Norwegian University of Life Sciences

Master's Thesis 2021 30 ECTS
Faculty of Bioscience

Disease development of *Chondrostereum purpureum* in common Norwegian plum cultivars

Mårten Wennerberg
Master of Science: Plant science

Abstract

Silver leaf disease, caused by the basidiomycete *Chondrostereum purpureum*, is a growing problem in Norwegian plum production. It affects the overall health and lifespan of the fruit trees. The inoculation experiments in this study set out to attain more knowledge regarding disease development of the fungus under Norwegian conditions. They were performed at NIBIO Ullensvang and Ås between fall 2019 and fall 2020. Different inoculation methods were used on the main plum cultivars used in Norwegian production (Edda, Jubileum, Mallard, Opal, and Reeves) utilizing five *C. purpureum* isolates of different origin. The results of the inoculations, using map-pins with mycelium placed in newly developed side shoots and 1-year old side shoots in an established field, were unreliable and the method was found ill suited for field inoculations. The map-pin method did work well in more controlled conditions with whips in pots. Map-pin inoculation of whips developed leaf symptoms after 45 days outdoors and 35 days in growth chamber experiments. Fruiting bodies developed in August on one root stump that was left in the field. In addition, two trees in the outdoor experiment had silver leaf infections that had come prior to inoculations.

Young graftings, inoculated via agar pieces with mycelium, showed leaf symptoms 77 days after inoculation no matter if the inoculation point being in the rootstock or scion. Almost all graftings displayed leaf symptoms at this time. The fact that the graftings were very small appeared to be the reason behind the many severe infections in this experiment. Five dead graftings, inoculated in the rootstock, developed fruiting bodies in the field in August before final assessments and were identified as *C. purpureum*. This is a good example of how infected trees can become inoculum sources in the field if not removed. Inoculations with mycelium, performed in fall and winter, developed leaf symptoms already at leaf emergence in May and did not seem to be inhibited by the colder conditions during inoculation and wintertime. Overall, the methods using agar pieces with mycelium caused the most infections and were the most severe in younger trees.

For future studies susceptibility of the different cultivars should be investigated to provide a preference for Norwegian fruit farmers. Studies investigating disease development spanning over multiple seasons should be done on well-established fields to see long term effects of the fungus. Such an experiment could also provide better insight to which cultivars are more resilient than others and would be of helpful for future farm practices in Norway.

Table of content

1. Introduction

1.1 Background

Since 2000 the area used for plum production in Norway gone up from 328 ha to a total of 432 ha in 2020, an increase of 104 ha. However, the increase of land utilized for plum production has not increased the total production to levels that would be expected. As of 2020 "Grønt produsentenes samarbeidsråd" have made a prediction that the production will be 1919 tonnes in 2023 (Øie, 2020). The significant drop in total yield 2020 was due to severe frost damage during flowering that year (Figure 1).

Clearing of plum orchards has also been seen to be done at an earlier time than before. Many different factors may be accredited to the earlier clearing of plum orchards and the lower-than-expected yield in Norwegian plum production, but one suggested explanation behind it has been the overall health state of Norwegian stone fruit orchards (Anna Birgitte Milford & Veggeland, 2021). Because of this development, the project "Better tree health: An improvement in stone fruit tree health" was started in 2018. The project involves several aspects of the most important stone fruit diseases and how to best increase the efficiency in Norwegian stone fruit production by broadening the knowledge level (NIBIO, 2017). This thesis will focus more specifically on the stone-fruit *Prunus domestica* commonly known as plums and the wood decay fungus *Chondrostereum purpureum* (pers.) Pouzar, commonly known as purple crust fungus causing the disease silver leaf.

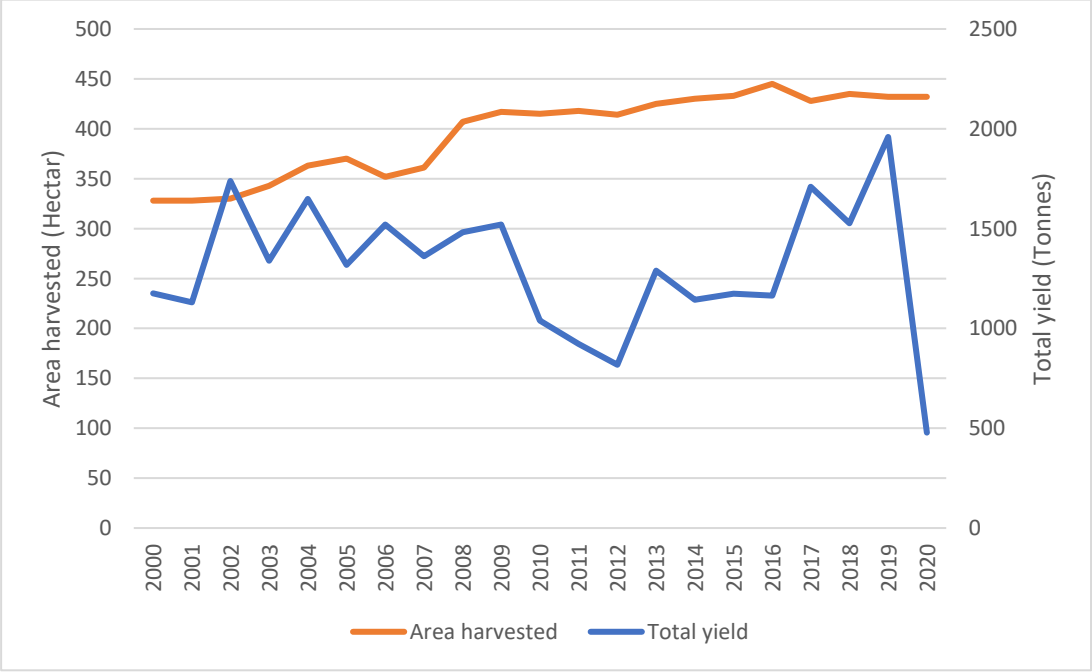

Figure 1. Total plum production in Norway displayed in tonnes and total area used for plum production in hectar between 2000 and 2020. The big drop in 2020 was due to frost during the flowering that year (FAOSTAT, 2021; Øie, 2020).

1.2 The basidiomycete *Chondrostereum purpureum*

Chondrostereum purpureum is a white-rot fungus (WRF) causing the disease commonly known as silver leaf, named after its silver hue like leaf symptoms. The fungus is naturally occurring in most temperate forests in the world (Figure 2). It has a wide host range of deciduous trees and has been shown to occur both as a saprotroph and a phytopathogen (Bishop, 1978; Pike, 2011; Reina et al., 2019).

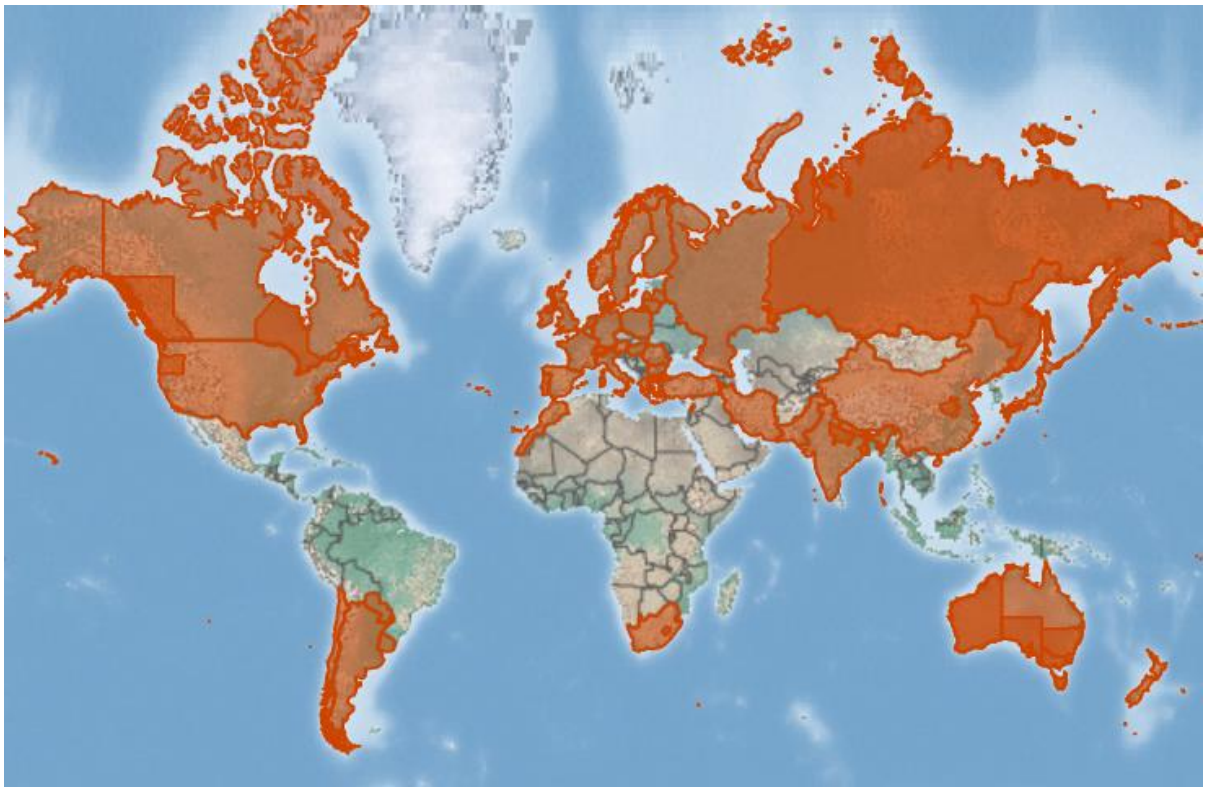

Figure 2. Distribution map for Chondrostereum purpureum *showing countries where the fungus has been recorded marked red. Retrieved from cabi.org*

The fungus spreads via basidiospores produced by basidiocarps appearing on dead previously infected wood in fall and can spread spores until early spring. The inoculum sources vary and can, e.g. be other infected trees in the vicinity or a pile of dead wood such as pruned infected branches that have not been properly removed or destroyed (Dye, 1974). Developed fruiting bodies can spread spores for at least a year and are disseminated with the help of wind and rain splash, which also are important factors for dispersal and successful infection. The fungus is considered to only enter via fresh wounds up to a month old (Spiers, 1985).

Released spores landing on open wounds germinate and mate with another compatible and germinated basidiospore to form heterokaryotic mycelium. The mycelium then starts to grow and spread into the woody tissue (Hamberg & Hantula, 2016). *Chondrostereum purpureum*

produces multiple compounds that break down the otherwise tough woody tissue of the tree that makes it available as nutrients for the fungus to grow and spread inside the tree. Among these compounds are enzymes known as endopolygalacturonase I (EndoPG I) that has been shown to act as a toxin and when transported via the xylem are believed to cause the very characteristic silvering of the leaves giving the disease its name (Miyairi et al., 1977; Miyairi, 1988; Reina et al., 2019; Simpson et al., 2001). This is the first visible symptom of the disease. The leaves exhibits this silvering because the toxin detaches leaf epidermis to detach from the palisade cells and thereby changes the way the light is reflected (Spiers et al., 1987). Because the toxin is transported via the xylem the observed symptoms do not necessarily indicate that the fungus is growing in that part of the tree and it can therefore be difficult to locate in some instances (Bishop, 1978).

Leaves on branches that have developed silver leaf symptoms will eventually start to develop necrosis, and dieback of affected branches will follow. When cutting an infected branch, the presence of the fungus can be seen as an irregular brown/greyish staining that goes through the infected wood (Pike, 2011). The reason for the dieback is that the fungus obstructs and seals off the xylem which consequently stops the flow of water and nutrients. Ultimately if no measures are implemented, symptoms will spread throughout the entire tree and kill it (Butler & Jones, 1949; Setliff et al., 1973; Williams & Cameron, 1956).

There are some cases where similar leaf symptoms have been observed, without finding the fungus inside the tree. This is then referred to as false silver leaf disease and is said to be due to different types of abiotic stressors such as nutrient deficiency or drought. Since it already can be difficult to find where in the tree the fungus is located the identification of false silver leaf also can be difficult to determine. Since it already can be difficult to find where in the tree the fungus is located the identification whether it is false silver leaf or not is difficult (Bintner, 1919; Brooks & Storey, 1924). The possibility of using molecular methods for detection is an option that is being studied but needs to be tested further on plum (France et al., 2016). Molecular methods can also be a costly procedure.

1.3 Silver leaf in stone fruit orchards

Silver leaf was first recorded in 1885 (Prillieux, 1885) and has since been considered a major pest in fruit orchards around the world including Norway (Bintner, 1919; Schøyen, 1946). The fungus has many different hosts where several fruit trees are known to be susceptible such as the pip-fruits apple (*Malus domestica*) and pear (*Pyrus communis*). However, stone-fruits such

as plum (*prunus domestica*), peach (*Prunus persica*), apricot (*Prunus armeniaca*), sweet and sour cherry (*Prunus avium, Prunus cerasus*) are more susceptible to the pathogen. Among the stone-fruits plum appears to be the most susceptible to the silver leaf disease (Bishop, 1978). In addition, it has been shown that C. purpureum grows about ten times faster in stone-fruits than pip-fruits (Beever, 1970).

Susceptibility of silver leaf disease has been demonstrated to differ between seasons. The trees seem to be the most susceptible in spring and early summer. Susceptibility then decreases in late summer and the beginning of fall with trees appearing to be the least susceptible in the middle of winter. This has been reported to be the same for *Prunus*, *Malus* and *Pyrus* species (Spiers et al., 1998).

In Norwegian plum orchards cultivars (cvs.) Avalon, Edda, Exalibur, Jubileum, Mallard, Opal, Reeves, Valor and Victoria are the most used in commercial production. Cvs. Jubileum, Mallard, Opal and Reeves are the ones that stand for the largest quantities and represent around 80% of the total plum production in the country (Øie, 2020).

From 2004 planting of plum trees in Norway increased noticeably. Most trees planted at this point were imported from Europe and still is the case and is now the most common when buying new plum trees. The silver leaf disease is considered one of the main diseases occurring in Norwegian plum orchards and thought to be a main contributor to the declining health in Norwegian plum orchards (Anna Birgitte Milford & Veggeland, 2021).

