTLs for canker resistance have been found recently (Bus et al. 2019). Resistance against diseases may perform as either a qualitative or quantitative trait. It involves either a single major gene or several genes that differ in their resistance effect where some have a major effect and some with less effect (minor genes) (Press 2014). Cultivars with complex resistance genes (quantitative resistance) to diseases are preferred for long term resistance (Schoonhoven et al. 2005).

2.3. Fruit tree canker (*Neonectria ditissima*)

Fruit tree canker is a woody tree disease on apple (*Malus*) and several other host plants for example pear (*Pyrus*) poplar (*Populus),* hawthorn (*Crataegus*) and beech (*Fagus*) (Flack and Swinburne 1977) and is an important disease in apple production (Sutton et al. 2014). It has potential to have great impact by reducing yield for the apple producer (Weber 2014). The host tree can be infected and colonized by *N. ditissima* throughout the year (Saville and Olivieri 2019). The pathogen infects the stem through leaf scars or wounds and can infect the fruit via lenticels and calyxes (Schumann and D'Arcy Cleora 2019; Sutton et al. 2014). This fungus can grow on the main stem, but it can expand further to other parts such as side branches, leaves and fruits. It can develop rapidly and the whole tree can be damaged (McCracken et al. 2003).

The canker was first described approximately 1880 by Goethe and in 1882 by Hartig. In 1921 it was reported about pathway to enter plant tissue through leaf scars after leaf fall by Wiltshire (1921), and in 1926 was published more details of *N. ditissima* and anatomy of the fungus (Zeller 1926).

Neonectria ditissima belonging to the Ascomycetes, can reproduce sexually or asexually. Fruiting bodies of the sexual stage are called perithecia. Perithecia are ovoid -pyriform shaped, 250-350 µm in diameter and 300-450 µm long, colonizing on surfaces of the host trees (Figure 1). The color of the perithecia is orange/red to dark brown (Figure 1) depending on age of the perithecium, when young the color is light orange and when old it is red to dark brown (Sutton et al. 2014). The fruiting bodies can produce ascospores with size 6-9 × 14-22 µm (Sutton et al. 2014), mainly in late summer and autumn, but ascospores may be seen the whole year for some regions, and they can disperse and germinate whenever the environment is favourable (Wenneker et al. 2017). Season and time to release ascospores can also vary from place to place depending on region and climate, ascospores are able to disperse whole year (Weber and Børve

2021). Ascospores can be dispersed by wind, or they may ooze from the perithecia in wet weather and be dispersed by insects that can transport the pathogen from the infection sites to other areas (Swinburne 1975; Weber 2014).

Figure 1. Typical symptom of infection by *Neonectria ditissima* (left) on apple. Fruiting bodies (perithecia, right). Photo: Kurab Røen.

The asexual stage is called *Cylindroncarpon heteronemum*, and it develops microconidia and macroconidia in sporodochia (a small, compact hyphal mass). Macroconidia develop on short cylindrical phialides (2-2.5 × 12-16 µm) on branched conidiophores. The shape of the macroconidia is straight or curved with rounded ends (4.5-5.5 × 52-62 µm), depending on the number of septa and can have up to 7 septa. Microconidia are smaller than macroconidia with a size of 2-3 × 4-8 µm; the shape is aseptate and cylindrical with rounded ends (Sutton et al. 2014). Conidia can differ in size and shape depending on the environment. Conidia developing from sporodochia (Figure 2) can appear in spring and can be produced in the summer and early autumn as well if the conditions are favourable (Sutton et al. 2014).

Figure 2. Sporodochia of *Neonectria ditissima* containing conidiphores and conidia appear on the bark. Photo: Kurab Røen.

2.4. Symptoms

Typical early canker symptoms are sunken areas with light brown to dark brown colour in the bark, and over time it will develop brown necrotic lesions depending on cultivar and development stage (Swinburne 1975). When *N. ditissima* colonize a tree, cankers can grow and girdle the stem and side branches, which dry out (Figure 3) and lead to death of the main stem and side branches (Saville and Olivieri 2019). Symptoms can also appear on the leaves (Naqvi 2004), and flower buds can get damaged and result in loss of flowers. The fungus can infect the host tree without any symptom development until the following year. *Neonectria ditissimma* colonizes and damage the plant tissue in the areas around the infection point, and then centric rings are developed. Infection areas develop and spread faster along the stem than sideways (Swinburne 1975). Favorable conditions during the infection are important to stimulate for the growth rate of the lesion areas (Van de Weg 1989). During the first period of infection, it can be easy to separate margins of the cankers, but after a while the infected area develops irregularly and is sunken into the stem (Sutton et al. 2014). The infection areas form flakes, dark brown color lesions, and the plant tissues are swollen around old canker wounds (Swinburne 1975).

Figure 3. Trees infected *with Neonectria ditissima*, typical symptoms of canker that can colonize on the main stem (left) and then spread to side branches (right). Photo: Kurab Røen.

2.5. Infection and life cycle

Fruit tree canker is an important apple disease, especially in regions with high humidity and regular rainfall (Beresford and Kim 2011; Swinburne 1975; Weber 2014). Climate conditions strongly influence the impact of the pathogen (Swinburne 1975; Beresford and Kim 2011; Weber 2014; Sutton et al. 2014; Madden 1997). The main pathway to enter the plant material is through natural openings such as leaf scars, e.g. when rosette leaves detach in the late spring, and it can enter inside all kind of wounds by artificial wounding from management of the trees (Saville and Olivieri 2019). Leaf scars in the autumn after leaf fall seem to be a major pathway to entering infection (Dubin and English 1974) and bark cracks in the winter, damage from growth and development and even damage from insect attack can be infection sites (Swinburne 1975; Weber 2014).

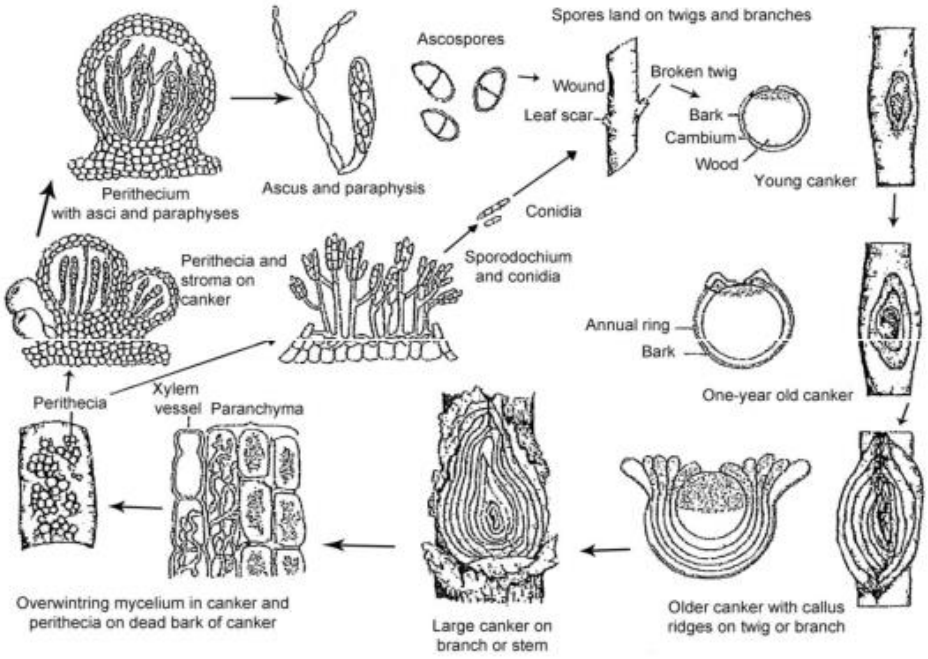

Figure 4. The life cycle of fruit tree canker caused by the fungus *Neonectria ditissima* (Agrios 2005).

New infections of *N. ditissima* can occur on the host tree by conidia or ascospores (Figure 4) in all growth and development stages (McCracken et al. 2003). *Neonectria ditissima* can establish in the wood tissue and penetrate into the xylem, and be transported along the vascular system, but the pathogen cannot enter through to the phloem (Crowdy 1949; Zeller 1926).

In the autumn, spores can land on the leaf scars, which are highly susceptible for infection 24-48 hours following leaf drop, and the spores are being absorbed into the vascular tissue (Cross et al. 2013). Leaf scars have increased resistance 48 hours after leaf fall (Cross et al. 2013). Conidia have a short life span and can only be dispersed a short distance by rain splash. The young apple fruit can be infected after full bloom and develop a fruit rot, symptoms can occur in the field and then continue to develop in storage (Xu and Robinson 2010).

The fungus can disperse from tree to tree within the orchard, ascospores can be airborne while conidia are mainly spread by rain or water splash. The main infection period occurs in the autumn through leaf scars, and symptoms will become visible the following season (Saville and Olivieri 2019). Airborne spores can initiate new infection points while waterborne spores mostly infect in the same tree. Trials in vitro have shown that ascospores can germinate more rapidly than conidia, indicating that ascospores can infect faster than conidia in regions with

favorable conditions during leaf fall in the autumn (Saville and Olivieri 2019). Ascospores can disperse up to 125 metres by wind, supporting the finding that ascospores are the major cause of long distance dispersal (Madden 1997). Wetness is associated with infections in leaf scars, because rain and high humidity stimulates infection due to the effect on spore release and germination (Dubin and English 1974).

Neonectria ditissima can expand within the orchard and can introduce infection in new orchard from infected plant material (Beresford and Kim 2011). In young orchards one can often see the symptoms within a few years after planting (Gomez-Cortecero et al. 2016; McCracken et al. 2003). It is reported that trees can be infected already during propagation in the nurseries, and infection can spread further to apple trees in the new orchard. Infection in the nursery can enter the young tree from an infected rootstock (Borve et al. 2018).

This study consists of three experiments at Njøs fruit and berry centre: 1. An experiment with 10 genotypes in 2018-2019 with inoculation of whole trees with *N. ditissima* conidia solution in the greenhouse. 2. An experiment in 2019-2020 with 16 genotypes, inoculation on whole trees with conidia of *N. ditissima* in the greenhouse. 3. An experiment with three seedling populations in 2020-2021 with inoculation of detached shoots with conidia of *N. ditissima* in growth chamber.

The objective of this study was to evaluate the level of resistance to fruit tree canker in apple genotypes, the goal is to find genotypes with high disease resistance that can be used as parents in crossing and for apple production in Norway and the other Nordic countries. A second objective was to investigate methods to determine resistance level that give reliable results. Here, methods were applied to record symptom development during the experimental period, such as measuring of the external lesion length. Furthermore, at the last observation several characters were recorded, including flaking, bubbles, ring pattern, discoloring, sporulation, wilting and internal canker lesion length to better describe the response to infection of the fungus *N. ditissima* and classify characteristic symptom development among genotypes. It was a purpose to find a standard method for discriminating susceptibility levels to fruit tree canker in apple during the selection process in apple breeding lines. Apple genotypes for these experiments were partly promising selections from the Norwegian apple breeding program and partly cultivars and selections from a common Nordic core and diversity collection (PPP Nordfruit (D. Røen, pers. comm.).

3. Materials and Methods

3.1. Plant material

3.1.1. Potted tree experiment in 2018

Ten different genotypes were chosen to compare the level of resistance against fruit tree canker caused by *N. ditissima* in a potted tree trial. One-year old whips in pots of 10 genotypes on M9 rootstock were used. The plants were from Åberge planteskule (plant nursery) in Sogndal. The trees were grafted in February-March 2018. The inoculation experiment was carried out at Njøs fruit and berry centre in Sogn in western Norway. The centre is a national site for breeding of fruits and berries in Norway, located at 61.1859° N, 6.8080° E. The graftwood was elite material from the Elite plant station Sagaplant AS in southeastern Norway, except 'Aroma'. 'Aroma' graftwood were collected from Åberge planteskule. The genotypes used are shown in Table 1. Except for 'Aroma', the genotypes used in this trial were cultivars or advanced selections from the Norwegian plant breeding company Graminor AS. Cultivars and selections from Graminor were chosen in order to gain more knowledge on their level of susceptibility to fruit tree canker. The Swedish cultivar 'Aroma' was included as a "control cultivar". 'Aroma' is commonly grown in Norway and in the Nordic countries and is known to have low to medium susceptibility to *Neonectria* canker. Graminor is now releasing MA982 05043, MA992 35005 and MA992 39008 as cultivars (D. Røen, pers.comm.).

For each cultivar/selection, five trees were potted in 3 L plastic pots. The potted trees were delivered to Njøs in beginning of August 2018, placed in the greenhouse early August - late September and then stored in the ventilated plant storage at Njøs from late September until December 2018. All potted trees were transferred to a room with temperature approx. 20 °C for temperation 24 hours before inoculation.

Table 1. Parentage and origin of the apple genotypes used in the experiment in 2018.

Genotype	**Parentage**	**Origin**
'Aroma'	'Ingrid Marie' × 'Filippa'	Sweden, SLU Balsgård
MA962 02073	'Discovery' × ARX 49-18 ('Aroma' o.p.)	Norway, Graminor Breeding Ltd.
MA992 39008	'Aroma' × 'Rubin'	Norway, Graminor Breeding Ltd.
'Tiara'	'Pink Pearl' × K 2-24 ('Alkmene' × 'Burgundy')	Norway, Graminor Breeding Ltd.
MA042 10041	'Martaeple' × 'Rubinstep'	Norway, Graminor Breeding Ltd.
'Idunn'	'Katja' × 'Buckley Giant'	Norway, Graminor Breeding Ltd.
'Oye'	'Discovery' × NY 18491	Norway, Graminor Breeding Ltd.
MA982 05043	'Discovery' × ARX 49-18 ('Aroma' o.p.)	Norway, Graminor Breeding Ltd.
NB 6-4	'Prins' × 'Carroll'	Norway, Graminor Breeding Ltd.
MA992 35005	'Tohoku 2' × 'Rubinstep'	Norway, Graminor Breeding Ltd.

3.1.2. Potted tree experiment in 2019

The trees used in the trial in 2019 were one-year old whips in pots of 16 genotypes on 'Antonovka' seedling rootstock, from grafting at Njøs in March 2019. 'Aroma', 'Elise' and 'Discovery' were used as "control cultivars". 'Aroma' is considered to have low to medium susceptibility to canker, while the other two cultivars are considered highly susceptible. Genotypes used are shown in Table 2. Following grafting, all genotypes were potted in 3 L plastic pots and were fertilized and watered with automatic irrigation system and further grown in the greenhouse.

Table 2. Parentage and origin of the apple genotypes used in the experiment in 2019.

