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Apple rootstocks may become infected by *Neonectria ditissima* during propagation

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Summary

The ability of apple rootstocks to become infected by *Neonectria ditissima*, the cause of European canker, was studied over two years. Rootstocks B9 and M9 with a size suitable for grafting (6 - 10 mm stem diameter, termed rootstocks), and smaller sized rootstocks (<5 mm stem diameter, termed transplants) of B9, M9, M26, MM106 and Antonovka were inoculated with *N. ditissima* at different times, either with contaminated map pins or with spore suspensions. In addition, the rootstocks were either defeathered (side shoots removed), topped (top shoot headed) or both, to create wounds that would normally occur during propagation, while wounds on transplants were made by removing leaves. One month after inoculation, slightly sunken canker lesions had developed around the inoculation points of the map pins or wounds. No lesions developed on the non-inoculated controls. Map pin inoculation resulted in 30 % to 89 % infection and spore suspension sprayed on wounds from 5 % to 45 % infected. When the cankered areas were split open, brown lesions with necrotic tissue due to infection by *N. ditissima* appeared. The transplants of M9, M26 and MM106 inoculated with contaminated map pins in 2014, developed necrosis on 40 % to 67 % of the plants, but there were no differences in incidence or severity among the different types. On the transplants of B9, Antonovka and M9 inoculated in 2015, there was more necrosis on B9 (42 %) than on

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Antonovka (11 %) and more sporulating lesions on B9 (29 %) than M9 (9 %) or Antonovka (4 %). It can be concluded that rootstocks used for apple trees may become infected by *N. ditissima*, and wounds should thus be protected during propagation.

Keywords: European canker; inoculation experiments; *Malus* × *domestica*; *Nectria galligena*; susceptibility

Introduction

European canker caused by *Neonectria ditissima* (Tul. & C. Tul) Samules & Rossman (syn. *Nectria galligena*) is an important disease in apple in cool, wet production areas as in northwestern Europe. Cankers on newly planted apple trees may originate from infections established during propagation that stay latent for up to three years after planting (McCracken et al. 2003). Latent infections of *N. ditissima* on young trees is a major concern for fruit growers and may cause great economic losses for both growers and the nurseries (Weber 2014). Latent infections of rootstocks may be a possible pathway for infection of apple trees during propagation. Susceptibility of commercially available rootstocks to Phytophthora crown rot, fireblight, apple scab, powdery mildew, latent viruses and wooly aphid is reported (Jackson 2003), but such information does not exist for European canker. However, in a recent experiment, susceptibility of two clones of M9, MM106, EMR001 and M116 was tested by inoculating detached shoots with *N. ditissima*, and clone 337 of M9 was more susceptible than the other rootstocks (Gòmez-Cortecero et al. 2016).

The European canker pathogen *N. ditissima* requires a wound to infect apple trees (Swinburne 1975). Possible entry points during propagation are when rootstocks are defeathered (side shoots removed) or topped, the roots are cut back, or at the time of budding

or grafting. During production of rootstocks, defeathering may be performed two or three times in each season. Rootstocks are topped once when final size for delivery is reached, and topped again at time of grafting or after the bud is established/starts to grow. Roots of rootstocks are pruned, both when lifted for overwinter storage and during grafting the following winter and spring. Rootstocks may also become injured during field treatments. All wounds are potential entry sites for *N. ditissima*, but the risk of infection is dependent on presence of inoculum and related to temperature and wetness. Optimal disease development occurs in the interval between 5 and 20 °C (Latorre et al., 2002). There may also be a possible risk of spread with internal growth of *N. ditissima* in stool beds, as documented for *Phythophthora* sp. (Tidball and Linderman, 1990). The objective of the present investigation was to determine the risk of infections of *N. ditissima* in apple rootstocks during propagation. Part of the experiments were carried out as a master thesis (Kolltveit 2015).

Materials and methods

Experiments were performed in a temperature regulated greenhouse at Ås (in 2014) and in a ventilated high plastic tunnel at Ullensvang (in 2014 and 2015) in southeastern and southwestern Norway, respectively. In the first series of experiments in 2014, the plant material consisted of rootstocks at a size normally used at time of grafting (termed rootstocks), including B9 (6-8 mm in diameter) and M9 Lancep (8-10 mm in diameter). The diameter was measured 20 cm above ground. The rootstocks were lifted from the production fields the preceding autumn and stored over winter at -1°C and delivered from a commercial supplier in time for the experiments. In a second series of experiments (in 2014 and 2015), smaller plants (< 5 mm in diameter, measured about 5 cm above ground, termed transplants, 1 - 1.5 years before time of commercial delivery) of rootstocks Antonovka, B9, M9 Lancep, M26 and MM106 were used.

The rootstocks were provided by NORGRO AS, and the transplants were from the Norwegian Elite plant station.

