Phenology and Spatial Dynamics of *Cacopsylla melanoneura* (Homoptera:Psyllidae) in Western Norway, an Insect Vector for Apple Proliferation

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Front page: Cacopsylla melanoneura at Loftesnes, Sogndal, 26.4.2016. Photo: Åsne Brede

Photos by Bjørn Arild Hatteland (Fig 9,12), all other photos are by the author.

Preface

This thesis is a part of a research project called «Plant quality adapted to a modern and sustainable Norwegian strawberry and apple industry (PlantQuality)», conducted by NIBIO (Norwegian Institute of Bioeconomy Research) and funded by the Norwegian Research Council.

I would like to give a great thanks to my supervisor *Bjørn Arild Hatteland*, for all the help in conducting the study, in driving me around, in writing, and keeping up with me in our travels from Bergen to Hardanger, and Sogn.

I would also like to thank *Marianne Hotl*e and *Marianne Bøthun* for collecting sticky traps, and *Monica Skogen* for the PCR analysis. Thank you, *Ynghild Gilje Storha*ug for collecting traps, for helping in the lab and for keeping me company in Lofthus. Thanks to *Olav* and *Sissel Opedal, Olav Utne, Odd Eirik Loftesnes* and *Njøs Næringsutvikling* for letting me use their orchards for research. Thanks to my family, and friends for being supportive and cheering me on, especially *Camilla Elmar, Maren Elise Nordheim* and *Marit Stranden* for feedback on my writing.

Bergen, June 1st, 2017

Abstract

In the last decade, the plant disease apple proliferation has caused problems for Norwegian fruit farmers, resulting in smaller and un-tasty apples, rendering them unsellable. The disease is caused by the phytoplasma *Candidatus* phytoplasma mali, a bacteria residing in the phloem of the plant. Apple proliferation is spread by infected propagation material, vascular connections and insect vectors. There is no cure for the disease, and to limit further spread, infected trees have been removed. Nonetheless, apple proliferation remains a severe problem. In Norway, the phytoplasma is believed to be spread by an insect vector; *Cacopsylla melanoneura*, as the main European vector *Cacopsylla picta* is not found in Norway. The aim of this study was to map the phenology of *C. melanoneura*, and to find if their occurrence in apple orchards can be predicted based on infected and healthy apple trees. This was done by monitoring populations of *C. melanoneura* in four apple orchards in western Norway by using yellow sticky traps and the beat-tray method, from March to June. The results show that *C. melanoneura* leaves their overwintering habitat early in the spring before bud break, when the temperature reach somewhere between 7 and 8.4°C. There was a population peak in mid-April, and the old generation of psyllids was gone by the first weeks of June. The main reproductive period seemed to occur in the April-May transition, and nymphs appeared from the end of May, when the apple trees were flowering. There was no indication of attraction towards infected or healthy trees in the overwintered generation of *C. melanoneura* in this study, but there was a significant result of larger trees hosting more psyllids than smaller trees in the same field, and thus may be a source of large quantities of infected nymphs if the larger trees are infected.

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Introduction

Fifty years ago, cell wall lacking mycoplasma like organisms (MLO's) was discovered in the phloem of plants carrying yellows-type diseases (Doi et al. 1967). As these types of diseases were previously believed to be caused by viruses, this discovery had a large impact on the field of plant pathology (Bertaccini & Duduk 2009). Later this group of prokaryotes was placed in a new taxon called *Candidatus* phytoplasma, commonly called phytoplasma. Phytoplasmas are obligate parasites as they lack the means of synthesizing several compounds necessary for survival, and they need to acquire these components from the host species (Bertaccini & Duduk 2009). The bacteria can be introduced and spread between host plants due to propagation or grafting of infected plant materials, vascular connection between plants trough parasitic plants or coalescence of roots, or by phloem-feeding insect vectors (Weintraub & Beanland 2006). The phytoplasmas are the smallest among the bacteria in both size and genome (Hoshi et al. 2007), and because of their uneven distribution and low concentration in the phloem, the disease is hard to detect. Today, the polymerase chain reaction (PCR) technique is redeemed sufficient for detecting the bacteria in both plant material and in their insect vector (Bertaccini & Duduk 2009).

Over 700 plant species have been found to suffer from phytoplasma infection, where the bacteria cause a variety of symptoms among them; abnormal growth, greening of flowers, small and un-tasty fruits, yellowing and withering of plants (Hoshi et al. 2007). Abnormal growth include late shoots, witches brooms, enlarged stipules, and root shoots (Blystad et al. 2014), Many of these species are important agricultural plants, and phytoplasma diseases causes severe economic damage all over the world. Among these diseases are the European stone fruit yellows, pear decline and apple proliferation, causing significant financial loss to the fruit industry in Europe. Apple proliferation (AP) is caused by *Candidatus* phytoplasma mali, and was first discovered in northeastern Italy in the Trentino region, in the middle of the 1990's (Tedeschi et al. 2012). AP has since then caused serious economic damage to the apple production industry. This disease cause changes in the tree's priorities, investing more energy in the growth of shoots and leaves, and less in the production of fruit, which has an impact on both apple quality and quantity (Jarausch et al. 2011). Fruit size might be reduced as much as 50 %, and weight by even more as a result of this disease (Malagnini et al. 2013). Color and taste is also influenced, making as much as 80 % of the harvest unsellable (Tedeschi et al. 2012). Throughout the year, the concentration of the bacteria fluctuates in the

tree sections above ground. Seemüller et al. (1984), showed that the phytoplasma dies in all aerial parts of the tree during winter, but survives in the roots and recolonize the trees from April/ May in Germany. A study of the seasonal colonization of *Ca.* P. mali in north-eastern Italy by Baric et al. in 2004-2005, showed that the concentration of the bacteria is highest in the shoots from September to February, and in the roots from February to April. How sufficient the phytoplasma spreads from the roots to the rest of the tree varies according to weather conditions and growth (Blystad & Brurberg 2016). Symptoms of phytoplasma diseases varies from year to year depending on the concentration of bacteria in the phloem tissue of the shoots and stem, and may not be evident at all even if the tree is infected, as the symptoms is independent of the concentration of the bacteria in the roots (Baric et al. 2011).

Insect vectors are important in the spread of phytoplasma diseases. As the phytoplasma resides in the phloem of the plant, vectoring the disease is restricted to phloem feeding insects, and thereby limited to certain subgroups within the order Hemiptera. Only insects within three superfamilies have been confirmed as vectors of phytoplasma. These are the Membracoidea, the Fulgoroidea (planthoppers) and the Psylloidea (the jumping plant lice). The Membracoidea contains the family Cicadellidae (the leafhoppers) where you find the largest number of phytoplasma vectors. The Psylloidea are found within the suborder Sternorrhyncha, where there are two families of phytoplasma vectors, the Psyllidae and the Triozidae (Weintraub & Beanland 2006). AP and *Ca*. P. mali are proven to be spread by the psyllid *Cacopsylla picta* (Foerster) (Syn. *C. costalis*) in northeastern Italy, Germany, northern France, and northern Switzerland (Jarausch et al. 2007), and by *Cacopsylla melanoneura* (Foerster) in northwestern Italy (Tedeschi et al. 2012; Jarausch et al. 2011). In Spain, both species are associated with the presence of AP, and they are not present in areas without the disease (Laviña et al. 2011).

The symptoms we today associate with AP has been observed in Norwegian apple orchards since the 1970's. The first registration of AP in Norway was in 1996, but only on singular trees (Blystad et al. 2011). It wasn't before 2010 that AP became registered as a problem in several Norwegian apple orchards. The disease was then reported in ten orchards in Sogn, and one in Telemark county. In these orchards, the symptoms of AP were very apparent in the entire field and on several cultivars of apple. The disease was especially evident on the apple cultivar "Discovery", which has shown significant size reduction, bad taste and poor coloration in the fruit (Hatteland et al. 2016). A risk evaluation by the Norwegian Scientific Committee for Food Safety (VKM) in 2012 concluded with high possibility for further

transmission and economic consequences of AP in Norway (Sletten et al. 2012). AP is marked as a quarantine pest. According to the guidelines of plant health, you are required to notify the Norwegian Food Safety Authority if there is a suspicion of AP (Forskrift om plantehelse 2000). It is recommended to remove the infected trees, and the farmers are not allowed to distribute infected propagation material (Ihlebekk Hauger 2017). During a survey in 2013, AP was found in all fruit districts in Norway (Blystad et al. 2014). How the disease is spreading in Norway is still not established; the pattern of infected trees does not indicate a spread through the coalescence of roots, and the testing of grafted trees from the same plant material, does not indicate the original plant material being infected (Blystad et al. 2014). This leaves an insect vector as a feasible candidate for spreading AP. A survey of potential vector candidates was conducted in 2013, through the project "PlantQuality" (Nestby et al. 2014), using yellow sticky traps and the beat-tray to collect insects from orchards in Hardanger, Lier, Sogn and Telemark. The main European vector, *C. picta,* was not found in any of the districts, but *C. melanoneura* was well represented in most orchards, causing this species to be the main candidate as an insect vector for AP in Norway (Hatteland et al. 2016). Using real-time PCR (Nikolić et al. 2010), *C. melanoneura* proved to be infected with *Ca.* P. mali in all four districts. In western Norway 30 % and 21 % of the population of *C. melanoneura* carried the phytoplasma in Sogn and Hardanger, respectively (Hatteland et al. 2016). This is very high compared to studies in Italy, where 3.6% of the population in northwestern Italy (Tedeschi et al. 2003), and 6.25 % at a field in northeastern Italy (Tedeschi et al. 2012), carried the phytoplasma. However, the actual transmission of the phytoplasma from the insect into the phloem of trees has not yet been confirmed in Norwegian populations of *C. melanoneura* (Hatteland et al. 2017). This does not exclude the psyllid as a vector, and transmission trials are still ongoing. As *C. melanoneura* at present is the only candidate insect vector for transmitting the disease in Norway, understanding the phenology and spatial distribution of this species is of importance.

C. melanoneura, also known as the Hawthorn psyllid, was reported by Ossiannilsson in 1992 as being widespread in the county of Akershus, but also found in the counties Telemark, Buskerud, Hordaland, and Nordland. The species was referred to as uncommon by Edland in 2004. However, according to a survey of potential vectors for AP, the species is currently common in all fruit districts of Norway (Hatteland et al. 2016). The species is oligophagous, and reproduce mainly on hawthorn (*Crataegus monogyna, C. oxyacatha, C. maximowiczii*), but is also found on apple (*Malus communis*) and pear (*Pyrus communis*) (Ossiannilsson

1992). *C. melanoneura* were reported as a pest on apple in Norway in the late 1980's in Telemark, but the species has not been shown to cause any direct damage to trees (Edland 2004). However, *C. melanoneura* have occurred in large numbers the last years (Hatteland et al. 2016), and as they produce large quantities of honey dew that could create a gateway for fungi such as botrytis (Edland 2004), their presence might cause damage to the fruit trees.

C. melanoneura has one generation per year, and hibernates as adults on conifers from July to early spring, when they re-migrate to hawthorn / apple / pear. According to Tedeschi et al. (2012), migration is dependent on a temperature threshold, which in northwestern Italy has been found to be around a temperature of 9.5°C. In Norway, the temperature threshold is assumed to be lower (around 8°C), since they have already migrated into the field when sticky traps have been collected in early March (Hatteland et al. 2016). According to Ossiannilsson (1992) and Edland (2004), the Scandinavian populations of the species start their egg-laying mid-May and this lasts to the end of June. The first instar nymphs are found at the end of May, and the last of the latest instar in late July. New adults emerge in June and July, and the migration of the new generation from the reproductive host plant to conifer trees in July and August. The old and the new generation does not overlap, and are distinguishable due to coloration difference. The young adults are orange with some white coloration on pronotum and genal cones, and yellow veins on the forewing, while the overwintered adults are darker, more red in the coloration, with brown to black forewing veins (Fig. 1) (Ossiannilsson 1992). Males and females are easily distinguishable by the terminalia, the final segment of the insect abdomen (Fig. 1). The male terminalia consist of a paramere pointing upwards creating a triangle with the proctiger hiding the aedeagus (Fig.2). The female terminalia consist of a proctiger and a subgenital plate protecting the ovipositor. The females are often larger than the males as they measure 2.95-3.3 mm, whereas the males are 2.52-3.1 mm (Ossiannilsson 1992).

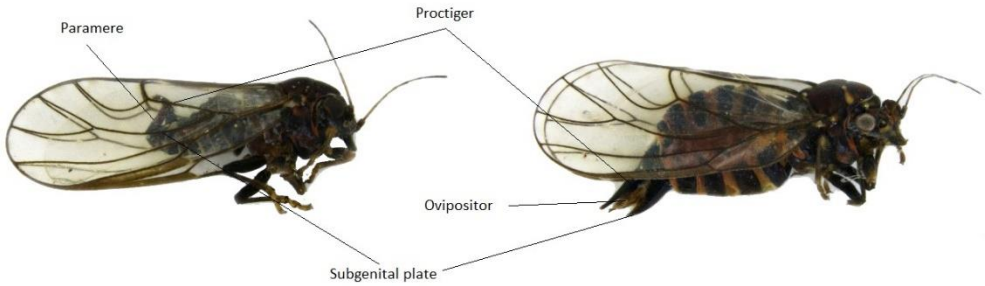

Figure 1: *C. melanoneura* male (left) and female (right) found at the Opedal field in 2017.

Another psyllid that could be found on both hawthorn and apple is *Cacopsylla affinis*, a species almost identical to *C. melanoneura*. Only male specimens of the two species are distinguishable by a small difference in the paramere and the terminal part of the aedeagus (Fig. 2) (Ossiannilsson 1992). The species is rare, but has been observed in Hardanger (Bjørn Arild Hatteland 2016, pers. com.). *C. affin*is has one generation per year, and hibernates during the autumn and winter on conifer trees like *C. melanoneura*. The adults of the species could possibly be found in the orchards from early/mid spring to June. Little is known about the vector capabilities of *C. affinis*, but the species is not currently regarded as a vector of AP, as *Ca.* P. mali has not been found in collected specimens (Tedeschi & Nardi 2010).

Figure 2: Aedeagus of *C. melanoneura* (left) and *C. affinis* (right). The Insects were found in Opedal 2017.

The main vector of AP in Europe is *C. picta*, a species not observed in Norway to date, but has been documented in Sweden (Ossiannilsson 1992) and in Finland (Lemmetty et al. 2011). *C. picta* has a life cycle similar to *C. melanoneura,* with one generation per year and a hibernation period on conifer trees. *C. picta* is monophagous to apple, and migrates into the fields and start oviposition later than *C. melanoneura*. *C. melanoneura* is the main vector for

AP in northwestern Italy (Tedeschi et al. 2002). In Germany however, *C. melanoneura* is proven not to be a vector for the disease even though both *Ca*. P. mali and *C. melanoneura* is present (Mayer et al. 2009). Experiments in Germany using DNA extraction and PCR analysis, shows that only 0.07 % of *C. melanoneura* (n=409) collected in AP afflicted orchards carried the disease, and even these did not have a high enough titer of the bacteria to transmit the disease, having 10 000 times less concentration of the bacteria than *C. picta* (Mayer et al. 2009). This suggests genetic differences within *C. melanoneura* populations in the efficiency of acquiring and transmitting the disease. Or alternatively, different strains of *Ca.* P. mali could favor different vectors (Baric et al. 2010). *C. picta* is not observed in northwestern Italy, as it is in the Trentino region in northeastern Italy where *C. picta* is the main vector (Tedeschi et al. 2002). It might be that *C. melanoneura* only develops the ability to transmit the disease when *C. picta* is absent, as *C. picta* is a much more efficient vector. This could mean that *C. melanoneura* has become more efficient at spreading the disease in Norway in the last decade, because of the absence of a more efficient vector.

As the concentration of bacteria is low in the aerial sections of the tree in the spring (Seemüller et al. 1984; Blystad et al. 2014), the vector most likely will have to acquire the phytoplasma from the shoots in the summer, and the bacteria multiply in the gut of the insect (Weintraub & Beanland 2006) during the psyllid's hibernation period. Tedeschi and Alma (2004) showed that nymphs of *C. melanoneura* can acquire the bacteria, but no transmission by nymphs was observed. In the same experiment, 70 % (n=10) of naturally infected, overwintered adults in a heavily infected orchard (85 % of the trees showing symptoms of AP) transmitted the disease, compared to eight less infected orchards (0-85 % trees showing symptoms) where 4.5 % (n=63) of the insects transmitted the disease. Multiplication of *Ca*. P. mali within the host *C. picta* and *C. melanoneura* was shown by Pedrazzoli et al. (2007), where specimens reared on infected plants for only one to six days and then moved to healthy plants for three to four weeks showed a significant increase in the titer of *Ca*. P. mali, compared to specimens that was frozen just after acquisition. However, the experiment also showed few infected adults of *C. melanoneura* at the beginning of migration (Pedrazzoli et al. 2007). The same results are apparent in experiments from Torino in 2000 and 2001 (Tedeschi et al. 2003), where very few adults collected early in spring tested positive for *Ca*. P. mali using PCR, and that the number of infected *C. melanoneura* increased during the season. In contrast, Jarausch et al. (2011) described a high titer of *Ca*. P. mali in the remigrants of *C. picta*, which showed no significant increase throughout the season. Either way, it is the

overwintered adults that seems to be important in the transmission of *Ca*. P. mali (Tedeschi & Alma 2004). Many questions have been raised the last thirty years about the relationship between the vector and the phytoplasma that remains unanswered. This includes the effect the phytoplasma has on the insect, whether this relationship is harmful, helpful or neutral to the vector. Earlier research suggested that the phytoplasma had a negative effect on the vector, but more recent studies believe the relationship is mutualistic (Weintraub & Beanland 2006). If the phytoplasma makes the host plant more suitable for the vector in terms of reducing the chemical defenses, or increase the availability of nutrients, vectoring the disease will increase the fitness of the insect. In addition, such mechanism will likely increase the attraction between the vector and the infected plants (Weintraub & Beanland 2006). This attraction might also benefit the phytoplasma as the vector acquire the disease for further distribution. *Ca*. P. mali has been shown to cause higher emissions of the natural sesquiterpene β-caryophyllene in the period between hatching and migration of the vector *C. picta*, altering the odor of the tree. This proved to attract young adult specimens of the vector (Mayer et al. 2008). This indicates that infected trees in the field will serve as hosts to more young specimens of *C. picta* than uninfected trees, and thus have a higher chance of acquiring the disease before the insect emigration. The opposite behavior is shown when they return to the field and prefer to oviposit on healthy trees, thereby spreading the disease (Eben & Gross 2013). β-Caryophyllene has not shown to alter the behavior of *C. melanoneura* (Eben & Gross 2013).