Genotype	**Parentage**	**Origin**
'Alkmene'	'Oldenburg' × 'Cox's Orange'	Germany
'Aroma Fagravoll'	Red sport of 'Aroma' ('Ingrid Marie' × 'Filippa')	Sweden, SLU Balsgård (red sport from Norway)
'Bramley's Seedling'	unknown parentage	United Kingdom
'Discovery'	'Worcester Pearmain' × 'Beauty of Bath'	United Kingdom
'Elise'	'Septer' × 'Cox's Orange'	The Netherlands, Wageningen
'Ellis Bitter'	unknown parentage	United Kingdom
'Fosseple'	unknown parentage	Norway, local cultivar
MA983 05002	unknown parentage	Norway, Graminor selections of *Malus sieversii*, selected in seedlings raised from seeds collected by a USDA gene bank in wild populations in Kazakhstan
MA985 03023	unknown parentage	
MA992 03006	unknown parentage	
MA992 37013	'Freedom' × 'Realka'	Norway, Graminor Breeding Ltd.
MA992 47003	'Pink Pearl' × 'Pristine'	Norway, Graminor Breeding Ltd.
NY 18491	'Macoun' × 'Antonovka'	USA, Geneva
'Sansa'	'Akane' × 'Gala'	Japan
'Silva'	'Melba' × 'Stenbock'	Sweden, SLU Alnarp/Öjebyn
X 4876	'Jonathan' × *Malus pumila niedzwetskyana*	France, INRA Angers

3.1.3. Detached shoot experiment in 2020

Three seedling populations were used to compare the level of resistance against fruit tree canker in detached shoot trial. The populations used are shown in Table 3. 'Elise' and 'Kanzi' were chosen because those genotypes are reported as highly susceptible to canker (Ghasemkhani 2015; Weber and Hahn 2013) while 'Golden Delicious' is shown as highly resistant to canker (Ghasemkhani 2015). Graftwood collected from one year old seedling trees were grafted on 'Antonovka' seedling rootstock at Njøs on March 2019, producing one single tree per individual in the populations. All trees were propagated in the greenhouse with automatic watering and fertilizing system and planted out in the field on 9 June 2020 with drip automatic irrigation system. On 3 and 4 November 2020 detached shoots were collected from the field to incubation room. The shoots were placed in 10 L plastic buckets with water and 5 ml per 1 L water Floralife Express Clear Ultra 200 before transferred into 1 L brown glass bottles. The detached shoots were in dormancy state/leaf fall. All detached shoots were collected from the top of main stem (Figure 5) for each tree with 40 cm shoot length.

Table 3. Populations used in detached shoots experiment in 2020.

Population	**Comment**
'Elise' × 'Stølen'	Susceptible genotype crossed with moderately resistant genotype
'Golden Delicious' × 'Stølen'	Resistant genotype crossed with moderately resistant genotype
'Kanzi' × 'Stølen'	Susceptible genotype crossed with moderately resistant genotype

Figure 5. Three seedling populations were planted out in the field on 9 June 2020. Detached shoots were collected from these trees. Photo: Kurab Røen.

3.2. Inoculum preparation

3.2.1. Potted tree experiment

The inoculum used in the experiments was an isolate of *N. ditissima* collected by Dr. Larisa Garkava-Gustavsson at Swedish University of Agricultural Sciences at Alnarp, in southern Sweden. Inoculum was previously used in inoculation experiments on detached shoots at Njøs in Nordic Public -Private partnership (PPP) projects for pre-breeding funded by the Nordic

Council of ministers. The inoculum was stored frozen and maintained at Njøs for further use since 2013. Prior to use of the inoculum, it was inoculated on three trees of 'Antonovka' rootstock. The trees were wound-inoculated with a spore suspension, kept at room temperature under cover of plastic bags to increase humidity and thus rapid development of the fungus. Macroconidia of *N. ditissima* for the inoculation experiments were collected after about 6-8 weeks, from the sporulating inoculation points (Figure 6) on the rootstocks. *Neonectria ditissima* sporodochia were diluted with sterile water, and macrospores were identified morphologically with light microscope (Figure 6). Comparison of morphology of macroconidia showed similarity with the original inoculum. The rest of the inoculum from the 'Antonovka' rootstock was stored in deep freezer at -20 °C for future use the following year, activated and maintained every year by the same procedure as described here (Figure 7).

Figure 6. The isolate of *Neonectria ditissima* inoculated in a rootstock of 'Antonovka' (left) and producing typical multiseptated macrospores that are normally straight or curved with rounded ends (right). Photo: Kurab Røen.

Spores of *N. ditissima* were collected by cutting the bark containing sporodochia from the 'Antonovka' rootstock with a scalpel and mixing it in autoclaved distilled water in 20 mL glass tubes. The tubes were shaken for 20-30 seconds and then spore suspensions were filtered with sterile cotton cloth (Mesoft 10 x10 cm, Mölnlycke health care, Göteborg, Sweden). A drop of conidia solution was pipetted on a slide and covered with cover glass for identification of the fungus. A solution of 10^5 macroconidia per mL was used in the inoculation experiments, and microscope counts in a hemocytometer were used to adjust the concentration correctly.

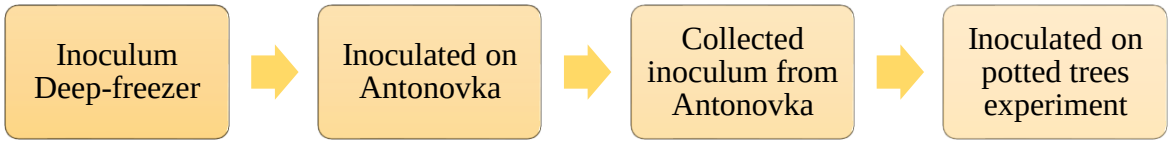

Figure 7. An overview of inoculum preparation at Njøs.

3.2.2. Detached shoot experiment

Plant material from the experiment with potted trees in 2019-2020 were kept in deep freezer after evaluation for future analyses. Some of the genotypes sporulated during experiment period. Inoculum used in the detached shoot trial was collected from three trees in experiment 2019-2020, genotypes used being 'Alkmene', 'Aroma Fagravoll' and 'Silva'. One day before inoculation the plant material was moved from deep freezer and kept in the laboratory hood. Inoculum was prepared from this material. Spores of *N. ditissima* was collected from cankered areas of three points from each tree by cutting the bark with scalpel and mixing with sterile distilled water in 40 mL glass tube and shaked for about 20-30 seconds on Vortex mixer (VWR Vortex Mixers). Spore suspensions was filtered by use of sterile cotton cloth (Mesoft 10 x10 cm, Mölnlycke health care, Göteborg, Sweden). Inoculum was counted under light microscope for determining concentration of conidia using a hemocytometer, and diluted with sterile water to wanted concentration. Spore suspensions with 10^5 macroconidia per mL were used to inoculate artificial wounds in the detached shoots trial. *Neonectria ditisima* isolates collected from plant materials were also placed on potato dextrose agar (PDA) to confirm isolate of *N. ditissima* (Figure 8) by microscopy.

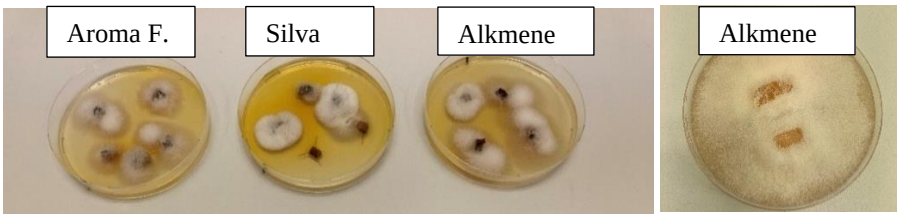

Figure 8. Isolates of *Neonectria ditissima* developing after 10 days on potato dextrose agar (PDA); isolated from infected trees of cultivars 'Aroma Fagravoll', 'Silva' and 'Alkmene' in the experiment in 2019 (left) and reisolated after 13 days on PDA (right). Photo: Kurab Røen.

3.3. Inoculation method

3.3.1. Inoculation method in 2018

The experiment was arranged in the greenhouse with pots placed in plastic trays with drip irrigation, in a randomised block design with 1 tree per cultivar per replicate (5 trees per tray and 2 trays per replicate). Four replicates were inoculated with *N. ditissima*, one replicate was inoculated with autoclaved distilled water. Inoculation was done in four days, starting on 18 December and finishing on 21 December 2018, one replicate inoculated with *N. ditissima* per day. The control replicate (inoculated with water) was inoculated first on 18 December.

At each inoculation point a wound was made with a scalpel removing the bud and the leaf scar (Garkava-Gustavsson et al. 2016; Van de Weg 1989). There were three inoculation points per tree, using buds number 10,14 and 18 or 11, 14 and 17 (depending on tree size) counted from grafting point. The wounds were labelled to easily recognize inoculation points when checking lesion development. A 10 µL conidial suspension (10^5 conidia per mL) was placed on each wound with a pipette. In the control replicate 10 µL autoclaved water was used (Figure 9).

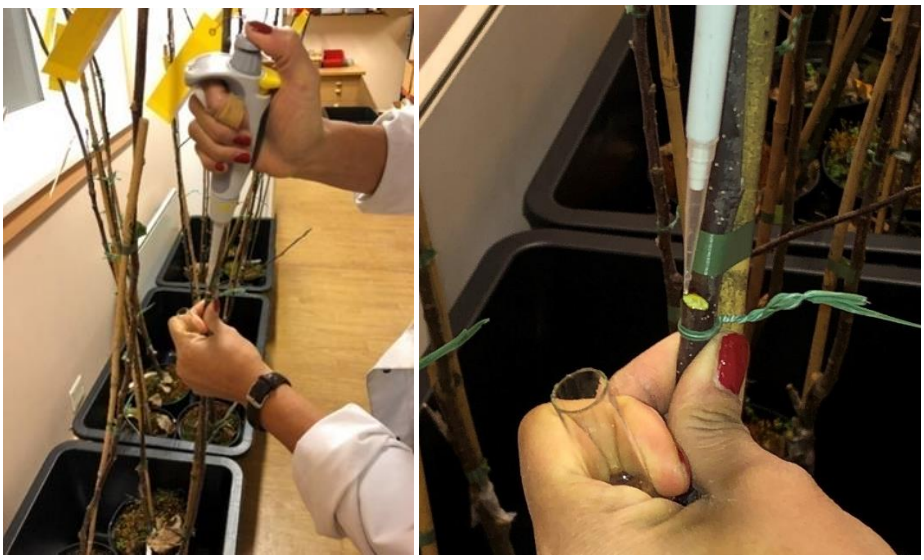

Figure 9. Inoculation of potted trees with *Neonectria ditissima* conidia solution on artificial wound made with a scalpel. Photo: Dag Røen.

Ten to 15 minutes after inoculation the wound was covered with Vaseline. The time it took before adding the Vaseline depended on how fast the conidial solution was absorbed into the wounded area, and absorption rate was different from cultivar to cultivar. Inoculation was performed in a room outside the greenhouse, where temperature was around 20 C°. After

inoculation was finished, the potted trees were kept in the same room at 20 C° for 24 hours before they were transferred to the greenhouse. The Vaseline was removed after 5 days. The experimental trees were checked every week from week 1-2019 to discover when the first disease symptoms were visible and decide when the first recording should be done.

3.3.2. Inoculation method in 2019

The experiment was carried out in the same greenhouse as the experiment in 2018 (Figure 10). The potted trees were placed on pallets with drip irrigation in a randomised block design with 10 replicates of 16 cultivars and one tree per cultivar per replicate. The 16 trees per replicate were all placed on the same pallet. Of the 10 replicates, 8 replicates were inoculated with *N. ditissima*, 1 replicate inoculated with autoclaved distilled water as control and 1 replicate untouched / unwounded. The trees of almost all cultivars had dropped the leaves before start of inoculation (Figure 10).

Inoculation was done on 21 December 2019. Artificial bud scars were made with a scalpel on the main stem (Garkava-Gustavsson et al. 2016; Van de Weg 1989). There were three inoculation points per tree on bud position numbers 10, 16 and 22 counted from grafting point. Some trees were short and small, and then bud position numbers 6, 10 and 14 were used.

Figure 10. Potted trees trial in the greenhouse 2019-2020, dormant stage in early January 2020 (left) and the trees in growth stage late spring 2020 (right). Photo: Kurab Røen.

It is similar inoculation procedure as used in 2018 experiment. The Vaseline was removed 4 days after inoculation in 2019.

3.3.3. Inoculation method in 2020

Detached shoots trial was performed in incubator room at Njøs with temperature and humidity control. Detached shoots were placed in a 1 L brown glass bottle, two shoots per bottle. The bottle contained water with Floralife express clear ultra 200 concentration 0.5 %, which is similar concentration as Chrysal Clear Professional 2 used by several other authors (Garkava-Gustavsson et al. 2013; Ghasemkhani et al. 2015; Scheper et al. 2018). All detached shoots were cut with 45°angle before transferred into the bottles with 300 mL water Floralife solution. The Floralife water solution was changed weekly during the whole experiment period (Ghasemkhani et al. 2015) to avoid developing of algae and other microorganisms. The detached shoots were not cut at the base part weekly because of the short initial length (only 40 cm long).

Detached shoots were collected from the field on 3 and 4 November and transferred directly to incubator room. The shoots that were used in the control replicate was collected on the first day and the shoots for replicate 1-4 were collected on the second day. Shoot length was 40 cm harvested from the top shoot of the main stem of the one-year-old trees. Wounds for inoculation were made by removing bud and leaf scar with scalpel on bud position 5 and 9 counted from base of detached shoot on 5 November (Figure 11). The one-year-old trees developed partly large side branches that were not easy to remove with scalpel. In some cases, a side shoot was formed at bud positions 5. and 9. Then a small scar was made on upper side of the side branch. There were two inoculation points per shoot.

Conidia solution with macroconidia concentration 10^5 per mL was pipetted with auto pipette and 10 µL dripped on the wounded area and covered by a drop of Vaseline within 5-10 minutes after inoculation to ensure conidia solution was absorbed into the wound before covering with Vaseline. The Vaseline was removed 4 days after inoculation. A randomized block design was used, with 5 detached shoots per population per replicate, 4 replicates inoculated with *N. ditissima*, 1 replicate inoculated with water and 1 replicate untouched.

Figure 11. Inoculation of cut shoots with *Neonectria ditissima* at inoculation day on 5.11.2020 (left and middle) and 10 days after inoculation when leaf fall was 100% (right). Photo: Kurab Røen.

3.4. Experiment conditions

3.4.1. Temperature and humidity during experiment period in 2018-2019

Temperature varied between 4° C (minimum) and 21 ° C (maximum) and the relative humidity varied approximately from 40 to 80% during experiment period from late December to mid-May in the greenhouse. A week before and after inoculation the temperature in the greenhouse was set to 18 C° then kept at 4 C° during January and February. From February temperature was increased to 16 °C day temperature and 10 °C night temperature with ventilation starting at 18 °C. Facility was regulated by automatic climatic control system (SENMATIC DGT-Volmatic version 104). The relative humidity and temperature were observed additionally with temperature and humidity monitoring equipment (Testo 174-H, Testo, Lenzkirch, Germany), placed within the potted trees in the greenhouse.

3.4.2. Temperature and humidity during experiment period in 2019-2020

Temperature varied between 4.9 ° C (minimum) and 22.5 ° C (maximum) and the relative humidity varied approximately from 48 to 85% in the greenhouse during experiment period. A week before and after inoculation the temperature in the greenhouse was set up to 18 C° then lowered to 4 C° during January and February similar to experiment in 2018-2019.

3.4.3. Temperature and humidity during experiment period in 2020-2021

The temperature in the incubator room varied from 16-22 °C and relative humidity varied from 60 to 80 % during experiment period. The room buildup was with greenhouse light and temperature control. In the room was placed 3 humidity machines (Ultraschall-Vernebler Boneco 7131) for keeping the optimal humidity for the inoculation. On 3 and 4 November 2020 when the detached shoots were transferred into the room, temperature was set to 18 °C and relative humidity was 80% (Figure 12). During inoculation day the temperature was kept at 18 °C while humidity decreased to approximately 40-50 %. After inoculation, the temperature and the relative humidity were kept around 16-22 °C and 60 to 80% during experiment period. The temperature and the relative humidity were observed with monitoring equipment (Testo 174-H, Testo Lenzkirch, Germany).