Inoculum and inoculation methods

The isolate used for inoculation originated from mycelia in a canker wound sampled in a commercial apple orchard in spring 2014. In 2015, a re-isolate (isolate made from inoculated rootstocks in 2014) of the same isolate was used for all inoculations, except for the last inoculation on transplants, where a new isolate obtained from a dead shoot on a newly planted tree was used. The isolate was kept viable by transferring it regularly to new PDA. Inoculations were performed by applying spore suspensions of *N. ditissima*, or by using map pins dipped in a culture on potato dextrose agar (PDA) containing the fungus (Talgø and Stensvand 2013). Spore suspensions were made from cultures on PDA (about 4 to 6 weeks old) by pouring a small amount of autoclaved water on the culture and gently removing the spores from the cultures with a glass rod. The spore suspensions were adjusted to approximately 10^5 spores/mL based on haemocytometer counts. The spore suspensions were applied by a hand held sprayer to run off, except for one experiment where droplets (20 µl) of a suspension were applied by a pipette onto freshly cut wounds. At Ås, the rootstocks were enclosed in plastic bags for 48 hours after inoculation to maintain high RH. This was not done at Ullensvang. At both locations, non-inoculated rootstocks were used as controls. Spore suspensions were tested for germination by placing droplets of the suspensions on microscope slides for 24 h at 20 °C in water saturated air. The germination varied from 84 % to 91 %.

Map pins with the fungus were inserted about 2 to5 mm into the wood of the rootstocks. The pins were inserted directly through the bark of 1-year-old tissue (Figure 1A), in defeathering wounds (Figure 1B), or into newly emerged shoots (Figure 1C). In addition, map

pins were placed at the base of side shoots (Figure 1D). On the transplants, the map pins were inserted in wounds after leaf removal on the main stem of current year growth. As controls, map pins without inoculum were placed in similar positions in some of the experiments.

Experiments with rootstocks

At Ås, M9 Lancep were topped at 15 cm above the base, potted in 1.5 L pots and placed in an experimental greenhouse compartment set to 16 hour of day light, 50 % relative humidity (RH) and 20 °C. The rootstocks were irrigated manually after need. Manual removal of insect larvae feeding on the leaves was performed twice, and it was sprayed against aphids once with imidacloprid at a recommended rate. The rootstocks started to grow 2-3 weeks after they were delivered from the cold store. One experiment was started April 7 and a replicate the following day, ca. five weeks after the rootstocks started to grow (Table 1). In both experiments, the following treatments were included on 3 (replicates) × 4 plants; 1: defeathering (side shoots removed at the base) and inoculation by applying a spore suspension, 2: no removal of side shoots and inoculation by applying a spore suspension, and 3: no removal of side shoots and inoculated by two contaminated map pins, one in the 1-year-old stem (Figure 1A) and one in the newly emerged growth (Figure 1C). Each treatment had a corresponding 3 (replicates) × 4 non-inoculated control plants. Duration of the experiments was until 18 July, 15 weeks after inoculation.

At Ullensvang, rootstocks of B9 and M9 Lancep were potted (one plant in each) in 4.6 L plastic pots, topped at 25 cm and placed in a ventilated high plastic tunnel (size 4 × 20 m and 2.5 m in height) without heating. The rootstocks started to grow ca. three weeks after potting and were inoculated in the period from 9 to 15 weeks after being taken out of cold store. Water was applied by drip irrigation in each pot. The rootstocks started to grow from several buds,

forming side shoots and a leading top shoot. The plants were treated twice against apple leaf midge and twice against powdery mildew during the experimental period in 2014, with recommended rates of spirotetramat and penconazol, respectively. Five experiments were started at May 20, May 21, June 2, June 18 and June 27, respectively (Table 1). Final assessment was made at the same time in August, and thus the duration from time of inoculation was from 7 to 11 weeks.

In the first experiment, started May 20, the following treatments were performed on 4 (replicates) × 5 plants of B9; 1: defeathering, topping of the leader shoot at about 5 cm above the base and inoculation with a spore suspension immediately afterwards, 2: as 1, but inoculation one day after defeathering and topping, 3: as 1 but inoculation three days after defeathering and topping, 4: defeathering, but no topping of leader shoot and inoculation with spore suspension, 5: defeathering, topping of leader shoot and inoculation with contaminated map pins in two defeathering wounds in two different heights of the stem, 6: defeathering, no topping of leader shoot, and inoculation with two contaminated map pins, one in a defeathering wound and one in the leader shoot. There were 4 (replicates) × 5 non-inoculated controls in each of treatments 1, 4 and 5.

In the second experiment started May 21, the following treatments were included on 4 (replicates) × 5 plants of M9; 1: defeathering, top shoot left non-topped and inoculation with a spore suspension immediately afterwards, 2: no defeathering, no topping and inoculation with a spore suspension, and 3: as 1, but inoculated with contaminated map pins in two defeathering wounds. There was 4 (replicates) × 5 non-inoculated controls of treatment 1.

In experiment three, started June 2, the following treatments were included on 4 (replicates) × 5 plants of M9; 1: defeathering, top shoot left non-topped and inoculation with a spore suspension immediately afterwards, 2: as 1 but inoculated one day after defeathering, 3:

defeathering and topping of leader shoot, inoculation with spore suspension immediately afterwards, 4: as 3, but inoculation after one day, 5: defeathering and topping, inoculation by contaminated map pins in two defeathering wounds, 6: side shoots and leader shoot left untouched, inoculated with two contaminated map pins in each of two side shoot bases. There were 4 (replicates) × 5 non-inoculated controls in each of treatment 1 and 3.

Experiment four started June 18 included only one treatment of 4 (replicates) × 5 M9; defeathering at June 2, topping of leader shoot June 18 and inoculation with a droplet of spore suspension directly on the freshly made wound in the top. There were five non-inoculated control trees.