If *C. melanoneura* is a vector of AP in Norway, understanding the movements of the overwintering generations is central in giving control and pest-management recommendations. This thesis is a part of a larger study (2013-2016) conducted by the Norwegian Institute of Bioeconomy Research (NIBIO) called «Plant quality adapted to a modern and sustainable Norwegian strawberry and apple industry (PlantQuality)». The main aim is to further describe population dynamics and behavior of *C. melanoneura* in Norway. This survey focuses on the overwintered generation, since the hibernated adults are potentially the main distributor of disease. This study was split into two main parts, describing the phenology, and the spatial distribution of the insect. In the phenology survey, the insects were collected by yellow sticky traps and the beat-tray, and compared between four fields. The aim of this survey was to i) analyze the temperature data when *C. melanoneura* entered the fields, to see if remigration can be predicted, and ii) describe the change of population densities, including the male and female ratios. In the spatial distribution study, the insects were

collected by the beat-tray per tree, and compared with other trees within the same field. The aim of this study was to look at the pattern of distribution of psyllids within the field, to test whether AP-infected trees attract psyllids to increase the uptake of the disease, or deter the psyllids onto healthy trees for increased transmission of the disease.

Material and Methods

Insects were collected in the spring of 2016 in four different apple orchards in western Norway. All the fields are located in areas of intense fruit farming in the Hardanger and Sogn districts in Western Norway. The fields are sloped with the lower end in the direction of the fjord, and the upper end in the direction of the forest. The fields were selected based on previous knowledge of AP-infected trees as well as high abundance of *Cacopsylla melanoneura* (Nestby et al. 2014; Hatteland et al. 2016). To collect the insects the beat-tray and yellow sticky traps were used, in addition to a leaf collection.

The study sites

Opedal

In Lofthus, Ullensvang, Hordaland (Coordinates WGS84 60°19'23"0N 6°39'51" E,), the Opedal orchard was used for both the spatial distribution study and the phenology survey. The closest forest of spruce was located 300 meters to the east (Fig. 3). The field was planted in 2005 and consisted of 799 apple trees of the cultivar "Discovery" in five rows of apple (158 - 161 trees per row). The trees were of approximate the same size. The field was mapped for symptoms in 2014 and 151 samples of roots and branches from the field were tested for *Ca.* P. mali, using real time PCR (Nikolić et al. 2010), 64 of these samples detected the bacteria (Blystad & Brurberg 2016). *C. melanoneura* has also been sampled from this field and tested for *Ca*. P mali in 2016 of which 6 % (N=375) were found to be positive for the phytoplasma (Hatteland et al. 2017). Both beat-tray samples and yellow sticky traps were collected in the field, and the latter extended into the neighboring apple field to the right (Fig. 4)

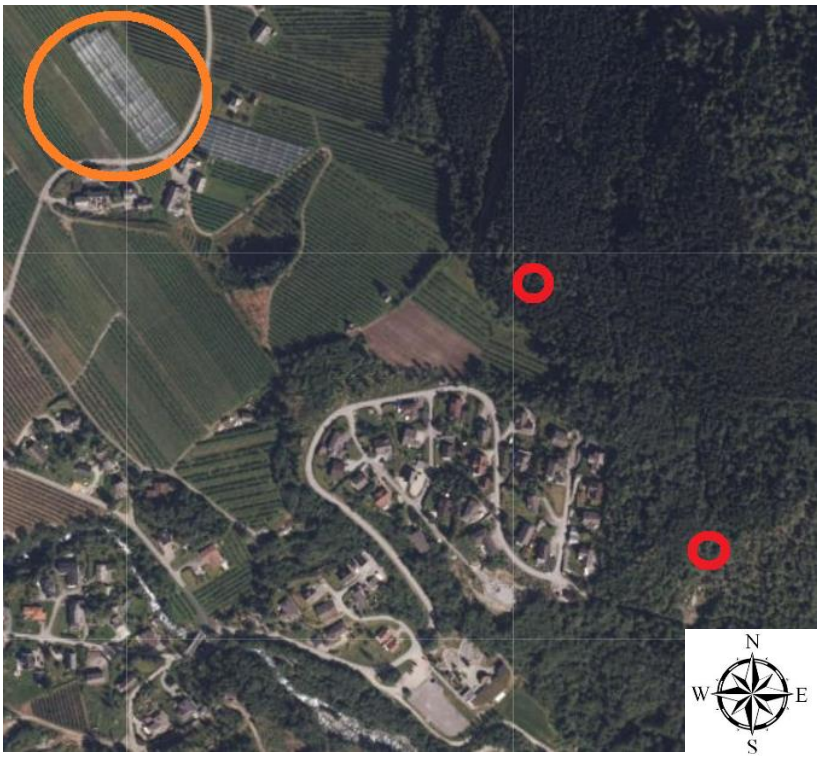

Figure 3:The Opedal field (orange circle) in proximity to the forest. The red circles indicate where the temperature loggers were installed. The red circle closest to the field is referred to as the “lower forest”, the other as the “upper forest”. The fjord is located to the west of the picture.

Figure 4: The Opedal field from above. The yellow spots mark the positions of the yellow sticky traps. The red spot mark the temperature logger. The field is sloped uphill from the northern to the southern end of the field.

Utne

A second orchard was selected at Utne, Ullensvang, Hordaland (Coordinates WGS84 60°25’15” N 6°37’41” E), which is situated on the other side of the fjord relative to the Opedal orchard. The Utne-orchard was used for the phenology survey. The field consisted of 28 rows of “Discovery” apples. The trees in this field were similar to each other, and small in size. The forest was located just 40 meters to the south. Yellow sticky traps were placed at three locations (Fig.5), and the beat-tray were used to collect insects.

Figure 5: The Utne field from above. The yellow spots marks the approximate position of the yellow sticky traps. The field was sloped from the fjord as the lower end, towards the forest (north to south).

Loftesnes

In Sogndal, Sogn og fjordane (Coordinates WGS84 61°14’21” N 7°08’04” E), eight rows of the Loftesnes-orchard were used for both the spatial distribution study and the phenology survey; four rows consisted of “Discovery” apples, and four rows of “Aroma” apples. The forest was located directly to the east of the field (Fig. 6). Three yellow sticky traps were used to collect insects for the phenology survey, and the beat-tray to collect for the spatial distriution study (Fig. 6). The trees in this field was highly variable in size and age (Fig. 7).

Figure 6: The Loftesnes field from above. The green line mark the part of the field used for distribution survey, and the yellow spots mark the approximate position of the sticky traps. The field was sloped from the “lower” sticky trap to the “upper”.

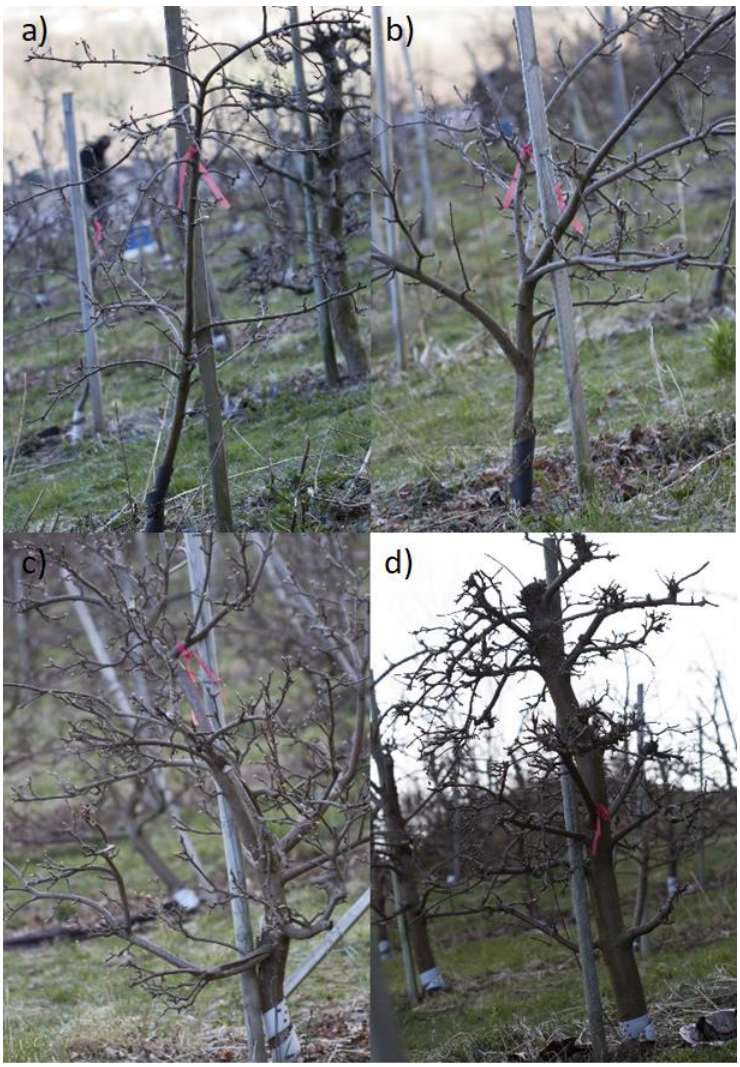

Figure 7: Apple trees in the Loftesnes field in the end of April, categorized from small to large sizes a) 1 (smallest), b) 2, c) 3 and d) 4 (largest). Larger trees having more branches and thicker stems.

Njøs

An additional site was chosen at Njøs, Leikanger, Sogn og Fjordane (Coordinates WGS84 61°10’43”N, 6°51’39”E). A total of three yellow sticky traps were used to collect data for the phenology survey (Fig.8). The forest was located directly to the east of the field.

Figure 8: The Njøs field from above. The yellow spots mark the approximate position of the sticky traps. The field was sloped from the “lower” sticky trap to the “upper”.

Collection methods

The beat-tray method

The beat-tray method was used to collect insects in both the phenology survey and the spatial distribution study. The beat tray is a cloth funnel of 45x64 cm connected to a handle (Fig.9). The funnel leads into a paper bag attached by a rubber band. A stick covered in styrofoam was used to tap branches making the insects fall into the funnel. A total of three branches were beaten per tree for the spatial distribution study and one branch per tree for the phenology survey. Each branch was beaten three times. The branches were selected according to the criteria of being easy to reach, having room to place the cloth funnel underneath, and being able to withstand the beating. All collections using this method were done by the same individual to eliminate the error of collecting differently based on person. The collected insects were killed by freezing and stored at -20 ºC. The beat-tray samples were used to estimate population densities.

Figure 9: The beat-tray and styrofoam stick in action at the Utne field. No paper bag is attached to the funnel in the picture.

The Sticky traps

Yellow sticky traps of the type REBELL Amarillo, from Andermatt Biocontrol, were used for the phenology study. The traps were hung from a branch by a wire (Fig. 10), approximately in the middle of the tree. The traps consisted of two plates inserted into each other creating a cross, where all the plate surfaces were vertical. The plates were covered in glue which can endure even extreme weather conditions, according to the producer. When collecting the traps, the plates were detached from each other and wrapped in plastic film before frozen. Insects collected with the sticky traps were used to estimate activity in the apple orchards.

Figure 10: A newly assembled yellow sticky trap (Andermatt biocontrol), ready to collect insects at the Opedal field in April 2017.

Leaf collection

Twenty-five leaves were collected per tree on sixty trees for the spatial distribution study and placed in separate paper bags. This was done to estimate densities of *C. melanoneura* nymphs. The approximate same amount of leaves were taken from the lower, middle and higher part of the tree.

Identification

The beat-tray samples were sorted using a stereo microscope and *Cacopsylla* species identified using Ossiannilson F. (1992). Furthermore, the sex of all *C. melanoneura* specimens were identified. *C. melanoneura* was distinguished from the similar *C. affinis* by looking at the terminalia of all the male specimens. The aedeagus of the genitalia (Fig.2) was looked at in specimens of doubt. Only one *C. affinis* individual were found among the male specimens that was examined this way in a sample from the Opedal field. This confirms *C. affinis'* presence in very low numbers in the field. This small proportion is not accounted for, and they are all referred to as *C. melanoneura* for the rest of this thesis. Other clearly distinguishable species were removed from the sample, and not counted.

C. melanoneura collected on the yellow sticky traps were identified and counted trough the plastic film with the insects still on the trap using a stereo microscope. The sex of all the *C. melanoneura* specimens on the traps were also determined.

No "alive" *C. melanoneura* nymphs were found in the leaf collection, but empty remains of the last instar nymphs (Fig.11b) were counted trough the stereo microscope.

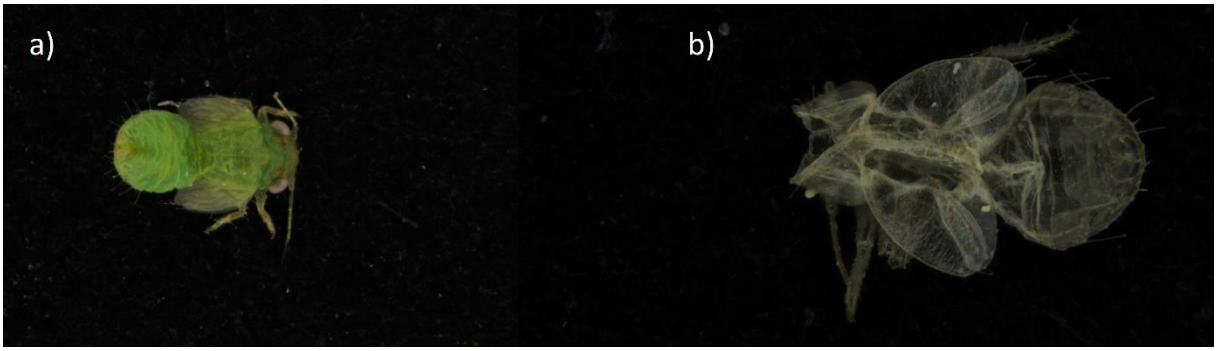

Figure 11: a) Nymph of *C. melanoneura* found in the beat-tray sample from the 7th of June, b) Remains of nymph found in the leaf collection the 22nd of June.

The Surveys

Phenology survey

Insects were collected from mid-February to the end of June 2016, using both the beat-tray and the sticky trap method.

The yellow sticky traps were used in all four orchards.
The total of nine sticky traps were set out in mid-February in Opedal (Fig. 4), because previous samplings have shown that *C. melanoneura* was already present in the orchards in the beginning of March (Hatteland et al. 2016). The traps were checked daily from the 7th of March when the temperature approached the assumed threshold for migration (8°C) to the 11th and then regularly from the 12th to the 18th of March. After the 18th, they were replaced with a new trap every week until the 27th of April, and then every other week until the 9th of June.
At the Utne field (Fig. 4) three traps were first set out the 17th of March, and then replaced with a new trap roughly every other week until the 9th of June.
At the Loftesnes field (Fig. 6) and the Njøs field (Fig. 7), three traps were set out the 23rd of March and the 17th of March, respectively, and replaced every week until the beginning of June. In all fields, the traps were spread out to cover the different parts of the orchard.

The beat-tray method was used to collect insects every second week from the 17th of March to the 9th of June in the Opedal, and the Utne field.
In the Opedal field, 66 different trees (Steiner 1972) were used for each collection date, resulting in the use of 462 trees. At Utne, which was a smaller field, 33 trees (Steiner 1972) were used, adding up to 231. One tree was never used twice for the phenology collection. All insects from the 66 / 33 trees were collected in the same paper bag (two bags in the flowering season, due to a lot of plant material falling into the bags).

Temperature was recorded using the software "Tiny Tag talk 2 data loggers" from Gemini data loggers, from 16.12.2015 to 30.06.2016 in the Utne- and the Opedal field, as well as in the forests close to Opedal (Fig. 3). The temperature loggers were placed approximately in the middle of the Utne field, and in the south end of the Opedal field (Fig. 4). Temperature data

from the Norwegian Meteorological Institute retrieved from https://www.yr.no were used to interpret phenology data from the fields in Sogn, from 27.2.2016 to 20.5.2016.