Figure 12. The climate condition in the incubator room at Njøs (right and left). The temperature and the relative humidity in the growth chamber were kept at 16-22 °C and 60-80 % during experiment period. Photo: Kurab Røen.

3.5. Disease symptom measurement

3.5.1. Measurement / assessments in 2018-19 experiment

First recording of external canker lesion length was on 28 February 2019 (week 8), 11 weeks after inoculation. Lesion length on the bark was measured in millimeters (mm) with a digital caliper (MarCal 16 EWRi, Mahr GmbH Standort Esslingen, Germany). The intervals between measurements were 5-10 days from week 9 in 2019. The last recording was done on 16 May 2019. Total experiment period from inoculation to last recording was 149 days.

The measurements ended when diseased area girdled the main stem especially for some genotypes and the main stem dried out in the area above inoculation point 3. The inoculation points 2 and 3 were difficult to measure for some cultivars when lesions in point 2 and 3 merged together and it led to measurement error. Such measurements were not included in data analysis.

By the last recording date, the appearance of several characters of disease symptoms and signs of the fungus in the bark were included in the recording, i.e. flakes, bubbles, ring pattern, discoloration (Figure 13), wilting, internal canker lesion length and presence of sporodochia of *N. ditissima*. The internal lesion length of the main stem was measured with digital caliper in millimeters. Details on additional observations are shown in Table 4.

Table 4. Additional observations and scales used in the last recording of potted tree trials.

Scale used by scoring	**1**	**2**	**3**	**4**	**5**	**6**
Flakes	Absent	Sparse	Some	Medium	Abundant	Severe
Bubbles	Absent	Sparse	Some	Medium	Abundant	Severe
Ring pattern	Absent	Sparse	Some	Medium	Abundant	Severe
Color (Figure 13)	Unchanged	Light	Light brown	Brown	Dark brown	Very dark brown
Sporulation	Absent	Present				
Girdling	No	Yes				
Wilting (Potted tree)	0 %	< 25 %	25 %	50 %	75 %	100 %
Wilting and Girdling (Detached shoot)	No = 0 and Yes = 1					
Swelling	Scoring with 1-5 scale as shown in Figure 14.					
Internal lesion length in millimeter	Split the main stem in two parts as shown in Figure 14.					

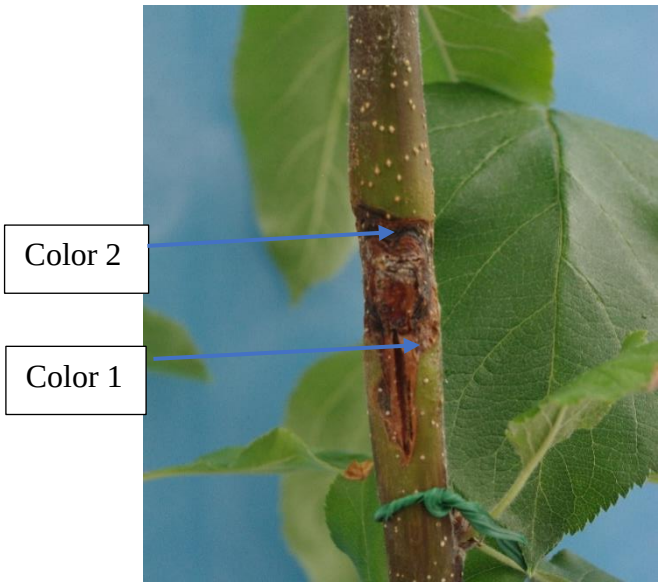

Figure 13. Color Scale used for color 1 and color 2 at the inoculation point. Color 1 is color when outer part of bark loosen (flakes etc.) and color 2 is color of sunken lesion. Color 1 was recorded in all three experiments, while color 2 was used only in potted tree experiment in 2018-2019. Photo: Kurab Røen.

3.5.2. Measurement / assessments in 2019-2020 experiment

Recording of visible lesion length started on 17 March 2020, 12 weeks after inoculation. Total length of external visible lesion was measured using a digital caliper with two weeks intervals (13-15 days). The last observation was on 9 June 2020. As in 2018 experiment, several characters were recorded by this last recording, with the recording procedure being similar as the procedure in 2018.

In 2019 also was implemented more characters to be observed, such as swelling (Figure 14) girdling, diameter of the stem 5 centimetres above the grafting point, diameter at the inoculation point and diameter below the inoculation point (mm). Total experiment period from inoculation to last recording was 170 days.

Figure 14. Scale used for scoring of swelling at the inoculation point (left) and splitting of stem for measuring internal lesion length with a digital caliper (right). Photo: (left) Elinde Natchanan Røen, (right) Kurab Røen.

3.5.3. Measurement / assessments in 2020-2021 experiment

After inoculation the shoots were checked and observed 3 times a week to ensure that the temperature and humidity were kept at optimal range for developing of canker, and to observe when the visible symptoms appeared. Detached shoots were measured weekly from week 48 in 2020 and the first measuring of external lesion length was on 24 November The canker lengths were measured by using a digital caliper in millimeters (mm). The first visible lesion areas are often brown to dark brown color with sunken bark on the main stem (Borve et al. 2019; Xu and Robinson 2010). The last observation was on 7 January 2021. The detached shoots trial arranged in the incubator room lasted 62 days after inoculation.

Observation of several characters were applied on the last measuring of the detached shoots trial, similar as potted trees trial. These characters included flakes, bubbles, ring pattern, miscoloring, wilting, girdling, sporulation, diameter (mm) of the stem and internal canker symptom area. After observation of all characters, the shoots were split into two pieces for recording of internal lesion length. The internal canker areas were recorded in millimeters with a digital caliper.

3.6. Statistical analysis

3.6.1. About Datasets

The number included in the statistical analysis was mean of the three inoculation positions on the main stem of each potted tree. The size of the artificial wound was approx. 3 mm. It was decided to set a threshold at 5 millimeters to ensure that the measured lesion was from the infection, not from wounding of the stem. Therefore, measured lesions less than 5 mm were not included in the data analysis.

The length of some lesions did not increase during the last period of observations, especially for the two last observations. The values of lesion length from such cases were included in the data analysis.

Inoculation points 2 and 3 were often influenced from the lower inoculation point 1. The stem above inoculation point 3 was often dead and dried out more than above the lowest inoculation point (Figure 15). When the main stem died and dried out, one could no longer separate the infection area from the healthy area because the discoloration caused by infection of the pathogen and wilting and death of the stem caused by strongly reduced water transport were sometimes difficult to separate. In such cases we therefore decided to use lesion length size from the last record before the main stem died.

Figure 15. Wilted shoots from inoculation at point 3 of ‘Elise’ (left), MA992 47003 (middle) and ‘Discovery’ (right). Photo: Kurab Røen.

In some cases, lesions from neighboring inoculation points merged. It made the measurement complicated and resulting in that the lesion area for each inoculation point was not measured precisely. Such data were not included in the statistical analysis. This resulted in one value of lesion length size missing in 2018 and four values missing in 2019.

In other cases, the recorded measurements could fail, e.g. if measured lesion size decreased from one recording date to the following. In such cases, there was obviously a wrong measurement, and this was corrected by including a value based on interpolation of the previous and the following measurement. Such wrong measurements may have been caused by difficulties in recording lesion growth with a digital caliper.

All data from detached shoots trial were included in data analysis.

3.6.2. Data analysis

1. Percentages of infection on each cultivar and the whole experiments were calculated with Pivot tables in Excel (Microsoft Excel for Microsoft 365, version 2103). Infection percentage per tree was calculated and two-way analysis of variance (ANOVA) was applied on these datasets (arcsin transformed) to determine significant differences ($P<0.05$) among genotypes using the GLM procedure of SAS software program (version 9.4).

2. Area under the disease progress curve (AUDPC) was calculated as sum of trapezoids, using the formula (Shaner and Finney 1977)

$$AUDPC = \sum_{i=1}^{n} \left[\frac{Y_{i+1} + Y_i}{2}\right] [X_{i+1} - X_i]$$

Y_i is lesion size at observation time *i*
X_i is number of days from start of experiment until observation time *i*
n is total number of observation times

Lesion size per tree was calculated as mean lesion size for the three inoculation points and used when calculating AUDPC. Two-way analysis of variance of the AUDPC values was performed using the GLM procedure of SAS software program (version 9.4). Mean separation was done by the Duncan test (P = 0,05) to determine significant differences between genotypes and replicates.

Number of days from inoculation until first symptom visible, wilting, girdling, diameter the inoculation point (mm) and diameter below the inoculation point (mm) were analyzed by two-way analysis of variance using GLM procedure of SAS software, with mean separation by Duncan test (P = 0,05). In addition, for experiment in 2019-2020, three parameters (AUDPC, external and internal lesion length) were also analysed on a dataset including inoculation points with no lesion development.

3**.** Additional recordings at the last day of observations were analyzed by two-way analysis of variance (cultivar and replicate), and principal component analysis (PCA), using Panel Check program v1.4.2 (Software program package collaboration between Nofima, Technical University of Denmark and University of Copenhagen). The results from PCA were interpreted to find patterns of characters among genotypes in PCA plot. The Panel Check program was also used for illustrating characteristics of cultivars in Spiderweb Plots, showing character profiles of the genotypes.

4. Results

4.1. Potted tree experiment in 2018-2019

Disease development

Visible disease symptoms were observed every week during the experimental period. At 82 days after inoculation the bark around the wounded area showed discoloration on genotype MA982 05043. Disease symptoms appeared on ‘Aroma’ (used as control) after 98 days. The overall mean of symptom appearance for all genotypes were 93 days after inoculation for this trial (Table 5). Statistical analyses showed that there were significant differences among genotypes in the number of days from inoculation until first symptom were visible. MA962 03073 and NB 6-4 developed symptoms significantly later than ‘Idunn’, MA982 05043 and ‘Oye’. Only MA982 05043 developed symptoms significantly earlier than ‘Aroma’.

Table 5. Infection percentage, area under the disease progress curve (AUDPC), symptom development, external and internal lesion lengths and wilting after inoculation with *Neonectria ditissima* on potted trees of 10 apple genotypes in 2018.

Genotype	Infection (%)	AUDPC	Number of days until first symptom	External lesion length (mm)	Internal lesion length (mm)	Wilting (1-6)
'Aroma'	92 ab	1877.5 abc	98 ab	62.5 abc	147.0	4.2 a
'Idunn'	100 a	1958.8 ab	86 bc	59.4 abcd	107.4	5.0 a
MA042 10041	67 bc	826.3 d	95 abc	31.4 d	44.5	1.0 b
MA962 02073	42 c	1709.9 bcd	102 a	59.1 abcd	80.1	4.0 a
MA982 05043	67 bc	2712.2 a	82 c	82.2 a	106.4	4.5 a
MA992 35005	92 ab	1405.0 bcd	93 abc	48.4 bcd	161.4	4.3 a
MA992 39008	100 a	998.3 cd	95 abc	42.1 cd	98.8	6.0 a
NB 6-4	100 a	1538.3 bcd	101 a	76.6 ab	152.0	5.2 a
'Oye'	83 ab	1621.3 bcd	84 bc	58.2 abcd	118.3	4.7 a
'Tiara'	67 bc	1619.0 bcd	89 abc	51.4 bcd	65.8	3.2 ab
Mean	81	1626.7	93	57.1	108.2	4.2
Probability levels of significance by Two-way analysis (Pr > F)						
Source of variance						
Genotype	0.0016	0.0052	0.0077	0.0156	0.1132	0.0275

The data are the means of four replicates with one tree per genotype inoculated with *N. ditissima* and mean of three inoculation points per tree. Mean separation by Duncan test (P = 0.05). External and internal fruit tree canker lesion length (mm) on the last observation date for 10 apple genotypes, measuring visible external lesion length and recording other outer characters before measuring internal lesion length.

Scale wilting: 1 = no wilting, 6 = whole tree wilted above the upper inoculation point.

There was significant difference between replicates for infection percentage and first day of visible symptom

Infection percentage

There were significant different infection percentages among the 10 genotypes (Table 5). Inoculation was done over four days in this experiment, and the statistical results showed that there was also significant effect of the replicate on infection percentage. The overall infection level indicate that the inoculations were functioning. Infection percentage varied from 42 % to 100 % depending on genotype, with an overall mean of 81 %. The genotype MA962 02073 had the lowest infection percentage at 42 %. The genotypes MA042 10041, MA982 05043 and 'Tiara' scored 67 %, 'Oye' 83 %, while MA992 35005 and 'Aroma' scored 92 %. The highest infection percentage was observed on 'Idunn', MA992 39008 and NB 6-4, all at 100 %. On uninoculated controls, no lesions developed around the inoculation points. Infection percentages of genotypes MA962 02073, MA982 05043, 'Tiara' and MA042 10041 were significantly lower than those of genotypes NB 6-4, 'Idunn' and MA992 39008. Altogether only one genotype (MA962 02073) was significantly different from 'Aroma' on infection percentage.

Wilting

Wilting was observed at the last recording date on the main stem above upper inoculation point (inoculation point 3) of each tree (Table 5). The genotypes used in this experiment showed a range in wilting from no wilting to wilting of all trees above the upper inoculation points, depending on genotype. MA042 10041 showed the lowest wilting and was wilting significantly less than 'Aroma' and the other genotypes, except for 'Tiara'.

Area under the disease progress curve AUDPC

The results in the Table 5 for AUDPC values show significant difference between genotypes. The genotype MA982 05043 had the highest AUDPC values, followed by 'Idunn' and 'Aroma'. The lowest value of AUDPC was on MA042 10041, which was significantly lower than 'Aroma'. There was no significant difference between replicates on AUDPC value. Potted trees developed canker lesions easy to measure (Figure 16).

Figure 16. Disease symptoms of *N. ditissima* on the wounded trees. Genotype MA042 10041 (A) had the lowest AUDPC and infection percentage, while 'Aroma' (B) represented higher values than MA042 10041. Picture A and B show the three inoculation points per tree (to the right is lowest inoculation point, and to the left is highest inoculation point. Photo: Kurab Røen.

Analysis of variance was calculated on external lesions for each recording date separately. A selection from Graminor, MA982 05043, developed larger lesions earlier than other genotypes (Figure 17). In comparison, MA042 10041 developed symptoms slowly over time, while on 'Aroma' symptoms appeared late but then symptoms developed rapidly (Figure 17). In general, lesions developed slowly until 94 days after inoculation (recording date 4, early April). Then lesions started to grow faster, especially on MA982 05043 and 'Aroma'. The genotype NB 6-4 developed lesions rapidly after recording no. 7 (after 14 April) and at the end of the experiment, lesions on this genotype developed to a similar level as MA982 05043.

