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Susceptibility of different apple cultivars to Codling moth (*Cydia pomonella*) and the factors that influence the infestation level

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Abstract

Apple is the most cultivated fruit tree in Norway. However, due to many challenges, apple production is insufficient regarding the consumption requirements. Codling moth (*Cydia pomonella*) is one of these challenges, a highly destructive pest in the apple orchards. The reported resistance against pesticides of *C.pomonella* emphasizes the need for more advanced management methods based on a better understanding of the pest`s behaviour. In our experiment, we investigated the susceptibility of six apple cultivars to codling moth: Aroma, Discovery, Elstar, Gravenstein, Summerred and Sunrise. The results reveal that Elstar and Gravenstein are more vulnerable to *C.pomonella* than Aroma, Discovery, Summerred and Sunrise. The results strongly indicate that codling moth less prefers early harvested cultivars. The chemical and mechanical properties of the different apple cultivars may have a crucial role in determining this susceptibility. The infestation ratio was also evaluated in the same cultivar (Aroma) in two different farming methods (conventional and untreated). The damage rate was 0.00% in the conventional Aroma and 16,60% in the untreated Aroma. The tree morphology and management practices could be the reason for this variation.

Sammendrag

Eple er den viktigste og mest produserte frukten i Norge. Likevel er avlingen utilstrekkelig i forhold til forbruket. En av utfordringene man møter i epleproduksjonen er det svært destruktive skadedyret Eplevikler (*Cydia pomonella*). Eplevikler har vist resistens mot sprøytemidler. Dette understreker behovet for utvikling av plantevernsmetoder, basert på bedre forståelse av skadegjørerens atferd. I dette forsøket ble det undersøkt mottakeligheten for *C.pomonella* på seks eplesorter: Aroma, Discovery, Elstar, Gravenstein, Summerred og Sunrise. Resultatene viser at Elstar og Gravenstein er mer sårbare for *C.pomonella* enn Aroma, Discovery, Summerred og Sunrise. Dette indikerer at epler som høstes tidligere er mindre foretrukket av eplevikler. De kjemiske og mekaniske egenskapene til de ulike eplesortene kan ha en stor innvirkning på skadeomfanget. Infestasjonen ble også evaluert i samme sort (Aroma) i ulike produksjonsmetoder (konvensjonelle og ubehandlede), der skadefrekvensen varierte fra 0,00 % i konvensjonell kultivert Aroma, til 16,60 % i ubehandlet kultivert Aroma. Denne betydelige forskjellen skyldes antakeligvis sprøytingsrutiner og treets morfologi.

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1. Introduction

Apples are essential in Norwegian fruit production. However, apple production is insufficient and doesn't meet the consumption requirements; apple production in Norway covers only 13.11% of the consumption demands (Rebnes & Angelsen, 2021).

The short growing season, in addition to spatial limitation, bound the production. Therefore, enhancing the orchard's efficiency is needed, e.g., higher tree density instead of the traditional planting system, more efficient pest management, tree pruning and fertilization.

Pests and diseases in the orchards are serious problems that can significantly harm the crops, thus causing substantial economic loss. According to FAO, the annual losses in plant crop production are 20-40% of the total crops production worldwide. FAO also published that plant pests cause 220 billion dollars of losses every year to the producers. Moreover, considering pesticides restrictions and the fact that integrated pest management became mandatory in the EU in 2014, we need to develop our biological control techniques and our knowledge of pests and their behaviour.

Integrated pest management (IPM) is a concept found in 1970 describing sustainable farming practices as the use of resistance seeds. Nowadays, IPM is focusing more on monitoring the pests to evaluate the necessity of pest management, promoting the use of non-chemical control and demoting chemical pesticides usage (Fao.org, 2019). The development of better management programs requires more research about pests’ behaviour. Therefore, we need to improve our knowledge about pests' biology, which may reveal some vital information, for example, the most vulnerable stage in the insect's lifespan that can profit our pest management methods.

Codling moth (*Cydia pomonella*) is one of the most destructive pests that feed on the fruit, and their international seriousness triggered many experiments and investigations. Thus, much information about the codling moth is published. E.g., the relationship between sugar content and moth fertility (Wenninger & Landolt, 2011), egg deposition location (AL Bitar et al., 2012; Wildbolz, 1958), cultivars vulnerability (Joshi et al., 2015), lifespan, and so forth. However, some of these studies should be re-evaluated in terms of Norwegian conditions. Moreover, many cultivated apple varieties in Norway aren`t included in these studies.