Spatial distribution survey

The spatial distribution of *C. melanoneura* within apple orchards was sampled two times from the fields of Opedal and Loftesnes using the beat-tray method and once at the Opedal field by collecting leaves. At Opedal, beat-tray samplings were carried out at the 1st, and 27th of April, and the leaf collection the 22nd of June. While the Loftesnes site was sampled the 4th and 25th of April. A total of 60 trees where used in the Opedal field, and 40 trees in the Loftesnes field. The insects were collected from individual trees and stored in separate paper bags. The same trees were used for all collection dates in both fields.
At the Opedal field, some of the trees were tested by real-time PCR for the AP disease in 2014, as well as surveyed for symptoms (Blystad & Brurberg 2016). Based on this data, 60 trees in the Opedal field were separated into four categories of 15 trees each: 1) Trees with clear symptoms and tested positive for disease, 2) Trees neighboring the trees in the previous point, but not tested for the AP disease, 3) Trees without clear symptoms, but have tested positive for disease, 4) Trees that have tested negative for disease and showing no symptoms.

At Loftesnes, no previous testing had been done, and the samples were collected from every fifth tree independent of how the tree appeared. Two branches from these trees were collected the 23rd of September to test for AP using molecular methods (real-time PCR) (Nikolić et al. 2010), but some of the tags were rendered unreadable between the collection date and the analysis, so only 30 of the 40 trees were tested. All the trees used for this study in both the Opedal and the Loftesnes field, were checked for symptoms in September 2016, as this is the period where the symptoms are the most evident (late shoots visible, and there are apples on the trees). Trees were scored for obvious symptoms like late shoots (Fig.12), shoots from roots, and small apples.

Figure 12: Shoots with a clearly different and lighter color than the rest of the tree, in the Opedal field, September 2016.

Statistical methods

Microsoft Excel was used to analyze the data for the phenology survey.

RStudio version 1.0.136 was used for statistical analyses for the spatial distribution survey (RStudio Team 2016)

The data collected for the spatial distribution survey was not normally distributed, and had an over-dispersion. To analyze this data, a generalized linear mixed-effects model (GLMM) with a quasi-poisson distribution was used. To create the spatial map, a grid (x, y) was made based on the actual position of the apple trees, where each coordinate had a distance of 0.5 m. These coordinates were incorporated into the model to make a spatial GLMM, adding the distance between the collection positions as a random effect, and adjusting for a possible spatial dependent result. This was done using the functions corSpatial and glmmPQL, in the respective packages "nlme" (Pinheiro & Bates 2000), and "MASS" (Ripley & Venables 2002).

The spatial GLMM was used to look at the differences in the population count of *C. melanoneura* per tree, using the explanatory variables of *positively tested / negatively tested / not tested* of AP by PCR in 2014, and *yes / no* to showing symptoms of AP in 2016 in the Opedal field. The same model was used on the samples from the Loftesnes field with size (1-4) as explanatory variables, as well as symptoms or not in 2016, and apple cultivar (Discovery or Aroma) as additional variables.

To analyze the correlation between the different collection dates, a Pearson's product-moment correlation coefficient was generated. The number produced with this test is between -1 and 1, where 1 would prove a total positive linear relationship, -1 a total negative linear relationship and 0 no relationship. The numbers of ±0.1 - ±0.3 would be a weak relationship, ±0.3-±0.5 a moderate relationship and ±0.5-±1 a strong relationship (Explorable.com, 2009).

Results

Phenology

A total of 2026 adult specimens of *C. melanoneura* were collected using the sticky traps from the beginning of March until the beginning of June 2016 in the Opedal field, while 196 individuals were sampled in the Utne field, 236 in the Loftesnes field, and 456 in the Njøs field. Similarly, 2373 specimens were collected with the beat-tray for the phenology study at Opedal, and 366 at the Utne site. All data collected for the phenology survey are found in appendix I.

First observations at the Opedal site

The 7th of March the first observation was made of eight specimens of *C. melanoneura* in the Opedal field. These insects had entered the field in the period between the 3rd, and the 7th of March. The highest temperature in this period was on the 4th of March with 8.41°C measured in the field and 7.03°C in the lower forest. The 5th and the 6th of March were rather cold days (<5°C), so the insects probably entered the field the 4th of March (Fig. 13). Apart from one extra individual found the 8th of March, no *C. melanoneura* entered the field until the 14th of March, when 79 specimens were found on the traps. This day the lower forest has a maximum temperature of 8.53°C, and the upper forest 5.59°C.

The highest temperature reached with no apparent activity in the field was the 10th of March, where the maximum temperature was 7.9°C in the field and 6.9°C in the forest.

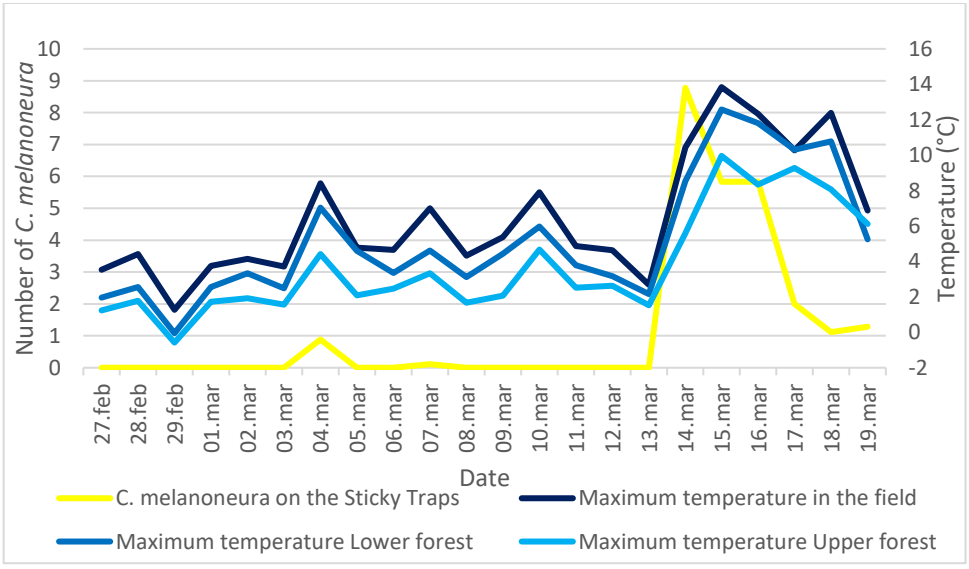

Figure 13: The first observation of *C. melanoneura* in the Opedal field, and the associated temperature measured at three locations. The yellow line shows how many *C. melanoneura* where found per sticky trap per day (primary y-axis). The blue lines are the maximum temperature measured in the field (dark blue), the lower (medium blue) and the upper forest (light blue) (secondary y-axis).

Phenology at the Opedal site

The first beat-tray sample from the 17th of March already collected 301 specimens of *C. melanoneura* (4.46 per tree). The number steadily increased before reaching a population peak in mid-April (Fig. 14). The 25th of May, only 32 specimens were sampled by the beat-tray method and the 9th of June, only one specimen was found. In the first five beat-tray samples the males made up approximately 55% of the population, only in the collection from the 25th of May was the female population higher (62.5%). The yellow sticky traps, however, showed the highest amount of activity in the middle of March (when they migrated from the forest) and in the period from the 28th of April to the 26th of May. This increase in activity later in spring was made up of male specimens, whereas the female activity was more stable throughout the entire period. The first nymphs were found in the beat-tray sample from the 25th of May in the apple blossom period, and they were very prominent in the collection from the 9th of June.

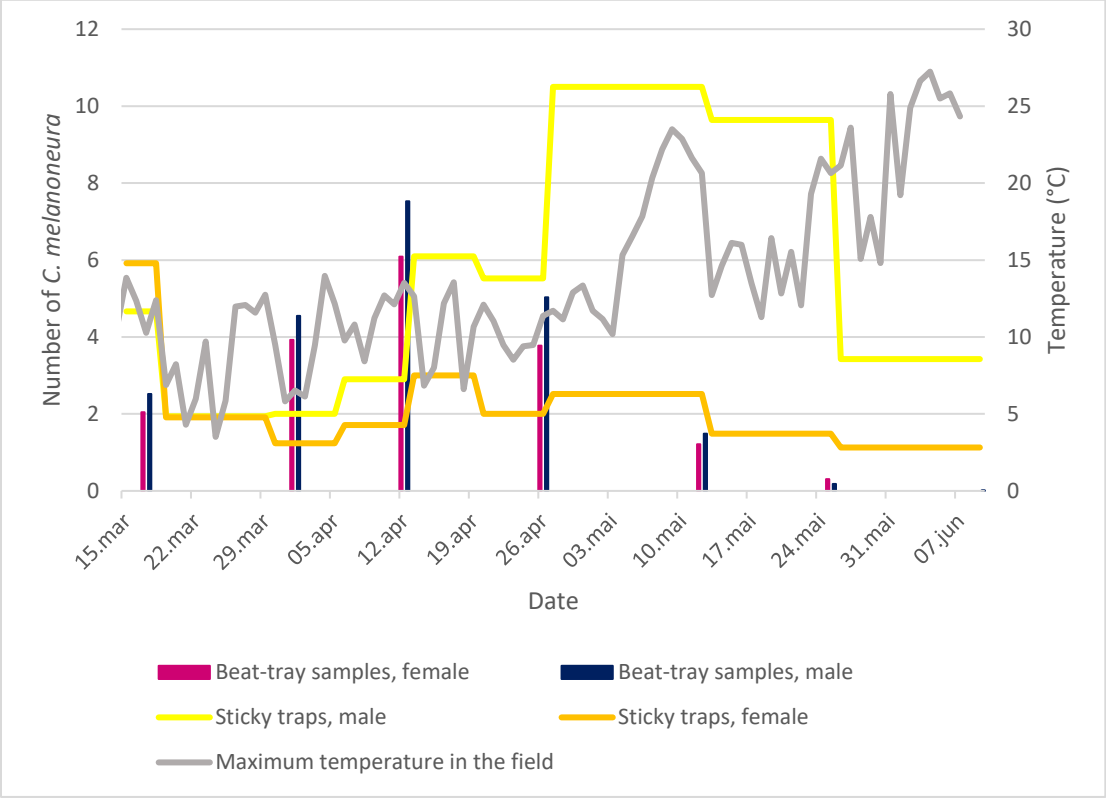

Figure 14: Phenology of C. melanoneura in the Opedal field based on yellow sticky traps (yellow for male and orange for female) and beat-tray samples (dark blue bar for male and pink for female). The Yellow and orange lines describe the mean number of C. melanoneura on three traps, per day (primary y-axis). After the 18th of March the yellow/orange lines describe a mean over a 1-2-week period. The pink and dark blue bars represent the number of C. melanoneura per tree. The grey line describes the maximum temperature per day as measured the field (secondary y-axis).

Phenology at the Utne site

The 17th of March was the first day that surpassed 8°C in temperature (max: 10.57°C), but the 14th and 15th had maximum temperatures of 7.86°C and 7.63°C, that might have triggered *C. melanoneura* to start migration. On the first trip to the Utne field the 17th of March, beat-tray samples gave seven specimens of *C. melanoneura*, showing that they recently entered the field. Of these seven specimens, six were female. The population peak in this field, was as in the Opedal field, in the middle of April (Fig. 15). The three sample dates in April all gave an approximate male ratio of 55 %, while in mid-May the female ratio was 63.5 %. The sticky trap samples showed an activity peak in the late March period, before decreasing during the rest of spring, excluding a very slight increase in male activity in the second half of May. Female activity in the field was in general higher than for males, except for in the latter part of May. The temperature was quite low, with maximum daily temperatures from 3.7°C - 8.53°C until the 26th of March, which could explain the increase of activity later in March.

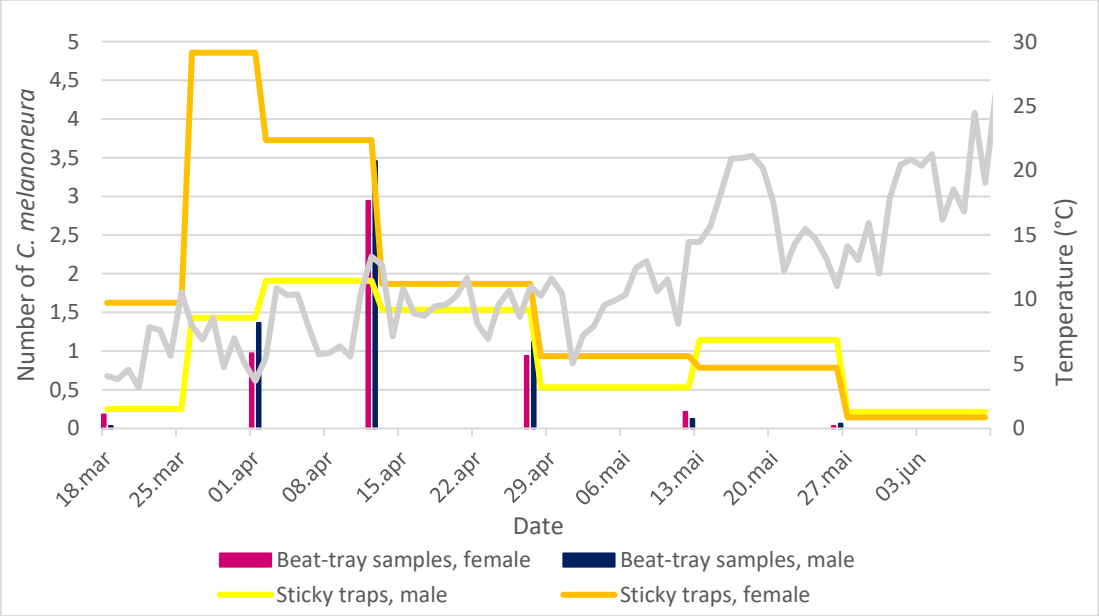

Figure 15: Phenology of *C. melanoneura* in the Utne field based on yellow sticky traps (yellow for male and orange for female) and beat-tray samples (blue for male and pink for female). The yellow and orange lines describe a mean number of *C. melanoneura* on three traps per day over a 1-2-week period (primary y-axis). The pink and blue bars represent the mean number of psyllids in the beat- tray samples per tree. The grey line describes the maximum temperature as measured in the field (secondary y-axis).

Phenology at the Loftesnes site

The first traps were collected and replaced in the Loftesnes field the 18th of March. Data from The Norwegian Meteorological Institute suggest that the 15th of March was the first day where the psyllids were likely to enter the field (Fig.16). Even though the temperature dropped quite quickly to below 5°C, the activity in the field stayed high. Female activity began high in March, before it steadily dropped after the first week of April. Male activity began quite modest and increased in the April-May transition. The beat-tray collection from the spatial distribution study (Appendix II, table x) gave 51% females at the 4th of April and 62% males at the 25th of April. The population peak was probably somewhere in between these dates.

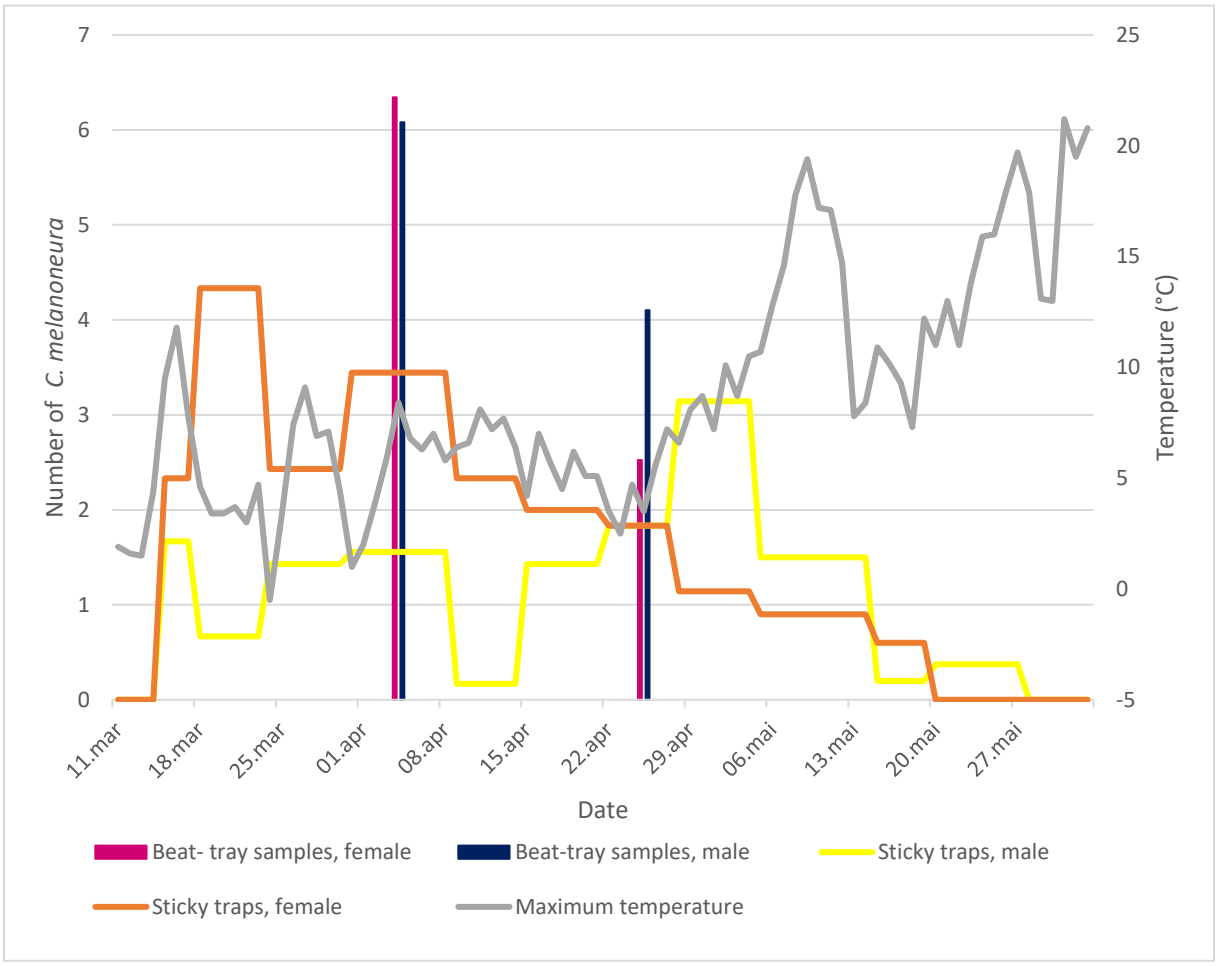

Figure 16: Phenology of *C.melanoneura* in the Loftesnes field based on yellow sticky traps (yellow for male and orange for female) and beat-tray samples (blue for male and pink for female). The yellow and orange lines describe the mean number of *C. melanoneura* on three traps per day over a one-week period. The pink and blue bars represent the mean number of insects in the beat-tray sample per tree (primary y-axis). The grey line describes the maximum temperature at a measurement point 8.2 km away from the field, based on data from The Norwegian Meteorological Institute (secondary y-axis).