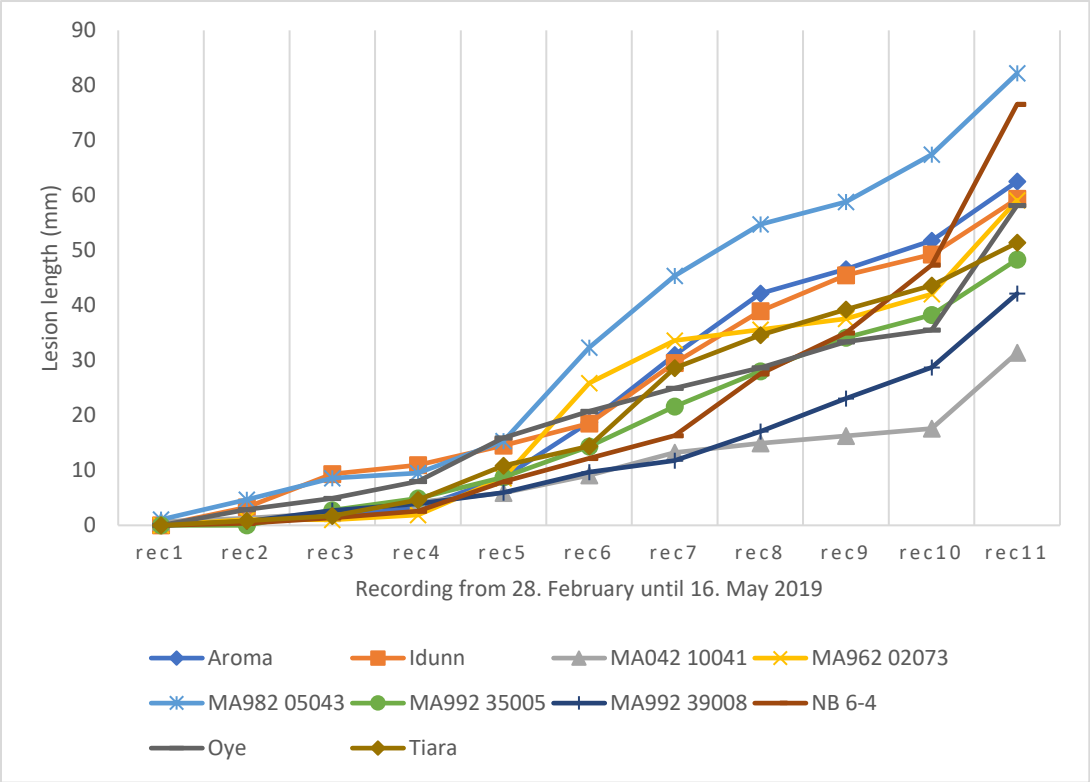

Figure 17. Developing of canker lesions in one-year old potted trees in 2018-2019 in the greenhouse during the experimental period. Recording (rec) of canker lesion lengths (mm) weekly from the day with first visible symptoms to the end of the experiment. Mean values of four trees per genotype and 3 inoculation points per tree.

All genotypes had internal canker lesion values higher than external lesion length values (Table 5). The genotypes MA982 05043 and NB 6-4 had the longest external canker lesions, while MA992 35005, NB 6-4 and 'Aroma' had long internal canker lesions. MA042 10041 had the shortest external and internal lesions of all genotypes in this experiment. The genotype MA992 35005 had low external lesion length but the results showed long internal canker lesions. The statistical analysis showed significant difference between genotype in external lesion length at last record date but no significant difference in internal lesion was observed in experiment 2018-2019.

Characteristic profiles

Significant differences in characters among genotypes were found by ANOVA and the Duncan test (P = 0.05) and the results are shown in the Table 6. No difference between genotypes were observed for ring pattern, bubbles and sporulation. Flaking and coloring was significantly different among genotypes, MA042 10041 and 'Tiara' showed significant difference to 'Idunn'

on flakes, while ‘Aroma’ was significantly different to MA962 02073 on color. No significant difference was observed between replicates for flakes (Table 6).

Table 6. Character profiles of ten apple genotypes after infection with *Neonectria ditissima*, with several characters on appearance of disease symptoms recorded by scoring at the end of the experiment.

Genotype	Flakes (1-6)	Ring pattern (1-6)	Bubbles (1-6)	Color (1-6)	Sporulation (1-2)
‘Aroma'	4.7 ab	1.8	2.8	3.8 ab	1.2
MA962 02073	3.1 b	1.0	1.7	2.3 c	1.0
MA992 39008	3.8 ab	1.0	3.7	4.2 a	1.2
‘Tiara'	3.3 b	1.4	2.3	3.1 bc	1.1
MA042 10041	2.7 b	1.3	2.2	3.3 ab	1.1
‘Idunn'	5.6 a	1.8	2.6	4.1 a	1.4
‘Oye'	4.5 ab	1.8	2.3	3.6 ab	1.3
MA982 05043	4.3 ab	1.4	2.2	3.0 bc	1.2
NB 6-4	5.8 a	2.4	3.0	4.0 ab	1.3
MA992 35005	4.8 ab	1.3	3.0	3.5 ab	1.2
Mean	4.2	1.5	2.6	3.5	1.2
Probability levels of significance by Two-way analysis (Pr > F)					
Source of variance					
Genotype	0.0278	0.3862	0.1055	0.0050	0.7106

The data are the means of each genotype, inoculated with *N. ditissima* in four replicates and values were the means of four trees and each tree inoculated on 3 positions. Control replicates did not develop disease symptoms and were not included in data analysis. There was significant difference between replicates for ring pattern, bubbles and color. Higher scores means darker color, or more flakes, ring pattern, bubbles or sporulation.

The results from Principal component analysis (PCA) showed that PC1 and PC2 presented 75.5 and 18.7% (in total 94.2 %) of the variation among the genotypes (Figure 18). Flakes described most of the variation in PC1 while bubbles and color described the variation in PC2. NB 6-4 and’ Idunn’ were characterized by high levels of flakes compared with other genotypes while MA992 39008 was characterized with more bubbles and MA962 02073 was lowest in this characteristic. The results from PCA indicate that flakes character was not correlated with color and bubbles, but flakes showed correlation with ring pattern.

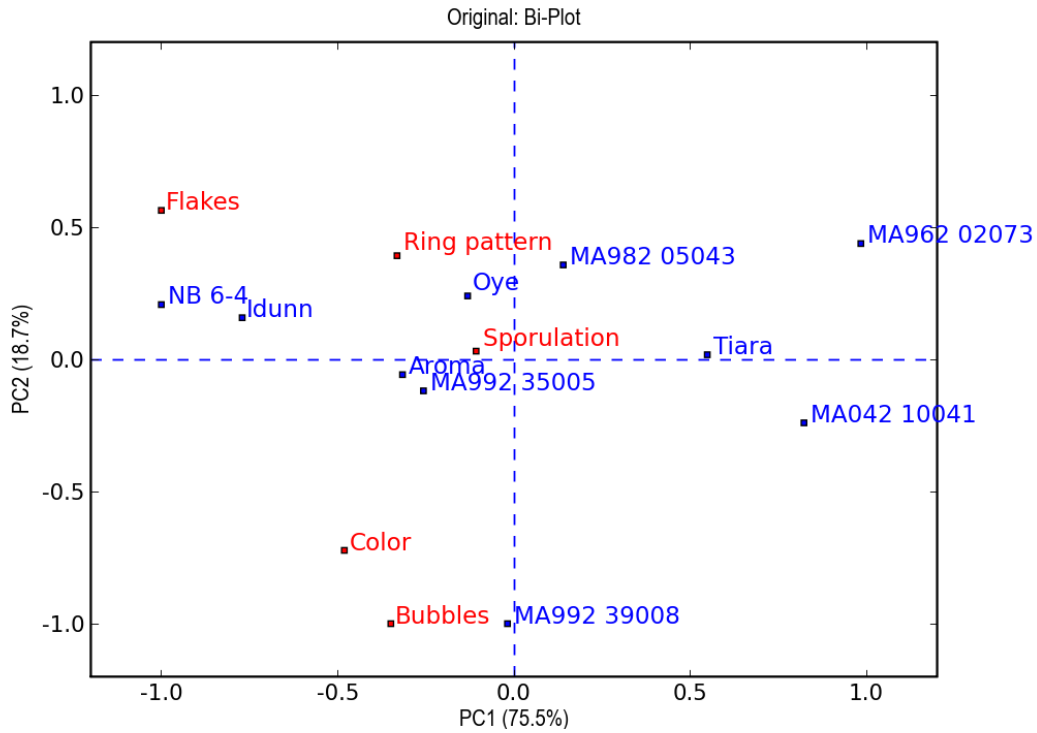

Figure 18. Principal component analysis (PCA) interpreted the values from scoring to find patterns of characters among the tested genotypes in PCA plot. PCA bi-plot of 10 genotypes, results on five characters (red) of ten genotypes (blue) represent the character profiles, significance can be read in Table 6.

4.2. Potted tree experiment in 2019-2020

Disease development

External lesion lengths (mm) were measured at two weeks intervals during the experiment. There were significant differences among genotypes for the first day visible symptoms appeared (Table 7). 'Silva' developed visible fruit tree canker lesions on the bark 100 days after inoculation, and it was the first genotype that showed disease symptoms while 'Discovery' 'Elise' and 'Aroma Fagravoll', used as controls, developed canker symptoms 102, 107 and 110 days after inoculation, respectively. There was not significant difference among these control cultivars. The latest cultivar to develop symptoms was MA992 03006, which developed canker lesions significantly later than the control cultivars. The overall average for symptom appearance for all genotypes were 108 days after inoculation.

Table 7. Infection percentage, area under the disease progress curve (AUDPC), symptom development, external and internal lesion length (mm) and wilting after inoculation with *Neonectria ditissima* on potted trees of 16 apple genotypes in 2019.

Genotype	Infection (%)	AUDPC	Number of days until first symptom	External lesion length (mm)	Internal lesion length (mm)	Wilting (1-6)	Girdling, No=1 and Yes=2
'Alkmene'	54 b	2478.0 de	115 ab	60.8 d	193.0 bcd	4.1 abcde	1.6 abcde
'Aroma Fagravoll'	100 a	2393.7 e	110 bcd	56.1 d	215.2 abcd	2.6 defg	1.2 fg
'Bramley's Seedling'	75 ab	2000.2 e	117 abc	53.3 d	237.7 abcd	1.8 fg	1.3 defg
'Discovery'	92 a	4425.7 b	102 cd	96.6 abc	142.6 cd	4.9 abc	1.7 abc
'Elise'	83 a	6023.9 a	109 bcd	124.2 a	113.5 d	5.5 ab	1.9 a
'Ellis Bitter'	83 a	2062.4 e	114 bcd	56.7 d	141.5 cd	1.6 g	1.2 efg
'Fosseple'	75 ab	3446.1 bcde	105 bcd	73.6 cd	304.2 ab	3.8 bcdefg	1.3 cdefg
MA983 05002	79 ab	2230.6 e	108 bcd	45.1 d	256.1 abcd	3.0 cdefg	1.3 defg
MA985 03023	92 a	4107.0 bc	102 cd	80.9 bcd	126.1 cd	5.4 ab	1.6 abcd
MA992 03006	90 a	2062.4 e	123 a	56.4 d	355.6 a	4.8 abcd	1.6 abcde
MA992 37013	83 a	4279.1 b	105 bcd	107.4 ab	220.4 abcd	5.4 ab	1.8 ab
MA992 47003	88 a	2788.1 cde	106 bcd	66.0 cd	225.6 abcd	5.4 ab	1.7 ab
NY 18491	100 a	2328.7 e	101 d	44.9 d	195.9 bcd	2.1 efg	1.1 g
'Sansa'	96 a	2689.5 cde	108 bcd	53.2 d	182.4 bcd	3.4 bcdefg	1.5 abcdef
'Silva'	100 a	3322.5 bcde	100 d	72.9 cd	295.9 ab	3.9 abcdef	1.4 bcdefg
X 4876	100 a	3900.3 cbd	110 bcd	121.3 a	266.3 abc	6.0 a	1.8 a
Mean	87	3292.5	108	73.1	217.0	4.0	1.5
Probablility levels of significance by Two-way analysis (Pr >F)							
Source of variance							
Genotype	0.0208	<.0001	0.0017	<.0001	0.0045	<.0001	<.0001

The data are the means of eight replicates with one tree per genotype per replicate inoculated with *N. ditissima* and mean of three inoculation points per tree. Mean separation by Duncan test (P = 0.05). External and internal fruit tree canker lesion length (mm) on the last observation date for 16 apple genotypes, measuring visible external lesion length and other outer characters before measuring internal lesion length.
Scale wilting: 1 = no wilting, 6 = whole tree wilted above the upper inoculation point.
There was not significant difference between replicates for any character.

Infection percentage

The results in Table 7 show that infection percentage was significantly different among the 16 genotypes while infection percentages were not significantly different between replicates. Inoculation of all replicates was done in one day in this trial. Infection percentage varied from 54 to 100% depending on genotype. 'Alkmene' had the lowest infection percentage, while control cultivars 'Elise', 'Discovery' and 'Aroma Fagravoll had 100, 88 and 83% infection, respectively. There was no significant difference between the three cultivars that were used as control ('Aroma Fagravoll', 'Discovery' and 'Elise') on infection percentage. There was one cultivar ('Alkmene') with significant difference from these three control cultivars. On the control trees (on NY 18491), there was one wound point that developed fruit tree canker.

Wilting

The genotypes showed variation in wilting, from small amounts to wilting of all areas above the upper inoculation point (varied from 1.6 to 6 on the scale) by the last recording date (Table 7). 'Bramley's Seedling', 'Ellis Bitter' and NY 18491 showed the lowest wilting. 'Aroma Fagravoll' showed some wilting, while 'Discovery' and 'Elise' had much wilting of the trees. 'Aroma Fagravoll' showed significant difference in wilting from 'Discovery' and 'Elise'. The highest wilting was observed on genotype X 4876 (score = 6) and was significantly different from 'Aroma Fagravoll'.

Stem diameter and swelling

To determine possible factors to influence susceptibility to canker, it was measured also diameter of the main stem 5 centimeter above the grafting point, the results showed a range in thickness from 7.3 to 11.5 millimeter (mean value). 'Bramley's Seedling' and NY 18491 had the thickest shoots at 11.1 and 11.5 mm, and in contrast MA992 37013 and 'Elise' had the thinnest shoots at 8.1- and 7.3-mm. NY 18491 shoots varied from 9.9 mm to 13.4 mm, while Elise had a range from 4.5 to 12.1 mm. There was a significant correlation between the main stem size and the external canker lesions at last recording date ($P = 0.0009$, $r = -0.299$). Correlation was also found significant between the main stem and AUDPC values ($P = 0.0020$, $r = -0.278$).

Table 8. Development of the main stem on inoculation points after inoculation with *N. ditissima*, measuring both diameter on the wounded points and below the wounded points. Ratios between diameter on the inoculation points and diameter below the inoculation points are also shown in this table.