Assessments done in Norway on disease incidence showed that 21% of the plum orchards had at least one tree with the disease. In younger orchards that were between 1-5 years old almost all fields had silver leaf to some extent: There was however a big variation between the orchards ranging between only a few trees in a field to 40% of the trees infected in another. Some cultivars seemed to have more infections than others. Mapping of plum tree health in the eastern region Viken in Norway showed that cvs. Jubileum, Mallard, Reeves, Valor and Victoria all had silver leaf as one of the main diseases observed in the field. Cv. Opal in this survey had no reported silver leaf, while Jubileum and Reeves appeared to be the most affected (Talgø et al., 2017). It has also been observed before that cvs. Jubileum and Reeves both are some of the more susceptible cultivars that are used in Norway (Børve & Stensvand, 2018).

As part of the project “Better tree health” this thesis sets out to improve the knowledge regarding silver leaf disease in Norway. There is little research done on silver leaf under Norwegian conditions and there is therefore a need to further investigate how the pathogen

behaves in a Norwegian climate. The performed inoculation experiments in this thesis specifically investigate disease development in some of the most common plum cultivars used in Norwegian plum production. Inoculation experiments are done on trees of different age as well as side branches from different growing seasons. Differences in disease development between cultivars are studied as well as differences between the used *C. purpureum* isolates. By using isolates of isolated *C. purpureum* from different commercial fruit orchards in Norway the experiments also wish to investigate whether isolate origin and/or age has any influence when it comes to disease development.

In the present work, we study the disease development under Norwegian weather conditions during one growth season. Furthermore, we investigate factors behind the disease development. This knowledge can support the understanding of the fungus in Norwegian fruit orchards and help with the future control of silver leaf disease in the field.

2. Materials and methods

Several experiments were performed looking at different aspects of the growth and development of *Chondrostereum purpureum* in plum cvs. Edda, Jubileum, Mallard, Opal and Reeves between September 2019 and October 2020. These experiments included outdoor pot experiment and field trials at NIBIO Ullensvang (N:60.31853, E:6.65381) as well as growth chamber pot experiments performed at NIBIO Ås (N:59.66622412, E:10.774039885). All trees used in the experiments were grown on St. Julien A rootstock.

2.1 Fungal isolates used for inoculations

Five isolates of *C. purpureum* from a collection at NIBIO Ås were used for the inoculations both in 2019 and 2020 (Table 1). All isolates' identities were confirmed by sequencing of the ribosomal Internal transcribed spacer (ITS) region.

Table 1. Isolates of Chondrostereum purpureum used for inoculations in 2019 and 2020.

Isolate number	**Isolate ID[a]**	**Source material**	**Location**	***C. purpureum* isolated from**
Isolate 1	250729	Apple, cv. Santana	Rygge, Viken	Necrotic woody tissue
Isolate 2	251589	Plum, cv. Avalon	Åsgårdstrand, Viken	Fruiting bodies
Isolate 3	252329	Plum, cv. Reeves	Hole, Viken	Necrotic woody tissue
Isolate 4	252382	Plum, cv. Jubileum	Ullensvang, Vestland	Fruiting bodies
Isolate 5	252395	Plum, cv. Jubileum	Ullensvang, Vestland	Fruiting bodies

[a]ID number used by NIBIO

Isolates 1-4 were made in 2019. Isolates 4 and 5 were taken from the same tree in the same commercial fruit orchard. The difference being that isolate 4 was made in 2019 while isolate 5 was made in 2020 and thereby being newer when starting the field trials.

Table 2. Overview showing which isolates were used for inoculations of the different experiments; numbers resemble the isolate from the isolate collection (Table 1).

Experiment	Isolate(s)
Isolate difference on 1-year old side shoots	1,2,3,4,5
Different inoculum age on 1-year old side shoots	4, 5, and fruiting bodies[c]
Different inoculum age on newly developed side shoots	4, 5, and fruiting bodies[c]
Development time on 1-year old side shoots	3
Development time on newly developed side shoots	1,2,3,4
Field trial; different inoculation methods	4
Outdoor/indoor pot experiments, whips[a]	3,5
Outdoor pot experiments, graftings[b]	5

[a] Young fruit tree without side branches and not yet pruned

[b] Bench grafting that was grafted in March 2020

[c] Fruiting bodies used in field trials with different inoculum age were taken from the same tree that isolate 4 and 5 was isolated from.

2.2 Map-pin field trials with cvs. Jubileum, Reeves, and Opal

Outdoor field trials in an established field were started in May and June of 2020 at NIBIO Ullensvang and conducted throughout the summer and fall of 2020. By using map-pins, a total of 121 trees of plum cvs. Jubileum, Opal and Reeves were inoculated using *C. purpureum* from culture (Table 1). Plum trees used for the outdoor map-pin experiments were planted in 2018 and the trees were four years old when inoculated. The trees had been pruned in May to stimulate new shoot growth before the experiments were initiated. Cv. Jubileum already had new shoots at this point and was therefore not pruned. In total, five map-pin field trials were started; three were started in May and two were started in early June, all which aimed to investigate how *C. purpureum* develops over time in the selected cultivars (Table 2).

Inoculations of the trees were performed by taking map-pins and scraping mycelium from the prepared pure cultures with its needle. Selected branches were punctured with the needle and thereby applying the *C. purpureum* mycelium into the woody tissue. The map-pins were left in the wound site throughout the entire experimental period. Throughout the entire experimental period all initiated map-pin field trials were visually assessed in the field every two weeks looking for leaf symptoms on inoculated side shoots.

2.2.1 Isolate differences on 1-year old side shoots

This field trial was started 16 May 2020 and included all isolates (1-5). This experiment was initiated to examine differences between the five different isolates when inoculated in plum cvs. Jubileum, Opal and Reeves (Table 1). A total of 27 plum trees was selected for inoculation, nine trees from each of the cultivars. These were divided into three repeats with three trees of each cultivar in each repeat. The three repeats 1, 2 and 3 were cut down and assessed in August, October, and November, respectively. Map-pin inoculations were performed as described above, and the needles were placed into one-year old side shoots that had emerged in 2019.

2.2.2 Different inoculum age on 1-year old side shoots

Development of *C. purpureum* with isolates from the same location but with different age in culture was investigated in this trial. In addition, cut pieces from spore bearing *C. purpureum* fruiting bodies from the same tree as isolate 4 and 5 (Table 2). Isolates 4 and 5 originated from the same source material but the cultures differed in age (0). Inoculations started 16 May 2020 and involved an aggregate of nine trees with three trees of each cultivar. Every tree received four treatments in four different side shoots as follows: one clean map-pin as control, one map-pin with isolate 4 mycelium, one map-pin with isolate 5 mycelium, and a pinned 5mm piece of *C. purpureum* fruiting body that was cut with a scalpel. Inoculations for this field trial were set into side shoots that emerged in 2019 and were placed approximately 30 cm out from the stem. Visual assessments were carried out every second week throughout the experimental period until it was concluded in October.

2.2.3 Different inoculum age on newly developed side shoots

This field trial was built up in the same way as the previously described experiment using the same set-up with isolates 4, 5 and spore bearing fruiting bodies. The experiment started 15 June 2020 and the map-pins were placed into new branches that had started to emerge that same year (Figure 3). It was also concluded in October and visually assessed every two weeks.

Figure 3. Example picture of map-pin inoculation with a piece from a Chondrostereum purpureum *fruiting body in a shoot that started its growth in 2020.*

2.2.4 Disease development over time on 1-year old side shoots

The fourth map-pin inoculation used isolate 3 (Table 1) and a clean map-pin as control and was set to examine symptom development over time on leaves and internal wood rot in shoots that had emerged in 2019. Inoculated side shoots were removed and assessed at regular intervals after inoculation, looking for development of necrotic woody tissue inside the shoots as well as leaf symptoms. A total of 90 trees was used in this experiment including 30 trees from each of the three cultivars. Each tree had two side shoots that were used for inoculations, one with a clean control map-pin and one map-pin utilizing mycelium from isolate 3. Inoculations of all the trees were done the 16 May 2020. The first assessment was conducted in June, four weeks after inoculation and then again at four-week intervals until the last one in October 24 weeks after inoculations. For each assessment time three trees of each cultivar had their two inoculated side shoots removed adding to nine trees each time.

2.2.5 Disease development over time on newly developed side shoots

The last of the five map-pin field trials was also set up to be assessed with a time interval, to assess leaf symptoms as well as wood rot inside the side shoots. Isolates 1-4 were used in addition to the clean map-pin needle as control. Inoculations were done 12 June 2020, and the map-pins were placed into side shoots that had started growth in 2020. The first side shoot assessment was conducted four weeks after inoculation and thereafter with two-week intervals until October, 18 weeks after inoculation.

2.2.6 Sample collection and lab assessment

Collection and assessment of samples was done in the same way for all the map-pin field trials. The side shoots were first visually examined in the field looking for potential leaf symptoms. Inoculated shoots were cut at the base of that year's growth with secateurs for further visual assessment in the laboratory. The total shoot length and position of the inoculation site in relation to shoot ends was measured. The shoots were visually examined sequentially for necrotic staining in the woody tissue. To ensure that the wood rot was not overlooked but correctly measured, cutting of the shoots started at the ends where the cut surface was visually checked for wood staining. Once wood staining was found, the shoots were split using the secateurs. The shoots that were visually examined had a minimum length of 10 cm. If necrotic wood symptoms were observed the length of the staining from the needle in both directions was measured with a pair of mechanical calipers. Once the initial visual assessment had been done, re-isolation from selected symptomatic shoots were performed for confirmation of the presence of *C. purpureum*.

2.3 Isolation procedure

The described disinfection, isolation, and re-isolation procedures were performed in the same fashion for all the different experiments (Table 2). The procedures were used when identifying and confirming the presence of *C. purpureum* from collected field trial samples and was conducted as follows.

Isolation of *C. purpureum* was done by cutting infected plant material with symptoms into approximately 5 mm pieces, using secateurs. Once cut, the bark of the wood pieces was removed to lower the risk of contamination from other undesirable fungi or bacteria that could outcompete *C. purpureum.* To further ensure the growth of *C. purpureum* on the agar plates, a swift surface disinfection of the sample pieces was performed.

Disinfection of the sample pieces was carried out in a fume cupboard. The cut sample pieces were kept in a tea strainer and were first submerged into 70% ethanol for 10 seconds followed by submersion into 0.5% sodium hypochlorite for 90 seconds. The sample pieces were then transferred to sterile filter paper and carefully moved over into a laminar flow hood for drying. After about 20 minutes of drying, the sample pieces were transferred to acidified Potato Dextrose Agar plates (aPDA) in 9 cm Petri plates. The aPDA was used to further suppress unwanted bacterial growth. Five sample pieces were placed in each agar plate, making sure there was sufficient space for growth and limiting the risk of mycelium from the different pieces

to overgrow one another before transferring the isolates. aPDA plates were then sealed with parafilm and placed in a growth room with 24 hours of light and a constant room temperature of 20°C. In general, it took three to four days until the first signs of growth of *C. purpureum*. Mycelium that was identified as possibly being *C. purpureum* was then transferred to normal PDA plates in a sterile bench for further identification.

2.4 Field trials with cvs. Mallard and Edda using different inoculation methods

In fall 2019, a total of 50 cv. Mallard and 18 cv. Edda plum trees planted spring 2019 were used in an inoculation experiment using eight different methods. An aggregate of 18 trees of cv. Edda and 26 trees of cv. Mallard were used for inoculations with *C. purpureum* cultures on the 19 September 2019. The remaining 24 cv. Mallard trees were inoculated two months later, on 21 November. Isolate 4, isolated in 2019 (Table 1) was used for all inoculation methods. An overview of the inoculations is provided below (Table 3).

All plum trees included in this experiment were visually assessed in the same way throughout the entire experiment. Because the two first assessments were carried out early in the season, leaves had not yet developed at the time of the first two assessments on 31 March 2020 and 21 April 2020. Assessments at these two occasions only involved recording the health of the tree, checking the inoculation point and evaluating leaf development using the BBCH-scale for stone-fruit (Meier et al., 1994). Later assessments were done every second week for the rest of the experimental period taking notes of silver leaf symptom development and overall tree health. Final assessments were done at different times due to the disease development for the different inoculation methods. Methods for the final registration also varied due to the different inoculation methods.

Table 3. Overview of the eight different methods used for inoculations with Chondrostereum purpureum in 2019 *on cvs. Edda and Mallard plum trees planted in spring 2019*[a].

Inoculation Method	Cultivar	Number of inoculated trees	Number of uninoculated control trees	Inoculation point	Inoculation date	End date experiment
Inoc.1: Mycelium covered with moist cotton placed onto wound on main stem	Edda	4	4	Main stem, ~40cm above ground	19.09.2019	07.08.2020
Inoc.2: Mycelium with agar placed onto wound on main stem	Edda	5	5	Main stem, ~40cm above ground	19.09.2019	07.08.2020
Inoc.3: Mycelium with agar placed onto cut wounds made on main stem bark	Mallard	4	4	Main stem, ~40cm above ground	19.09.2019	07.10.2020
Inoc.4: Mycelium covered up with moist cotton placed onto three newly pruned side shoots	Mallard	3	3	Straight cuts on side shoots from 2018, pruned ~10cm from main stem	19.09.2019	07.09.2020
Inoc.5: Mycelium with agar placed onto three newly pruned side shoots	Mallard	3	3	Straight cuts on side shoots from 2018, ~10cm from main stem	19.09.2019	19.08.2020
Inoc.6: Mycelium with agar placed onto two-centimeter wounds on three side shoots	Mallard	3	3	Side shoots from 2019, bark wound ~10cm from main stem	19.09.2019	26.09.2020
Inoc.7: Mycelium with agar placed onto a wound made on main stem	Mallard	8	4	Main stem, ~40cm above ground	21.11.2019	21.10.2020-23.10.2020
Inoc.8: Mycelium with agar placed onto three newly pruned side shoots	Mallard	8	4	Straight cuts on side shoot from 2018, ~10cm from main stem	21.11.2019	21.10.2020-23.10.2020

[a]In total 9 cv. Edda and 29 cv. Mallard were inoculated in the experiment, another 9 cv. Edda and 21 cv. Mallard were used as control trees were used in the field trial.

2.4.1 Cv. Edda inoculated in September 2019

For the cv. Edda trees two different inoculation methods with isolate 4 were used (Table 3). Four trees were inoculated by scraping mycelium with a needle from the PDA containing the isolate. The mycelium was then placed with the needle directly onto a ~4 cm wound made with a scalpel around 40 cm above the ground on the mainstem. A damp piece of cotton was placed on top of the applied mycelium and finally it was secured and sealed with parafilm for two days.