Phenology at the Njøs site

The traps were first collected and replaced the 18th of March in the Njøs field. The temperature data collected from the Norwegian Meteorological Institute measured at Njøs measurement station about 0.1 km away from the field, did not surpass 8°C before the 18th of March. The day before the maximum temperature was 7°C. Most of the specimens on the trap probably entered the field the 18th (Fig. 17). Female activity was very high in the period from the 18th to the 23rd of March, before being surpassed by male activity. The activity declined from the initial migration until the end of April, where the activity of both sexes increased in the last week of April (male activity increased more), before declining again in the second week of May.

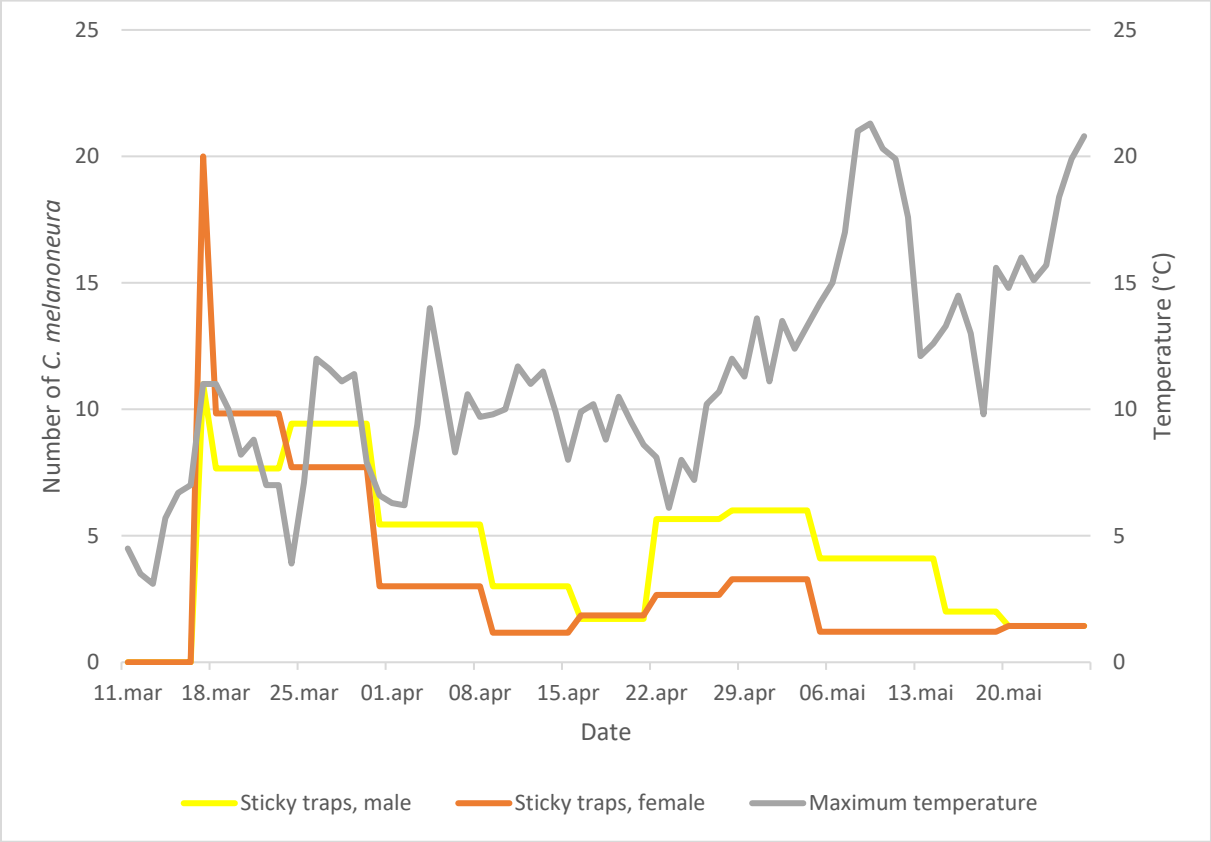

Figure 17: Phenology of *C. melanoneura* in the Njøs field based on yellow sticky traps (yellow for male and orange for female). The yellow and orange lines describe the mean number of *C. melanoneura* on three traps per day over a one-week period (primary y-axis). The grey line describes the maximum temperature at a measurement point 0.1 km away from the field based on data from the Norwegian Meteorological Institute (secondary y-axis).

Differences in catch-rate of *C. melanoneura* by the yellow sticky traps

The Opedal site

The eight specimens of *Cacopsylla melanoneura* that entered the Opedal field the 4th of March were found on the traps located in the southern and upper part of the field, closest to the forest. After the 14th of March, the traps situated at the lower part of the field generally collected more psyllids than the traps at the upper part. Trap number 2 caught significantly more psyllids than the other traps throughout the season (Fig. 18, and 19). As much as 26 % of all psyllids were found on trap 2, and in the late May burst (12.5 – 25.5) almost 40 % of the collected insects were found on this trap alone (Fig. 19). Trap 4 caught the second highest number of psyllids, collecting 18% of the total, whereas trap 8 only caught 4 %.

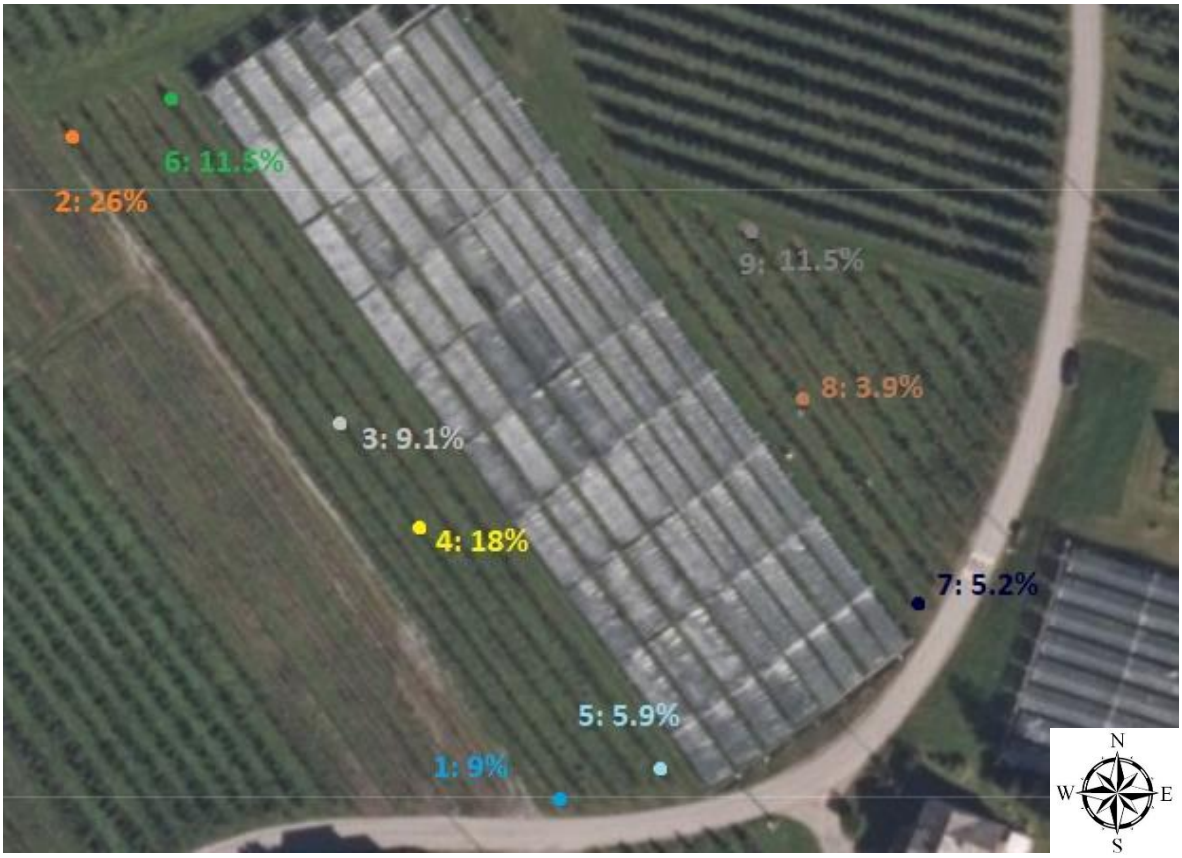

Figure 18: The Opedal field from above, Colored spots mark the positions of the yellow sticky traps, including the associated percentage of the total number of *C. melanoneura* caught by the trap. The south-eastern end of the field is closer to the forest, and is the upper edge of the sloped field.

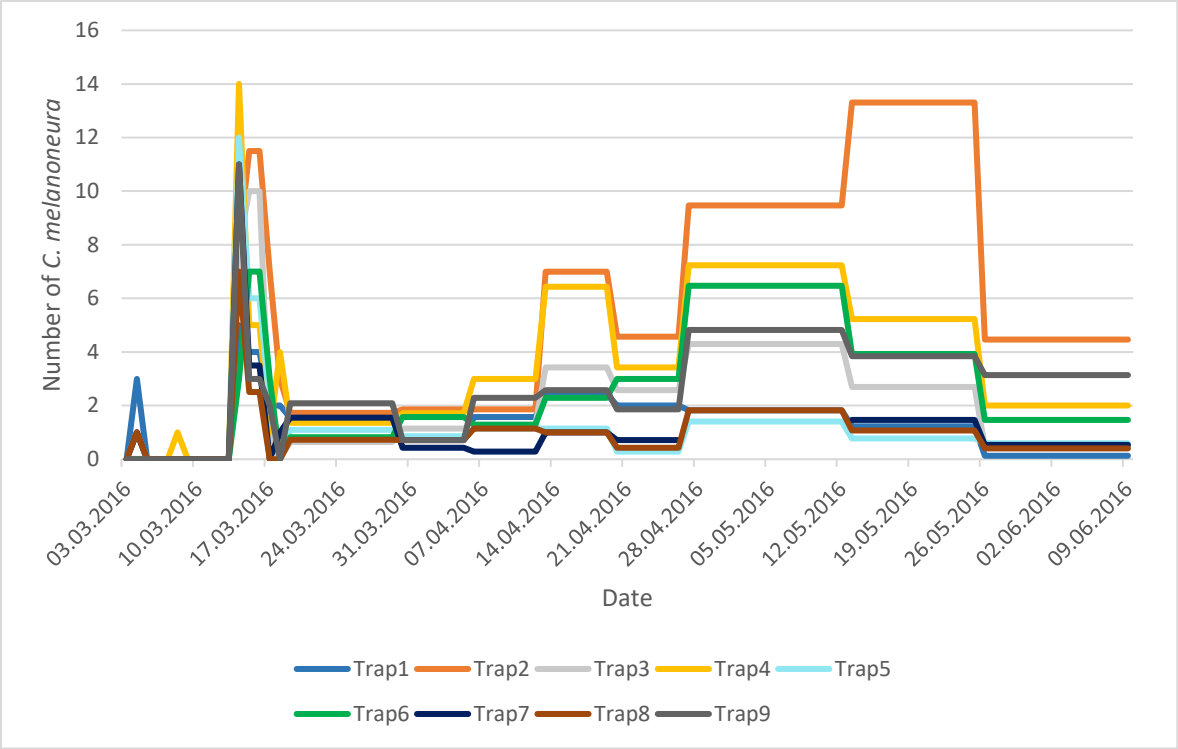

Figure 19: Differences in the catch rate of *C. melanoneura* by the nine traps located in the Opedal field troughout the spring. Colors are matched with figure 18.

The Utne site

At the Utne field, the lowest situated trap close to the fjord caught almost half (48.3%) of the collected *C. melanoneura*, and especially the activity in April were located around this trap (Fig. 20). The middle trap caught 33.3% of the collected psyllids, and most of the late March activity. The trap at the upper part, which were closest to the spruce forest, only caught 18.4% of the total psyllids, and no psyllids from the middle of May.

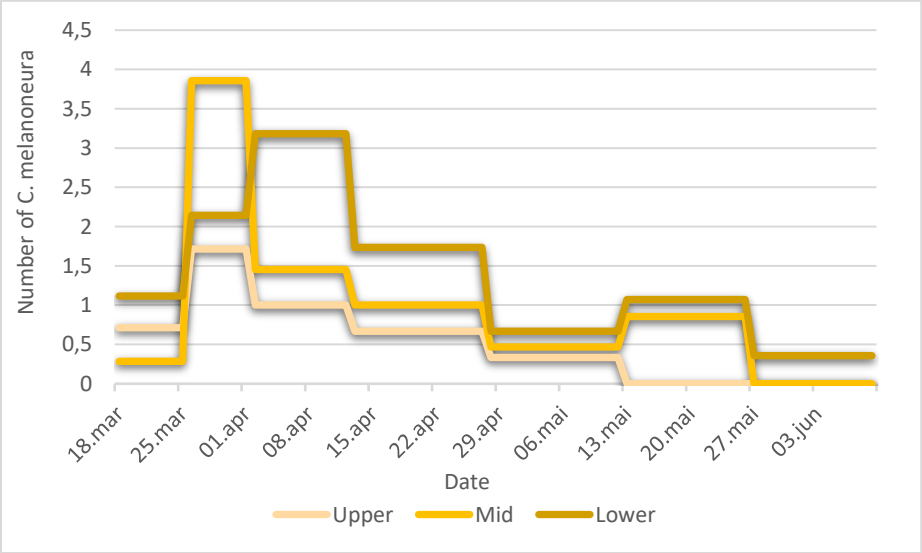

Figure 20: Catch rate of *C. melanoneura* by the three traps located in the Utne field troughout the spring.

The Loftesnes site

At the Loftesnes field, the upper trap caught the highest amount of *C. melanoneura* in the third week of March and in the first week of April. In all other periods the upper trap caught very little of the activity, and dropped to none one week before the other traps (Fig. 21). All together this trap caught 27 % of all the psyllids collected. The lower trap showed the opposite trend, and caught low numbers when the upper trap caught large numbers, and vice versa; 34.5 % of all psyllids are caught by this trap. The middle trap captured high amounts of psyllids in both early and in late spring, in total 38.5 % of the psyllids collected in the field.

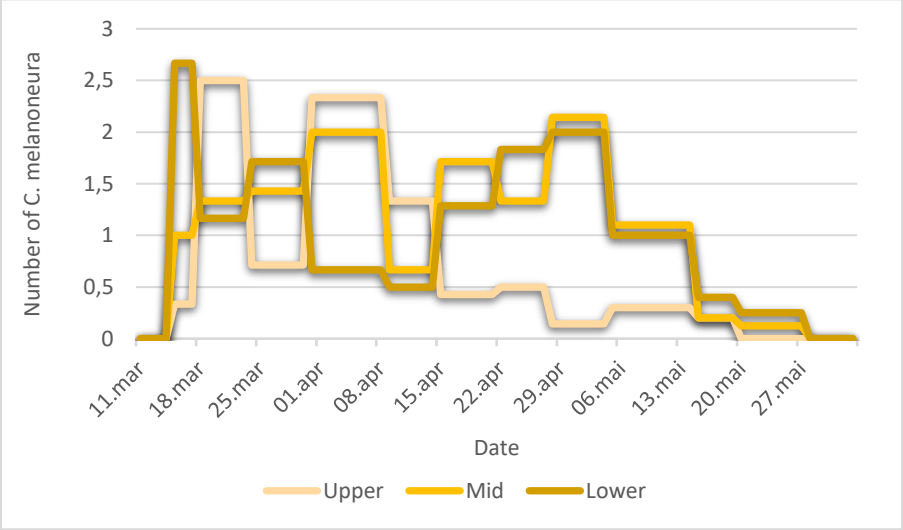

Figure 21: Catch rate of C. melanoneura by the three traps located in the Loftesnes field troughout the spring.

The Njøs site

The Njøs field however, did not show the same variety in catch rate between the different parts of the field (Fig. 22). All three traps caught approximately the same number of psyllids, and followed the same pattern the entirety of the season.