Experiment five started June 27 included one treatment of 4 (replicates) × 5 M9: defeathering, topping of the leader shoot at 7 cm and inoculation by two contaminated map pins in the leader shoot. There were no controls.

Experiments with transplants

In the high tunnel at Ullensvang, experiments with plug plants of M9, M26 and MM106, and of M9, B9 and Antonovka were carried out in 2014 and 2015, respectively. The plants were delivered in late June both years. The plants were potted in 1 L pots in 2014 and in 2 L pots in 2015. They were about 7 and 10 cm tall when potted in 2014 and 2015, respectively. All leaves except two or three full sized leaves in the top were removed to simulate defeathering and thus cause wounding. Leaf removal and inoculation experiments were repeated three times both years. For each rootstock there were three replicates of five plants in the experiments (Table 2). Plant protection during the two years was carried out as described above for Ullensvang for full

sized rootstocks in 2014 but only one out of two applications; in 2015 the transplants were not treated with pesticides or fungicides.

Assessments

At Ås, the rootstocks were assessed for symptom development five times. At the final assessment after 15 weeks, top shoot length, number of side shoots and diameter of the main stem 10 cm above the soil surface were recorded in addition to number of lesions of *N. ditissima* and growth of the pathogen on PDA. At Ullensvang, the number of wounds caused by defeathering were counted. Rootstocks were observed for fungal growth at one or two week intervals. At the end of the experimental period, each of the rootstocks were cut at the soil surface and brought to the laboratory. Length of the top shoot, number of emerging side shoots, number of wounds, diameter of the main stem (15 cm above ground) and top shoot (5 cm from the base), number of possible canker lesions (Figure 1E), and dead or desiccated tissue were recorded. On rootstocks inoculated with map pins (Figure 1F-H), length and width of the lesions around the pins were recorded, presence of sporulation of *N. ditissima* was observed with the use of a stereo magnifier and desiccated tissue recorded. On the rootstocks inoculated with spore suspensions, canker wounds and eventually other symptoms of disease such as dead shoots were enumerated, and sporulation was observed as mentioned above. To determine potential development of fungal growth on healthy appearing rootstocks and on lesions without sporulation, sections of the rootstocks were incubated separately on grids in water saturated air at 20 °C for up to 7 days. Sections developing signs of *N. ditissima* were assessed for presence of conidia as mentioned above. Either at the time of the main assessment or after incubation, all map pin inoculated rootstocks and some of the ones inoculated with spore suspensions, and their non-inoculated controls, were split open longitudinally and observed for presence and size

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of necrotic tissue inside the stems (Figure 1I). Length of necrotic tissue was determined. To confirm the diagnosis in the different experiments, a piece of the stem of one rootstock per treatment was cut in two halves, surface sterilized in 70 % ethanol for 10 seconds and 0.5 % NaOCl in 90 seconds and placed on PDA. Mycelial growth of *N. ditissima* was assessed after up to 7 days at 20 °C and confirmed by microscopy if necessary.

The transplants were assessed biweekly for symptoms of *N. ditissima* and finally in September both years, after 8-12 weeks. None of the plants had sporulating lesions at that time, and therefore all of them were cut off at the base, all leaves were removed, followed by incubation in water saturated air at 20 °C for 14 days. The plants were then assessed for presence of sporulating lesions of *N. ditissima*, split in two halves and assessed for necrosis in the wood tissue (Figure 1I).

Temperature and precipitation records were both years obtained from a climate station located 150 m from the site of the tunnel at Ullensvang. In 2015, a logger for temperature and air humidity (Testo 175H1, Testo, Lenzkirch, Germany) was in addition placed in the center of the tunnel, about 1.5 m from the ground.

Data were analysed with the GLM procedure of SAS (SAS Institute, Cary, NC, USA). All incidence data were arcsine-square root transformed prior to analysis. Only non-transformed means are presented. Mean values were separated by Student Newman Keuls method at P = 0.05. Disease development in differently aged tissue on map pin inoculated rootstocks was analyzed with repeated measurements, and AUDPC (area under the disease progress curve) was calculated.

Results

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Climatic conditions

At Ullensvang in 2014, the temperature was higher than the following year from April to the end of July. In August, temperatures were relatively similar between the two years (Table 3). Monthly mean temperatures inside the tunnel was higher than outside in 2015 (not recorded inside the tunnel in 2014). Number of days with daily mean temperature between 5 and 20 °C were fewer in July 2014 than in 2015. In August, the two years were similar. In the tunnel in 2015, there were fewer days within this temperature range during all months (Table 3).

Rootstocks

None of the non-inoculated controls developed infections in any of the experiments at Ås or Ullensvang. In both the experiments at Ås (with M9), there were more infections after inoculation with map pins than with spore suspensions, and there was a higher incidence of infections on rootstocks where the side shoots were removed (defeathered) compared to non-defeathered rootstocks ($P = 0.0001$, Figure 2A). There was a higher number of visible infections around map pins in newly emerged tissue (45 %) than in 1-year-old tissue (30 %) ($P = 0.0493$) prior to incubation, but not after ($P = 0.4848$). Development of symptoms around map pins started earlier in newly emerged tissue than on 1-year-old wood, giving different line patterns ($P = 0.0013$), and disease development recorded as AUDPC was more rapid in newly emerged tissue ($P = 0.0007$, Figure 3A).