The second method was performed on five cv. Edda trees. Agar pieces were stenciled out with a corkborer. The agar pieces with mycelium of *C. purpureum* were then taken out with a needle placed on top of a ~ 4 cm wound made with a scalpel on the mainstem. The agar with mycelium was then sealed with parafilm to ensure inoculation. In addition, four trees with only a damp cotton piece as well as five trees using clean agar pieces were used as control. The parafilm was removed after two days for both methods.

Final assessment was done 7 August 2020. All the inoculated trees were cut at the basis and brought indoors for examination. One control tree for each of the used methods was also collected. A visual examination of the tree was done assessing total number of side shoots, number of side shoots with silver leaf symptoms and number of dead/healthy side shoots. In addition, the presence and length of potential necrotic woody tissue symptoms inside the mainstem and branches was recorded and measured. The wood rot was measured both directions in relation to the inoculation point. The trees were systematically cut from the ends of the trees and then inwards towards the inoculation point to make sure the actual length of the wood staining was not missed during the visual assessment. Once wood rot was found, the tree was split in the middle to be able to measure its length. If no staining was found a 10 cm piece around the inoculation point was still split and visually examined.

2.4.2 Cv. Mallard inoculated in September 2019

The experiment was started 19 September 2020, inoculating 13 cv. Mallard trees. A total of four different methods was utilized for inoculations of different plant parts and age in this experiment (Table 3). Two of the four methods were identical to the previously mentioned methods for cv. Edda, either applying only mycelium covered with a moist cotton piece or a stenciled agar piece with mycelium placed onto wounds. But instead of inoculating only one wound on the mainstem, a total of three branches from 2018 were cut with secateurs about 10 cm out from the mainstem. Inoculations were then placed onto the straight cut on the branches. Three trees for each of these two treatments were used for inoculations.

The third method was inoculation with mycelium agar pieces placed onto ~4 cm cuts made with a scalpel in the mainstem bark. Cuts were made approximately 40 cm above the ground.

In the fourth inoculation method, two-centimeter-long wounds were made with a scalpel on three branches from the 2019 growing season. Mycelium agar pieces were then placed onto the wounds and sealed with parafilm as described earlier. For all the methods the parafilm seal was removed after two days.

All in all, three control and three inoculated trees were used for each branch inoculation method, while a total of four control and four inoculated trees were used for the mainstem inoculation method. Final assessments for these inoculation methods were done on three different dates, 26 August 2020, 7 September 2020, and 7 October 2020. However, the final assessments were performed in a similar manner. As described for cv. Edda, an initial visual examination was made for all cv. Mallard trees. Furthermore, tree height, branch position and the presence of necrotic woody tissue symptoms recorded. Methods using three branches as inoculation points had a few additional assessments. Length of inoculated branches was measured as well as presence and length of necrotic woody tissue symptoms. Finally, samples from different parts of the trees were selected for DNA extraction and subsequently real-time PCR analysis.

2.4.3 Cv. Mallard inoculated in November 2019

A total of 16 cv. Mallard trees were inoculated 21 November 2019 with two different inoculation methods (Table 3). One method was wounding of the main stem with a knife approximately 40 cm from the ground placing mycelium with agar onto the ~4 cm wound that had been opened in the bark. The second method also used mycelium with agar, but they were placed onto straight cuts made from pruning of three side shoots from 2018. For each method eight cv. Mallard trees were used for inoculations and four trees were kept as uninoculated controls.

Visual assessments throughout the experimental period were carried out at the same time and in the same way as the previously described inoculation methods (2.4.1 and 2.4.2).

The experiment was concluded 23 October 2020. Trees were cut down for assessment and further assessed in the laboratory as described for the inoculations done for cv. Mallard inoculated in September 2019 (2.4.2).

2.5 Pot experiments with whips of cvs. Jubileum and Reeves

Pot experiments using whips inoculated via map-pins with *C. purpureum* mycelium on cvs. Jubileum and Reeves were performed outdoors at NIBIO, Ullensvang and indoors in growth chambers at NIBIO Ås from April to August 2020. The whips were grafted in 2019 and reared in pots in a high tunnel and moved out 21 April 2020. Both outdoor and indoor inoculations were performed with the map-pin method as in the previously described field trials. Two needles with mycelium were placed in the main stem of the tree: one approximately 10 cm from the top and the other about 30 cm above the soil surface of the pot. Isolates 3 and 5 were used for the inoculations (Table 1).

2.5.1 Outdoor pot experiment with whips of cvs. Jubileum and Reeves

For the outdoors experiment a total of 20 whips, ten of each of cvs. Jubileum and Reeves were inoculated 17 April 2020. Half of the field trial was cut down after two months in June, cutting down five trees of each cultivar for assessment. Five cv. Reeves on 17 June and five cv. Jubileum on 25 June. The remaining ten trees were cut down and assessed 10 August 2020. Throughout the experiment, the development of leaf symptoms was recorded every second week until the final assessment.

Final assessments were done by cutting down the trees at the base near the soil surface and bringing them indoors to the lab. Leaf symptoms and length of necrotic wood staining in relation to the inoculation point was registered. Pots of the trees cut down in June were left outdoors with their stumps and were checked for possible development of fruiting bodies of *C. purpureum* until the final assessment in August. Additionally, fruiting bodies and wood samples were collected from the final assessments for isolation and real-time PCR analysis.

2.5.2 Pot experiment with whips of cvs. Jubileum and Reeves in growth chamber

Another five cv. Jubileum and five cv. Reeves whips that also reared at NIBIO Ullensvang were sent to be used for the map-pin inoculations indoors in a growth chamber at NIBIO Ås. The growing conditions in the growth chamber were 16 hours/8 hours day/night and a constant temperature of 16°C. The experiment started 17 April 2020 and ended one month later 22 May. The same assessment and sample collecting method was used as described for the outdoors experiment.

2.6 Pot experiments with graftings of cvs. Jubileum, Mallard and Reeves

On 3 March 2020, a total of 99 healthy graftings of cvs. Jubileum, Mallard and Reeves were grafted and propagated in pots inside a high tunnel at NIBIO Ullensvang. The 99 graftings were used for an outdoor pot experiment with mycelium of *C. purpureum* using three different inoculation methods.

2.6.1 Graftings using two different inoculation methods

The 99 graftings kept at NIBIO Ullensvang were used for an experiment using the following inoculation methods: (i) agar piece with mycelium placed onto a wound made on the scion and (ii) agar piece with mycelium placed onto a wound made on the rootstock. Out of the 99 graftings 31 were of cv. Jubileum, 33 of cv. Mallard, and 35 of cv. Reeves. Before inoculations, all shoots growing on the rootstocks were removed. Isolate 5 was used for all three inoculation methods (Table 1). Inoculations were initially started inside the high tunnel 17 April 2020, but the grafting pots were moved outside 26 May 2020 where they remained until the end of the experiment.

The inoculation methods for both the rootstock and scion were performed in the same way. By wounding the bark approximately in the middle of the rootstock or scion main stem using a scalpel, an open wound about 2 cm wide was created for inoculation. A corkborer with a diameter of 5 mm was used to stencil pieces of agar with mycelium from the agar plates. These were placed onto the wound, and thereafter sealed with parafilm to ensure that the mycelium stuck to the grafting. The parafilm was removed from the graftings after two days.

For inoculations of the scion 14 trees of cv. Jubileum and 15 each of cvs. Mallard and Reeves were used while for inoculations of the rootstock 14 trees were used of cv. Jubileum, 13 of cv. Mallard and 15 of cv. Reeves. Additionally, five trees of cvs. Mallard and Reeves and three of cv. Jubileum were used as controls. Controls for the three inoculation methods included clean map-pin needles and clean agar pieces that were placed onto the wounds.

Once inoculations were made, the disease development was followed up every two weeks looking for silver leaf symptoms, measuring of shoot length and checking if the scion was alive until the final assessments. Graftings with silver leaf symptoms appearing in June-July were finally assessed between 2 and 16 July 2020. The remaining trees were assessed the following month 14 and 17 August 2020.

Final assessments of the graftings were performed by cutting them just above the soil and visually examining them indoors. Health of the scion (alive/dead), leaf symptoms on shoots on

both rootstock and scion, as well as any necrotic staining inside the rootstock or scion was recorded. For graftings inoculated with map-pins spread of the necrotic staining in both directions from where the needles were placed was measured with mechanical calipers. Pots of trees cut down and assessed in July were left outdoors and rootstocks were checked for development of fruiting bodies until the last of the trees were assessed in August.

2.7 DNA extraction

Extraction of genomic DNA for real-time PCR analysis was performed on selected samples from the performed experiments. DNA extractions from 229 collected samples were made using the DNeasy Plant Mini Kit (QIAGEN) utilizing the manufacturers extraction protocol. The samples were taken from trees in the pot experiments involving whips, graftings, and the field trial inoculated in 2019 with different inoculation methods. In addition, leaf samples were taken from trees with and without silver leaf symptoms (Chapters 2.4, 2.5, 2.6).

Selected sample pieces were first cut into smaller pieces with secateurs and subsequently ground into a fine powder using liquid nitrogen and a mortar before starting the DNA extraction procedure. The DNA extraction protocol was followed with an exception to centrifuging that was done at 13,000rpm instead of 14,000rpm. The extracted DNA product (200µl kept in 1.5ml microtubes) was then stored in a freezer at -20°C.

2.8 Real-time PCR procedure for *Chondrostereum purpureum*

For the detection of *C. purpureum*, a TaqMan based assay developed at NIBIO was used (Brurberg MB, unpublished results). Real time PCR was performed using a CFX96™ Real-Time Detection System from Bio-Rad and Bio-Rad CFX Maestro 1.1 software for collection and analysis of data. The components and volumes used for the master mix when quantifying *C. purpureum* from samples is described. Both probe and the primers were custom made by Thermo-Fischer.

For each reaction well 2 µl of extracted DNA and 18 µl of real-time PCR reaction mix was pipetted adding to a total of 20 µl per reaction well. The real-time PCR reaction mix used for 1× DNA sample was composed as follows; 10 µl SsoAdvanced Universal Probes Supermix (Bio-Rad), 1 µl of both primers Cpur3-Forward/Reverse 10 pmol/µl, 0.8 µl Cpur3-Probe 5pmol/µl and 5.2 µl distilled water adding to a total of 18 µl. The reaction mix was prepared in a 2ml microtube and then kept on ice.

An 18S ribosomal RNA (rRNA) internal control was used as an amplification control to counter false negatives in the real-time PCR detection. Also, for the 18S rRNA internal control 18 µl of

reaction mix was used as well as 2 µl of extracted DNA for each reaction well. The 18S rRNA real-time PCR reaction mix contained 10 µl SsoAdvanced Universal Probes Supermix (Bio-Rad), 0.48 µl of 18S-forward primer: GACTACGTCCCTGCCCTTTG-10 pmol/µl, 0.48 µl of 18S-Reverse primer: AACACTTCACCGGACCATTCA, 10 pmol/µl, 0.64 µl 18S-Probe: 6FAM-ACA CAC CGC CCG TCG CTC -Tamra, 5 pmol/µl and 6.4 µl distilled water with a total of 18 µl.

To be able to easily present the C^t-values from the real-time PCR analysis a "*Chondrostereum purpureum* score" (C.p-score) from 0 to 6 resembling the number of cycles the reaction took to reach the threshold was created. Each number of the scale represents an interval of cycles used for the given sample in the real-time PCR analysis. In the scale 0 equals >40 cycles and no *C. purpureum* DNA detected and 6 equaling 17-19.9 cycles which resembles the highest amount of DNA present (Table 4).

Table 4. C^t-value scale and the given intervals for the C.p-score ranging from 0-6 used for presenting real-time PCR analysis results.

C^t-value	**<17-19.9**	**20-22.9**	**23-25.9**	**26-28.9**	**29-31.9**	**32-34.9**	**35-40**
C.p-score	6	5	4	3	2	1	0

2.9 Statistical analysis

Statistical analysis was performed by the GLM procedure of SAS (SAS Institute, Cary, NC, USA). All incidence data was arcsine-square root transformed prior to analysis. Only non-transformed means are presented. Mean values were separated by Student Newman-Keuls method (SNK) at $\alpha = 0.05$. Data from each experiment was analyzed separately and partially as pooled data.

3. Results

3.1 Map-pin field trials with cvs. Jubileum, Reeves, and Opal

Five field trials, using map-pin inoculations were tested in 1-year old and newly developed side shoots. The different experiments used isolates with different origin and age (Table 1 and Table 2). In addition, the development at different times after inoculation was assessed. The map-pin field trial using different inoculum age and fruiting bodies in newly developed side shoots had the only incidence of silvering leaf symptoms and was found on a cv. Reeves tree on a side shoot inoculated with isolate 5.

3.1.1 Isolate differences on 1-year old side shoots

There were no significant differences in wood discoloration between the different isolates, or cultivars in mean of three assessment times (Table 5). For cv. Reeves there were two shoots, assessed on the 12 August 2020 and 28 October 2020 inoculated with isolate 3, that were considerably longer, 283 mm and 170 mm, respectively. These two recordings increased the average for cv. Reeves (Table 5)

Table 5. Average wood rot length (mm) in last year's growth inoculated with Chondrostereum purpureum *by map-pins with different isolates in May 2020.*[a]

	Jubileum	**Opal**	**Reeves**	**P-value**
Isolate 1	5.0	4.3	5.4	0.989
Isolate 2	4.9	8.4	8.7	0.125
Isolate 3	2.9	9.6	58.3	0.102
Isolate 4	6.2	14.6	16.6	0.456
Isolate 5	5.2	6.0	5.0	0.905
Control	3.6	7.9	5.8	0.605

[a]Mean of three different assessment times (3, 5 and 6 months after inoculation) and three replicates (trees) each time.

3.1.2 Different inoculum age on 1-year old side shoots

In mean of cultivar, there was no difference in wood rot between the inoculum types. For each of the cultivars, there was a significant difference for cv. Jubileum (P = 0.028), but not for cvs. Opal and Reeves. When comparing inoculum type, there was significantly longer rot when using fruiting bodies as inoculum sources on cv. Opal than on cvs. Reeves and Jubileum (P = 0.023). For isolate 5 made in 2020, there was a significantly longer wood rot on cv. Reeves than on cvs. Jubileum and Opal (P = 0.008). There was no difference among the cultivars when comparing non-inoculated controls and when inoculated with isolate 4 from 2019 (Table 6).

Table 6. Average wood rot length (mm) in 1-year old wood inoculated May 2020 with Chondrostereum purpureum *mycelium from isolates with different ages by map-pins or with fruiting bodies.*

	Jubileum[a]	**Opal**	**Reeves**	**P-value**
Isolate 4	4.2	22.3	21.6	0.555
Isolate 5	6.3	8.0	21.3	0.008
Fruiting bodies	3.8	15.5	5.0	0.023
Control	1.7	6.2	7.0	0.379
P-value	0.044	0.441	0.251	

[a]Mean values of three replicates five months after inoculation.