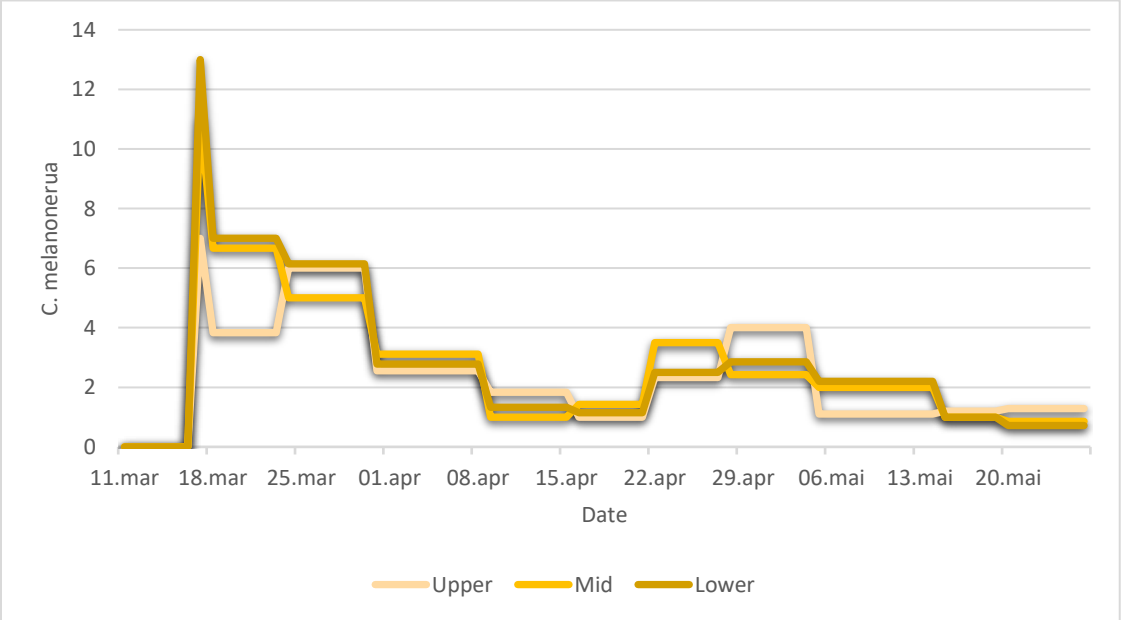

Figure 22: Catch rate of C. melanoneura by the three traps located in the Njøs field troughout the spring.

Spatial distribution

All data collected for the spatial distribution study are found in appendix II.

Opedal

A total of 1183 specimens of *C. melanoneura* were found in the first collection in the beginning of April, with a mean of 19.72 ± 1.49 (standard error) (Fig. 23a). In the second collection from the end of April, 943 specimens were found, with a mean of 15.72 ± 1.3 (Fig. 23b). In the leaf collection, no alive nymphs were found, only empty remains of the last instar (Fig. 11b), 330 of these were found, with a mean of 5.5 ± 1.13 (Fig. 23c). Of the 60 trees used for this study, 21 showed symptoms of apple proliferation in 2016, 16 of these had previously tested positive for AP, only one had tested negative. All data

The two beat-tray collections (early and late April) only showed a weak correlation in the spatial distribution of *C. melanoneura* (0.25, p = 0.05135, Pearson´s product-moment correlation), as only a quarter of the trees host the approximate same number of psyllids in both collections (Fig. 24a). The first sampling and the nymph collection showed a moderate correlation in the spatial distribution (0.39, p=0.002, Pearson's product-moment correlation) (Fig. 24b). The second sampling and the nymph collection had a stronger, but still only a moderate correlation (0.48, p=0.00012, Pearson's product-moment correlation), still less than half the trees followed a pattern (Fig. 24c). In the data from the first collection, the psyllids seemed to be randomly spread in the field (Fig. 25a), but in the second collection the samples contained more psyllids in the western part of the field (Fig. 25b).

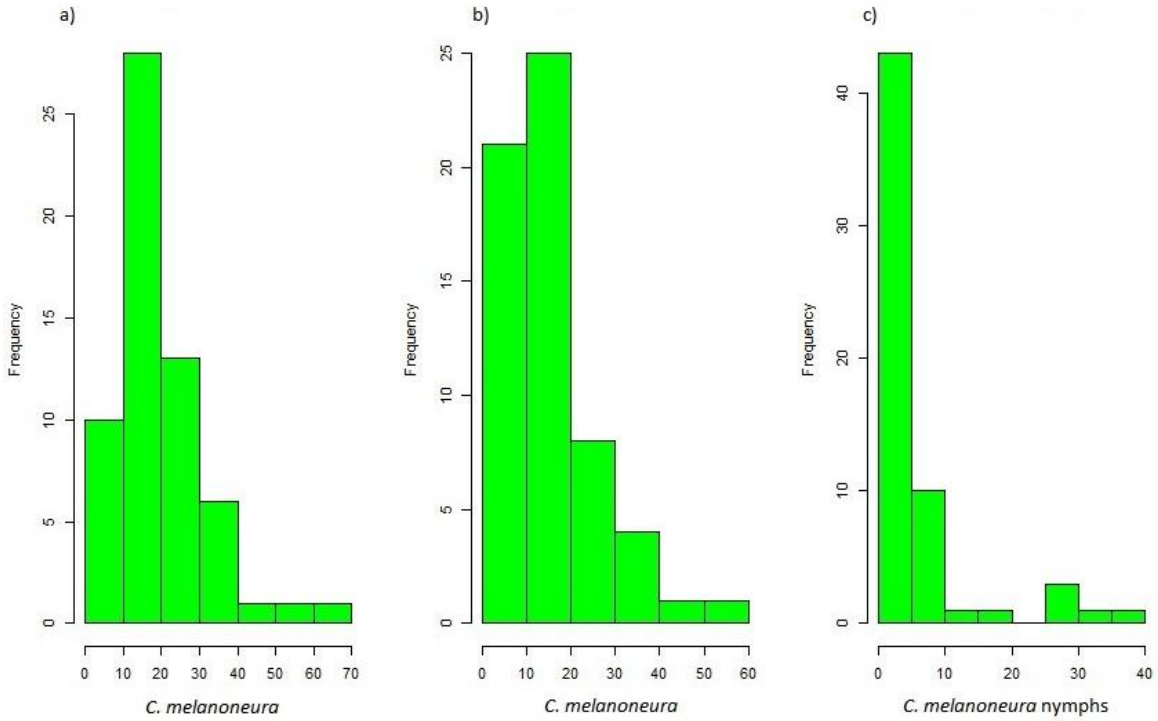

Figure 23: Frequency in the number of *C. melanoneura* hosted per tree for the three collection dates a) 1st of April b) 27th of April c) 22nd of June in the Opedal field.

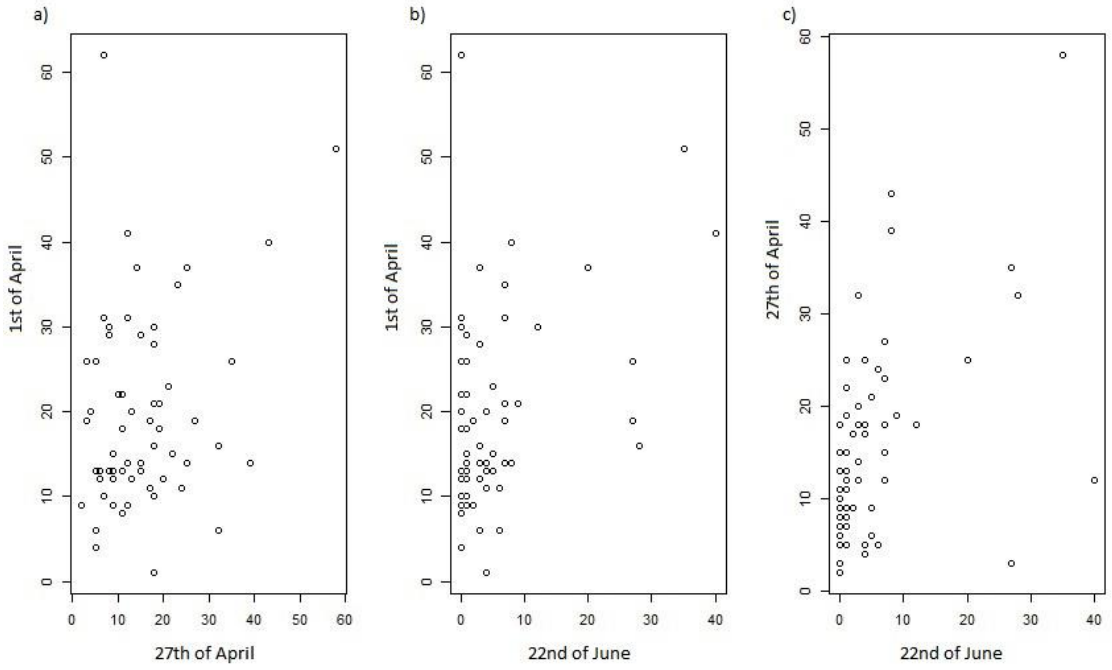

Figure 24: Correlation between the collection dates in the Opedal field between the a) 1st and 2nd collection, b) 1st and last collection, c) 2nd and last collection.

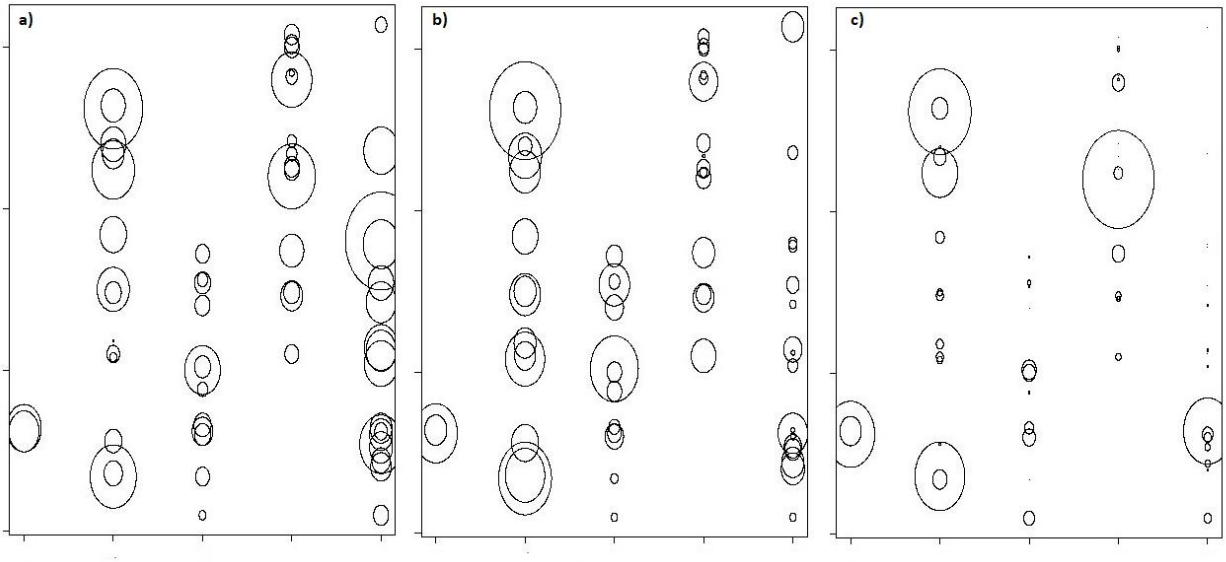

Figure 25: Spatial map of the Opedal field, mapping the distribution of *C. melanoneura* collected the a) 1st of April, b) 27th of April c) 22nd of June. The size of the circles indicates the number of *psyllids/ nymphs (larger circles equals a higher number)*.

Trees were regrouped into new categories based both the PCR results from 2014 and the symptoms in the field in 2016, as this might give the most accurate picture of the current conditions of the trees. This separates the trees into six categories (Table 1). This set up creates groups of trees with different uncertainty of carrying the AP disease, where group 1 (tested negative, without symptoms) are the least likely to carry the disease, while trees in group 6 (tested positive, with symptoms) were infected by AP, and were the most likely to have higher concentrations of phytoplasma in the shoots. If there is a difference in psyllid attraction to the trees, the difference should be most prominent between these two groups. In the first collection (Fig. 26), ignoring group 4 as it only contains one sample, the group of trees that had tested positive for AP but showed no symptoms (group 3) had the lowest number of psyllids of 16.43± 2.7. The four trees that had not been tested but showed symptoms (group 5) had the highest number of psyllids with 30.25±7.43, followed by the group that tested positive and showed symptoms (group 6) with 21.44±3.37. This shows tendencies towards that symptoms in the trees might attract psyllids, and that latent infection and / or healthy trees might not. However, the difference in numbers of *C. melanoneura* between trees that showed symptoms (group 4,5,6) and trees that did not (1,2,3) was not significant (p=0.19, GLMM, quasi-poisson). In the second collection (Fig. 27), group 5 still had the highest number of psyllids (20.25±12.75), followed by group 3 (16.57±2.96), while group 6 had the lowest (14.69±2.11) contradicting the results from the first sampling. This time there was no difference in symptomatic trees and asymptomatic trees (p=0.96, GLMM,

quasi-poisson). In the last collection (Fig. 28), group 5 had the highest number of nymphs as well, with 15.55±9.1 nymphs, followed by group 1 (negatively tested without symptoms) with 7.38±3.09. Group 6 sampled the lowest number of nymphs with a mean of 3.44±0.88. There was no difference between the trees with and without symptoms in the amounts of nymphs collected (p=0.93, GLMM, quasi-poisson).

Table 1: The trees in the Opedal field divided into six groups based on the variables "tested for PCR in 2014", and "symptoms in 2016", included number of trees in the group (N) and their mean numbers of C. melanoneura per tree in the three collections.

Group	PCR 2014	Symptoms 2016	N	First collection	Second collection	Nymph collection
Group 1	Negative	None	13	20.15 ±3	14.85±3.29	7.38±3.09
Group 2	Not tested (neighbor)	None	12	18.17±2.4	15.08±2.7	4±2.18
Group 3	Positive	None	14	16.43±2.7	16.57±2.96	4.86±1.96
Group 4	Negative	Yes	1	9	12	1
Group 5	Not tested (neighbor)	Yes	4	30.25±7.43	20.25±12.75	15.55±9.1
Group 6	Positive	Yes	16	21.44±3.37	14.69±2.11	3.44±0.88

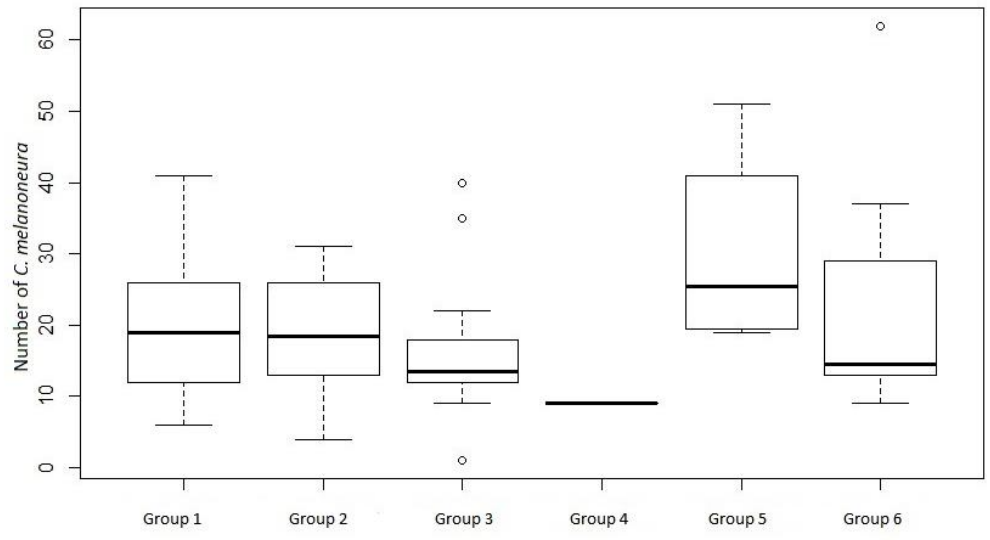

Figure 26: Number of *C. melanoneura* caught on trees the 1st of April tested for AP by PCR in 2014, and checked for symptoms in 2016 divided into six categories representing from the left: 1) Negative without symptoms (n= 13), 2) Not tested without symptoms (n= 12), 3) Positive without symptoms (n=14), 4) Negative with symptoms (n=1), 5) Not tested with symptoms (n=4), 6) Positive with symptoms (n= 16).

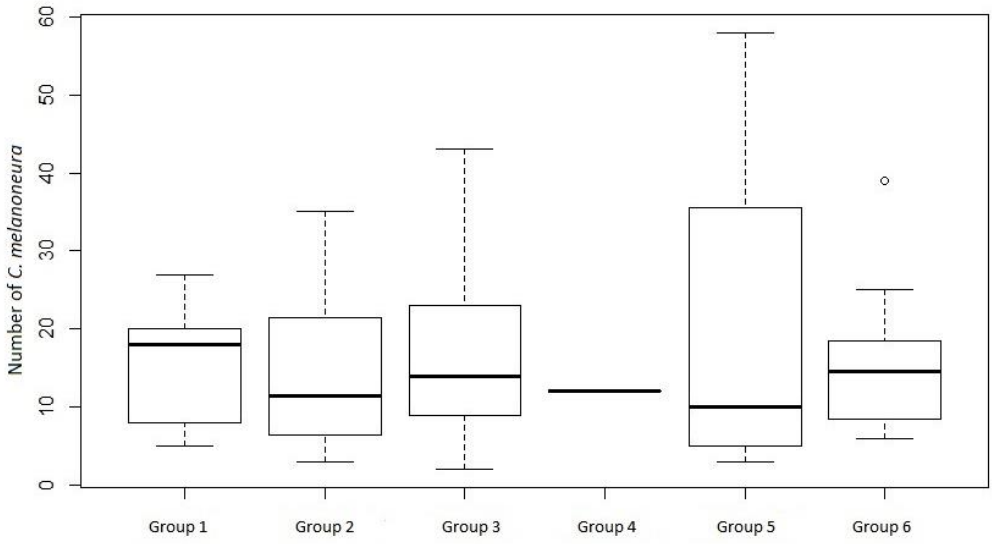

Figure 27: Number of *C. melanoneura* caught on trees the 27th of April tested for AP by PCR in 2014, and checked for symptoms in 2016 divided into six categories representing from the left: 1) Negative without symptoms (n= 13), 2) Not tested without symptoms (n= 12), 3) Positive without symptoms (n=14), 4) Negative with symptoms (n=1), 5) Not tested with symptoms (n=4), 6) Positive with symptoms (n= 16).