In the B9 experiment at Ullensvang, there was a higher incidence when inoculated with map pins than with a spore suspension in mean of topped and non-topped plants ($P = 0.0001$). There was no difference in infection of non-topped and topped rootstocks when inoculated by map pins ($P = 0.3910$) or with spore suspensions at day 0 ($P = 0.1979$). There was a significant

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difference between inoculation immediately following defeathering and inoculation performed one or three days afterwards, but no difference between one and three days (P = 0.0374, Figure 4A). The number of sporulating lesions on non-topped and map pin inoculated rootstocks of B9 was higher on newly developed tissue than in 1-year-old wood prior to incubation, but not after (Table 4). There was a higher incidence of necrosis and larger lesion width in newly emerged tissue compared to 1-year-old wood (Table 4), but there were no differences in lesion length between new and old tissue of B9 (Table 4). Development of symptoms around map pins started earlier in new tissue, giving different line patterns (P = 0.0470), and disease development expressed as AUDPC was higher in new compared to 1-year-old tissue (P = 0.0003, Figure 3B).

If inoculated at day 0 (immediately after wounding) in both the second started May 21 and the third experiment started June 2 at Ullensvang with rootstock M9, there was a higher incidence of infections after inoculation with map pins (56 or 58 %) than with spore suspension (0 or 20 %) (P = 0.0070 and 0.0002 in experiments 2 and 3, respectively). On M9 in experiment 3 there were fewer infections if inoculation took place one day after defeathering than if inoculated at day 0, both on topped (P = 0.0113, Figure 4B) and on non-topped rootstocks, the latter with an incidence of 15 % on day 0 and no infections on day 1 (P = 0.0420). There was no difference between non-topped and topped rootstocks inoculated at day 0 (P = 0.4347).

All rootstocks became infected after inoculation by applying a drop of spore suspension on a wound after topping of the leader shoot (fourth experiment started June 18). In the fifth experiment started June 27, map pins placed in the leader shoot resulted in 89 % infected rootstocks. Top shoot length was lower and number of side shoots emerging after defeathering was higher on the topped rootstocks in all experiments where that could be compared (data not shown). The mean number of side shoots removed per plant at time of defeathering at Ullensvang were 5.2 on B9 and 7.6 on M9. All infections observed after inoculation with spore

suspensions were located in the defeathering wounds (Figure 1E), except one infection of a non-wounded M9 at Ås. That infection was observed as brown tissue inside when splitting open a rootstock, and it developed *N. ditissima* when isolated on PDA. In total for all the experiments, the incidence of infections per defeathering wound inoculated the same day as defeathering took place was 9 % on B9 and 2 % on M9.

Transplants

Inoculation with spore suspensions at time of leaf removal in three experiments in July 2014 resulted in no infections. Inoculation with map pins resulted in necrotic tissue (infections) inside the plants (Figure 1I), but no sporulation occurred on the surface of the plants either before or after incubation. An average of 48.9 % of the map pin inoculated plants developed lesions and none on the non-inoculated control plants. There were no significant differences in infection level between the different transplant genotypes in 2014 (Table 5).

There was a significantly higher incidence of sporulating lesions on transplants of B9 than of M9 and Antonovka, and the number of plants with internal necrosis was higher on B9 than on Antonovka, but there was no significant difference between B9 and M9 (Table 5). In the fourth experiment (with newly obtained inoculum), internal necrosis developed on 72.5, 23.3 and 8.3% of B9, M9 and Antonovka, respectively (P = 0.0396), but sporulation on the surface of lesions only developed on B9, on 16.8 % of the transplants (P = 0.0381).

Discussion

In the present experiments, it was documented that both rootstocks B9 and M9 Lancep at a size ready for propagation (> 6 mm stem diameter) became infected by *N. ditissima* after

inoculation (Figure 2). Transplants or smaller sized rootstocks (< 5 mm stem diameter) of B9, M9 and Antonovka developed sporulating lesions after inoculation. In the year before M9, M26 and MM106 developed internal necrosis only (Table 4). In the experiment with transplants, B9 developed more sporulating lesions than M9 and Antonovka. In general, the amount of infections were also higher on final sized rootstocks of B9 compared to M9. As far as we are aware, this is the first documentation that indicates differences in susceptibility to *N. ditissima* among commonly used rootstocks when tested *in vivo*. Recent *in vitro* testing showed that M9 clone 337 was more susceptible than the other rootstocks tested, including MM106 (Gòmez-Cortecero et al. 2016). Rootstock B9 was not included in that investigation.

A wound or a natural opening is required for infection of *N. ditissima* (Swinburne 1975). In the present experiments, wounds on both transplants and final sized rootstocks became infected after inoculation (Table 4, 5, Figure 1, 2, 3, 4). During production of rootstocks, both natural openings as leaf scars and wounds after treatments such as defeathering, heading back and root pruning as well as mechanical damage occurs. The risk of wounds becoming infected was indicated by the experiment with spore suspensions where an average of 25 % of the rootstocks became infected and developed symptoms during a short experimental period (up to 15 weeks). The risk of infection was strongly reduced if the inoculum was in contact with the wound one day after wounding vs. immediately after wounding (Figure 4). In experiments with inoculation of leaf scars with *N. ditissima*, they rapidly became less susceptible than if inoculated immediately after leaf removal (Crowdy, 1952).