When looking into the treatments for each cultivar separately, only isolate 5 within cv. Jubileum was significantly different from the control treatment (P=0.044). There were no significant differences among any of the inoculation methods within cv. Jubileum. Within cvs. Opal and Reeves there was no significant differences between any of the inoculations nor did the un-inoculated controls.

No growth of *C. purpureum* was obtained after isolation of samples with wood rot on.

3.1.3 Different inoculum age on newly developed side shoots

In mean of three cultivars inoculation by isolate 4 from 2019 resulted in longer wood rot after four months (P = 0.039) than the non-inoculated control. There was no difference between control and inoculation with a piece of fruiting body or isolate 5 from 2020 (Figure 4).

Figure 4. Average wood rot length (mm) per inoculum four months after inoculations with Chondrostereum purpureum mycelium using map-pins on newly developed side shoots. *Mean of cvs. Jubileum, Opal and Reeves combined and consists of three repeats each adding up to a total of nine trees per inoculation. Different lower-case letters indicate significant differences between means according to student Newman-Keuls test at P ≤ 0.05.*

Isolate 4 in this map-pin experiment had one inoculated shoot that displayed silver leaf disease symptoms on 2 October 2020. This was the only shoot that developed any leaf symptoms from the map-pin inoculations on trees planted in 2018 (Figure 5).

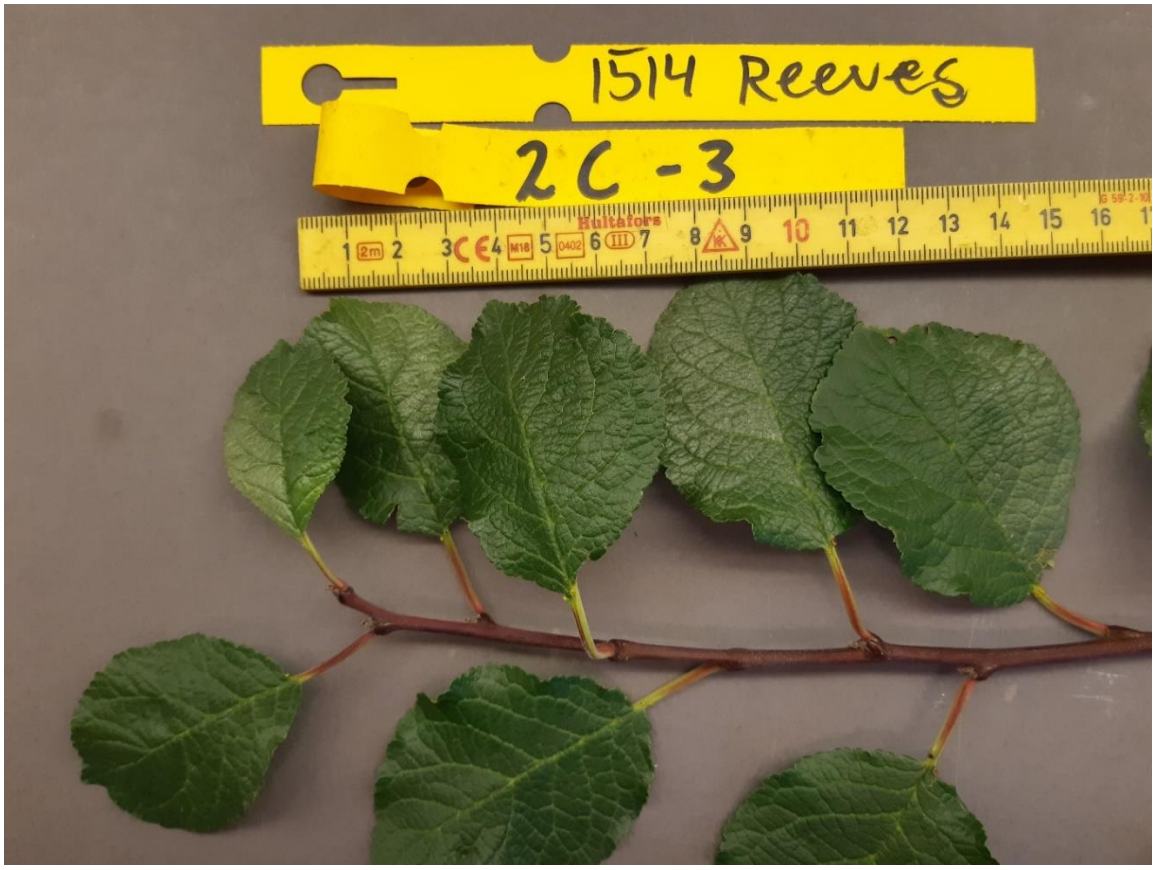

Figure 5. Side shoot developed in 2020 on cv. Reeves tree that exhibited silver leaf symptoms after map-pin inoculation with mycelium of Chondrostereum purpureum *isolate 5. Symptoms can be seen as sections of silvering on the leaves and were present on the entire shoot in October one week before the final assessment of the experiment.*

3.1.4 Disease development over time on 1-year old side shoots

For the map-pin experiment investigating disease development on 1-year old side shoots with assessments every four weeks, there was no silver leaf symptom development throughout the course of the experiment. In mean of treatments for inoculations of the three cultivars showed no significant difference. There were also no significant differences in wood rot length among any of the shoots inoculated with isolate 3 within the cultivars for any of the six assessment weeks. Only the control treatment on cv. Opal, assessment week 12 showed a significant difference in wood rot length compared the other assessment weeks 4, 8, 16, 20 and 24 ($P = 0.010$). Control shoots for week 12 on cv. Opal had a mean of 17mm being almost 10mm longer than the second longest wood rot mean (assessment week 16) for the control treatment within cv. Opal. For cv. Reeves, assessment week 20 of the control treatment had the longest wood rot

length and was significantly longer compared to the control on assessment weeks 4 and 24 (P = 0.020). Weeks 8, 12 and 16 average wood rot were not significantly shorter than week 20.

3.1.5 Disease development over time on newly developed side shoots

Investigating the wood rot length, the combined mean per isolate showed that none of the isolates were significantly different; however, control was significantly shorter than all the used isolates (P = 0.006) (Figure 6).

Figure 6. Mean wood rot (mm) in newly developed side shoots after map-pin inoculations with Chondrostereum purpureum *isolates 1-4 (Table 1) as well as a clean map-pin used for control. Mean of three cultivars and the eight assessment times every 2 weeks over 18 weeks with three trees per replicate. Lower-case letters that are the same indicate that there is no significant difference between the means according to student Newman-Keuls test at at $P \leq 0.05$.*

Comparison between the cultivars shows that cv. Jubileum had the highest mean of 25.8 mm and was significantly different to both cvs. Opal and Reeves (P = 0.014). There was no significant difference between cv. Opal and Reeves (Figure 7).

Re-isolations conducted at each assessment week after the measuring of wood rot resulted in 16 out of 96 of the shoot samples from the inoculated shoots being identified as *C. purpureum*.

Many inoculated side shoots had water that had entered via the map-pin and had bacterial infections (Figure 8).

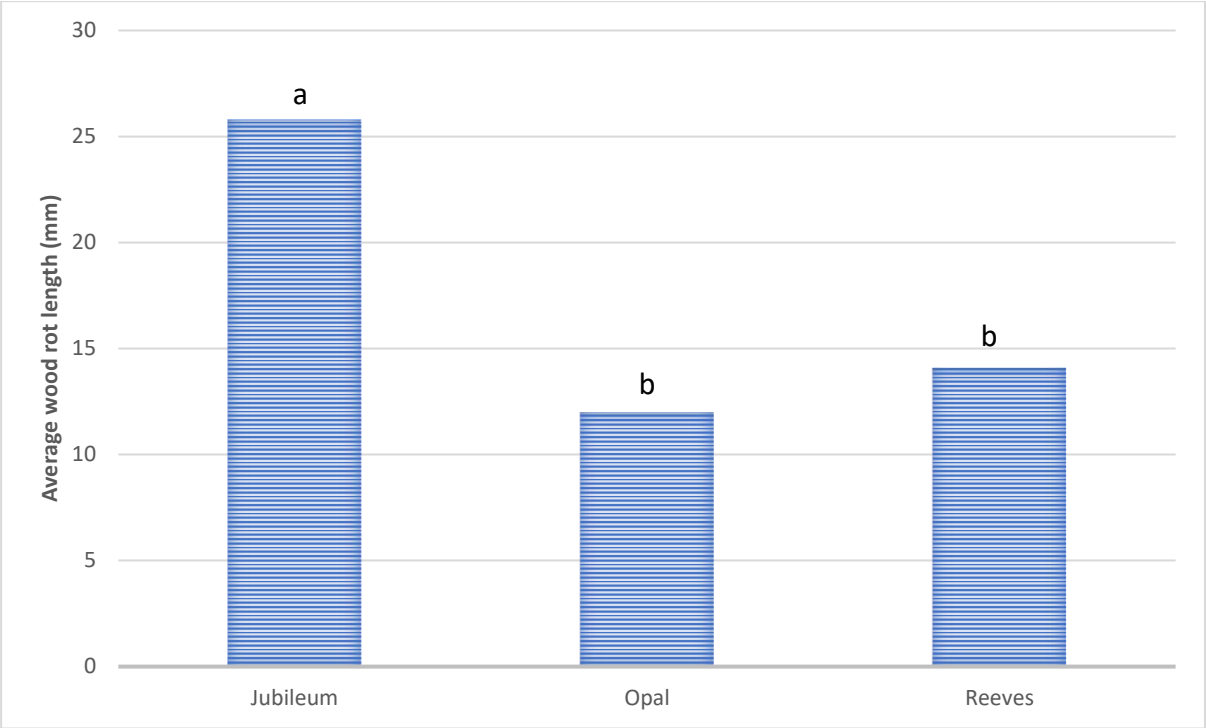

Figure 7. Average wood rot (mm) after inoculation by map-pins with different Chondrostereum purpureum *inoculum from the eight different assessments done until 18 weeks after inoculations. Mean of all isolates (isolates 4, 5 and fruiting bodies) used on the three cvs. Jubileum, Opal and Reeves. Different lower-case letters indicate significant differences between the means according to student Newman-Keuls test at* $P \leq 0.05$.

Figure 8. Newly developed side shoot cleaved and assessed twelve weeks after inoculation with map-pin in spring 2020. Puncture wound with water and bacterial infection. Photo: M. Wennerberg

3.2 Field trials using different inoculation methods with cv. Mallard and Edda

For the experiment with eight different inoculation methods on a total of 38 cvs. Edda and Mallard trees in 2019, 21 of the trees showed silver leaf symptoms. The 21 trees that developed symptoms occurred in methods 1-7. Silvering leaf symptoms did not develop on trees inoculated with method 8 (Table 7) where mycelium with agar was placed onto newly pruned side shoots.

Five out of the seven inoculation methods that displayed silver leaf symptoms had at least one symptomatic tree on 15 May 2020, i.e., inoculation methods 1, 3, 4, 5 and 6. Inoculation methods 2 and 7 showed their first leaf symptoms two weeks later 28 May. Inoculation method 2 on cv. Edda inoculated 19 September 2019 took the longest before showing the first silver leaf symptoms, which was after 252 days. Inoculation method 7 on cv. Mallard inoculated 21 November 2019 had the shortest time between inoculation and first symptoms appearing, with 189 days. Inoculation methods 1, 3, 4, 5 and 6 inoculated 19 September 2019 all took 239 days before first leaf symptoms appeared (Table 7).

All cv. Mallard trees inoculated 19 September 2019 with methods 4, 5 and 6 had leaf silvering already fully developed on the fourth field assessment 28 May 2020 (Table 7, Figure 9).

Table 7. Silver leaf development for the field trial with cvs. Edda and Mallard using different inoculation methods (Table 3) with Chondrostereum purpureum *isolate 4 (Table 1).*

			Silver leaf development			Recovery of *C. purpureum* after isolation or real-time PCR	
Inoculation method No.[a] **(**Table 3**)**	Cultivar	No. of inoculated trees	Incidence (%)	First recorded leaf symptom (date)	Days between inoculation and symptoms	Mainstem	Branches
Inoc.1	Edda	4	75	15.05.2020	239	+[b]	+[b]
Inoc.2	Edda	5	80	28.05.2020	252	+[b]	+[b]
Inoc.3	Mallard	4	75	15.05.2020	239	+[c]	+[c]
Inoc.4	Mallard	3	100	15.05.2020	239	+[c]	+[c]
Inoc.5	Mallard	3	100	15.05.2020	239	+[c]	+[c]
Inoc.6	Mallard	3	100	15.05.2020	239	+[c]	+[c]
Inoc.7	Mallard	8	25	28.05.2020	189	+[c]	+[c]
Inoc.8	Mallard	8	0	N/A	N/A	-[c]	+[c]

[a]Table 3 for fully explained inoculation method.

[b]Confirmation of the presence (+) or absence (-) of *Chondrostereum purpureum* with isolation on agar

[c]Confirmation of the presence (+) or absence (-) of *Chondrostereum purpureum* with real-time PCR

Both inoculation method 7 and 8 (Table 3) performed on cv. Mallard inoculated 21 November 2019 had the least incidence of foliar symptoms of all the methods. The first tree with silver leaf symptoms for inoculation method 7 was observed 28 May 2020, and the second was observed at the last assessment two months later 20 July. Method 8 did not develop any symptoms throughout the entire experiment (Table 7, Figure 9).