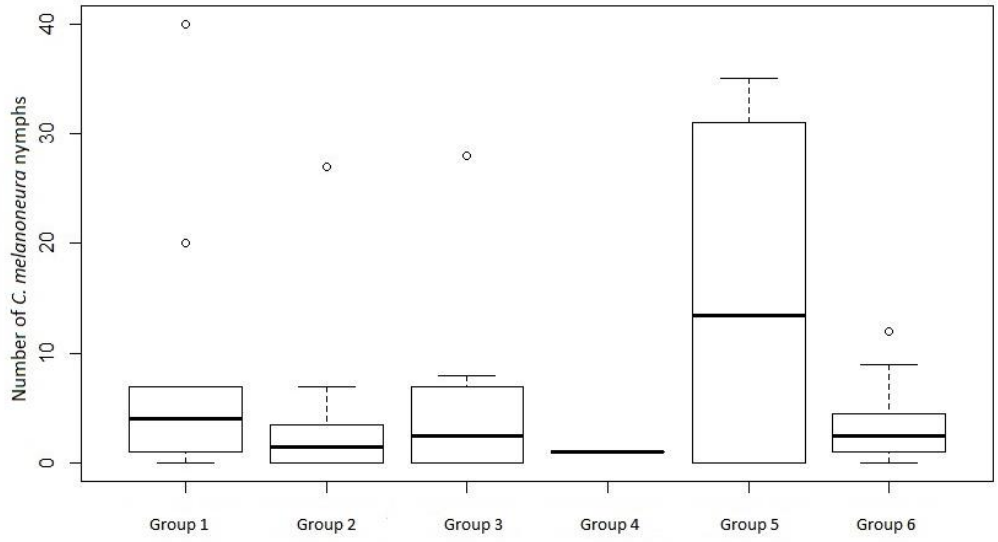

Figure 28: Number of *C. melanoneura* nymph shells caught on trees the 22nd of June tested for AP by PCR in 2014, and checked for symptoms in 2016 divided into six categories representing from the left: 1) Negative without symptoms (n= 13), 2) Not tested without symptoms (n= 12), 3) Positive without symptoms (n=14), 4) Negative with symptoms (n=1), 5) Not tested with symptoms (n=4), 6) Positive with symptoms (n= 16).

Loftesnes

In the first beat-tray collection 472 *C. melanoneura* were collected at the Loftesnes orchard with 12.32±1.73 specimens per tree (Fig. 29a). In the second collection 265 psyllids were found with 6.625±0.71 specimens per tree (Fig. 29b).

Only five samples contained more than 30 psyllids the first collection, and only two samples more than 15 psyllids in the second (Fig. 29). The trees that sampled high amounts in the first and last collection were not the same (Fig. 30), and the correlation for the field in general between the two collection dates was only moderate (0.39, p = 0.01, Pearson´s product-moment correlation).

Of the 30 trees that were tested for AP, 16 showed symptoms. Two of the trees tested positive on both collected branches, and one tree tested positive on one branch. The latter had shown no symptoms of AP, and the two others had some late growing shoots and small apples. This proved that AP existed in the field.

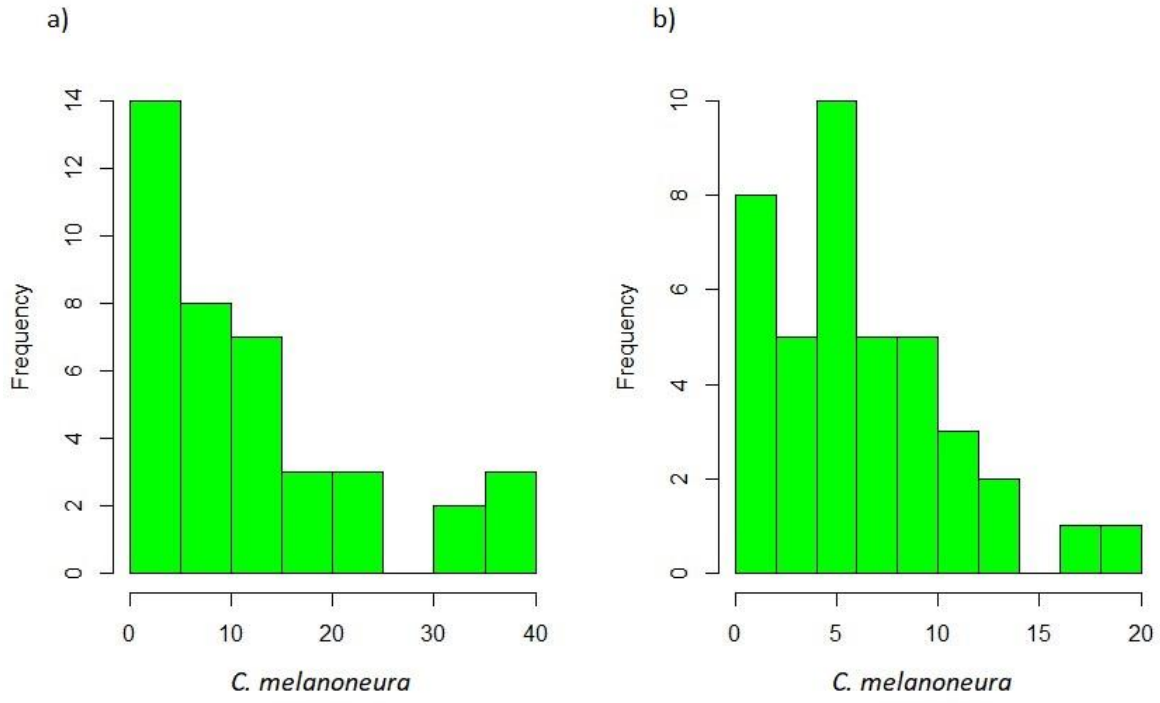

Figure 29: Frequency in the number of *C. melanoneura* hosted per tree in the two collection dates a) 4th of April b) 25th of April in the Loftesnes field.

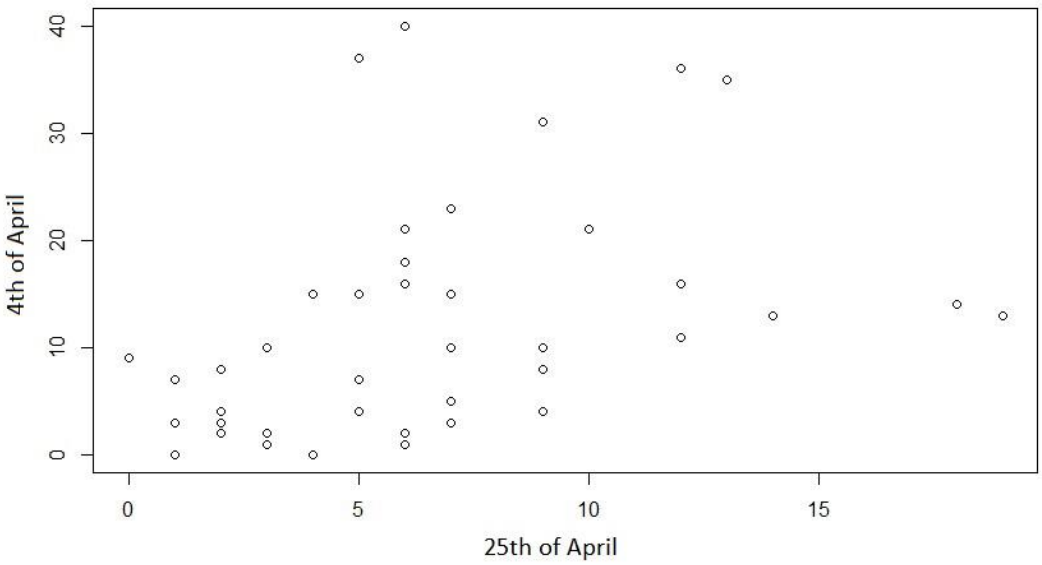

Figure 30: The correlation between the numbers of *C. melanoneura* sampled in beginning and late April in the Loftesnes field.

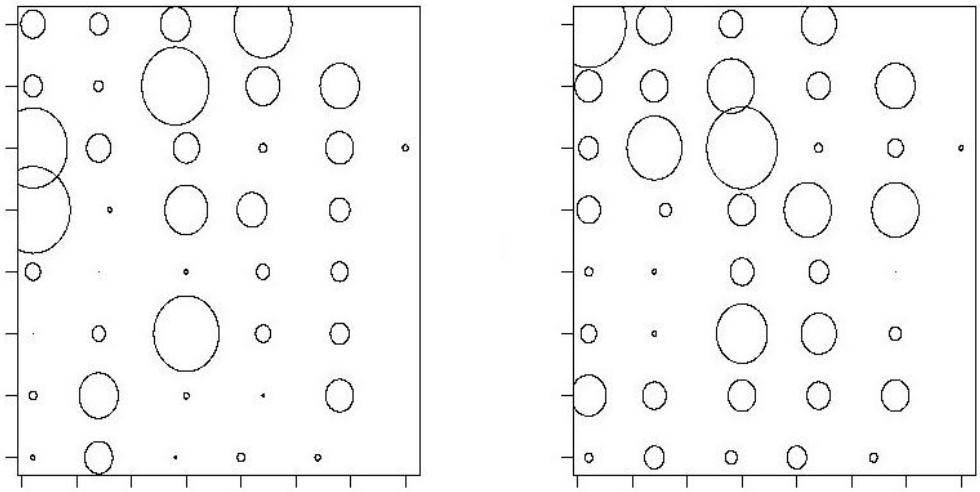

Figure 31: Spatial map of the Loftesnes field, mapping the distribution of *C. melanoneura* collected the a) 4th of April, b) 25th of April. The size of the circles indicates the number of *psyllids (larger circles equals a higher number)*.

As the size difference between the trees in this field is prominent, this needs to be accounted for. The trees were separated into four size categories from smallest to largest (Fig. 8) (Table 2)

Table 2: The size categories of trees from 1 (smallest) to 4 (largest) in the Loftesnes field, including number of trees in the groups (N), and the mean number of *C. melanoneura* for each collection.

Size	N	First collection	Second collection
Size 1	3	3.33±2.4	2±0.58
Size 2	19	6.32±1.29	4.63±1.24
Size 3	12	18.33±3.1	9.58±1.54
Size 4	6	23.83±4.64	9.33±1.36

In the first collection, there was no significant difference in the number of *C. melanoneura* between size 1 and 2 (p=0.3, GLMM, quasi-poisson), but there was a difference between size 1 and 3 (p=0.0067, GLMM, quasi-poisson), and size 1 and 4 (p=0.0026, GLMM, quasi-poisson). In the second collection, there was still no significance between the smallest tree sizes (p= 0.12, GLMM, quasi-poisson), and the difference between size 1 and the two other sizes were still significant (p=0.007, p=0.012, GLMM, quasi-poisson family, respectively). Because of this, the four size groups were simplified into two groups: small and big (Table 3).

Table 3: The trees in the Loftesnes field separated into two groups based on size difference, including number of trees in the groups (N), and the mean number of *C. melanoneura* for each collection.

Size	**N=**	**First collection**	**Second collection**
Small	22	5.9±1.17	4.27±0.57
Big	18	20.17±2.58	9.5±1.1

The difference between the size groups small and big were significant at both sampling dates (p=0, and p= 0.0001, GLMM, quasi-poisson) (Fig. 32).

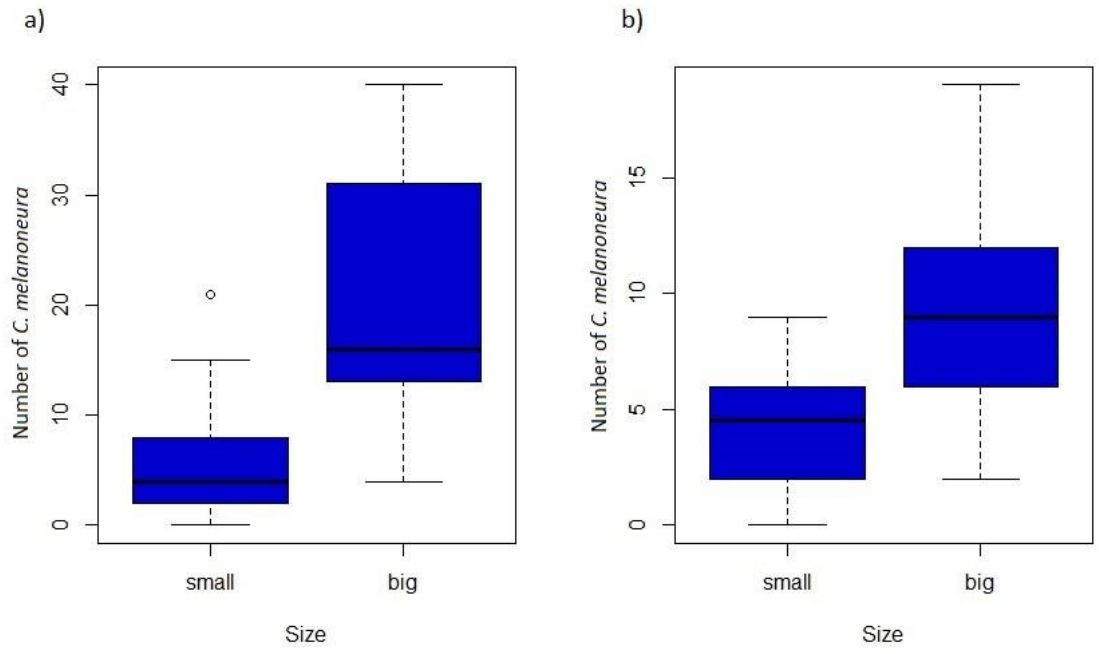

Figure 32: The difference in the number of *C. melanoneura* cought on smaller and bigger trees in the Loftesnes field the a) 4th of April b) 25th of April

The group of small trees included 22 of the 40 samples (Table 4).

Table 4: The small trees in the Loftesnes field separated into four categories based on cultivar and symtoms, including the number of trees (N), and the mean number of *C. melanonerua* for the two collection dates

Cultivar	**Trees with symptoms**			**Trees without symptoms**		
	N	First collection	Second collection	N	First collection	Second collection
Aroma	5	6.6±1.72	3.2±1.5	10	4.5±1.45	3.5±0.58
Discovery	1	3	1	6	8.16±3.25	7±0.45

Even though the two-way interaction between cultivar and symptoms was not significant on either sampling date (p=0.3153 and p=0.1419, GLMM, quasi-poisson), including both factors described the differences better than looking at them separately.

In the first collection, none of the groups showed a significant difference in *C. melanoneura* numbers. The biggest difference was found between the Discovery group without symptoms and the Aroma group without symptoms (p=0.25, GLMM, quasi-poisson) (Fig. 33a).

In the second collection, asymptomatic Discovery trees hosted significantly more insects than both the Aroma groups (p<0.02, GLMM, quasi-poisson), but there was no difference between the two groups of Aroma (p=0.8, GLMM, quasi-poisson) (Fig. 33b).

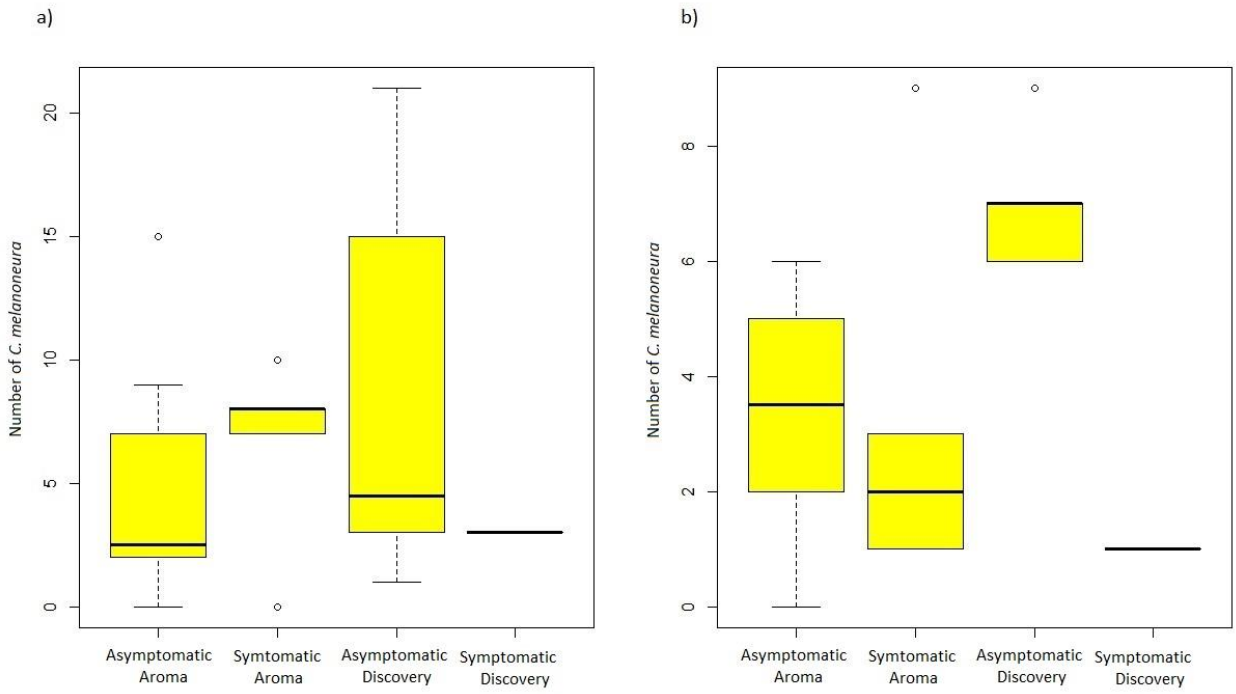

Figure 33: Specimens of *C. melanoneura* on small trees the a) 4th of April and b) 27th of April, within the categories of (1) Aroma trees without symptoms, (2) Aroma trees with Symptoms, (3) Discovery trees without symptoms, (4) Discovery trees with symptoms.