In the present experiments, there were differences in growth patterns of topped and non-topped and of defeathered and non-defeathered rootstocks. The topped and defeathered rootstocks developed new side shoots, while the non-topped and defeathered rootstocks developed longer top shoots and fewer side shoots. For none of the two inoculation methods

used, were there any differences in incidence of *N. ditissima* after inoculation of topped or non-topped rootstocks. Total regrowth was probably nearly similar for the different pruning strategies and they were similar in growth vigor. In general, it is expected that young and vigorous apple trees are more susceptible to *N. ditissima* than older trees (Swinburne, 1975; Weber, 2014).

When rootstocks were inoculated with map pins, there was a clear difference between tissues of different age (Figure 3, Table 3). Current season growth developed more infections after inoculation than 1-year-old tissue, expressed as more necrosis inside the stems, earlier formation of conidia on the lesion surface and wider lesions. This clearly indicates that the pathogen grows more rapidly in younger tissue. When inoculum was applied directly on a fresh made cut of a current season top shoot, it resulted in 100% infection. Conidia on the cut surface were likely transported into the tissue by capillary suction, as documented for *N. ditissima* on leaf scars in apple (Crowdy, 1952) and on heading back wounds following pruning of scion during production of apple (Mc Cracken et al. 2003).

Optimal temperatures for conidial germination of *N. ditissima* was reported to be 20 - 25 °C, but may occur from 6 - 30 °C, and disease incidence increased linearly between 5 and 20 °C (Latorre et al. 2002). In 2015, the daily mean temperatures outside the plastic tunnel in the experimental period from May to August 2015 at Ullensvang, were between 5 and 20 °C (except one day). In 2014, July had 13 days with daily mean temperatures above 20 °C. No infections occurred on transplants inoculated with suspensions in July 2014, contrary to in July 2015. High temperatures in July 2014 may thus have completely stopped the disease development.

Root primordia forming on the rootstock part at the tree base, have been proposed as possible entry points for canker infections due to their rapid growth, formation of cracks and

the prolonged periods of wetness at the tree base (Swinburne 1975). Root primordia did not develop during the period of assessments of the rootstocks in the present experiments and were thus not assessed.

In UK, artificial inoculation with *N. ditissima* was carried out following defeathering of rootstock M9 (McCracken et al., 2003). They applied spore suspensions directly into three defeathering wounds on each rootstock, followed by budding with cv. Queen Cox. Depending on inoculum concentration, 80 to 100 % of the trees developed cankers prior to planting, i.e. 21 months after inoculation. Of the trees with cankers, 94% were dead before planting. Even at the lowest inoculum concentration, only a few trees survived, indicating how fatal infections of *N. ditissima* on rootstocks can be on young trees.

The risk of infection during propagation is dependent on the level of available inoculum, the presence of open wounds in the plant tissue and climatic conditions. If an infection takes place, it may develop visible symptoms already four weeks after inoculation. Careful monitoring of rootstocks prior to propagation would probably reveal most of such infections. Even on 1-year-old wood, rootstocks developed infections after inoculation in the present experiments. It is thus likely that infections may establish at time of propagation of trees if inoculum is present.

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Figure 1. Positions of map pin inoculation of *Neonectria ditissima* in final sized apple rootstocks; in 1-one-year old wood of M9 (A), in a defeathering wound on B9 (B), in a newly emerged shoot on M9 (C), and at the base of a side shoot of M9 (nine weeks after inoculation) (D). Cankers of *N. ditissim*a as a result of inoculation; with spore suspension on defeathered rootstock B9 (E), with a mycelia contaminated map pin in 1-year-old wood of B9 (F) and M9 (G), and on a newly emerged and topped shoot of M9 (H). Necrotic tissue inside a small rootstock of M9 inoculated with a map pin (I).