2. The aim of the experiment

The considerable damage done by the codling moth made it crown the list of most harmful pests worldwide (Chambers et al., 2006). The aim of this thesis is investigating the codling moth preferences regarding cultivated apple varieties in Norway. The study may reveal the relation between the different properties of apple cultivars and their vulnerability to *C.pomonella.*

The investigation will be done theoretical and experimental. The theoretical part is essential because it will be the reference for discussing the results and the hypotheses. It describes the codling moth`s behaviour, apple cultivars properties, the effect of temperature and global warming and the correlation between our results and earlier observations.

The experimental part evaluates the infestation ratio in six different apple cultivars, Aroma, Sunrise, Discovery, Gravenstein, Summerred, Elstar, and organic/untreated Aroma. The different infestation reflects codling moth preferences and the susceptibility of the different apple cultivars.

Additionally, the infestation by codling moth was evaluated in trees of the same cultivars but with different farming methods. Such comparison may also provide vital information about the impact of tree architecture, age and farming practices on susceptibility to *C.pomonella*.

3. Literature

3.1. Apple, *Malus domestica*

Apple is one of the oldest fruit trees, *Malus domestica* belongs to *Rosaceae* family (Vik, 2016).

Apple production is essential in the total fruit production in Norway. The primary output is distributed along the east, south, and west coast (to Molde). In Inland, the trees are cultivated around Nordsjø, Fiskumvannet, Tyrifjorden and Mjøsa lakes (figure 1). Approximately the total production was 7469 and 8302 tons in 2018 and 2019, respectively (Myren, 2020). Still, apple production is insufficient to meet the consumption demands. The Norwegian apple in 2020 was 5768 tons compared to 43996 tons imported and fulfilled only 13.11% of the market requirements (Rebnes & Angelsen, 2021). The production depleted noticeably from 2019. Nevertheless, apple production declined massively in a larger time scale; the production depleted more than 87% since 1961 (Gerritsen, 2017). Organic production supplied the market with 1.04% of the total apple produced in 2019 (Myren, 2020).

Figure.1. The distribution of apple production in Norway (Myren, 2020).

3.2. Apple's cultivars

The ancient interest and the early existence of apples contributed to an abundant apple diversity. Nowadays, there are many thousands of apple cultivars around the world. Cultivars differ in colour, resistance to pests and diseases, chemical compounds, and other mechanical and sensory properties (Oplysningskontoret forfrukt og grønt, n.d.).

Apple cultivars also differentiate in their maturity time. Some varieties require more time to ripen. Basically, apple varieties can be divided into early cultivars (e.g., Eir, Idunn and Julyred), moderate early cultivars (e.g., Discovery and Summerred) and Late cultivars (e.g., Aroma, Elstar, Gravenstein and Lobo) (Frukt og bær lecture notes, 2019). Apple diversity is very advantageous; the differences in harvesting times supply the market with fresh fruit for an extended period (Oplysningskontoret forfrukt og grønt, n.d.).

Norway's most cultivated apple varieties are Discovery, Aroma, Summerred, Gravenstein, Lobo, Kronprins, Caroll, Åkerø, Julyred and Geneva (Oplysningskontoret forfrukt og grønt, n.d.). However, the focus in this study will be on the selected cultivars for the experiment (Aroma, Discovery, Elstar, Gravenstein, Summerred and Sunrise). This selection was based on the variation in harvesting dates (table 1) and the availability of these cultivars in the orchard (Åsbakken) at the university.

3.2.1. Discovery

Discovery, a hybrid of Worcester Pearmain and Beauty of Bath from England, came to Norway in 1974. The tree is small and grows weakly. It has a medium early bearing and gives a medium-rich yield.

The fruit has medium size. The red colour under the skin is one of the characteristics of this variety. The base colour is yellow-green to yellow, covered with a warm red colour. The fruit has a sweet taste of white, firm, and juicy flesh under relatively thick skin. Discovery is resistant against scab and powdery mildew, but this variety is not winter hardy.

Harvesting is from the beginning of September, and ripening is from September to October (Nilsen, 2020).

3.2.2. Sunrise

Sunrise is one of the best early-season apples, initially developed in Canada, a mix of Golden Delicious, McIntosh, and other unnamed verities.

The fruit is round-shaped and has a medium size. Sunrise has firm flesh and smooth skin (Sunrise apples, n.d.). The base fruit is a pale yellow to light green covered with red colour and has a sweet and juicy taste. It can be harvested between September and October (Oplysningskontoret forfrukt og grønt, n.d.).

3.2.3. Aroma

Aroma is a product of crossing Ingrid Marie with Filippa done in Sweden in 1947. This cultivar has an early and heavy bearing.