Genotype	A=Diameter on the inoc.point (mm)		B=Diameter below the inoc.point (mm)		Ratio A/B	
'Alkmene'	10.2	bc	10.6	bc	1.0	c
'Aroma Fagravoll'	11.8	ab	10.8	ab	1.1	a
'Bramley's Seedling'	11.8	ab	11.1	ab	1.1	abc
'Discovery'	7.8	e	7.8	fgh	1.0	abc
'Elise'	5.0	g	5.5	i	1.0	abc
'Ellis Bitter'	10.5	bc	10.3	bcd	1.0	abc
'Fosseple'	9.8	cd	9.6	bcde	1.0	abc
MA983 05002	9.7	cd	9	cdef	1.1	abc
MA985 03023	7.8	e	8.3	efg	1.0	bc
MA992 03006	8.4	de	8.8	def	1.0	bc
MA992 37013	6.1	fg	6.7	hi	0.9	c
MA992 47003	8.0	e	8.5	efg	1.0	c
NY 18491	12.6	a	12.2	a	1.1	abc
'Sansa'	9.9	cd	9.8	bcde	1.0	abc
'Silva'	10.9	bc	10.1	bcd	1.1	ab
X 4876	7.1	ef	7	gh	1.0	abc
Mean	9.2		9.1		1.0	
Probability levels of significance by Two-way analysis (Pr>F)						
Source of variance						
Genotype	<.0001		<.0001		0.0263	

There was significant difference between replicates for diameter around inoculation point and diameter below inoculation point. Ratio A/B value < 1, sunken lesion, Ratio A/B value = 1, not swelling, Ratio A/B value > 1, swelling

In addition, thickness on the inoculation point and the area below inoculation point were measured. There were significant differences between genotypes for both parameters and for the ratio between the two parameters (Table 8). A ratio above 1.0 indicate swelling around inoculation point, while a ratio below 1.0 indicate sunken lesions. The cultivars can be divided into two groups, with the first group having smaller or similar diameter around infection areas than the diameter below the inoculation point (group A), and the second group showing larger thickness around infection areas than the area below the inoculation point (group B). The genotypes in group A had in general thinner shoots than those in group B. Swelling was also observed visually by scoring (Table 9).

Girdling

In the 2019-2020 experiment, girdling was included as another parameter to determine susceptibility levels to *N. ditissima.* Girdling of the stems varied among the tested genotypes and significant differences were found. The cultivars 'Elise' and 'Discovery' had significantly more girdling than 'Aroma Fagravoll', 'Bramley' s Seedling', Ellis Bitter' MA983 05002 and NY 18491 while NY 18491 was ranked lowest in girdling compared with the other genotypes in this test.

AUDPC and lesion development

AUDPC was significantly different among the genotypes,' Elise' had the highest AUDPC value followed by 'Discovery' and MA992 37013, while 'Bramley's Seedling' 'Ellis Bitter', MA992 03006 and had the lowest AUDPC values. AUDPC for 'Elise' was significantly higher compared with all other genotypes. 'Aroma Fagravoll' scored low to medium high for AUDPC value compared with other genotypes in this experiment. There was a significant difference between control cultivars 'Elise', 'Discovery' and 'Aroma Fagravoll'. There was no significant difference between replicates for AUDPC. Canker symptoms developed clearly on inoculation points (Figure 19).

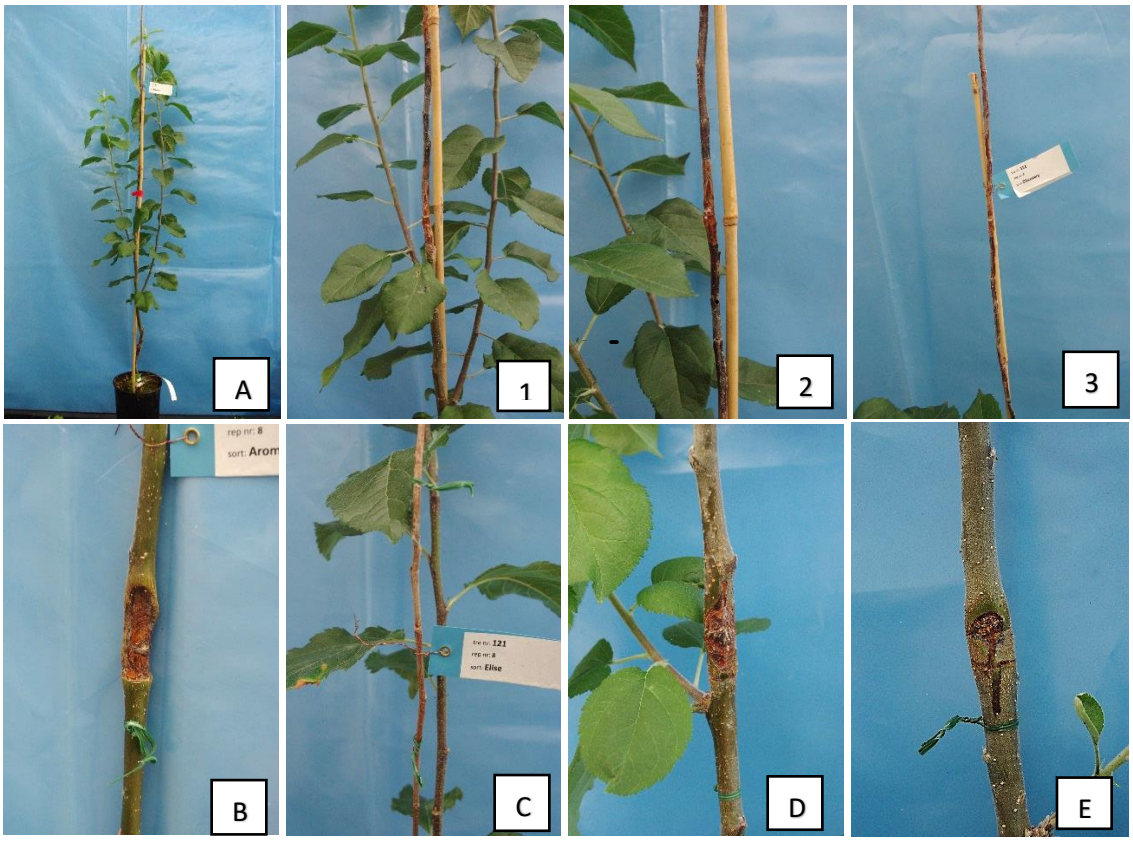

Figure 19. Infection on the wounded areas caused by *Neonectria ditissima* on genotypes 'Discovery' (A with inoculation point 1, 2 and 3), 'Aroma Fagravoll'(B), 'Elise'(C), 'Ellis Bitter'(D) and 'Bramley's Seedling'(E). Photo: Kurab Røen.

Analysis of variance was calculated on external lesions for each recording date separately. Disease symptoms appeared earliest on 'Silva' and then 'Discovery' developed the lesion surface size similar to 'Silva' (on recording date 3, significant difference between genotypes on external lesion (mm), (P = 0.0007). There were observed 'Discovery' canker lesions before 'Aroma Fagravoll' and 'Elise' (Table 7). 'Elise' developed disease symptom fastest over time while in NY 18491, 'Aroma Fagravoll' 'Bramley's Seedling', MA992 03006 and 'Ellis Bitter' lesion length increased slowly compared with other genotypes (Figure 20). The genotypes 'Discovery', X4876, MA992 37013 and 'Elise' had developed the same level of disease symptoms at the last recording date (P = <0.0001), but the genotype MA992 37013 developed canker lesions faster after recording no. 5 (after 29 April). In general lesions developed rapidly from 90 days after inoculation (recording date 3, end of March).

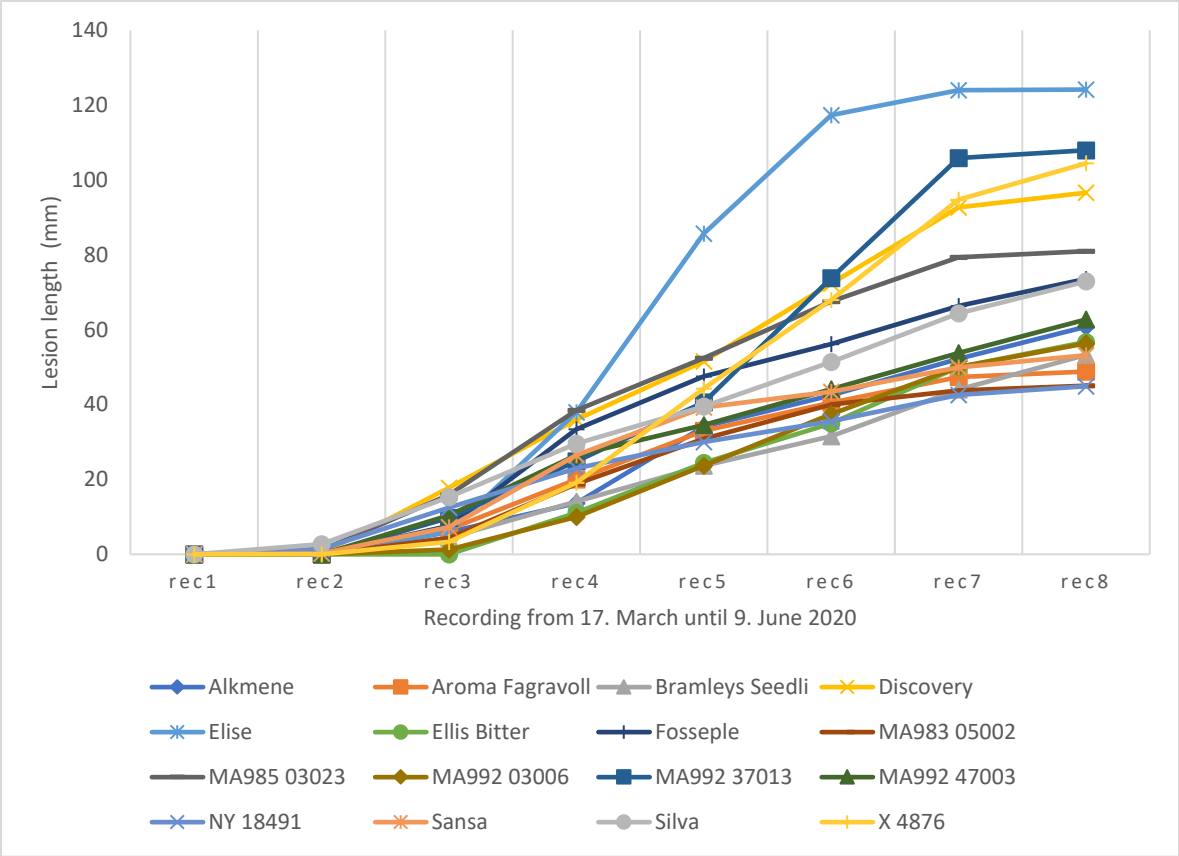

Figure 20. Developing of canker lesions in one-year old potted trees in 2019-2020 in the greenhouse during the experimental period. Recording (rec) of canker lesion lengths (mm) with two weeks interval from the day with first visible symptoms to the end of the experiment. Mean values of 8 trees per genotype and 3 inoculation points per tree.

External and internal lesion length on the end of the experiment is shown in Table 7, the results showed that there were statistically significant differences between genotypes for both external and internal canker lesions. The genotype 'Discovery', MA992 37013, X 4876 and 'Elise' had the longest external lesions, while NY 18491 had the shortest external lesions.

MA992 03006, 'Fosseple' (Figure 21) and 'Silva' had long internal lesions length. In contrast, 'Elise' 'Discovery' and MA985 03023 had short internal lesions length.

All genotypes, except for 'Elise', had longer internal lesions than external canker lesions. Statistical analyses showed that 'Aroma Fagravoll' was significantly different from 'Discovery' and 'Elise' for external lesion length, while 'Discovery' was similar to 'Elise', but no difference between these three apple genotypes were observed for internal lesion length (Table 7).

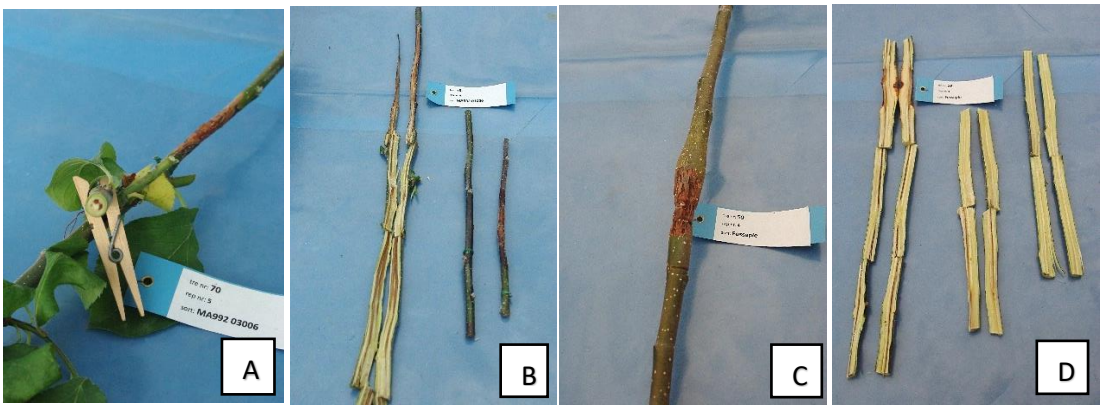

Figure 21. Disease symptoms developing on and inside the stem after inoculation with *Neonectria ditissima*. Genotype MA992 03006 (A and B, B: to the right is lowest inoculation point, and to the left is the uppermost inoculation point) and 'Fosseple' (C and D, D: to the right is lowest inoculation point, and to the left is the uppermost inoculation point) had long internal lesion lengths. Pictures show canker lesions, and splitting of stem on the last day of recording. Photo:Kurab Røen.

Characteristic profiles

There were significant differences among genotypes for the characters flakes, bubbles, ring pattern and swelling but no significant difference in color 1, color 2 and sporulation (Table 9). 'Elise' had high scores for flakes and low scores for bubbles and medium swelling, while 'Aroma Fagravoll' had more ring pattern and swelling but had similar flakes to 'Elise'.

Results from the PCA showed that PC1 and PC2 represented 48.5 and 20.9%, respectively of the variation among the genotypes (Figure 22). 'NY 18491' showed a high level of ring pattern. 'Silva' and Sansa were characterized by high level of flakes. The finding from PCA plot in this test indicate that flakes, bubbles and swelling are not correlated with color 2 and ring pattern, and flakes seem to be negatively correlated with bubbles and swelling.

Table 9. At the last record date was observed appearance of disease symptoms on the canker lesions by scoring. Character profiles of 16 different genotypes after inoculation with *Neonectria ditissima*.

Genotype	Flakes (1-6)	Ring pattern (1-6)	Bubbles (1-6)	Color 1 (1-6)	Color 2 (1-6)	Sporulation (1-2)	Swelling (1-5)
'Alkmene'	3.3 c	1.1 c	1.0 b	3.6	4.8	1.0	2.1 bc
'Aroma Fagravoll'	4.3 abc	2.4 abc	1.2 b	4.0	5.7	1.2	3.8 a
'Bramleys Seedling'	3.7 bc	1.9 bc	1.1 b	3.1	4.6	1.1	2.8 b
'Discovery'	4.9 ab	1.5 bc	1.4 b	3.8	5.0	1.0	2.2 bc
'Elise'	4.5 abc	1.0 c	1.1 b	3.8	4.5	1.0	2.7 b
'Ellis Bitter'	3.2 c	1.8 bc	2.1 a	3.5	5.1	1.1	2.3 bc
'Fosseple'	4.7 abc	1.9 bc	1.2 b	3.3	4.3	1.1	2.3 bc
MA983 05002	4.8 abc	1.7 bc	1.0 b	3.6	5.0	1.0	2.6 bc
MA985 03023	4.3 abc	2.0 bc	1.4 b	4.0	5.3	1.1	2.0 bc
MA992 03006	3.6 bc	2.3 bc	2.0 a	3.7	4.9	1.3	2.3 bc
MA992 37013	4.3 abc	1.8 bc	1.3 b	3.5	4.3	1.1	2.3 bc
MA992 47003	3.6 bc	1.3 bc	1.2 b	3.8	4.8	1.2	2.2 bc
NY 18491	5.2 ab	3.6 a	1.0 b	3.9	5.6	1.2	2.2 bc
'Sansa'	5.2 ab	2.1 bc	1.1 b	3.9	5.5	1.1	1.7 c
'Silva'	5.5 a	2.6 ab	1.1 b	4.0	5.8	1.4	1.6 c
X 4876	3.8 bc	1.0 c	1.3 b	4.0	5.4	1.1	2.3 bc
Mean	4.3	1.9	1.3	3.7	5.0	1.1	2.3
Probability levels of significance by Two-way analysis (Pr > F)							
Source of variance							
Genotype	0.0130	0.0064	0.0001	0.4196	0.1538	0.0677	0.0010

The data are the means of each genotype, inoculated with *Neonectria ditissima* in eight replicates and values were the means of eight trees inoculation points per tree. Control replicates did not develop disease symptom and not included in data analysis. There was significant difference between replicates for flakes, bubbles and sporulation. Higher scores means darker color, or more flakes, ring pattern, bubbles, sporulation or swelling.