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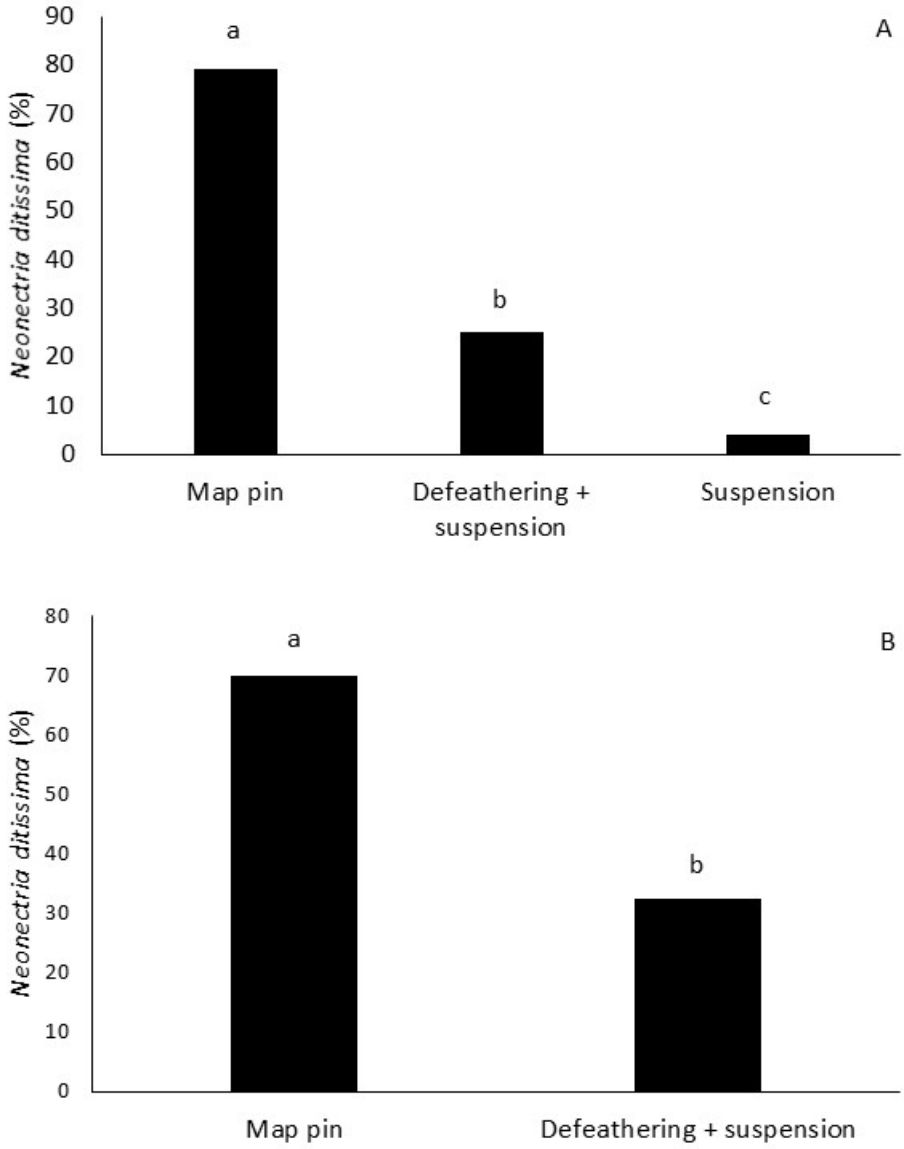

Figure 2. Incidence (%) of *Neonectria ditissima* after inoculation with contaminated map pins (Map pin) or spore suspensions applied to rootstocks M9 at Ås or B9 at Ullensvang, and wounded by removal of side shoots (Defeathering + suspension) or non-wounded (Suspension, only at Ås). The leader shoot was topped or kept non-topped at Ullensvang. Mean of two experiments, each including three replicates with four plants in each at Ås and four replicates with five plants in each at Ullensvang. Assessments made 15 (A) or 11 (B) weeks after inoculation. Bars with different letters are significantly different according to Student Newman Keuls test at P = 0.05.

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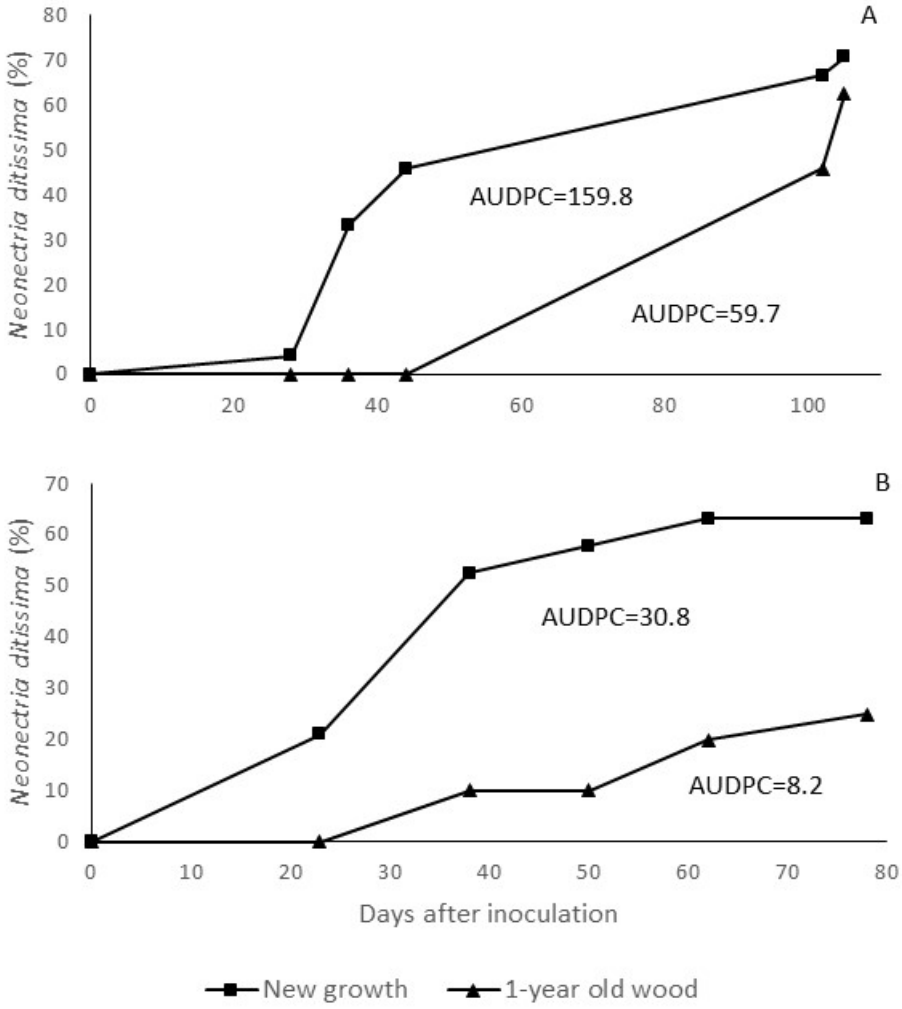