The fruits are round with no edges. Aroma apples have a medium to large size. The base colour of the fruit varies from yellow to green-yellow, covered with a pink-red colour on the skin. The skin is dry and medium thick. Light yellow flesh, moderate firm, juicy, and a balanced acid/sugar taste. Aroma is harvested between late September and early October, and the ripening time is between November and December.

Aroma is winter hardy and has good storing properties, and it can be held at a lower temperature and last longer (Nilsen, 2020).

3.2.4. Summerred

Canadian establishment created by free pollination of Summerland (Where the parents are McIntosh and Golden Delicious) came to Norway in 1964.

The tree grows vigorously but declines gradually through the years. Summerred is an early and rich bearing variety, but the tree is vulnerable to scabs (Nilsen, 2020).

The apple has an oval shape, little edged, and medium-size. The fruit has a yellow-green to the green base colour, covered with dark red colour over a large part. Thick and greasy skin and the flesh is firm and has a light green to light yellow colour. The harvest time is from mid to late September, and the ripening time is from October to December (Nilsen, 2020).

3.2.5. Gravenstein

The cultivar's origin is unknown, but it came to Norway early in 1792. The tree is tall with a broad crown. Gravenstein tree grows strongly and bears abundantly. The tree can be prone to fruit tree cancer, powdery mildew, scab, and rust (Nilsen, 2020).

The fruit has a cylinder shape with edges, but it varies according to climate, apples are flatter in eastern Norway and taller in the west. The base colour varies from greenish-yellow to yellow or even gold, covered with red stripes. Gravenstein apples emit strong odours and have a sour-sweet taste. The skin is greasy and thin, with whitish-yellow and moderate firm flesh underneath.

Harvesting time is from middle to late September and ripens from October to December (Nilsen, 2020).

3.2.6. Elstar

Elstar is a dutch crossing between Golden Delicious and Ingrid Marie and came to Norway in the eighties. The tree has a moderate-strong growing, and the bearing is early and heavy.

The fruit has a round shape and medium size. The base colour is yellow, covered with orange-red stripes. The skin is medium-thick, and the flesh is moderate firm. The fruit flesh is light yellow, juicy with good taste. This variety can be vulnerable to scrab.

It can be harvested from the beginning to the middle of October. The ripening period is from November to January (Nilsen, 2020).

3.3. Sensory and mechanical properties

3.3.1. Sensory properties

Sensory properties describe the taste, texture, size, shape, and colour of the fruit. These properties vary between apple varieties and are very critical regarding consumer choices. For instance, the taste can be sweet, sour, bitter, or salty. The taste is a result of interaction between many factors. Therefore, it's complicated to measure the taste by instruments (Karlsen et al., 1999). Furthermore, the different endogenous compounds interact and alter the fruit properties. For example, despite the fruit size and colour, harvesting time can be indicated by firmness, soluble solids, starch, and sugar contents. There are some scales that can be used to measure some properties, such as starch, size, colour, hedonic, and intensity scales (Watada, et al., 1980).

3.3.2. Mechanical properties

Firmness is a critical factor regarding harvesting time. Firmness and many other properties are linked. E.g., firmed fruit reflects higher juiciness and maturity and lower softness. Declined firmness correlates with lower water content and softening, which is a critical problem for producers. Loss of firmness happens due to changes in turgor pressure, cell wall, and cell membrane deformation (Fathizadeh et al., 2020).

Fruits consist of a complex of different cells; thus, the mechanical properties are based on macroscopic mechanical properties, like the cell's properties and thus tissue properties. Cell wall, turgor pressure, cell size, and the intercellular space contribute to determining the texture properties, such as firmness, failure load, toughness, and deformation (Kubik & Kazimirova, 2015).

The primary test of firmness is by penetrometer, which measures the needed force to push a metal piece a determined distance into the apple flesh. Greater force reflects higher firmness (Guo et al., 2007).

We can also measure the force needed to penetrate the flesh with or without the skin, which provides more details about the skin's properties. Grotte et al. (1999) measured the contribution of skin to the overall firmness. They found differences in the overall firmness among the different cultivars. During senescence and storage, the skin's contribution to the overall firmness in apple increases due to changes in the apple flesh by turgidity loss (Grotte et al., 1999).

Despite the high accuracy of the penetrometer, this method, like the compression methods, destroys the apples after evaluation. We can use nondestructive methods to measure apple firmness without any waste. Nondestructive methods are classified into acoustic vibration, optical, resistance, and electrical capacity techniques (Fathizadeh et al., 2020).