There are 18 trees in the group of larger trees (Table 5).

Table 5: The bigger trees in the Loftesnes field separeted into four categories based on cultivar and symtoms, including the number of trees (N), and the mean number of *C.melanoneura* for the two collection dates

Cultivar	**Trees with symptoms**			**Tree without symptoms**		
	N	First collection	Second collection	N	First collection	Second collection
Aroma	5	25±5.5	10±1.45	0	-	-
Discovery	10	15.1±2.31	8.6±1.56	3	29±7.5	11.66±3.76

In the first collection, there was a significant difference between Discovery with and without symptoms (p= 0.037, GLMM, quasi-poisson), where the trees without symptoms contained more psyllids than the symptomatic trees (Fig.34a). The difference between Aroma and Discovery with symptoms was almost significant, Aroma trees having the highest amount (p=0.076, GLMM, quasi-poisson).

In the second collection, there was no significant differences, the biggest difference was still found between the Discovery with and without symptoms, where the trees without symptoms hosted more psyllids (p=0.35, GLMM, quasipoisson) (Fig. 34b). The difference between Aroma and Discovery with symptoms was not as prominent this time (p=0.59, GLMM, quasi-poisson).

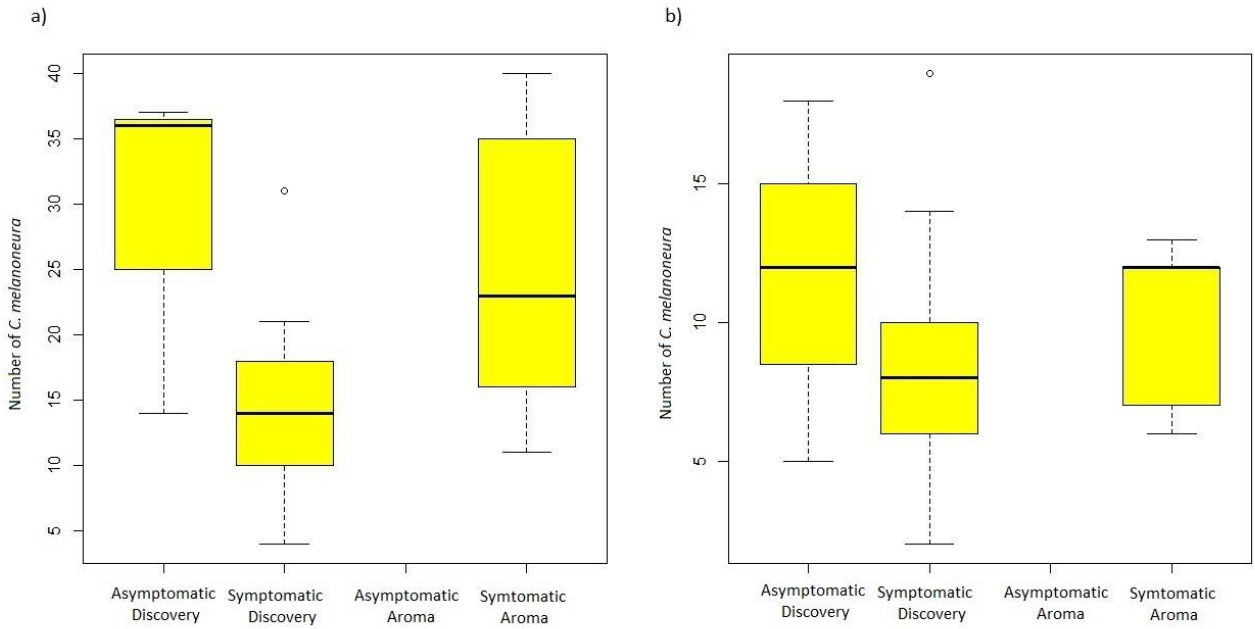

Figure 34: Number of *C. melanoneura* on big trees in a) the beginning of April, b) late April, within the categories of (1) Discovery trees without symptoms, (2) Discovery trees with symptoms (3) Aroma trees without symptoms, (4) Aroma trees with symptoms.

Discussion

Psyllids were present in all four fields form early/ mid-March before bud break, which was earlier than described by Edland (2004), but in correspondence with a recent survey of psyllid phenology in Norwegian apple orchards (Hatteland et al. 2016). The first specimens of *Cacopsylla melanoneura* entered the Opedal field in early March (Fig. 4), but after the first specimens arrived, no psyllids were found until ten days later. This might be because the threshold temperature for migration was reached in overwintering sites of spruce trees closer to the field earlier than in the main sites in spruce forest (Fig.3). Probably, this threshold was reached in the spruce forest when the main migration began in mid-March. In all other fields (Fig. 15,16,17), the migration continued even though the temperature dropped after the threshold was reached for the first time. This supports the assumption that the migration into the Opedal field did not truly begin before mid-March. Based on these results, the threshold temperature for *C. melanoneura* to start the springtime migration into apple orchards seems to be somewhere between 7°C and 8.4 °C in Western Norway. In Italy, this threshold was shown to be around 9.5°C (Tedeschi et al. 2012).

According to the beat-tray samples, the migration continued until mid-April, when the highest numbers of psyllids were found in the Hardanger orchards (Figs. 13 and 14). This peak was found during the bud burst, when the green leaves become visible in the buds. According to the sticky traps, the activity started to increase after the population peak. Mostly males were sampled by sticky traps after the population peak which may indicate that this period is the main mating period of *C. melanoneura*. This was more evident in the Opedal field, where there was a very high increase in the number of *C. melanoneura* found on the sticky traps, even though the beat-tray collected very little (Fig.14). This might be because the Opedal field had more psyllids in general, and because more of the field was covered, by using nine traps instead of three. This activity peak in the April-May transition was found in all the sites except for Utne. At the Utne field, the lowest number of psyllids was collected, which might be why the three traps in this field did not capture the reproductive activity as in the other fields. The results from the Opedal field were in accordance with the sticky traps collected in the same field in 2015 (Hatteland et al. 2016), that also collected high amounts of psyllids in mid-March, and in late April/early May. The same female / male ratio was also found in Hatteland et al. (2016). Psyllids were also collected by sticky traps in Lier, Buskerud in 2015,

that collected high amounts in early / mid-April and mid / late-May (Hatteland 2015, unpublished data).

The last adult of *C. melanoneura* found by the beat-tray method was one singular male in the Opedal field at the beginning of June. The last sticky traps collected in Hardanger in the beginning of June still had quite a few psyllids, but the activity was much lower than in the previous period. The use of one or two more weeks of sticky traps in the Opedal field could have marked the end of the season. In the Loftesnes orchard and the Utne field, no more *C. melanoneura* were found after the 27th of May. The last sticky trap collected at the Njøs site the 26th of May still had a few adult psyllids. This corresponded well with Edland (2004), who described that overwintering adults of *C. melanoneura* could be found until the beginning of June. Replacing the traps every week in Hardanger, as in the Sogn fields, would have given a more precise pattern of the activity, including a more representative number of psyllids as some of the traps were filled with insects and particles and might not have been able to collect for the full time-period in the field. In addition, it was more difficult to count the psyllids accurately when they were that abundant on the trap.
The nymphs started to appear in the beat-tray samples from late May, after the flowering started, which match the phenology described by Edland (2004), but in contrast to the same description, only the remains of the nymphs were found in late June, while Edland (2004) suggested that alive nymphs might be found until the end of July. This had implications for the nymph collection for the spatial distribution study, as the leaf collection should have taken place earlier to find alive nymphs. As there seemed to be no more nymphs in late June, beat-tray samples could find young adults of the species in June, even though Edland (2004) suggest they emerge only late in June until the end of July.

Exploring how *C. melanoneura* is distributed between the sticky traps, it becomes clear that the placement of the trap in the field is very important in terms of how much insects they collect (Fig. 18). The uppermost traps in the fields collected more psyllids in the beginning of the season than later. These traps are closer to the forest, and might therefore capture more psyllids in the migration period. In the Opedal, Utne and the Loftesnes fields, the traps situated lower in the field generally collected more insects than the upper ones, especially later in the season. This could mean that when the psyllids are moving around, they move downward in a sloped field, and thus the concentrations of psyllids are higher in the lower part of the fields. The same result was seen in the three sticky traps that collected psyllids in the Opedal field in 2015 (Hatteland, 2015, unpublished data.). However, this spatial

relationship was not found in the beat-tray samples, which rather showed a movement towards the western part of the Opedal field (Fig. 25), and if any movement at all, upward towards the forest (eastward), in the Loftesnes field (Fig. 32).

Even though the different traps in the Opedal orchard collected very differently, their catch rates relative to each other (Fig.19), was quite even during the entire spring, where the traps 2 and 4 generally caught more, 3, 6 and 9 caught a medium amount, and trap 5, 7 and 8 caught very little. Trap 1, did however, catch a medium amount earlier in the spring before dropping in early May. This indicates that using only three traps to measure activity in the field is probably not sufficient. The actual location of the activity might be missed this way, as might be the case at the Utne site. As the late season activity seemed to be located at the middle to the lower end of the sloped fields, more traps located here might measure the activity in the reproductive period more accurately. This has implications for the monitoring of psyllids and sampling of insects in general. It would be interesting to survey the activity distribution in orchards of even height. Three traps were used in the less sloped field in Lier, Buskerud in 2015, and the catch rate between these were quite even (29, 32 and 39 %) (Hatteland 2015, unpublished data). When phenology has been mapped in Italy, three sticky traps have been used in a diagonal line(Tedeschi et al. 2002; 2003; 2012). When looking for potential vectors of AP in Spain, one and two sticky traps have been used (Laviña et al. 2011), that also showed different catch rates in one orchard.

No significant difference was found in numbers of *C. melanoneura* between AP-symptomatic and non-symptomatic trees in the Opedal field, in neither of the three collections. Trees with symptoms showed a trend towards hosting more insects in early April, but the latter collections did not follow this result, which might be expected as the correlation between the collection dates were low. This could be because *C. melanoneura* was not attracted or repelled by unhealthy trees, or it could be that the symptoms that would cause a change in host-selecting behavior were not sufficiently prominent this year.

In the first collection at the Loftesnes site, non-symptomatic Discovery trees among the bigger trees hosted significantly more psyllids than the symptomatic ones. However, the sample size for non-symptomatic Discovery trees was very small, making this result less reliable. The group of non-symptomatic Discovery trees did collect the most psyllids in both collection dates, in both small and big trees, which could indicate an attraction towards healthy, Discovery trees. Among the smaller trees, the group of Discovery trees hosted more

insects than both Aroma groups, indicating a preference for Discovery over the Aroma cultivar, but this was not evident among the bigger trees.

Cacopsylla picta acquires *Ca.* P. mali as nymphs and / or young adults in the summer, and the phytoplasma multiplies over the winter (Jarausch et al. 2011). In *C. melanoneura* populations in Italy however, very few specimens had a high enough titer of the phytoplasma for detection just after migration, and the number of infected specimens increased during the spring (Tedeschi et al. 2003). This could be because the phytoplasma needs more time to multiply within the psyllids in spring, or the infected insect might have delayed migratory responses. An additional hypothesis is that the psyllids might acquire the phytoplasma as adults in spring, which could happen if the phytoplasma is present in the aerial parts of the trees in this period as shown by Baric et al. (2011).

If the Norwegian vector acts the same way as *C. picta* does in Germany, we would expect an attraction towards healthy trees in the overwintering adults. If *C. melanoneura* as overwintered adults acquires the phytoplasma in the spring, we might expect an attraction towards infected trees. However, if the acquisition of the bacteria is set to the nymph stages, maybe the lack of attraction is the most effective way to spread the disease, as this will increase the probability that some infected psyllids will stay and oviposit on healthy trees and vice versa. *C. picta* has shown a higher mortality when developing on AP-infected trees (Mayer et al. 2011) and if this trait could be transferred to *C. melanoneura*, a higher number of nymphs should be found on healthy trees, independent of adult numbers on the same trees.

In summary, the results of this study showed a small incline towards attraction to symptomatic trees early in the Opedal field, and a small incline towards attraction to healthy trees in the Loftesnes field. As these results were contradicting and not repeated, there was all in all no significant differences in *C. melanoneura* numbers in these trees because of AP-symptoms, in either field. The nymph collection gave no indication that there was a higher mortality on infected trees. However, only the remains of the last instar nymph were found, and these remains could have disappeared from some trees, and was difficult to detect. Because of this, these samples did not provide sufficient and reliable data of which trees had the highest nymph development. These results does correspond with previous studies that have not shown any attraction towards infected or uninfected plants in *C. melanoneura* populations (Eben & Gross 2013). As the choice of reproductive host plant within the field seems to be random, this could also support the theory of phytoplasma acquisition in the nymph stage.

It could be interesting to look at the spatial distribution of the young, and emigrating adult-generations in the summer, as this is the period where β-caryophyllene is emitted to attract the other European vector *C. picta* (Mayer et al. 2008). This could however be difficult if the new generation of *C. melanoneura* only stays for a few days on apple, before changing hosts (Mayer & Gross 2007).

Four of the trees in Opedal that had been reported as symptomatic in the 2014 study (Blystad & Brurberg 2016), had no visible signs of AP in 2016. Fourteen of the trees that were proven to be infected based on genetic analyses, had no symptoms. In the Opedal field, the late growth was very limited and hard to detect. Furthermore, the small apples could just be a symptom inadequate pruning, as the apples grew close together in clutches, and there were no signs of root shoots. This means that the symptoms in this field were not a reliable way to confirm or disprove the presence of the bacteria in the tree. The lack of obvious symptoms in 2016 might indicate that because of environmental conditions this year, the bacteria had not colonized the trees in the same way as earlier years, and thus might explain the lack of differences in psyllid numbers between the trees. Infected trees may be symptom-less and act as healthy trees for several years, and only carry the phytoplasma in the roots (Carraro et al. 2004).

Symptoms were somewhat more evident in the Loftesnes field. This field showed rather clear signs of root shoots, late growth, and some of the trees showed small apples. It is important to keep in mind that showing symptoms does not mean that the tree is a definite carrier of the phytoplasma. Of the 30 trees that were tested for AP by molecular methods, 15 showed symptoms of AP, and only three tested positive. Two of these tested positive on both branches and showed signs of small apples and late growth, the last one tested positive on one branch and showed no symptoms of AP. As symptoms in the trees are linked to high concentrations of phytoplasma in the shoots (Baric et al. 2011), we would expect a higher number of positive samples. This result could mean that what is regarded as “abnormal growth” in this study was indeed not associated with AP, or could be that the branches collected did not have phytoplasma, even though the main stem did. Furthermore, shoots from the roots was noted as a symptom, but this might not be associated with phytoplasma in the aerial parts. Very few of the sampled trees showed this symptom alone, and it would not significantly change the result to only look at symptoms in the fruit and aerial shoots.

A highly significant difference was found between smaller and larger trees at the Loftesnes site on both collection dates. As larger trees had a greater surface area, this result was

somewhat intuitive, but it does have implication in orchards that consist of trees of different sizes and age, like the Loftesnes field. As the larger and older trees are inhabited by more psyllids, they are at a higher risk of being infected (given that *C. melanoneura* does vector the disease). If the larger tree is infected, more psyllids of the new generation will become carriers of AP, as more nymphs will acquire the phytoplasma. This would have an impact in the spread of the disease on a regional level, as an increased number of new and infected adults migrate into other fields after the hibernation period.

When compensating for the size in the Loftesnes field, the other variables were unevenly distributed, and the sample sizes became very small. For future spatial studies, making sure the trees being compared are of approximately the same size would be more optimal if the aim of the study is to look at differences between infected and healthy trees, or between cultivars.

C. melanoneura has not yet been confirmed as a vector for AP in Norway 2017 (Hatteland et al. 2017), even though it is our only known viable candidate vector. Should *C. melanoneura* prove to be a vector for the AP disease in the ongoing trials, efforts to regulate the population will be put to action, and knowing the phenology of the species will be helpful in doing so. Biological control by natural enemies have only shown limited success because the overwintered generation of the species start to spread the disease before any of the predators becomes active (Baldessari et al. 2010). Controlling and manipulating populations of psyllids could be done by killing or sterilizing the adults using pesticides, reduce mobility and egg anchorage (Baldessari et al. 2010), luring and trapping them, or removing/ killing the eggs or the young stages of the psyllids. In Italy, they usually treat for *C. melanoneura* by applying organophosphates or pyrethroids three times during the spring, to limit flight / migration activity, oviposition, and treat for the springtime generation. This, however, have large negative impacts on beneficial species as well (Baldessari et al. 2010). Treating for psyllids by using pesticides early in the spring during the migration period will limit the damage done to other species, as the psyllids are very early present in the fields. This study did not include chemical sampling and analysis, but *C. melanoneura* are using plant odours to find their way to and from the overwintering sites and their reproductive hosts (Mayer & Gross 2007). This knowledge can be exploited to create species-specific traps that can attract psyllids by chemical compounds during migration, as β-caryophyllene does *for C. picta*(Gross 2011). Such traps can be beneficial in both the surveillance and the pest management of psyllids.