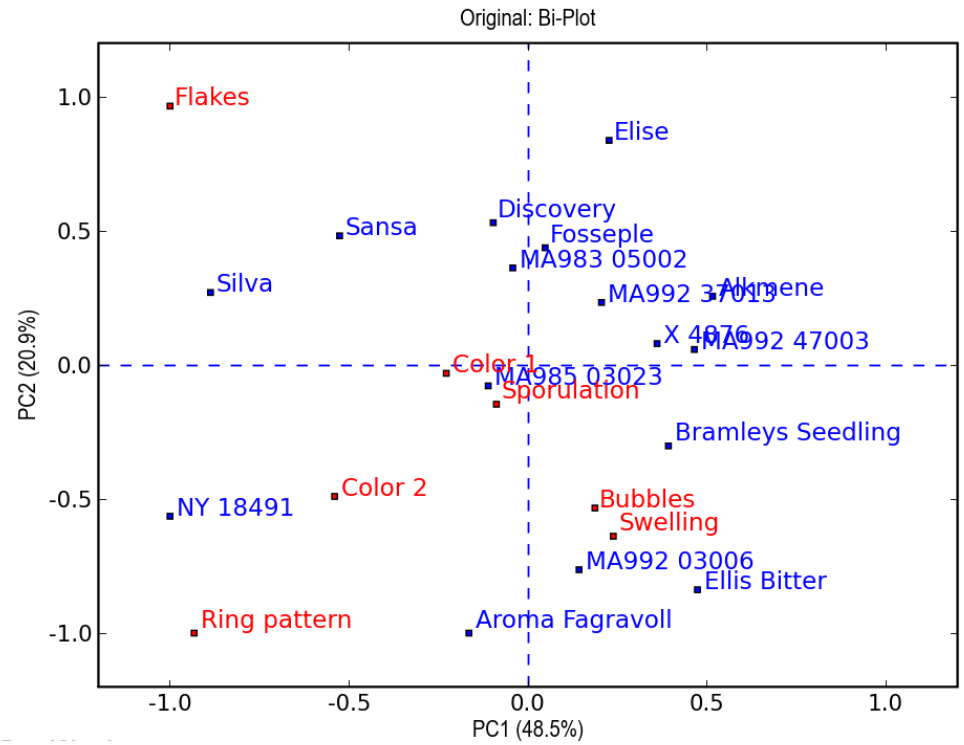

Figure 22. Principal component analysis (PCA) interpreted the value from scoring to find patterns of characters among the tested genotypes in PCA plot. PCA bi-plot of 16 genotypes, results on 7 characters (red) of 16 genotypes (blue) represent the character profiles, significance can read in Table 9.

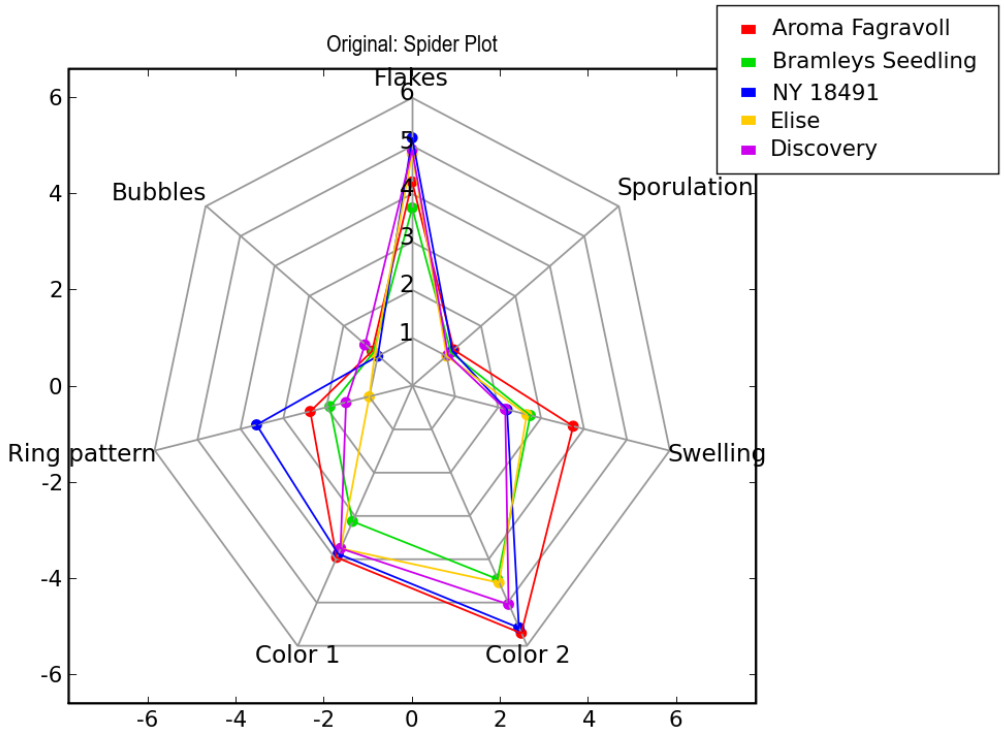

Figure 23. Character profiles of 'Aroma Fagravoll', 'Bramley's Seedling', 'Discovery', 'Elise', and NY 18491 in 2019-2020 experiment. These characters were observed on the last measuring date of the experiment, significance can read be in Table 9.

A spider plot can be used to show the character profile of cultivars in the test, and is showed as an example here (Figure 23). The character profiles of 5 genotypes are visualized in a spider plot. The plot includes also characters where cultivars did not differ significantly in ANOVA, with significant differences shown in Table 9.

4.3. Potted tree experiment in 2019-2020 including points with no lesion development

Table 10. Area under the disease progress curve (AUDPC), external and internal lesion length (mm) on wounded area for 16 apple genotypes of potted trees inoculated with *Neonectria ditissima* in 2019.

Genotype	AUDPC	External lesion length (mm) at the last date	Internal lesion length (mm) at the last date
'Alkmene'	1307.3 f	31.4 d	167.4 bcd
'Aroma Fagravoll'	2393.7 cdef	56.1 bcd	215.2 bcd
'Bramley's Seedling'	1628.3 f	40.0 cd	231.6 abcd
'Discovery'	4188.7 ab	90.3 ab	137.0 cd
'Elise'	5303.8 a	112.8 a	117.0 d
'Ellis Bitter'	1694.7 ef	46.3 cd	141.5 cd
'Fosseple'	2631.0 bcdef	57.3 bcd	307.0 ab
MA983 05002	1777.0 ef	35.6 cd	241.6 abcd
MA985 03023	3766.7 abc	74.3 bc	126.1 cd
MA992 03006	1972.0 def	53.4 bcd	353.5 a
MA992 37013	3531.1 bcd	89.5 ab	228.0 abcd
MA992 47003	2478.5 cdef	59.4 bcd	195.6 bcd
NY 18491	2328.7 cdef	44.9 cd	195.9 bcd
'Sansa'	2553.1 bcdef	50.9 bcd	182.4 bcd
'Silva'	3326.4 bcde	72.9 bc	295.9 ab
X 4876	3900.3 abc	121.3 a	266.3 abc
Mean	2798.8	64.8	212.6
Probability levels of significance by Two-way analysis (Pr >F)			
Source of variance			
Genotype	<.0001	<.0001	0.0028

The data are the means of eight replicates (trees) and three inoculation points per tree with *Neonectria ditissima*. Mean separation by Duncan test (P = 0.05). Inoculation points with no lesions developed were included in this data analysis.

For the experiment in 2019-2020, data analysis was also calculated for a dataset including inoculation points with no lesion development. The results in Table 10 show significant differences among genotypes and no significant difference was found between replicates of the AUDPC values. ‘Elise’ and ‘Discovery’ had the highest AUDPC values while ‘Bramley’s Seedling’ and ‘Alkmene’ had the lowest AUDPC values. ‘Alkmene’ and ‘Bramley’s Seedling’ had approximately the same levels of AUDPC values as ‘Aroma Fagravoll’. ‘Aroma Fagravoll’ was significantly different from ‘Discovery’ and ‘Elise’ for AUDPC.

Significant difference between the 16 genotypes were observed on external and internal lesion length on the last record date. The results showed that ‘Discovery’, ‘Elise’ and X 4876 had long external canker lesions while ‘Alkmene’, MA983 05002 and ‘Bramley‘s Seedling’ had the lowest external lesions size. ‘Aroma Fagravoll’ had medium external lesions size compared to other genotypes in this ranking. The results showed that ‘Silva’ ‘Fosseple’ and MA992 03006 had the longest internal lesions while the genotypes such as ‘Discovery’, MA985 03023 and ‘Elise’ performed the lowest internal lesions length. The genotype MA992 03006 differed significantly in internal lesion length from these three control cultivars.

4.4. Detached shoot experiment in 2020-2021

Disease development

In the detached shoot trial, disease symptoms were observed weekly. The first canker lesions appeared on average 18 days after inoculation with *N. ditissima* and then developed rapidly during the experimental period. The first seedling population that showed visible disease lesions was ‘Golden Delicious’ × ‘Stølen’. The mean date for the first lesions were at the same range for all populatons in this experiment (Table 11), with a mean of 17 days for ‘Golden Delicious’ × ‘Stølen’, 18 days for ‘Elise’ × ‘Stølen’ and 20 days after inoculation for ‘Kanzi’ (‘Nicoter’ Kanzi®) × ‘Stølen’. No difference between populations were observed in the number of days from inoculation until first visible symptom.

Infection percentage

The detached shoots were all inoculated in one day and there was no significant difference observed among the seedling populations (Table 11). The three seedling populations showed a range in infection percentage, and it was highest in the seedling populations ‘Elise’ × ‘Stølen’ (80 %) and ‘Kanzi’ × ‘Stølen’ (79 %), while ‘Golden Delicious’ × ‘Stølen’ had 65 % infection

but there was no significant difference between populations in this parameter. None of the uninoculated control shoots developed canker lesions on the detached shoots.

Wilting and girdling

There was 8 % wilting in the seedling populations of ‘Golden Delicious’ × ‘Stølen’ while in the two other seedling populations there were no wilting (Table 11). Observation of the girdled shoot in this experiment showed that the populations of ‘Elise’ × ‘Stølen’ and ‘Golden Delicious’ × ‘Stølen’ had girdled at 8 % and none of the seedlings in population ‘Kanzi’ × ‘Stølen’ were observed girdled on the inoculation points.

Diameter of stem

Diameter (mm) of the stem showed that the population ‘Kanzi’ × ‘Stølen’ had the thickest shoots at 9.3 mm (mean value), with a maximum thickness of the shoots at 13.2 millimeter and minimum at 6.1 mm. Population of ‘Elise’ × ‘Stølen’ had the thickness size at 9.2 mm and maximum at 11.2 mm while minimum at 5.5 mm. ‘Golden Delicious’ × ‘Stølen’ had thinner shoots at 9.0 mm (mean value) and the maximum at 12.3 mm and the minimum at 3.5 mm. There was no significant correlation between shoot size and the external lesion length at last date and AUDPC values on ‘Elise’ and ‘Golden Delicious’ populations but correlation was found between the main stem size, the external canker lesions ($P = 0.0219$, $r = -0.522$) and AUDPC values ($P = 0.0222$, $r = -0.521$) on ‘Kanzi’ population.

Area under the disease progress curve AUDPC and lesion development 2020-2021

Inoculation with macroconidia 1000 spores per ml on the artificially wounded shoots showed AUDPC values that were not significantly different between the seedling populations (Table 11). There was a tendency that population ‘Elise’ × ‘Stølen’ had the highest AUDPC values in this experiment while the population ‘Kanzi’ × ‘Stølen’ presented the lowest AUDPC values. The population ‘Golden Delicious’× ‘Stølen’ performed in between AUDPC values to ‘Elise’ × ‘Stølen’ and ‘Kanzi’ × Stølen’. The statistical analyses showed that there was no significant difference between replicates on AUDPC values in detached shoots experiment.

Table 11. Infection percentage following inoculation of *Neonectria ditissima* and other observations on inoculation points for three seedling populations in a growth camber.

Population	Infection (%)	Number of days until first symptom	Wilting (%), No=0 and Yes=1	Girdling (%), No=0 and Yes=1	AUDPC	External lesion length (mm)	Internal lesion length (mm)
'Elise' x 'Stølen'	80	18	0	8	1011.8	26.2	34.4
'Golden Delicious' x 'Stølen'	65	17	8	8	880.7	23.5	30.5
'Kanzi' x 'Stølen'	79	20	0	0	677.4	21.4	26.8
Mean	75	18	3	5	856.6	23.7	30.5
Probability levels of significance by Two-way analysis (Pr > F)							
Source of variance							
Population	0.0457	0.3498	*	*	0.0713	0.5448	0.4116

The data are the means of four replicates (5 trees per population per replicate) each tree inoculated with *N. ditissima* and mean of two inoculation points per shoot. Mean separation by Duncan test (P = 0.05). External and internal fruit tree canker lesion length (mm) on the last observation date for three seedling populations, measuring visible external lesion length and other outer characterdrs before measuring internal lesion length.

Scale wilting: 0 = no wilting, 1 = whole shoot wilted above the upper inoculation point.

There was not significant difference between replicates for any parameter, except for infection percentage.

*Not analyzed in ANOVA

Figure 24. Detached shoots inoculated with *Neonectria ditissima*: External lesion lengths were measured weekly from week 48 in 2020, and internal lesion lengths were measured at the end of the experiment (A), disease symptoms of *N. ditissima* 'Elise' × 'Stølen' (B), 'Golden Delicious' × 'Stølen' (C), 'Kanzi' × 'Stølen' (D).

Table 11. show results on external and internal lesion length. There was no statistically significant difference between populations. The lesion surface developed clearly when using detached shoot method (Figure 24).

Analysis of variance was calculated on external lesion length for each recording date separately. Lesion growth curve of the three different populations of 'Elise' × 'Stølen', 'Golden Delicious' × 'Stølen' and 'Kanzi' × 'Stølen' are shown in Figure 25. Lesions of 'Elise' × 'Stølen' and 'Golden Delicious' × 'Stølen' grew at approximately similar rates. Lesion growth on populations 'Kanzi' × 'Stølen' developed slower than the other populations in the beginning of the recording time, and then started to grow faster after recording no. 4 (mid-December). Then lesions grew to almost the same level as population of 'Golden Delicious' × 'Stølen' at the last recording date. At recording date no. 3 and 4, there was significant difference ($P = 0.0035$ and $P = 0.0067$) between populations in lesion size, with population 'Kanzi' × 'Stølen' having shorter lesions than the other two populations.