Figure 3. Incidence (%) of symptomatic infections caused by *Neonectria ditissima* recorded in days after inoculation with map pins containing mycelia of the fungus; either in newly emerged growth or 1-year-old wood, directly in the shoots on M9 at Ås (A) or in defeathering wounds on B9 at Ullensvang (B). Mean of two experiments for each rootstock; three replicates with four plants in each for M9 and four replicates with five plants in each for B9. Area under the disease pressure curve (AUDPC) calculated for each category of tissue.

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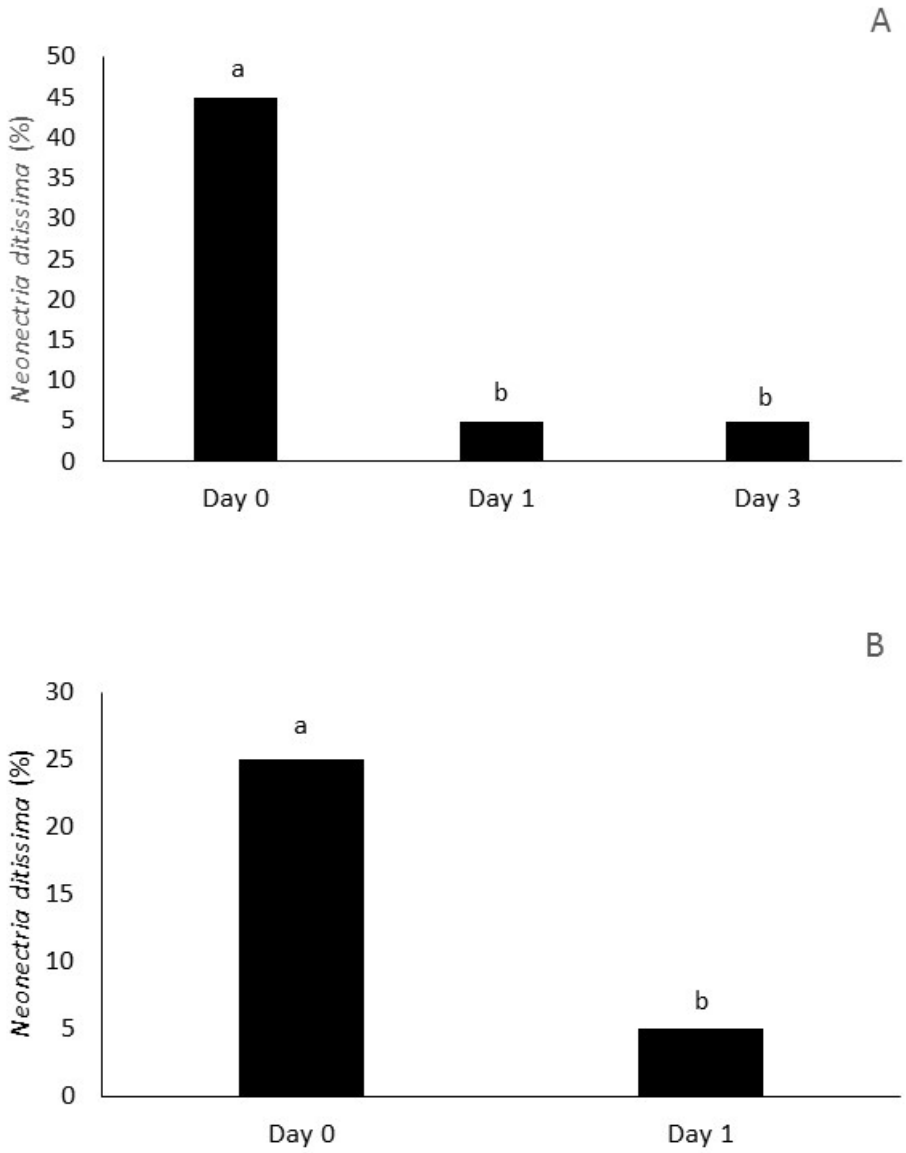

Figure 4. Incidence (%) of *Neonectria ditissima* when inoculation with spore suspensions took place after removal of side shoots (defeathering) and topping on rootstocks B9 (A) or M9 (B); either immediately after defeathering (Day 0), or one (Day 1) or three (Day 3, only B9) days after inoculation. Mean of one experiment including five plants in each of four replicates for each rootstock. Assessments made 11 (A) or 9 weeks after inoculation (B). Bars with different letters are significantly different according to Student Newman Keuls method at P = 0.05.

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Table 1. Overview of vigour (defeathering and topping) treatment and inoculation of *Neonectria ditissima* on commercial sized rootstocks in 2014.