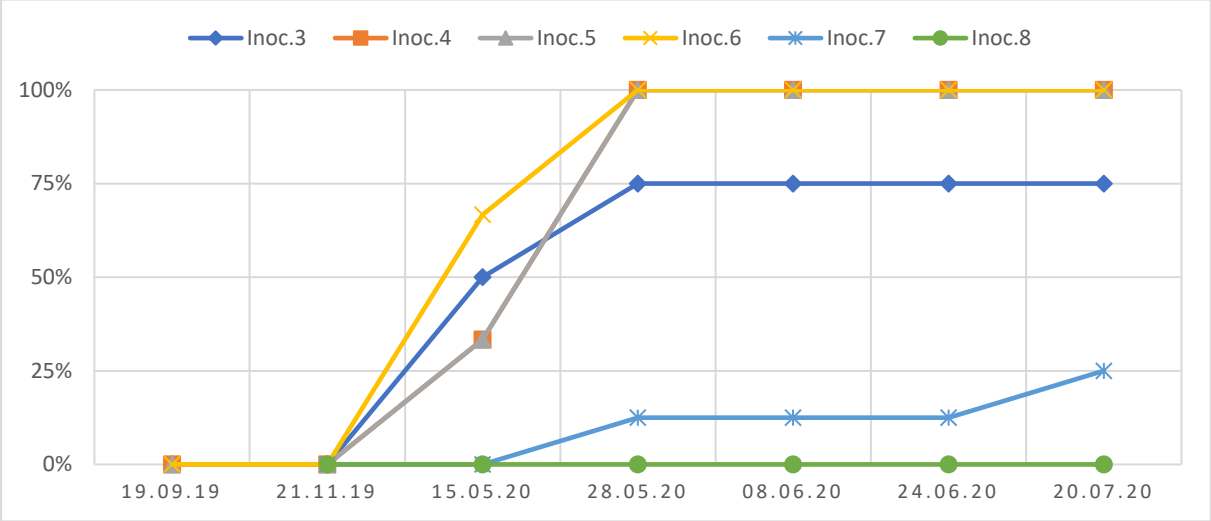

Figure 9. Development over time of silver leaf symptom incidence shown in percentage on cv. Mallard for the field trial inoculated in 2019 using different inoculation methods with Chondrostereum purpureum. *Inoculation methods 3, 4, 5 and 6 were used 19 September 2019 and methods 7 and 8 21 November 2019. Leaf symptoms were assessed at the given dates.*

Despite no visible silver leaf symptoms for method 8, real-time PCR analysis found that *C. purpureum* was present near the inoculation point of the trees and had a higher C.p-score than method 7 that showed symptoms. Inoculation methods 4, 5 and 6 had the highest average C.p-score and were all inoculated on side shoots (Figure 10).

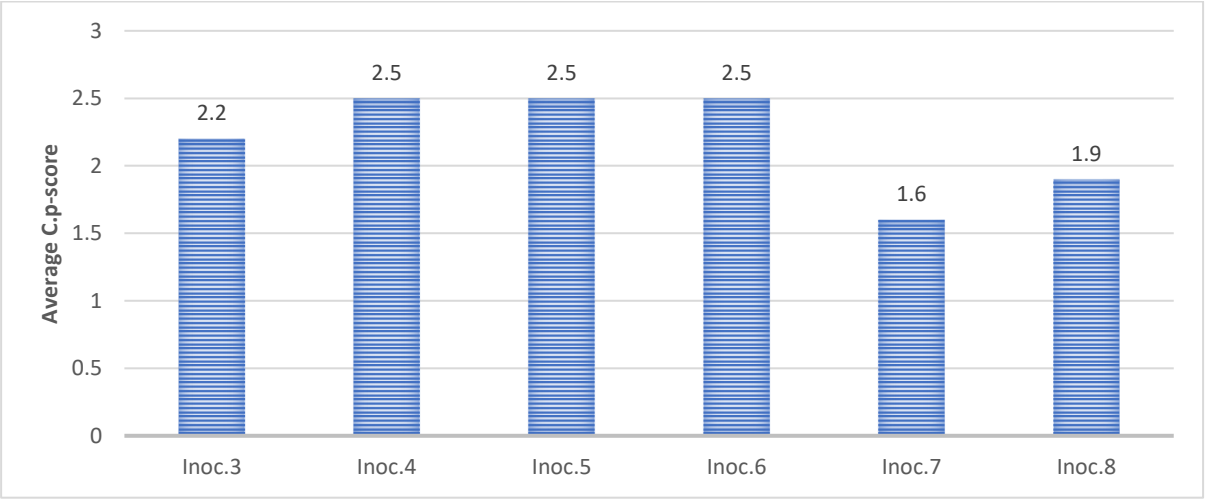

Figure 10. Average C.p-score (Table 4) after real-time PCR analysis of field trials with different inoculation methods (inoculation methods 3-8 (Table 3)) using Chondrostereum purpureum *isolate 4 on cv. Mallard. Values are only from samples taken around the inoculation point(s) of the tree and there are six samples behind each column from trees that showed silver leaf symptoms except for inoc.8 that had no symptoms.*

Real-time PCR analysis of tissue from inoculation points of symptomless trees of inoculation method 7 and 8 had similar C.p-score (Table 3). However, the average C.p-scores from those were lower than for trees exhibiting silver leaf symptoms (Table 8).

Table 8. Average C.p-score (Table 4) for symptomless trees and trees with symptoms on cv. Mallard trees inoculated in 2019 with different inoculation methods (Table 3) using Chondrostereum purpureum isolate 4 (*Table 1*). *C.p-scores come from values for inoculation point(s) for the given inoculation method 3-8.*

Inoculation method	**Method 3**	**Method 4**	**Method 5**	**Method 6**	**Method 7**	**Method 8**
C.p-score symptomless	0	N/A[a]	N/A[a]	N/A[a]	1.3	1.9
C.p-score w/ symptom	2.2	2.5	2.5	2.5	1.6	N/A[b]

[a]All trees showed silver leaf symptoms.

[b]None of the trees showed silver leaf symptoms.

For cv. Edda inoculated 19 September 2020, trees from inoculation method 2 showed symptoms when assessed 15 May the year after while inoculation method 1 had its first tree with silver leaf symptoms 28 May. Both inoculation methods for cv. Edda had developed symptoms on all but one tree on the next assessment carried out 8 June. The number of trees with symptoms was then unchanged until the experiment was concluded (Figure 11). One of the trees inoculated using method 1 was dead by 8 June but had not developed any leaf symptoms prior to that. The first tree with silver leaf symptoms (inoculation method 2) showed wilting signs 24 June and was dead 20 July. Several inoculated trees of cv. Edda were weaker in growth than the other used cv. Mallard. Whether this was because of the fungus or just weak growth after planting could not be determine.

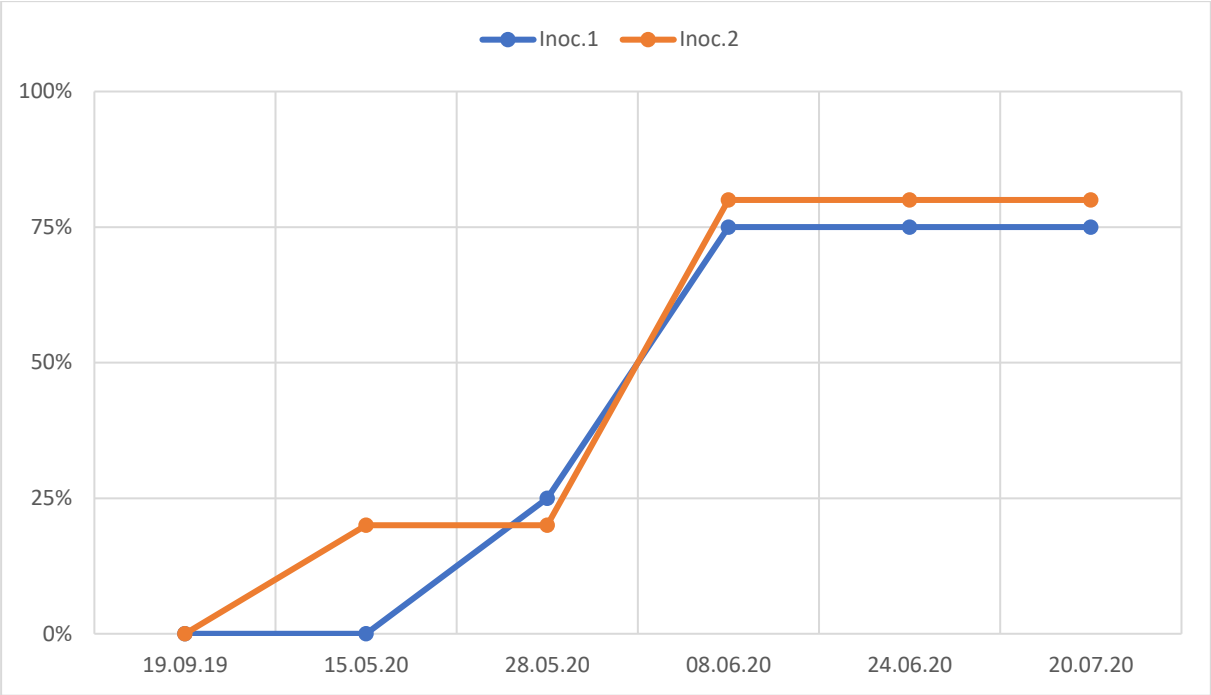

Figure 11. Mean incidence (%) of trees with silver leaf symptoms in spring 2020 on cv. Edda inoculated 19 September 2020 with different inoculation methods (methods 1 and 2) using Chondrostereum purpureum *isolate 4. Leaf symptoms were assessed at the given dates.*

3.3 Pot experiment with whips of cvs. Jubileum and Reeves

Pot experiments, both outdoors and in growth chambers were conducted using the map-pin inoculation method and isolates 3 and 5 (Section 2.5). Both experiments resulted in trees with silver leaf symptoms and were confirmed as *C. purpureum* via real-time PCR analysis or isolation on agar. Development was faster in the growth chamber compared to the outdoor pot experiment.

3.3.1 Outdoor pot experiment with whips of cvs. Jubileum and Reeves

Outdoor pot experiment with whips inoculated with map-pins had silver leaf disease development in both cvs. Jubileum and Reeves after the assessment 10 August 2020. Silver leaf symptoms were observed on four trees, three of cv. Jubileum and one of cv. Reeves. *Chondrostereum purpureum* was found in another two cv. Reeves trees after isolation of wood samples from the inoculation point but these did not display any leaf symptoms. Only trees inoculated with isolate 5 displayed silver leaf symptoms. This was also the only isolate that was retrieved from isolation on agar (Table 9). Two cv. Jubileum and one cv. Reeves out of the four trees in total that displayed silver leaf symptoms were symptomatic 1 June 2020, 45 days after inoculation. The fourth and last of the trees to display symptoms was of cv. Jubileum another 26 days after the first trees exhibited silver leaf symptoms.

Table 9. Overview of the outdoor pot experiment with whips of cvs. Jubileum and Reeves inoculated with *Chondrostereum purpureum* via map-pins performed at NIBIO Ullensvang.

Cultivar	**Isolate (**Table 1**)**	**Total no. of trees**	**Number of trees with leaf symptoms**	**Necrotic woody tissue[a]**	**Confirmed *C. purpureum* after isolation**
Jubileum	Isolate 5	4	3	2	3
Jubileum	Isolate 3	4	0	0	0
Reeves	Isolate 5	4	1	0	3
Reeves	Isolate 3	4	0	2	0
Jubileum	Control	2	0	0	0
Reeves	Control	2	0	0	0

[a]Staining observed inside the woody tissue of the whip

One tree from each cultivar that showed silver leaf symptoms had wood staining that seemed to come out of the rootstock and not the inoculation points. The section was pronounced in both cultivars but was somewhat more severe in cv. Reeves. The wood staining was the strongest close to the base of the rootstock and faded, the further away from the base of the tree it was. This fading was similar for both cultivars and could not be observed around the top inoculation made in the scion (Figure 12, Figure 13).

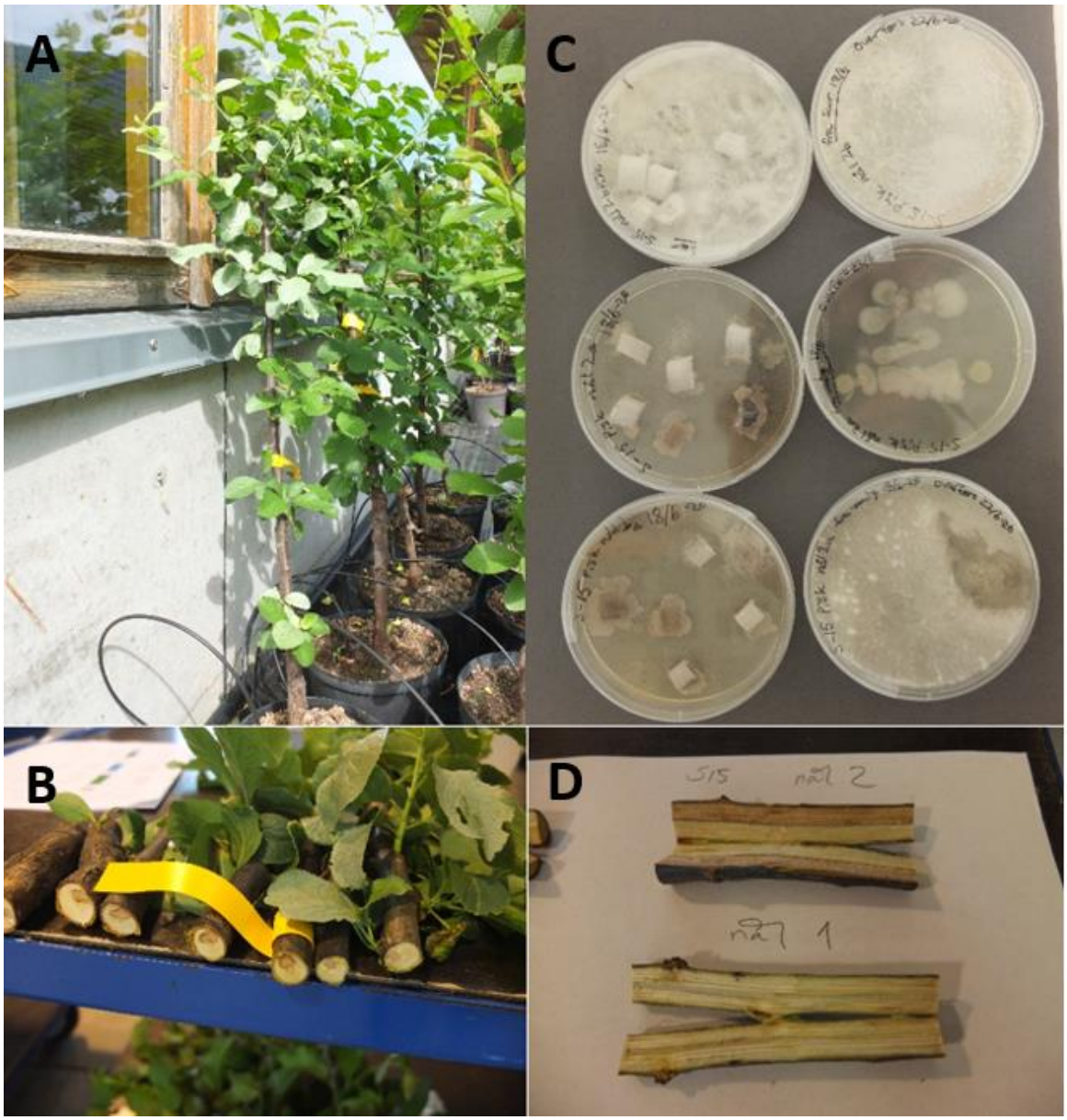

Figure 12. Cv. Reeves whip tree map-pin inoculated with Chondrostereum purpureum *that displayed silver leaf symptoms after inoculation in spring 2020 and had a section of wood staining going through the wood originating from the rootstock.* ***A:*** *Silver leaf symptoms on cv Reeves whip tree 17 June 2020.* ***B:*** *Wood staining seen after systematically cutting through cv. Reeves tree for measuring the length of the wood staining.* ***C:*** *Fungal growth after isolation of wood samples onto PDA after the initial assessment.* ***D:*** *Cleaved sections of the two map-pin inoculation points where the section with symptomatic woody tissue can be observed around the point of the lower needle ("nål 2") while the upper needle ("nål 1") has no staining from the inoculation. Photo: M. Wennerberg*