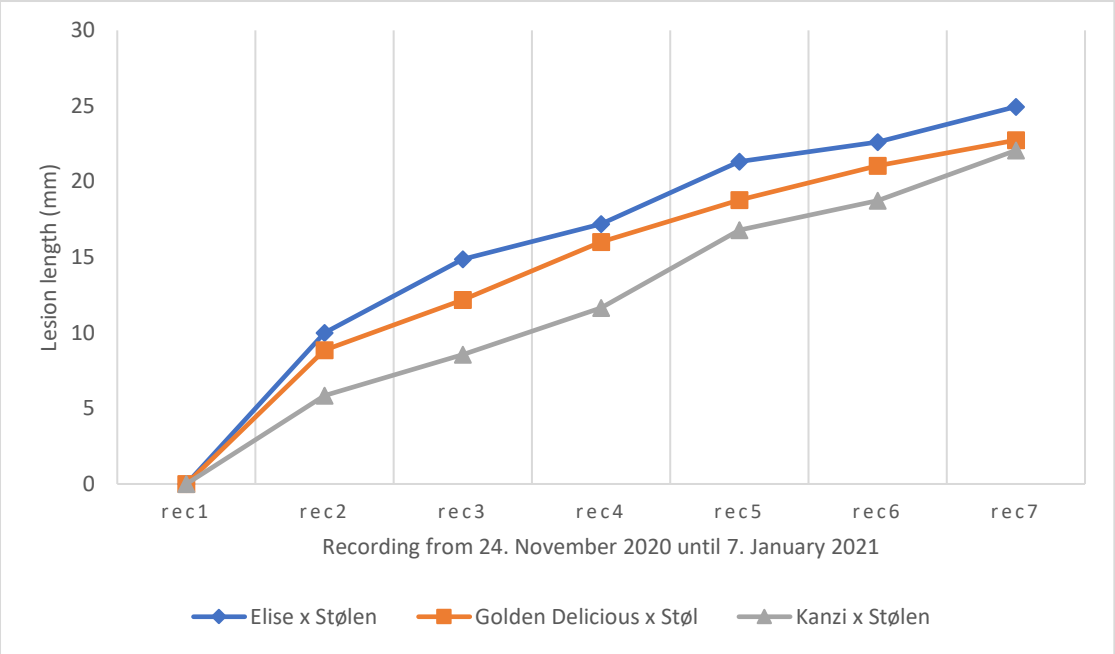

Figure 25. Lesion length (mm) development measured weekly during the experimental period in 2020 - 2021. Detached shoots experiment with three seedling populations inoculated with *Neonectria ditissima*.

Characteristic profiles

At the final assessment date, there were no significant differences in the four characters flakes, bubbles, ring pattern and lesion color among the three seedling populations. The results from PCA indicated that PC1 and PC2 covered 86.0 and 14.0% of the variation among these populations (Figure 26). Flakes described most of the variation in PC1, with the population of 'Elise' × 'Stølen' having more flakes compared with the other populations but anyway there was no significant difference in the characters flakes, bubbles, ring pattern and color observed in this seedling population trial. The mean for each character is illustrated in a spider plot in Figure 27. Miscoloring was also observed, the results showed that populations of 'Golden Delicious' had a bright brown color, populations of 'Elise' had brown color and populations of 'Kanzi' had tendency to dark brown color.

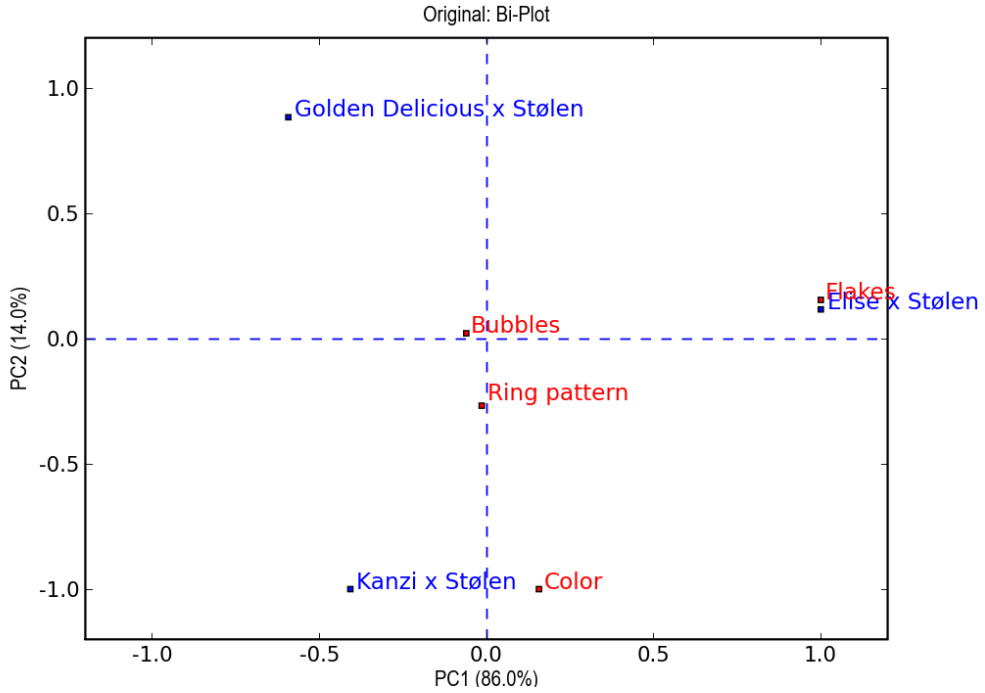

Figure 26. Principal component analysis (PCA) interpreted the value from scoring to find patterns of characters among the tested genotypes in PCA plot. PCA bi-plot of populations of three apple crosses (blue), results on four characters (red) represent the character profiles, no significance difference was found in these four characters.

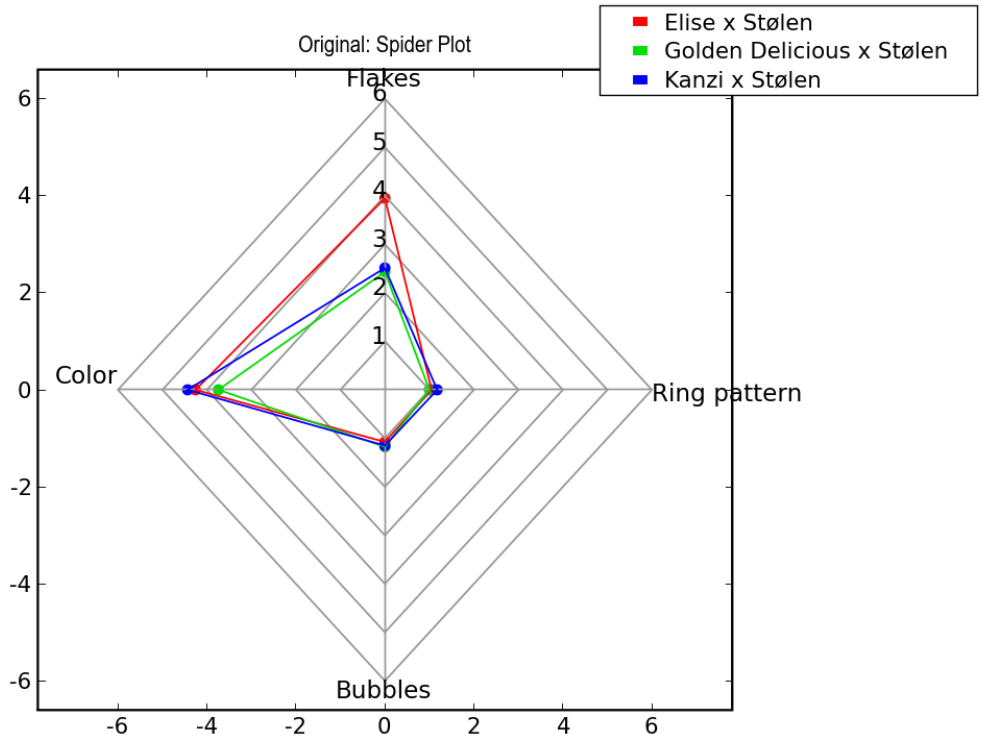

Figure 27. Character profiles of genotypes in an experiment in 2020-2021; detached shoots inoculated with *Neonectria ditissima*. The mean for each character is presented and displayed as a spider plot. These characters were observed on the last measuring of the experiment on 7 January 2021, no significant difference was found in these four characters.

5. Discussion

5.1. Evaluation method

The results presented in this study discriminate susceptibility of apple genotypes to fruit tree canker caused by *N. ditissima* and illustrate response of apple genotypes after infection with this fungus. Several characters were observed for illustrating characteristics among the test genotypes, the results being based on potted trees method and detached shoots method with inoculation of the main stem with macroconidia after artificial wounding under controlled conditions. For the two potted tree experiments in the greenhouse, the main stem clearly developed symptoms that were easy to measure, except at the last period of observations when it was difficult to conduct measuring for some genotypes, for example 'Elise' had problem with lesions from neighbouring inoculation points merging and stem being girdled. 'Elise' was described as a highly susceptible cultivar to fruit tree canker from previous studies (Garkava-Gustavsson et al. 2013; Ghasemkhani et al. 2015; Nybom et al. 2016a) and the results from this study was agreement with this statement. The detached shoot experiment was carried out in the growth camber, measuring was easier than in the potted tree trial because the shoots did not have problem with interference between inoculation points and were not dead or dried out (a small amount died maybe because these shoots had lower diameter of the stem).

Inoculation with *N. ditissima* after artificial wounding with scalpel (Nybom et al. 2016a) was an easy assay to perform. It was an effective and not time-consuming assay to use as an inoculation procedure. Inoculum used in these three experiments was kept in deep freezer and activated on rootstock before collecting conidia used in the inoculation trials. This method seems to give highly active inoculum with high number of macroconidia. The detached shoot trial was performed in the same year as the last potted tree trial. Frozen sporulating lesions from the last potted tree experiment was then used directly for making inoculum to use in the detached shoot experiment in the end of 2019. Inoculum of *N. ditissima* was stored frozen and was able to infect wounded points on the main stem of apple genotypes (Orchard et al. 2018). It is a suitable method to keep the inoculum to use in inoculation trials for screening disease resistance in the breeding program for apple.

In all genotypes in these experiments, inoculation with conidia suspension resulted in infection after inoculation, but each genotype responded to the canker differently in their susceptibility level as was also reported in other studies (Garkava-Gustavsson et al. 2013; Gomez-Cortecero

et al. 2016; Van de Weg 1989; Wenneker et al. 2017). In our experiments were found significant differences between genotypes on potted tree experiments, however in 2018-2019 experiment it was used only 'Aroma' as control cultivar, considered not to differ in canker related characteristics to the red sport 'Aroma Fagravoll' used as control in 2019-2020 experiment. The 2018-2019 experiment was a test of susceptibility level in cultivars and selections from Graminor. Genotypes used in experiment 2019-2020 were genotypes in Nordic core and diversity collections (decided in Nordic PPP pre-breeding project) that were not tested in previous canker trials.

There are several methods to screen susceptibility level to fruit tree canker. Other study (Scheper et al. 2018) indicated that the results from detached shoot method did not give reliable information about the test cultivars as the investigation did not give the similar results about test cultivars in comparison between potted tree method and detached shoot method. Whole tree assay showed more reliable and correct results when related to observation under field conditions for example 'Gala' performed resistance against fruit tree canker in detached shoot method but observation of disease risk under field conditions showed high susceptibility (Scheper et al. 2018). Test cultivars used in detached shoots treatment respond to infection differently from potted tree treatment. Common problems seen when using detached shoots is that shoots die, and challenging measurement caused by interference between lesions and dying of shoots. In our study on detached shoot treatment in this thesis, we used the main stem of one-year old tree to test susceptibility to fruit tree canker of apple genotypes. There was no significant difference among test genotypes in three seedling populations, on shoots collected in November and carried out over 6o days after inoculation. There was however observed only slightly drying out and death of shoots making it easy to measure with no challenge due to dying shoots. The results showed no significant difference in AUDPC values between seedling populations of 'Elise' and 'Kanzi'. These two cultivars are known as highly susceptible to canker, and these two seedling populations probably respond in a similar way to canker disease as the parents. The ranking in the experiment with detached shoot method showed that 'Elise' populations had highest AUDPC values and 'Kanzi' populations presented the lowest AUDPC level, but when considering the trend in Figure 25 it seems that 'Kanzi' could develop canker lesions larger than population of 'Golden Delicious' if the experiment continued two weeks extra. The cultivar 'Golden Delicious' is described as resistant to canker cultivar but this character seems not inherited to 'Golden Delicious' seedling populations in this trial. It may be because when selecting in clonal crops, one is normally selecting the extreme types different

from the parents. A seedling population with such extreme phenotype in the parents may have a mean less extreme than the parents (Press 2014) and it is possible that resistance gene from 'Golden Delicious' was recessive gene (Gómez-Cortecero et al. 2016). Furthermore, there was a low number of seedlings per population and only one tree per seedling tested and this can also impact the results.

The two potted trees experiments were carried out using the same procedure, and inoculum isolate. AUDPC level and external lesion length were at the same level for 'Aroma' ('Aroma Fagravoll') in both experiments. This indicate that one can rely on the results for 'Aroma' in experiment in 2018-2019. 'Aroma' is considered to be quite strong against fruit tree canker (Ghasemkhani et al. 2015). In the 2019-2020 experiment 'Aroma' performed at the expected level compared to the susceptible control cultivars 'Discovery' and 'Elise'. When 'Aroma' is one of the most susceptible genotypes in the 2018-2019 trial it then indicates that most of the selections/cultivars tested were similar to or even more resistant than 'Aroma'.

In this study the experiments were carried out only one year for each cultivar and each method. It should be repeated to validate the conclusions on susceptibility among the tested genotypes. Further research is needed.

5.2. Parameters

There was observed the number of days the first symptoms were visible after inoculation, using this parameter to discriminate level of resistance to fruit tree canker seem to be possible to find significant difference between genotypes (Van de Weg 1989). There was the same trend in development of the fungus both years, when 4 weeks after first symptoms visible, the lesion length developed rapidly. In 2018 first visible symptoms were seen earlier than in 2019. In both years 'Aroma' was used as control, the number of days to visible symptoms for this cultivar were found at the different level, it may be caused by difference in plant material, climate, soil quality, fertilizer and watering which can impact the results reasonably when testing susceptibility level against fruit tree canker (Swinburne 1975; Van de Weg 1989). Trees may differ in defence mechanism level, this mechanism is often related to encoding of genes (Willey 2016). Probably aggressivity level of inoculum may also influence the results. There was reported about this in previous study (Andrivon 1993). Of this results in general, shorter time after inoculation when symptoms were visible should indicate as susceptible cultivar and in

contrast longer time before disease symptom appeared should indicate resistance cultivar, but it was not the same trends for all genotypes. Anyway, one should evaluate together with other parameters to examine resistance level to get reliable results.