Locations, rootstock	Vigour treatment[a]	Inoculation method[b]	Date of inoculation and duration of experiment
Ås			
M9	Defeathered	Suspension d0	April 7 & 8 (exp. 1 and 2), 15 weeks
	No treatment	Suspension d0	
	No treatment	One map pin in stem and one in top	
Ullensvang			
B9, exp 1	Defeathered+topped	Suspension d0, d1, d3	May 20, 11 weeks
	Defeathered+topped	Map pins in two wounds	
	Defeathered	One map pin in wound and one in top	
M9, exp 2	Defeathered	Suspension d0	May 21, 11 weeks
	No treatment	Suspension d0	
	Defeathered	In two wounds	
M9, exp 3	Defeathered	Suspension d0	June 2, 9 weeks
	Defeathered	Suspension d1	
	Defeathered+topped	Suspension d0	
	Defeathered+topped	Suspension d1	
	Defeathered+topped	Map pins in two wounds	
	No treatment	In side shoot basis and top	
M9, exp 4	Defeathered June 2+topped June 18	Droplet on top wound	June 18, 8 weeks
M9, exp 5	Defeathered+topped	Two map pins in top	June 27, 7 weeks

[a]Defeathered was removal of sideshoots, topped was main leader headed back

[b]Suspension d0 means that the spore suspension was sprayed on at the same day as the vigour treatment, d1 the day after or d3 three days after. Spore suspension had about 10^5 spores per ml.

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Table 2. Overview of experiments in 2014 and 2015 on transplants of apple rootstocks wounded by removing the lower leaves and inoculated with *Neonectria ditissima*.

Year, rootstocks	Number of plants inoculated per rootstock[a]	Inoculation method	Start date (duration until assessment)
2014, M9, MM106, M26			
	15	Suspension[b]	July 1 (12 weeks)
	15	Suspension	July 8 (11 weeks)
	15	Suspension	July 22 (9 weeks)
	15	Map pin in wound	July 22 (9 weeks)
2015, M9, B9, Antonovka (Ant.)			
	15	Map pin in wound	July 8 (12 weeks)
	15 (M9 and B9), 6 (Ant.)	Map pin in wound	July 14 (10 weeks)
	15 (M9 and B9), 6 (Ant.)	Map pin in wound	July 29 (8 weeks)
	15 M9, 12 (B9 and Ant.)	Two map pins in wounds	July 30 (8 weeks)

[a]Non-inoculated controls per rootstock were 5 in 2014 and 3 in 2015.

[b]The suspension contained about 10^5 spores per ml.

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Table 3. Climatic conditions[a] during the experimental period in 2014 and 2015 at Ullensvang; number of days with daily mean temperature above 5 and below 20 °C (D), monthly mean temperature in °C (Temp) and number of days with precipitation above 0.2 mm per month (Prec) in April to August[b].

	April			May			June			July			August		
	D	Temp	Prec	D	Temp	Prec	D	Temp	Prec	D	Temp	Prec	D	Temp	Prec
2014	27	8	10	31	12	12	30	15.8	11	18	19.2	18	31	15.5	24
2015	19	5.9	13	31	8.8	23	30	12.1	13	30	14.4	18	31	15.0	25
Tunnel 2015	n.a.[c]	n.a.	n.a.	30	14.2		22	16.7		25	17.9		29	16.9	

[a]Data from a climate station 150 m away from the experimental site (in 2014 and 2015) and from a data logger inside the high plastic tunnel (in 2015).

[b]indicating days with less direct sun and less risk of high temperatures inside the plastic tunnel.

[c]n.a. = not assessed.

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Table 4. Effect of tissue age of rootstock B9 (new shoots or 1-year-old wood) on development of *Neonectria ditissima* assessed 11 weeks after inoculation with map pins in wounds after removal of side shoots (defeathering) and in non-topped leader shoots.

Tissue	Sporulation before incubation (%)[a]	Sporulation after incubation (%)[a]	Diameter of the stem (mm)[b]	Lesion width (mm)	Lesion length (mm)	Internal necrosis (%)
New shoot growth	61.1[c]	66.7	7.7	12.1	19.4	83.3
Wound in 1-year-old wood	25.0	45.0	7.4	9.4	17.8	40.0
P-value	0.0252	0.3746	0.7563	0.0023	0.9268	0.0072

[a]Incidence of rootstocks with formation of conidia (sporulation) at final assessment, before and after incubation.

[b]Measured below the infection site on the stem.

[c]Mean of one experiment including four replicates and five rootstocks of each treatment. P-values below 0.05 indicates that the two mean values in each column are different according to Student Newman Keuls method.

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Table 5. Incidence of internal necrosis and incidence of lesion forming conidia (sporulation) after incubation of transplants of apple rootstocks inoculated with map pins containing mycelia of *Neonectria ditissima* in 2014 and 2015.

2014		2015		
Rootstock	Necrosis (%)[a]	Rootstock	Necrosis (%)	Sporulation (%)[b]
M9	40.0[c]	B9	42.2 a[d]	28.9 a
M26	66.7	M9	26.7 ab	8.9 b
MM106	40.0	Antonovka	11.3 b	4.0 b
P-value	0.2247	P-value	0.0294	0.0032

[a]None of the rootstocks developed sporulation after incubation, 8-12 weeks after inoculation.

[b]After incubation of transplants in moist chambers for 14 days at 20 °C.

[c]Mean of one inoculation point on each of five rootstocks in three replicates per type in 2014 and three experiments each with 3 (replicates) × 2-5 plants per rootstock in 2015, assessed 8-12 weeks after inoculation.

[d]Values denoted with different letters are significantly different according to Student Newman Keuls method at P = 0.05.