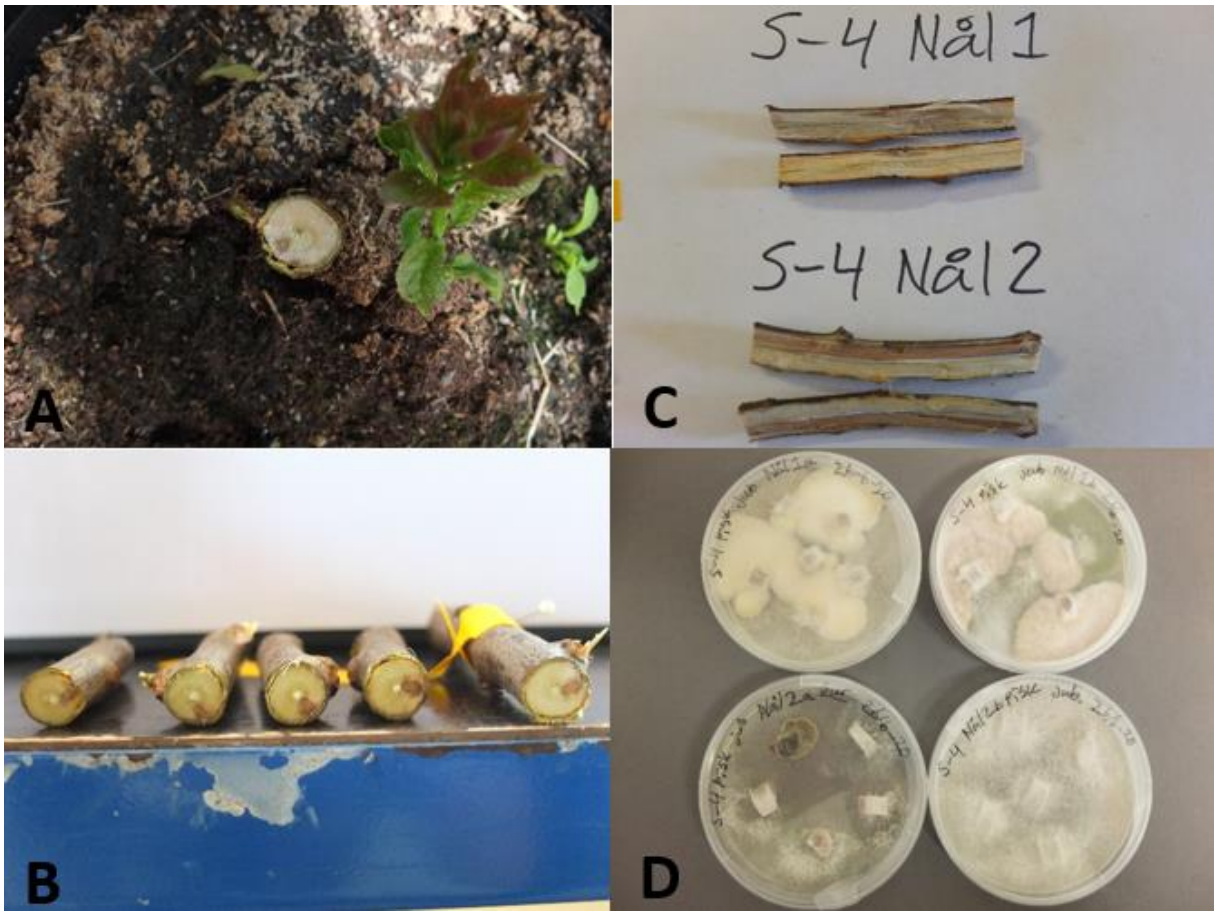

Figure 13. Cv. Jubileum whip tree map-pin inoculated with Chondrostereum purpureum *that displayed silver leaf symptoms after inoculation in spring 2020 and had a section of wood staining going through the wood originating from the rootstock.* ***A:*** *Root stump in a pot with visible wood rot right after it was cut down for assessment.* ***B:*** *Wood staining seen after systematically cutting through the tree for measuring the length of the wood staining.* ***C:*** *Cleaved inoculation points for cv. Jubileum tree during assessment where the wood staining section can be seen around the lower inoculation point ("nål 2") but not around the top inoculation point (nål 1).* ***D:*** Fungal *growth after isolation of wood sample pieces onto PDA after the initial assessment. Photo: M. Wennerberg*

The pots containing the root stump were left outdoors after the first assessment 17 June, and the root stump of cv. Reeves with the previously described wood rot section (Figure 12) developed fungal fruit ligaments. These grew from the tree stump and were first observed 23 days after on 10 August (Figure 14). A root shoot that had grown from under the soil surface had also developed silver leaf symptoms. The fruiting bodies were identified as *C. purpureum* after isolation on agar. The whip of cv. Jubileum with a wood rot section did not develop any fruiting ligaments before the experiment was concluded.

Figure 14. Fungal fruiting body that developed on root stump of cv. Reeves that had a wood rot section coming from the root. The fruiting bodies was observed 23 days after the tree was cut down for final assessment and was identified as Chondrostereum purpureum *after isolation on agar. Photo: J. Børve*

3.3.2 Pot experiment with whips of cvs. Jubileum and Reeves in growth chamber

The growth chamber experiment was inoculated with two map-pins using *C. purpureum* isolates 3 and 5 and developed silver leaf symptoms faster than the outdoor pot experiment. Symptoms were visible 35 days after inoculation. All trees inoculated with isolate 5 displayed silver leaf symptoms for both cvs. Jubileum and Reeves. The trees that displayed silver leaf symptoms all had them on the entire tree (Figure 15). For isolate 3 only one cv. Reeves tree showed symptoms.

Real-time PCR analysis revealed the presence of *C. purpureum* in all trees inoculated with isolate 5 also those that did not show any leaf symptoms. Trees inoculated with isolate 5 had a higher C.p-score than trees inoculated with isolate 3. For isolate 3 of cv. Jubileum the fungus was also shown to be present despite the lack of visible silver leaf symptoms. For cv. Reeves inoculated with isolate 3, real-time PCR showed that only the tree with symptoms had *C.*

purpureum present. All the trees where *C. purpureum* was detected had severe wood rot symptoms throughout the entire tree (Table 10).

Table 10. Overview of indoor growth chamber pot experiment with whips of cvs. Jubileum and Reeves map-pin inoculated with Chondrostereum purpureum *at NIBIO, Ås.*

Cultivar	Inoculation isolate	No. of trees	Trees with leaf symptoms	Trees with necrotic woody tissue	Trees with *C. purpureum* according to real-time PCR[a]
Jubileum	Isolate 5	2	2	2	2
Jubileum	Isolate 3	2	0	2	2
Reeves	Isolate 5	2	2	2	2
Reeves	Isolate 3	2	1	1	1
Jubileum	Control	1	0	0	0
Reeves	Control	1	0	0	0

Figure 15. Cv. Jubileum tree with silver leaf symptoms (left) inoculated with Chondrostereum purpureum *isolate 5 via map-pins kept in growth chamber at NIBIO ÅS. Tree on the right is a control. Photo: M. Wennerberg*

3.4 Outdoor pot experiments with graftings of cvs. Jubileum, Mallard and Reeves

The different inoculation methods using *C. purpureum* mycelium on either the rootstock or the scion of the graftings of cvs. Jubileum, Mallard and Reeves all developed silver leaf symptoms to some extent. Inoculations done onto rootstocks had the highest incidence percentage of silver leaf development on both new shoots from the rootstocks (32%) as well as new shoots coming from the scion (27%) compared to graftings inoculated in the scion. Adding up to a total of 51% of the inoculated trees displaying leaf symptoms for rootstock inoculations. Graftings inoculated in the scion had 16% with symptoms on new shoots from the rootstock while 23% had symptoms on new shoots growing from the scion. None of these differences were significantly different to one another (Figure 16).

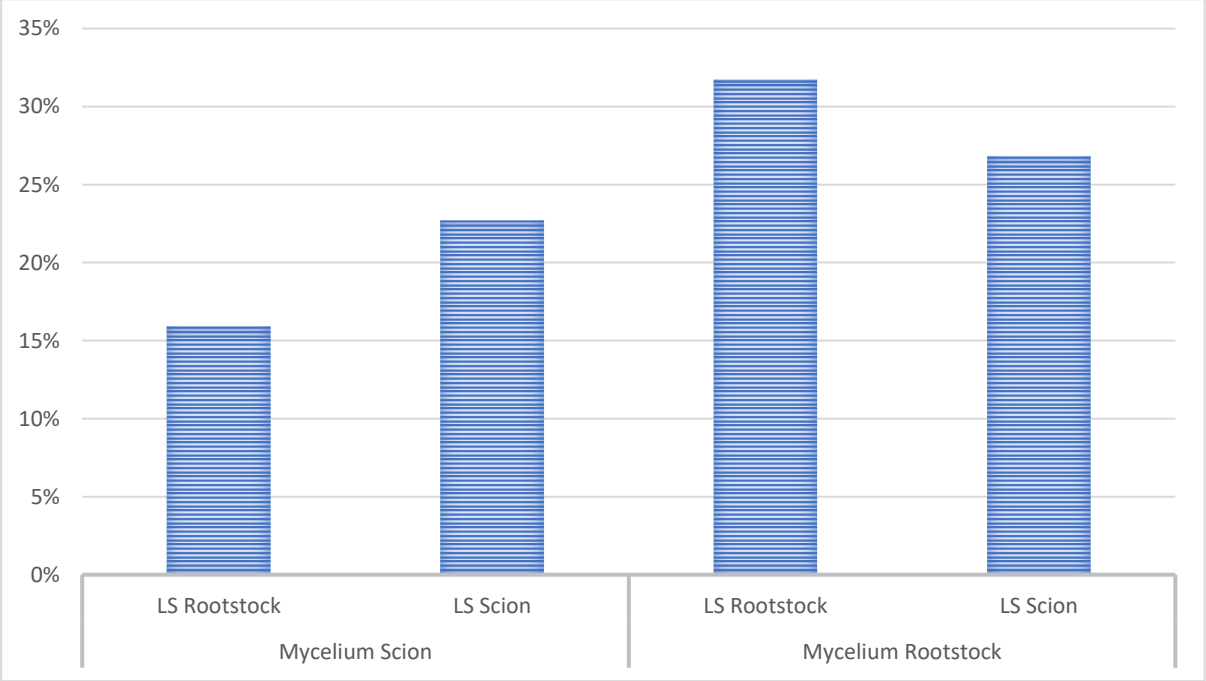

Figure 16. Outdoor pot experiment with bench graftings of cvs. Jubileum, Mallard and Reeves inoculated with Chondrostereum purpureum isolate 5 onto the rootstock or the scion main stem (2.6.1). *Incidence (%) of silver leaf symptoms (LS) for the two inoculation methods developed either on new shoots from the rootstock or on new shoots growing from the scion. A total of 41 graftings was used for inoculations onto the rootstock and 44 were used for inoculation of the scion.*

When comparing incidence of silver leaf symptoms per cultivar for each of the methods, none of the differences between the cultivars were significant. Incidence of silver leaf symptoms on new shoots from the scion was low for all cultivars for inoculation in the scion. Cv. Mallard had the highest incidence when looking at inoculation of the rootstock and leaf symptoms from new shoots growing out from the scion. (Figure 17).

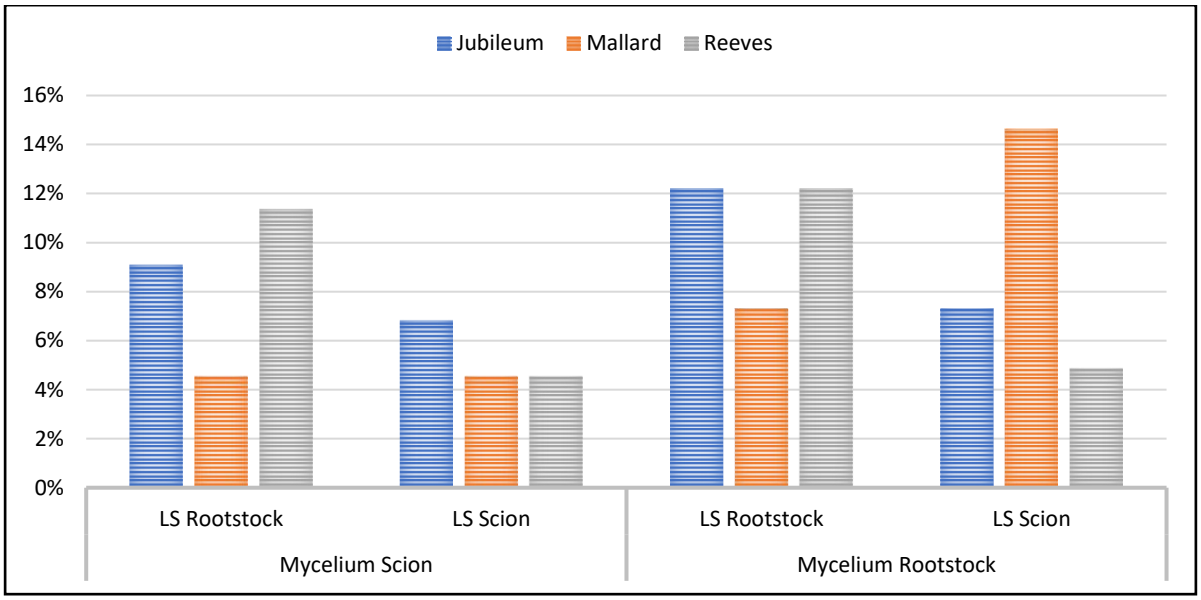

Figure 17. Silver leaf symptom (LS) incidence (%) per cultivar and inoculation method using Chondrostereum purpureum isolate 5 *on bench graftings in the outdoor pot experiment with cvs. Jubileum, Mallard and Reeves. Figure shows incidence (%) of silver leaf symptoms (LS) that occurred either on new shoots from the rootstock or new shoots from the scion of the grafting. A total of 41 graftings was used for inoculations onto the rootstock and 44 were used for inoculation of the scion.*

Real-time PCR analysis and isolation onto agar of wood samples from cvs. Jubileum and Reeves of both scion and rootstock inoculations showed that *C. purpureum* had spread throughout the entire grafting. This was also easily observed visually in several graftings where the woody tissue was clearly infected. The same was found for cv. Mallard, but only isolation onto agar was performed here (Figure 18).