Infection percentage is used to investigate the level of susceptibility against *N. ditissima* (Garkava-Gustavsson et al. 2016; Ghasemkhani et al. 2015; Scheper et al. 2018; Van de Weg 1989; Wenneker et al. 2017). The results from potted trees experiment in 2018-2019, potted trees experiment in 2019-2020 and detached shoots experiment showed infection percent with a range from 42-100 %, 54-100 % and 65-80 % (overall mean at 81, 87 and 75 %). The results showed high infection percent compared with studies by Van de Weg (1989), Garkava-Gustavsson et al. (2016) and Wenneker et al. (2017). It is important to point out that the infection percent in all experiments was high, probably as inoculum was activated on plant material before used in the inoculation trials. Inoculation was done within short time after wounding (Xu et al. 1998) and inoculated wound covered with Vaseline. Conidia can grow and develop in favourable conditions as temperature in a wide range between 5-25 °C and relatively high humidity, favourable conditions can increase infection level (Weber 2014) and it is reported from in vitro studies that conidia can germinate below 6 °C (Latorre et al. 2002). In our study, conditions for canker development were good for germination in the greenhouse and growth chamber (Latorre et al. 2002).

There were no significant differences observed among three seedling populations for all parameters such as infection frequency, AUDPC values and other characters as flakes, bubbles, ring pattern and lesion color. However, 'Elise' did have many flakes in potted trees experiment in 2019-2020, and seedlings of 'Elise' seem to inherit this character. 'Kanzi' seedling populations developed lesions later than the two other seedling populations, but at the end of experiment period lesions on 'Kanzi' seedlings developed faster and approached the lesion size to the other two families. If the experiment period had been expanded longer and the trend continued, 'Kanzi' seedlings might have turned to have the longest lesions in this trial.

The external lesion length, AUDPC level and infection frequency are often used to determine the level of susceptibility against *N. ditissima* (Garkava-Gustavsson et al. 2016; Ghasemkhani et al. 2015; Scheper et al. 2018; Van de Weg 1989; Wenneker et al. 2017). Each of these parameters alone seem not to be enough to conclude about susceptibility level in an apple genotype. The results in our study showed that the genotype with lowest AUDPC level (MA992

39008) and highest AUDPC values (‘Idunn’) presented the same infection percent (100%), while the genotype MA982 05043 had the highest AUDPC level but had one of the lowest infection percentages. It is important to look at several parameters together for example infection percent, AUDPC level, external lesion length, internal lesion length, wilting, girdling and other observations on the last observation to determine susceptibility of apple genotypes to fruit tree canker (Wenneker et al. 2017; Børve J. pers. comm.). The reason for recording all these parameters in phenotyping is to get a reliable picture on resistance in breeding material for use as parents and for introduction of new resistant cultivars. Reliable phenotyping is also necessary when searching for resistance genes or markers for resistance genes (Nybom et al. 2016b).

The results in these studies showed significant differences between genotypes on external lesion length at the last recording date, and this parameter can be used to investigate level of susceptibility to canker. The results of external lesion length seem to have good association with AUDPC level and number of days from inoculation until first symptom which also can indicate resistance ability to bark canker caused by *N. ditissima.* Recording of external lesion length was a parameter we observed from the beginning until the end of experiments, conducted weekly, except for potted trees experiment in 2019-2020 when it was measured with two weeks interval. The results from the potted tree trial recorded weekly and with two weeks interval might show similar results, indicating that recording with two weeks interval was a good enough approach to observe external lesion and calculate AUDPC. Reducing measuring time point from weekly to two weeks interval might be convenient and time saving. For detached shoots trial one may perform measuring weekly. It may not require shorter intervals between each recording time.

Considerable differences were found also on internal lesion length in 2019-2020 potted tree trial, but no differences were found in 2018-2019 trial. Even if there were significant difference among genotypes in last potted trial (in 2019-2020), it is quite difficult to find a clear pattern to correlate with other parameters. Using internal lesion length to investigate susceptibility of apple cultivars in these studies is difficult, for example in 2019-2020 results we cannot discriminate between ‘Aroma Fagravoll’, ‘Discovery’ and ‘Elise’. These three cultivars were similar in internal canker lesions. As we know from previous studies these three cultivars ranked differently in the level of resistance to fruit tree canker in apple (Garkava-Gustavsson et al. 2013) as they also do for other parameters in our study. Internal lesion and external lesion

length seem not to be correlated. As a result, internal lesion length cannot be used alone to conclude about susceptibility level to fruit tree canker, but observation of internal canker lesions might be an important character of phenotyping to investigate why the tree wilt or die even if the external and AUDPC values were not high. An example in this case is the genotype MA992 35005 and MA992 39008 which have very long internal lesion and high in wilting but low in external lesion length and AUDPC values. Another example is MA992 03006 which obtained the highest internal lesion values and low values in external lesion length and AUDPC, but was observed high in wilting. To better understand about the epidemiology of the fungus, studies showed that *N. ditissima* entering pathway was on wounded sites and then transported most upward by xylem vessels (Zeller 1926). Lesion growth expanded long from infection sites, and the fungus can also enter phloem vessels, but it might take longer time than to penetrate through xylem (Crowdy 1949). In this test the growth and development of canker on all apple genotypes, except for 'Elise', showed longer lesions of *N. ditissima* inside the plant tissue than visible canker lesions on surface from inoculation points. This disease is known to have latent infection with canker symptoms appearing a few years later after infection (McCracken et al. 2003). The parameter internal lesion length may not be of importance to use to separate significant difference among apple cultivars (do not has ability to discriminate control cultivar). This parameter also is a very time-consuming measurement, but anyway this character can represent or describe profile information for how the cultivar reacts on infection.

Several other parameters were used to estimate susceptibility to fruit tree canker in apple in this study. Wilting and girdling, were both observed in experiment in 2019-2020 (potted trees) and in 2020-2021 (detached shoots). In 2018-2019 (potted trees) was recorded only wilting, not girdling. Wilting and girdling might be important characters to include in the whole picture to determine resistance ability of apple cultivars. Wilting and girdling characters seem to be correlated in the potted trees trial, 'Aroma Fagravoll' being significantly different from 'Discovery' and 'Elise'. These characters can discriminate significant differences among the control cultivars, and this is also in agreement with observation in the orchard. 'Discovery' is known to have high susceptibility to fruit tree canker caused by this fungus, and 'Aroma' ('Aroma Fagravoll') is relatively resistant to canker. Our results relate to the results from others studies (Garkava-Gustavsson et al. 2013). The characters are related, as girdling can results in wilting of trees after attack of *N. ditissima*. In our study, 'Elise' had thinnest shoots while 'Aroma Fagravoll' shoots had similar thickness to 'Discovery'. The genotypes 'Bramley's Seedling' and NY 18491 had larger stem size and were the most resistance to canker. In our

study all trees/shoots were of same age. There was significant difference between genotypes on shoot diameter and the results showed correlation between shoot size, AUDPC level and the external lesion length at the last observation date.

The shoot size was smaller for the 'Golden Delicious' populations than for the other two populations. It is possible that shoot size may influence the results on resistance level. Wilting and girdling can develop faster on thin shoots than on thick shoots and for these characters we can overestimate level of susceptibility to fruit tree canker if shoot size is not similar in the genotypes we compare.

It is reported that wood age influence on infection by fruit tree canker, affecting on disease incidence and disease severity, showing more and enlarged lesion size on older wood than young wood. This can be caused by difference in growth and development mechanism, and older wood may can store food source that is necessary for the fungus more than young wood (Amponsah et al. 2017). In another study, young apple trees are more sensitive to fruit tree canker than older trees (Swinburne 1975), young trees probably had smaller size in diameter than older trees. However, in our study all trees/shoots in an experiment were of same age.

Recording of external symptoms as flakes, ring pattern, bubbles, lesion color, swelling or sunken lesions describe extra information on how cultivars respond to fruit tree canker. The results presented in this thesis indicate that much flakes seem to be correlated with high AUDPC values, infection percent and high in wilting. Including of these characters will help to find clear pattern to define characters of susceptibility or resistance against the disease. 'Elise' and MA985 03023 obtained much flakes, and characterized similar to 'Aroma Fagravoll', but 'Aroma Fagravoll' had more swelling than MA985 03023 and 'Elise'. It seems to be typical of the susceptible cultivars as 'Elise' to have much flakes after infected by canker and it might be inheriting this character to seedlings of 'Elise'. It seems that flakes character may indicate high susceptibility level. Swelling is probably a response from the plant to reduce damage that can occur after infection on resistant genotypes (Figure 28).

The results showed no significant difference among apple genotypes on bubbles, ring pattern and sporulation in potted tree trial in 2018-2019, however there was significant difference between the test genotypes in 2019-2020 but not significant difference among control cultivars. More research is necessary to give more information about how these characters correlate to

susceptibility level to canker and why the plant respond to canker with such symptoms, it is possibly caused by differences in genetic background (Gómez-Cortecero et al. 2016; Van de Weg 1989).

Observation of several parameters in the end of experiment are time consuming, and it can be a challenge to score correctly. Scale could be improved for more easy use because it is very easy to score not correctly if the recorder is not trained to use of scale. Our suggestion is to produce picture scales to use under recording which describe about what is difference between scale levels to ensure precise and reliable scoring. The recording method should be simple. Further work is necessary to improve scale descriptions.

Figure 28. Swelling character occurred on resistant genotypes such as 'Aroma Fagravoll' (A), NY18491 (B) and 'Bramley's Seedling' (C). Photo: Kurab Røen.

In addition to the results from ANOVA, it was conducted PCA plot to give information on relationship between characters in the test material in each experiment, and spider plot to visualize characters in the cultivars in an easy way to read (only a small number of the test cultivars should be included, especially in spider plot). The spider plot is unable to describe information on relationships between characters, but the two plots can reveal different information of each genotype and comparison among apple genotypes. It is reasonable to include in PCA and spider plots also characters that performed no statistically significant difference, in order to give a full profile for the test cultivars, while significance can be read from ANOVA tables.

On potted tree experiment in 2019-2020, data analysis was also calculated for a dataset including inoculation points with no lesion development. There was significant difference between the test genotypes for a dataset included no lesion development and for a dataset included only lesion development. However, when not including inoculation points with no infection, calculation separates better between genotypes on lesion growth. For example, for AUDPC values in dataset with only infected inoculation points we can sort the level of susceptibility to *N. ditissima* between the control cultivars 'Aroma Fagravoll', 'Elise' and 'Discovery', but in dataset with all inoculation points included 'Elise' and 'Discovery' were similar in susceptibility level to apple canker. On the other hand, the ranking of the genotypes for AUDPC levels were mainly similar for both datasets. 'Alkmene' had the lowest infection percent, but when calculating on dataset including only inoculation points with canker lesions, 'Alkmene' lesion growth was similar to 'Aroma Fagravoll'. In addition, 'Alkmene' canker lesions girdled the tree for almost all inoculation points. 'Alkmene' seem to be lower than the other genotypes on canker incidence (infection percent), but on canker severity characters 'Alkmene' had at the same level as 'Aroma Fagravoll' or even more susceptible than 'Aroma Fagravoll' (for example on girdling character).

The reason to run statistical analysis with two types of datasets is to avoid overestimating the susceptibility to *N. ditissima.* of apple cultivars with low infection percent when including only inoculation points with developing lesions. This study showed similar results for all genotypes except for some cultivars, ranking differently for some parameters, but the whole picture of the ranking was similar. The genotypes turning out resistant or susceptible, did not totally change from susceptible to resistance.

In our study, the tested apple genotypes varied in their level of susceptibility to fruit tree canker caused by *N. ditissima*. Low AUDPC values, low infection incidence and longer time until first symptoms visible after inoculation, no girdling and low wilting should indicate resistance, for example as found for MA042 10041 and 'Bramley's Seedling'. The selection number MA042 10041 is from a cross between 'Marteeple' × 'Rubinstep' and 'Rubinstep' is known as relatively resistant to fruit tree canker (Kühn 2004). This character seems inherited to MA042 10041. 'Bramley's Seedling' is known as a resistant cultivar to fruit tree canker (Lindhard Pedersen et al. 1994) and the result from this study was in agreement with this statement. In contrast, high AUDPC values, high infection incidence and shorter time until first symptoms visible after inoculation, and girdling of the whole trees, as found for 'Idunn' and 'Elise' should indicate

that they are highly susceptible. ‘Elise’ is from a cross between ‘Septer’ × ‘Cox’s Orange’, and Cox’s Orange was described as susceptible cultivar (Garkava-Gustavsson et al. 2013). The Swedish cultivar ‘Aroma’ presented low to medium susceptibility to *N. ditissima* compared to other genotypes based on evaluation for all parameters.

6. Conclusions

The tested apple genotypes in this study differ in their level of susceptibility to fruit tree canker caused by *N. ditissima*. The results showed differentiated ranking of the response after artificial wound inoculation. Inoculation of potted trees with conidia suspension was an easy and effective method, with inoculation in dormant period (December) and trees evaluated through 5-6 months. Inoculum used in the experiments was stored in deep freezer from one season to the next and activated by wound inoculation on one year old apple rootstock trees before start of a new experiment. Macroconidia was then collected from these rootstock trees for use in the experiments. This method resulted in a high infection level.

Infection percent and AUDPC level are commonly used to investigate resistance of apple genotypes to fruit tree canker. This study shows that it is necessary to examine the level of susceptibility to canker by using several parameters. Infection percent or AUDPC level alone are not enough to conclude about resistance level. Evaluation of both parameters together and including several more parameters seem to ensure more reliable results.

The number of days until the first symptoms appeared after inoculation seem to be a good parameter to include when determining susceptibility to canker, together with other parameters such as AUDPC level and lesion size at the end of experiment. The lesions started to grow rapidly approximately 4 weeks after first symptoms were visible in the potted tree trials, while in the detached shoot trial the lesions grew rapidly already from one week after the first symptoms appeared. From our study, internal lesion size measured after splitting of stem cannot be used alone to conclude on resistance to *N. ditissima*, but this may be a beneficial character to phenotype to get a reliable picture about susceptibility level to fruit tree canker.

Wilting and girdling are important parameters to include to examine resistance of apple genotypes, both parameters presented a good association with other parameters such as AUDPC level and external lesion size at last recording date. On the last recording date was implemented

several characters of external symptoms such as flakes, ring pattern, bubbles and swelling, to enhance the understanding of response after infection with *N. ditissima*. In this study, swelling was a good parameter that may discriminate significant difference among apple cultivars.

PCA plots give useful information on relationship between cultivars and characters, and spider plot is a good way to visualize characters in the cultivars in an easy way to read. PCA plot showed that there is a relationship between the characters swelling and bubbles. These two characters might be related to resistance, while flakes seem to be associated with susceptibility. Recording a range of symptoms after infection can give a clearer picture of susceptibility to canker in apple genotypes.

After analysing datasets including or not including inoculation points with no infection symptoms, it is clear that when including only inoculation points with infection in data analysis, susceptibility of cultivars with low infection percentage can be overestimated.

Low values for all examined parameters, as for example on the genotypes MA042 10041 and 'Bramley's Seedling' in our study, indicate quite good resistance to canker in these genotypes. On the other hand, high values of many parameters for a genotype indicate that it is highly susceptible, as for example 'Discovery', 'Elise' and 'Idunn'. In our study, MA042 10041 and 'Bramley's Seedling' showed even stronger resistance against the canker than 'Aroma', which responded at the expected level. MA042 10041 and 'Bramley's Seedling' may be good parents in breeding for increased canker resistance in apple cultivars.

7. References

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