Even though no attraction towards AP-infected or healthy trees were found in this study, future studies might still consider this possibility. Improvements of this study would include using fields that have clear differences in symptomatic and asymptomatic trees, and have trees of the same cultivar, age and size. This would give more reliable results reducing errors of the uncertainty of infection, and other factors that might change the distribution of psyllids between the trees. Doing more than two collections, and collecting nymphs earlier in spring will give more repetitions, and actual alive nymphs would give data on survival on infected trees versus healthy trees. Another way to get further knowledge of AP- induced attraction is to do laboratory trials where the plant material used is infected or healthy by absolute certainty, and see if collected *C. melanoneura* has a preference by using a Y- shaped olfactometer ((Mayer et al. 2008). This way, the psyllids can also be tested, to see if infected and non-infected psyllids have different responses. However, such trials might not represent the reality of what goes on in the field.

Conclusion

According to the findings of the present study, *Cacopsylla melanoneura* will migrate into the apple orchards when the temperature rise above the threshold of somewhere between 7 and 8.4°C. This might cause different onset of migration into the field due to different overwintering areas of conifer forest. The present study described a population peak of *C. melanoneura* in the field in mid-April. In and around this peak the male / female ratio was 55 / 45. After the peak is reached, the activity in apple fields started to increase, mainly by male specimens due to the mating period. This activity is seen until mid/late May, and the overwintered generation is probably completely gone within the first two weeks of June.

The pattern of distribution of psyllids between sticky traps, shows that some areas of the fields have higher activity than others, indicating a need for more traps to better survey the psyllid population than the three traps that is the current standard.

There is no indication from this study that AP-infected or healthy trees attract *C. melanoneura* specimens differently. Larger trees do however host more insects, and as the apple proliferation disease causes symptoms that can make the trees grow more branches / shoots, and this alone could result in infected trees hosting more insects. If larger trees are infected, this in turn will cause more psyllids to acquire the phytoplasma, and thus have an impact on the spread of the AP disease.

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APPENDIX I

Phenology, and temperature data

Table i: Total number of C. melanoneura found on sticky traps at Opedal in the beginning of March.

Trap nr	3 March	4/3 - 07/3	08 March	09 March	10 March	11 March	11/3-14/3	14/3 -16/3	17 March	18 March
1	0	3	0	0	0	0	5	8	2	2
2	0	0	0	0	0	0	8	23	7	3
3	0	1	0	0	0	0	8	20	1	0
4	0	1	1	0	0	0	14	10	1	4
5	0	1	0	0	0	0	12	12	2	0
6	0	0	0	0	0	0	3	14	3	0
7	0	1	0	0	0	0	11	7	0	1
8	0	1	0	0	0	0	7	5	0	0
9	0	0	0	0	0	0	11	6	2	0

Table ii: Number of female C melanoneura found on sticky traps at Opedal

Trap nr	18/03 - 29/3	29/3-5/4	5/4- 12/4	12/4-19/4	19/4-26/4	26/4- 12/5	12/5-25/5	25/5- 9/6
1	10	2	4	6	8	13	5	0
2	9	4	5	15	11	30	19	15
3	4	4	2	7	4	12	7	1
4	8	5	6	19	7	17	5	4
5	4	3	2	2	0	1	2	4
6	6	4	5	6	7	20	6	5
7	7	0	1	2	0		6	4
8	5	1	3	0	1	6	6	2
9	10	3	8	6	4	22	2	9

Table iii: Number of male C melanoneura found on sticky traps at Opedal

Trap nr	18/3 - 29/3	29/3-5/4	5/4- 12/4	12/4-19/4	19/4-26/4	26/4- 12/5	12/5-25/5	25/5- 9/6
1	7	3	7	11	14	18	11	2
2	10	9	8	34	21	131	152	52
3	3	4	7	17	18	61	28	6
4	7	7	15	26	24	96	63	26
5	8	3	6	6	2	23	8	5
6	3	7	4	10	21	90	45	17
7	10	3	1	5	5		13	4
8	3	4	5	7	2	25	8	4
9	13	2	8	12	9	60	48	38

Table iv: Number of female (f) and male (m) C. melanoneura caught on sticky traps in the Utne field.

Trap/date	17/03 - 25/3	25/3-1/4	1/4-12/4	12/4-27/4	27/4-12/5	12/5-26/5	26/5-9/6
Upper	4f1m	9f3m	11f	8f2m	5f	0	0
Mid	2f	15f2m	10f6m	6f9m	4f3m	3f9m	0
Lower	7f1m	10f5m (trap found on the ground!)	20f6m	14f12m	5f5m	8f7m	2f3m

Table v: Number of female (f) and male (m) C. melanoneura caught on sticky traps at Loftesnes, and the date of collection (The traps were in the field for a week).

Trap/ date	17 March	23 March	30 March	08 April	14 April	21 April	27 April	04 May	14 May	19 May	27 May	02 June
Upper	1m	11f4m	2f3m	15f6 m	7f1m	1f2m	2f1m	1f	3f	1f	0	0
Mid	2f1m	8f	6f4m	14f4 m	4f	7f5m	3f5m	5f10 m	3f8m	1m	1m	0
Lower	5f3m	7f	9f3m	2f4m	3f	6f3m	6f5m	2f12 m	3f7m	2f	2m	0

Table vi: Number of female (f) and male (m) C. melanoneura caught on sticky traps at Njøs, and the date of collection (The traps were in the field for a week).

Trap/ date	17 March	23 March	30 March	08 April	14 April	21 April	27 April	04 May	14 May	19 May	26 May
Upper	5f2m	13f10m	18f24m	9f14 m	1f10 m	4f3m	4f10 m	14f14 m	3f8m	3f3m	4f5m
Mid	6f5m	16f24m	14f21m	7f21 m	3f3m	6f4m	5f16 m	4f13 m	2f18 m	1f4m	3f3m
Lower	9f4m	30f12m	22f21m	11f14 m	3f5m	3f5m	7f8m	5f15 m	7f15 m	2f3m	3f2m

Table vii: Total female (f) and male (m) C. melanoneura in the beat-tray samples, from Opedal and Utne.

Date	Opedal	Utne
17 March	135f166m	6f1m
01 April	259f300m	32f45m
12 April	402f497m	211
26 April	249f332m	97f114m
12 May	80f98m	7f4m
25 May	20f12m	1f2m
09 June	1m	0

Table viii: Temperature data, all fields. Acquired by Tiny Tag loggers (Opedal, Lower forest, Upper forest, Utne), and by the Norwegian Meterological Institute (Loftesnes, Njøs).

Date	Maximum temperature Opedal	Maximum temperature Lower forest	Maximum temperature Upper forest	Maximum temperature Utne	Maximum temperature Loftesnes	Maximum temperature Njøs
27 Feb	3.54	1.96	1.23	4.07	1.9	4.5
28 Feb	4.41	2.56	1.78	3.82	1.6	3.5
29 Feb	1.27	-0.05	-0.58	4.55	1.5	3.1
01 March	3.74	2.55	1.72	3.15	4.4	5.7
02 March	4.15	3.33	1.93	7.86	9.5	6.7
03 March	3.72	2.48	1.56	7.63	11.8	7
04 March	8.41	7.03	4.42	5.62	7.8	11
05 March	4.78	4.59	2.08	10.57	4.6	11
06 March	4.65	3.35	2.46	7.97	3.4	10
07 March	7.01	4.62	3.33	6.88	3.4	8.2
08 March	4.32	3.11	1.67	8.53	3.7	8.8
09 March	5.37	4.43	2.06	4.75	3	7
10 March	7.9	5.98	4.67	6.99	4.7	7
11 March	4.87	3.78	2.52	5.01	-0.5	3.9
12 March	4.63	3.17	2.63	3.7	3.3	7.1
13 March	2.69	2.16	1.52	5.49	7.4	12
14 March	10.44	8.53	5.59	10.88	9.1	11.6
15 March	13.84	12.58	9.96	10.35	6.9	11.1
16 March	12.34	11.81	8.34	10.39	7.1	11.4
17 March	10.27	10.31	9.28	7.95	4.4	7.9
18 March	12.39	10.77	8.06	5.75	1.00	6.6
19 March	6.87	5.25	6.11	5.86	2	6.3
20 March	8.24			6.36	3.9	6.2
21 March	4.29			5.58	5.9	9.4
22 March	5.99			10.57	8.4	14

23 March	9.7			13.31	6.8	11.2
24 March	3.51			12.68	6.3	8.3
25 March	5.86			7.15	7	10.6
26 March	11.97			10.86	5.8	9.7
27 March	12.07			8.93	6.4	9.8
28 March	11.57			8.74	6.6	10
29 March	12.75			9.48	8.1	11.7
30 March	9.5			9.59	7.2	11
31 March	5.81			10.28	7.7	11.5
01 April	6.52			11.66	6.4	9.9
02 April	6.14			8.09	4.2	8
03 April	9.45			6.93	7	9.9
04 April	13.97			9.6	5.7	10.2
05 April	12.17			10.7	4.5	8.8
06 April	9.78			8.66	6.2	10.5
07 April	10.81			10.98	5.1	9.5
08 April	8.41			10.3	5.1	8.6
09 April	11.23			11.64	3.5	8.1
10 April	12.71			10.46	2.5	6.1
11 April	12.12			5.03	4.7	8
12 April	13.51			7.23	3.5	7.2
13 April	12.64			7.91	5.6	10.2
14 April	6.83			9.58	7.2	10.7
15 April	8.01			9.91	6.6	12
16 April	12.18			10.38	8.1	11.3
17 April	13.55			12.45	8.7	13.6
18 April	6.61			12.97	7.2	11.1
19 April	10.67			10.63	10.1	13.5
20 April	12.09			11.55	8.7	12.4
21 April	11.03			8.11	10.5	13.3
22 April	9.48			14.47	10.7	14.2
23 April	8.53			14.46	12.8	15
24 April	9.39			15.66	14.6	17
25 April	9.47			18.23	17.8	21
26 April	11.37			20.91	19.4	21.3
27 April	11.71			20.97	17.2	20.3
28 April	11.15			21.14	17.1	19.9
29 April	12.88			20.17	14.7	17.6
30 April	13.35			17.45	7.8	12.1
01 May	11.71			12.24	8.4	12.6
02 May	11.17			14.31	10.9	13.3
03 May	10.18			15.47	10.2	14.5
04 May	15.33			14.66	9.3	13

05 May	16.56			13.17	7.3	9.8
06 May	17.84			11.05	12.2	15.6
07 May	20.34			14.13	11	14.8
08 May	22.19			13.04	13	16
09 May	23.49			15.93	11	15.1
10 May	22.87			12.01	13.8	15.7
11 May	21.63			17.87	15.9	18.4
12 May	20.64			20.46	16	19.9
13 May	12.73			20.84	17.9	20.8
14 May	14.61			20.37	19.7	
15 May	16.11			21.26	17.9	
16 May	15.98			16.17	13.1	
17 May	13.5			18.54	13	
18 May	11.28			16.81	21.2	
19 May	16.42			24.46	19.5	
20 May	12.83			19.06	20.8	
21 May	15.53			25.98		
22 May	12.06			26.05		
23 May	19.29			26.07		
24 May	21.58			26.42		
25 May	20.64			23.52		
26 May	21.14			24.01		
27 May	23.61					
28 May	15.08					
29 May	17.79					
30 May	14.81					
31 May	25.79					
01 June	19.22					
02 June	24.89					
03 June	26.63					
04 June	27.24					
05 June	25.5					
06 June	25.83					
07 June	24.32					

APPENDIX II

Spatial distribution data

Table ix: Spatial distribution data, Opedal (Row number from east to west, and tree number from south to north). 2014 data from Blystad & Brurberg (2016).

Row	Tree	Total *C.melanoneura* 1/4-17	Total *C.melanoneura* 27/4-17	Nymphs 22/6-17	PCR 2014	Symptoms 2014	Symptoms 2016
1	5	13	5	4	Negative	No	No
1	20	18	19	1	Positive	No	No
1	22	16	18	3	Negative	No	No
1	26	20	13	0	Not tested	No	Yes, late shoots
1	27	37	14	3	Positive	Yes, witches broom	Yes, light shoots
1	30	20	4	4	Not tested	No	No
1	31	11	24	6	Positive	Yes, witches broom, small apples	No
1	32	19	3	27	Not tested	No	Yes, late shoots
1	52	29	8	1	Positive	No	Yes, late shoots
1	56	26	3	0	Not tested	No	No
1	57	29	15	1	Positive	Yes, small apples, much growth	Yes, late shoots
1	71	26	5	1	Negative	No	No
1	77	22	10	0	Positive	No	No
1	89	31	7	0	Not tested	No	Yes, late shoots, small apples
1	90	62	7	0	Positive	Yes, witches broom, small apples	Yes, small apples
1	118	30	8	0	Negative	No	No
1	157	10	18	0	Negative	No	No
2	154	13	9	0	Positive	No	No
2	151	12	9	1	Positive	Yes, small apples	No
2	150	13	8	1	Not tested	No	No
2	142	4	5	0	Not tested	No	No
2	141	10	7	1	Positive	Yes, witches broom, small apples	Yes, late shoots, small apples
2	140	35	23	7	Positive	No	No
2	121	8	11	0	Negative	No	No
2	117	9	2	0	Positive	No	No
2	113	13	11	0	Not tested	No	No
2	112	13	6	5	Positive	Yes, witches broom, small apples	Yes, small apples
2	110	41	12	40	Negative	No	No
2	87	21	18	7	Negative	No	No

2	74	14	12	3	Positive	Yes, late shoots, small apples	Yes, lots of late shoots, small apples
2	73	19	17	2	Not tested	No	No
2	55	12	20	3	Negative	No	No
3	5	6	5	6	Negative	No	No
3	17	12	6	0	positive	No	No
3	30	14	15	7	positive	Yes, late shoots, small apples	No
3	31	18	11	0	not.tested	No	No
3	33	15	9	5	positive	No	No
3	44	9	12	1	negative	No	Yes, late shoots, small apples
3	50	31	12	7	not.tested	No	No
3	51	14	39	8	positive	Yes, witches broom, small apples	Yes, small apples
3	70	13	15	0	positive	No	Yes, late shoots
3	77	14	25	1	not.tested	No	No
3	78	9	9	2	positive	No	Yes, small apples
3	86	12	13	1	positive	No	No
4	132	21	19	9	positive	Yes, late shoots, small apples	Yes, late shoots, small apples
4	131	51	58	35	not.tested	No	Yes, small apples
4	120	22	11	1	positive	No	Yes, late shoots, small apples
4	117	19	27	7	negative	No	No
4	112	37	25	20	negative	No	No
4	92	23	21	5	negative	No	No
4	75	28	18	3	not.tested	No	No
4	74	14	25	4	positive	Yes, small apples	Yes, late shoots
4	59	1	18	4	positive	No	No
4	55	11	17	4	positive	Yes,witches broom, small apples	Yes, small apples
4	54	6	32	3	not.tested	No	No
4	28	15	22	1	positive	No	Yes, late shoots, small apples
4	18	16	32	28	positive	Yes, small apples	No
4	17	40	43	8	positive	No	No
5	31	26	35	27	not.tested	No	No
5	32	30	18	12	positive	Yes, small apples	Yes,lots of late shoots, small apples

Table x: Spatial distribution data, Loftesnes (Row number from south to north, and tree number from west to east)

Row	Tree	Total *C.melanoneura* 4/4-17	Total *C.melanoneura* 25/4-17	Symptoms 2016	PCR 2016
2	1	2	2	No	-
2	7	15	5	No	-

2	14	1	3	No	-
2	20	4	5	No	negative
2	27	15	2	No	negative
3	29	15	7	No	negative
3	22	1	6	No	negative
3	15	3	7	No	negative
3	7	21	6	No	negative
3	1	4	9	No	-
4	1	0	4	No	negative
4	7	7	1	Root shoots, late shoots	negative
4	15	35	13	Root shoots, small apples	-
4	22	8	9	Root shoots	negative
4	29	10	3	Root shoots	negative
5	29	9	0	No	negative
5	22	7	5	No	positive
5	15	2	6	No	negative
5	7	0	1	Root shoots, late shoots	negative
5	1	8	2	Root shoots	-
6	1	40	6	Root shoots, late shoots, small and poor colored apples	negative
6	8	2	3	No	negative
6	15	23	7	Root shoots, new shoots	negative
6	21	16	12	Root shoots, late shoots, small apples	negative
6	29	11	12	Root shoots, late shoots, small apples	negative
7	35	3	1	Root shoots, late shoots	negative
7	29	15	4	Root shoots	negative
7	22	4	2	Root shoots, small apples	negative
7	15	14	18	No	-
7	7	13	14	Root shoots, late shoots, small and poor colored apples	negative
7	1	37	5	small apples	negative
8	1	10	7	small apples	negative
8	7	5	7	No	-
8	14	36	12	No	negative
8	22	18	6	Alot of root shoots, late shoots, small apples	-
8	29	21	10	Root shoots	-
9	22	31	9	late shoots, small apples	negative
9	14	16	6	late shoots, small apples	positive
9	7	10	9	late shoots, small apples	positive

9	1	13	19	Small apples	-