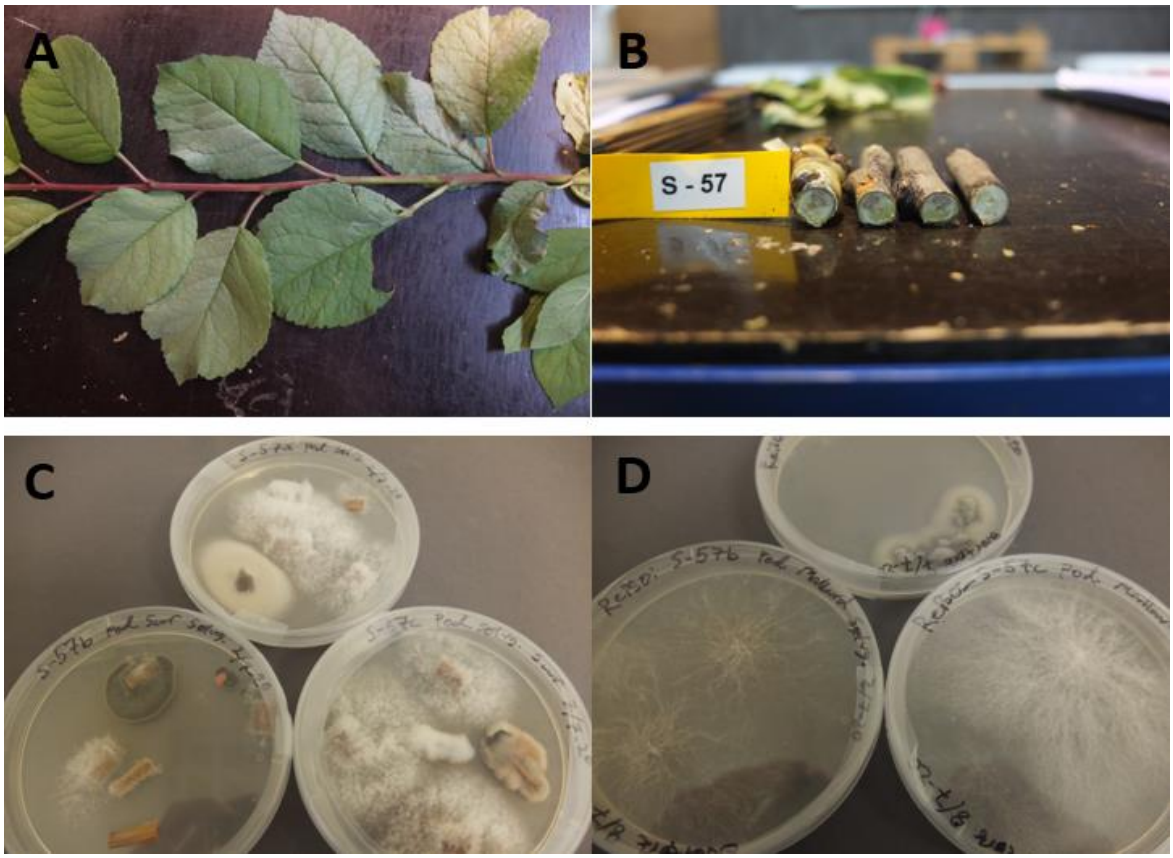

Figure 18. Cv. Mallard grafting inoculated 17 April 2020 in the in the rootstock with Chondrostereum purpureum *mycelium assessed 2 July.* ***A:*** *New shoot grown from the scion of the grafting showing silver leaf symptoms.* ***B:*** *Cv. Mallard grafting cut in sections showing wood staining going through the entire woody tissue of the grafting.* ***C:*** *Fungal growth after isolation onto agar with samples taken from both the scion and rootstock of one grafting.* ***D:*** *Mycelium taken from wood sample pieces visually identified as* Chondrostereum purpureum. *Photo: M. Wennerberg*

Of the total 41 graftings that displayed silver leaf symptoms in the experiment 39 had developed already 2 July, eleven weeks after inoculation with the rest showing at the end of the experiment in August. A total of 36 graftings had scions that were completely dead at the time of their final assessment and 20 of these had silver leaf symptoms observed on the rootstock.

Eleven graftings, three cv. Jubileum, one cv. Mallard, and seven cv. Reeves were completely dead at the end of the experiment. A total of five pots had developed fungal fruiting ligaments growing from the dead grafting right above the soil surface of the pot and were observed in August before final assessments. These were later identified as *C. purpureum* after isolation onto agar as well as one taken for real-time PCR scoring 6 on the C.p-score scale (Table 4). All five developed from graftings inoculated in the rootstock. Three graftings of cv. Jubileum, one from cv. Mallard and one from cv. Reeves developed fruiting bodies. All graftings were completely dead when the final assessment was done and had been dead for at least a month at this point. Additionally, none of the graftings that developed fruiting ligaments had any recorded silver leaf symptoms during the experimental period (Figure 19).

Figure 19. Dead cv. Reeves grafting from outdoor pot experiment with different inoculation methods with fungal fruiting ligament confirmed to be Chondrostereum purpureum *via real-time PCR. Fruiting ligament had developed on the dead grafting just above the soil surface of the pot in August right before the final assessment 7 August 2020.*

4 Discussion

4.1 Map-pin field trials

Experiments using map-pins for inoculation of side shoots of different age and with different isolates were supposed to follow the development of silver leaf symptoms throughout the season. However, from May 2020 to October only one side shoot inoculated with isolate 5, displayed any silver leaf symptoms. It developed on a newly developed side shoot on a cv. Reeves tree (3.1.3). There was, however, a noticeable wood rot inside the inoculated side shoots out of which a few resulted in *C. purpureum* growth confirming that there had been a successful infection. This was found for all the isolates no matter the origin or age.

The wood rot length varied a lot for all the field trials and isolates providing uncertainty surrounding the development of the fungus. Some assessment weeks could have barely any wood rot inside the side shoot only measuring to a few millimeters while the next assessment could have wood rot that was several centimeters long. In many cases, control and isolate wood rot length were very similar. Only a few of the field trials had isolate inoculations with significantly longer wood rot than the control with some trials only having it within one cultivar. Wood rot did not seem to follow a pattern when looking at the three cultivars and varied between the different field trials and isolates as well. Even though cv. Opal has shown to have lower silver leaf incidence in Norwegian plum orchards this could not be seen in the performed inoculation experiments.

As said, isolation rarely resulted in fungal growth of *C. purpureum* and often ended up with growth of bacteria or other fungi. These either had made it into the puncture wound or had not been removed during the sterilization process. From the almost 550 inoculated side shoots only a small part developed the fungus after isolation. The field trial investigating disease development over time in newly developed side shoots (3.1.5) had 16 out of 96 successful isolations of *C. purpureum* which by far was the highest success rate.

The low leaf symptom incidence in these field trials may have several explanations but it could indicate that there were some difficulties to achieve a successful infection with the map-pin inoculation method. Something that supports this is the variation in the measured wood rot length, which was the case for all the different map-pin field trials no matter the isolate or side shoot age. Additionally, the visual assessment of wood rot length and measuring of it had some flaws. It was in some instances difficult to determine whether the observed wood rot came from the growing fungus or if it was due to the mechanical damage from the map-pin. This was especially difficult if the discoloration of the woody tissue was restricted to only a few

millimeters around the map-pin. Here, isolation rarely resulted in growth of *C. purpureum* mycelium on the agar. Many times, the control map-pins inserted into side shoots were recorded with a wood rot longer than that of side shoots inoculated with *C. purpureum* isolates. This would imply that the mechanical damage causes wood rot that mistakenly was measured as rot caused by the fungus since it can be difficult to separate. Because the isolation on agar infrequently resulted in a visual identification of the fungus, the measured discoloration is difficult to use for any conclusion when it comes to how far inside the woody tissue the fungus developed.

When inoculating the side shoots there were some difficulties to confirm if any mycelium entered the puncture wound made with the map-pin. The problem being that there was no way of telling if any mycelium was in the wound or if it only slid up and stayed on the surface of the bark leaving it exposed. This was particularly difficult in newly developed side shoots since they were very fragile and easily could break if punctured multiple times. Likely this led to the amounts of mycelium entering the wound varied between the inoculations and could possibly increase or decrease the probability of infection.

Abiotic factors such as sun and rain could have contributed to failed inoculation as well. Sunny and warm weather could dry out the applied mycelium and kill it, only leaving the needle in the field. Rainfall could physically remove the mycelium before it had established if water droplets hit it. Another thing that was observed in some cases was that water had entered the wound via the map-pin needle and often resulted in other organisms establishing inside the inoculation point, rendering the side shoot invalid for measurement. Because the map-pin punctures the bark of the side shoot the needle might also bring organisms on the bark into the wound that potentially could outcompete *C. purpureum*. This combined with water entering the wound would probably contribute to this process.

Differences in pathogenicity between isolates have been observed in previous studies and cannot be excluded here as a factor. The same isolate could be seen to infect one host severely while another inoculated host with the same isolate barely showed an infection had taken place. This was seen across all used isolates and did not correlate with isolate origin (Ekramoddoullah et al., 1993; Spiers et al., 2000). This has even been reason enough to only included one isolate in inoculation experiments to eliminate this factor (Spiers et al., 1998).

The inconsistency of the mentioned wood rot length and the uncertainty surrounding whether the inoculation resulted in a successful infection, creates some doubt to the recorded results.

How the tree reacts to the puncture wound seems to depend on several factors and experience of the person assessing the inoculated side shoot is important when assessing the wood rot. The reaction to the puncture wound varied between the cultivars and could not be seen to follow any real pattern but this would be something that could be useful to know more about for further trials of this nature. It would also be interesting to investigate inoculated side shoots in the field until the next season to see if there would be any silver leaf symptoms at this point.

Map-pin needle inoculations were also done on whip trees grown outdoors and in a growth chamber which resulted in several clear infections and silver leaf symptoms. This will, however, be discussed in that section.

4.2 Field trials with cvs. Edda and Mallard using different inoculation methods

Inoculation with eight different methods performed in September and November 2019 on cvs. Edda and Mallard resulted in 21 out of 38 trees in total displaying silver leaf symptoms divided between inoculation methods 1-7 (Table 7). Of these 7 were of cv. Edda and 14 of cv. Mallard. The symptoms showed early in spring 2020 right after the leaves had emerged in May and it only took 187 days for inoculation method 7 to develop silver leaf symptoms. The other methods displayed their first symptoms on at least one tree two weeks later with an exception to inoculation method 8 that showed no silver leaf symptoms.

This development would suggest that inoculations done in 2019 were somewhat successful in all the methods. This could also be argued for inoculation method 8, even though leaf symptoms never occurred here. The reason being that real-time PCR analysis found that *C. purpureum* was present around the inoculation point of asymptomatic trees from this inoculation method although in lower quantities than infected trees.

Inoculation with mycelium has shown to provide good infection rates with the mycelium reaching deep into the woody tissue and it could explain the infection rate for the experiment and severity of infections (Spiers & Hopcroft, 1988). As mentioned previously trees of *Prunus*, *Malus* and *Pyrus* have been recorded to have a window where the resistance against *C. purpureum* increases in the fall and reaches a peak mid-winter.

It is of interest how early in the season the first silver leaf symptoms show for the different methods (Figure 9, Figure 11). Inoculation method 7 was one of the two methods performed in November when in theory the resistance should be higher than during the first inoculation time done in September. When looking at the number of trees that were successfully infected in this experiment it seems that this had no real influence on the virulence.

Although the inoculations were done late in the season with colder temperatures at the time it does not seem to have affected the inoculations much and the fungus seems to have establish well inside the trees. Since *C. purpureum* breaks down woody tissue to extract nutrients in addition to acquiring it from the xylem the seasonal variation of soluble nutrients might not affect the fungus as much if it is already well established.

Temperatures are lower in winter, but the fungus has been observed to grow in vitro down to 8°C with an optimum at 20 °C to 25 °C and a maximum at 32 °C following a similar pattern outdoors inside xylem tissue (Stanislawek et al., 1987). Temperatures could therefore be sufficient for growth long periods of the winter months close to the fjords in Vestland Norway and could have helped the fungus to establish. In spring when trees supposedly are at their most susceptible with temperatures rising and the amount of soluble carbohydrates and xylem flow increases. An already established fungus from last year could have a big advantage and developing very fast at this point. This would explain the quick leaf symptom development in May 2020 and would also be in line with the literature.

While inoculation method 7 was the quickest in developing silver leaf symptoms it had only developed symptoms on one more tree when the experiment was concluded out of the total eight inoculated trees. Methods 4, 5 and 6 however, had developed silver leaf on all inoculated trees about a month after the first observed silver leaf symptoms. However, comparison is difficult since there were only three inoculated trees in total for the other methods. One difference that should be pointed out is that inoculation method 7 is the only one of these four methods that was inoculated in the main stem of the tree while the other three methods were inoculated in pruned side shoots in some way.

The development of leaf symptoms on side shoot inoculated trees might have been influenced by it, since the silver leaf causing toxins have a shorter way to the leaves. This could also explain why real-time PCR analysis found *C. purpureum* in asymptomatic trees of inoculation method 7 and why trees with an infection still were asymptomatic. It could also, be that the fungus was not as established in these trees, but this would be difficult to determine. As discussed above, inoculations with the same isolate can result in great variation of infection level in the same experiment. Many factors such as weather and the general tree health can have effects on the infection success.

4.3 Outdoor and indoor pot experiments with whips of cvs. Jubileum and Reeves

Pot experiments were carried out both outdoors and indoors aimed to investigate development disease development and differences between the cultivars and the two isolates 3 and 5. Only trees inoculated with isolate 5 displayed silver leaf symptoms in the outdoor experiment for both cultivars, with cv. Jubileum having more trees affected. This would imply that isolate 5 is more aggressive than isolate 3 which could be related to the age of the culture. It is also supported by the results from the whips grown in growth chamber at NIBIO Ås where real-time PCR analysis showed that *C. purpureum* of isolate 5 samples was more abundant inside the infected trees. This has also been seen to be the case for this isolate in another study (Børve J., unpublished results). Due the low number of trees used in these experiments; only careful assumptions can be made about this.

The results in this experiment, does not indicate that there is a susceptibility difference between the two cultivars. The low number of trees also makes it difficult to show that one cultivar is more susceptible than the other.

Disease development time for the inoculated trees varied between the outdoor and indoor experiments. Development was 10 days faster in the growth chamber. This difference is not big, but the controlled environment in the growth chamber appears to have been somewhat better for development. Silver leaf symptoms also seemed to be more pronounced in the growth chamber. This could be due to the stability of the temperature in the growth chamber, stimulating the growth. Outdoors the temperature fluctuates during the day and could therefore slow the fungal growth down due to a lower average temperature. The steady climate inside the growth chamber combined with the lower amount of competition from outside organisms seems to have boosted the development of the fungus and infection rate of the fungus.

It is notable that due to space restrictions the whips in the growth chambers had to be cut off in the tops to be able to fit, and it cannot be ruled out that this wounding influenced the disease development. This difference in treatments makes a comparison difficult between the two experiments. This is important for future reference when planning to include growth chamber experiments.

Something else that was observed in the outdoor pot experiment was that two of the whips, one of each cultivar, had what seemed like a fungal infection that originated from the rootstock. Later this was identified as *C. purpureum*. Due to the severity and the fact that the wood rot was strongest near the base of the tree, would suggest that it had come in from the plant nursery.

The trees from the experiment were all imported from Europe. The whip of cv. Reeves later developed fungal fruiting bodies from the root stump were identified as *C. purpureum*. These observations highlight an important problem when it comes to the amount of silver leaf disease in Norway but also in general. Since most trees planted in Norway are imported, the importance of clean trees from the nurseries is crucial to keep Norwegian plum orchards healthy. The disease is almost impossible to spot before the leaf symptoms or fruiting bodies appear. This could therefore be a factor behind the amount of silver leaf in the plum orchards. NIBIO reported (Anna Birgitte Milford & Veggeland, 2021) that more trees of the four main cvs. Jubileum, Mallard, Opal and Reeves were removed more often before ten years had passed since planting between 2004-2009 compared to the period 1998-2003. An increased import of trees since 2004 was discussed but could not be verified. They did however conclude that the health of Norwegian plums should be monitored.

Map-pin inoculations in these experiments were more successful in comparison to the side shoot inoculations in the field and resulted in several trees with silver leaf symptoms. The differences here could be due to various factors. Some of the reasons could be age of the inoculated tree, age of woody tissue that was inoculated, or trees in the field being more exposed to weather. Additionally, the whips were inoculated inside in the high tunnel and indoors in the growth chamber, sheltering them from any abiotic factors, which might have played a major role for infection. Another plausible reason could be that it is generally easier to inoculate a main stem than a side shoot. The mass of the main stem is generally bigger, and it is therefore easier to make sure the mycelium gets into the puncture wound. Additionally, the reaction to the puncture wound is more easily separated from the actual fungal infection in the woody tissue.

4.4 Outdoor pot experiments with graftings of cvs. Jubileum, Mallard and Reeves

The two compared methods in this pot experiment did not have any differences between cultivars nor did it differ, where the inoculum was placed. Almost all graftings that displayed silver leaf symptoms had developed them at the start of July indicating that the disease development time was similar in all graftings, no matter the inoculation site.

Many of the graftings had severe woody tissue symptoms, which probably can be accredited to the size of the graftings, meaning that young and small trees, like the graftings are more sensitive to the fungus. Therefore, they are more likely overwhelmed by the fungus and killed at an early stage. A successful infection in a small grafting could have the potential to be more devastating than one in an older tree that overall has more mass. Once established inside a grafting the occlusion of the xylem may occur very quickly, potentially killing the tree. Seeing that eleven graftings were completely dead at the end of the experiment and 36 scions of the graftings had died during the experiment would suggest, that occluding of the xylem had occurred here.

Several graftings developed *C. purpureum* fruiting bodies directly from the dead grafting standing in the pot. This only occurred on trees inoculated in the rootstock and could be explained by the fact that their xylem was blocked at a very early stage after inoculations, thereby killing the grafting early on.

All newly developed shoots on the rootstock had been removed prior to the start of the experiment. This combined with a very successful inoculation in the rootstock could lead to effective blockage of the xylem stopping all nutrients from flowing to the only side shoots on the scion quickly starving the entire tree and killing it. All dead trees were dry throughout the entire woody tissue indicating that the fungus had totally taken over inside.

This development of fruiting bodies together with the ones in the experiments with whips shows that they can develop very quickly even during late summer if the fungus has established itself well enough. These results emphasize the importance of removing infected wood and trees in the fruit orchard and shows the potential of *C. purpureum* spreading in a nursery or fruit orchard.

5 Conclusion

Disease development in the different inoculation experiments outdoors and indoors had a wide variation in success. Inoculation with mycelium agar pieces had more successful and severe infections than the map-pin inoculation method carried out in the field. Inoculation experiments using agar pieces with mycelium resulted in successful inoculations in most cases and in accordance with the literature gave deep infections inside the wood.

Inoculation with map-pins of side shoots in an established field led to few successful infections and seems to be better suited for experiments in more controlled environments. Verification difficulties regarding whether the map-pin or the fungus caused the wood rot rendered much of the data difficult to interpret. The results show that the method needs to be revised if it is to be used in field trials again.

The Map-pin inoculation of cvs. Jubileum and Reeves carried out controlled environments showed that the method works but most likely is influenced a lot by abiotic factors in the field. Leaf symptoms from these experiments occurred after 45 days for the outdoor pot experiment and 35 days for the whips in growth chamber. Isolate 5 that was the youngest culture used for inoculation seemed to be more aggressive in these experiments. The outdoor experiment only had silver leaf on trees with this isolate. Real-time PCR analysis showed that *C. purpureum* was more abundant in trees inoculated with isolate 5 than isolate 3 in the growth chamber. Results were however not enough to confirm this.

Graftings used for inoculations with mycelium on the rootstock or scion had many severely infected trees and silver leaf symptoms were seen after 11 weeks. At this point 39 out of the total 41 graftings that displayed leaf symptoms were showing symptoms. This is thought to be due to the young age and size of the graftings making them more vulnerable once the infection has established. The field trial using eight different inoculation methods with a year older cv. Mallard trees also resulted in several severe infections but none of these resulted in the trees dying during the season. Inoculation done on smaller cv. Edda trees in September also died during the experimental period supporting this theory.

Inoculation of cvs. Edda and Mallard in the fall and winter of 2019 showed that successful inoculation had occurred in winter despite the lower temperatures and the by literature described lower susceptibility during this time of year. *Chondrostereum purpureum* had established enough to quickly start growing in spring 2020 and exhibiting foliar silvering symptoms right after leaf emergence in May for all but one inoculation method. The fast

development in spring is thought to be because of the increase of soluble carbohydrates in spring providing more nutrients for growth.

None of the results from the experiments could provide data to support that one cultivar was more susceptible than another although the literature has noted such differences. This should be a topic for future studies in Norway to establish the susceptibility of the common plum cultivars here. Differences between isolates could not be confirmed for any of the isolates that were used but all were seen to infect inoculated trees. Isolate 5 being the youngest *C. purpureum* culture showed signs of being more aggressive when analyzing results from the whip inoculation experiments.

The development of fruiting bodies on dead graftings and root stumps emphasizes the removal of infected trees and wood from the field or plant nursery to prevent these from becoming inoculum sources. It also shows that if the infection is severe enough, fruiting bodies can form quickly.

Reference list

Anna Birgitte Milford, J. B., Bjørn Arild Hatteland, Marianne Stenrød, Frode & Veggeland, A. V. o. G. B. (2021). *Verdsetting av reguleringen av norsk plantehelse fra et miljømessig, økonomisk og sosialt perspektiv: Regulering og overvåking av import, plantevernmidler og mykotoksiner*. Divisjon for matproduksjon og samfunn: NIBIO.

Beever, D. (1970). The relationship between nutrients in extracted xylem sap and the susceptibility of fruit trees to silver-leaf disease caused by Stereum purpureum (Pers.) Fr. *Annals of applied biology*, 65 (1): 85-92.

Bintner, J. (1919). Silver Leaf Disease. Stereum purpureum. *Bulletin of Miscellaneous Information (Royal Gardens, Kew)*, 1919 (6/7): 241. doi: 10.2307/4111529.

Bishop, G. C. (1978). *Studies on silver leaf disease of stone and pome fruit trees*: Adelaide.

Brooks, F. & Storey, H. (1924). Silver-Leaf Disease.—IV. *Journal of pomology and horticultural science*, 3 (3): 117-141.

Butler, E. & Jones, S. (1949). Silver leaf disease of plum, Stereum purpureum (Fr.) Fr. *Plant Pathology. London: MacMillian & Co*: 763-767.

Børve, J. & Stensvand, A. (2018). *Sølvglans i frukt*. NIBIO, Plantevernleksikonet: NIBIO. Available at: https://www.plantevernleksikonet.no/l/oppslag/1172/ (accessed: 11.04).

Dye, M. (1974). Basidiocarp development and spore release by Stereum purpureum in the field. *New Zealand journal of agricultural research*, 17 (1): 93-100.

Ekramoddoullah, A., Shamoun, S. & Wall, R. (1993). Comparison of Canadian isolates of Chondrostereum purpureum with respect to temperature response, virulence, and protein profiles. *Canadian Journal of Plant Pathology*, 15 (1): 7-13.

FAOSTAT. (2021). Food and agricultural data Plums and sloes Norway. In *FAO*. Food and Agriculture Organization of the United Nations (FAO) online database (FAOSTAT). Available at: http://www.fao.org/faostat/en/#data/QC (accessed: 2021.04.11).

France, A., Grinbergs, D. & Carrasco, J. (2016). *First detection of silverleaf (Chondrostereum purpureum) on rabbiteye blueberry (Vaccinium virgatum) and disease damages*. XI International Vaccinium Symposium 1180.

Hamberg, L. & Hantula, J. (2016). The efficacy of six elite isolates of the fungus Chondrostereum purpureum against the sprouting of European aspen. *Journal of environmental management*, 171: 217-224.

Meier, U., Graf, H., Hack, H., Hess, M., Kennel, W., Klose, R., Mappes, D., Seipp, D., Stauss, R. & Streif, J. (1994). Phenological growth stages of pome fruit (Malus domestica Borkh. and Pyrus communis L.), stone fruit (Prunus species), currants Ribes species and strawberry (Fragaria x ananassa Duch.). *Nachrichtenblatt des deutschen Pflanzenschutzdienstes*.

Miyairi, K., Fujita, K., Okuno, T. & Sawai, K. (1977). A toxic protein causative of silver-leaf disease symptoms on apple trees. *Agricultural and biological chemistry*, 41 (10): 1897-1902.

Miyairi, K. (1988). Biochemical studies on the silver-leaf inducing substance of apple silver-leaf disease. *Bulletin of the Faculty of Agriculture-Hirosaki University (Japan)*.

NIBIO. (2017). *Prosjektbeskrivelse: Bedre trehelse-Helsetilstanden til steinfrukttrær må forbedres*: NIBIO. Unpublished manuscript.

Pike, B. (2011). *The Fruit Tree Handbook*: Green books.

Prillieux, E. (1885). Le Plomb des arbres fruitiers. *Bull, des séances de la Soc. Nationale d'Agriculture de France*.

Reina, R., Kellner, H., Hess, J., Jehmlich, N., García-Romera, I., Aranda, E., Hofrichter, M. & Liers, C. (2019). Genome and secretome of Chondrostereum purpureum correspond to saprotrophic and phytopathogenic life styles. *PLOS ONE*, 14 (3): e0212769. doi: 10.1371/journal.pone.0212769.

Schøyen, T. H. (1946). *Skadedyr og sykdommer i frukt- og bærhagen*. Oslo: Aschehoug.

Setliff, E., EC, S. & EK, W. (1973). Stereum purpureum associated with sudden decline and death of apple trees in Wisconsin.

Simpson, R., Brewster, D., Christeller, J. & Spiers, A. (2001). Extracellular enzymes of Chondrostereum purpureum causal fungus of silverleaf disease. *New Zealand Plant Protection*, 54: 202-208.
Spiers, A. (1985). Factors affecting basidiospore release by Chondrostereum purpureum in New Zealand. *European journal of forest pathology*, 15 (2): 111-126.
Spiers, A., Edwards, W. & Hopcroft, D. (1987). Effects of silverleaf infection on ultrastructure of foliage of Prunus, Rosa, and Populus. *New Zealand journal of botany*, 25 (3): 411-423.
Spiers, A. & Hopcroft, D. (1988). Factors affecting Chondrostereum purpureum infection of Salix. *European journal of forest pathology*, 18 (5): 257-278.
Spiers, A., Brewster, D., Bus, V. & Hopcroft, D. (1998). Seasonal variation in susceptibility of xylem tissue of Malus, Pyrus, Prunus, and Salix species to Chondrostereum purpureum in New Zealand. *Mycological Research*, 102 (7): 881-890.
Spiers, A., Brewster, D., Slade, A. & Gardiner, S. (2000). Characterization of New Zealand isolates of Chondrostereum purpureum with regard to morphology, growth, pathogenicity and RAPD banding patterns. *Mycological Research*, 104 (4): 395-402.
Stanislawek, S. D., Long, P. & Davis, L. (1987). Sugar content of xylem sap and susceptibility of willow to Chondrostereum purpureum. *New Zealand Journal of Botany*, 25 (2): 263-269.
Talgø, V., Strømeng, G. M., Myren, G., Stensvand, A. & Børve, J. (2017). Sølvglans: En viktig sjukdom i plommetre. *Norsk frukt og bær*, 5.
Williams, H. & Cameron, H. (1956). Silver-leaf of Montmorency sour cherry in Oregon. *Plant disease reporter*, 40: 954-956.
Øie, L. (2020). *Fruktsesongen 2020*: Grønt produsentenes samarbeidsråd.

Norges miljø- og biovitenskapelige universitet
Noregs miljø- og biovitskapelege universitet
Norwegian University of Life Sciences

Postboks 5003
NO-1432 Ås
Norway