When determining the harvesting time, the purpose of consumption (fresh or industrial usage) and transport time should be concerned. Briefly, we can state that the early ripening stage is more suitable for longer transport distance and fresh consumption. Many indicators can be measured to determine the ripening stage, like starch contents\sugar contents, firmness, background colour, juice and acid content, seeds and flesh colour, and ethylene concentration. Premature harvesting will result in poor fruit quality due to off-flavour or lack of flavour. Moreover, delayed harvesting will result in softer fruits with shorter storage life (PennState Extension, 2017).
Determining the starch content, background colour, and firmness is crucial because they are related to the other indicators. For example, lower starch contents correlate with higher sugar contents because the starch degrades to sugar as the apple matures. Pale colour reflects low sugar content and immaturity, acidity decreases during ripening. We can evaluate the starch content by the Starch-iodine test, starting with cutting the apple in half and then applying an iodine solution to the flesh surface. The iodine will stain the starch with a dark blue colour. The percentage of the colourized area can be compared to a starch determining scale, e.g., the eight points scale:

A. total starch when the whole surface and core is dark blue, B. 50% of core colourized, C. 0% of core colourized and 100% of flesh dark blue, D.80% of flesh dark blue, E. 60% of flesh colourized, F. 40% of flesh colourized, G. 20% of flesh stained, H. No colour and thus no starch (PennState Extension, 2017).

Table.1. Important mechanical properties and ripening time of selected apple cultivars. Mechanical properties can provide some resistance against arthropods (Grotte et al., 1999).

Cultivar	Flesh	Skin	Ripening
Discovery	Firm	Thick	September-October
Sunrise	Firm		October
Aroma	Medium-firmness	Moderate thickness	November-December
Summerred	Firm	Thick	October-December
Gravenstein	Medium-firmness	Moderate thickness	October-December
Elstar	Medium-firmness	Moderate thickness	November-January

3.4. Apple ripening stages

Fruit maturating processes are divided into early ripening stages, such as starch degradation and late-ripening stages, like colour changes, flesh softening, and volatile biosynthesis. Later ripening processes are highly dependent on ethylene, while early ripening is ethylene independent (Johnston et al., 2009).

The fruit`s volatiles contribute to provide a specific taste and aroma. Every compound has its influence. Therefore, aroma variation in apple cultivars is driven by the differentiation of the volatile compound's ratios, including ketones, lactones, terpenoids, esters, alcohols, and apocarotenoids (El Hadi et al., 2013).

3.5. Codling moth (*Cydia pomonella*)

Class: Insecta

Order: Lepidoptera

Family: Tortricidae

Genus: Cydia

Species: Cydia pomonella

(CABI,2021)

3.5.1. Distribution and host plants

The Codling moth is a global problem due to its wide-ranged distribution. *Cydia pomonella* is registered in Africa, Asia, Europe, North and South America and Oceania (figure 2).

Their broad distribution may reflect their high tolerance and adaptation to various circumstances, especially climatic conditions. Consequently, there are an abundant diversity of hosts, such as apple, quince, apricot, almond, pears, maize, walnut, plum, peach, and pear (CABI, Plantwise Knowledge Bank, n.d.).

Figure.2. The distribution of codling moth (Pajač et al., 2011)

3.5.2. Description

The adults have a light grey colour, with a 15-22mm long wingspan with dark brown spots on the tip (the outer margins) with some coppery markings. The eggs are disks that are 1 mm in diameter. The larva is 18-20 mm long, white to pink colour with a brown head. There is also a shield on the segment behind the head (Pajač et al., 2011).

Figure.3. Adult codling moth (N.Trandem, NIBIO, 2021)

Figure.4. Matured larva of *Cydia pomonella* (N. Trandem, NIBIO, 2021)

3.5.3. Lifecycle

A single female can deposit somewhere between 30-70 eggs (Caprile & Vossen, 2011). Eggs are distributed separately on the fruits. Usually, the hatched larva penetrates the fruit and seeks the seeds within 24 hours. The covered fruits are preferable to codling moth's larva (Cranshaw & Hammon, n.d.) (figure 5). Larva has five instars, and full development takes approximately four weeks. The fully matured larva leaves the fruit and drops on the ground to pupate and overwinter in a covered place (Welter, 2009, s. 174).

Figure.5. Pale skin around the entrance reflects moth protective behaviour (G. Kaisoon)

The pupa overwinters under the bark or soil, and new adults' emergence is associated with temperature, humidity, and fruit development. In general, the adults appear between the end of May until late June.

Codling moth has between 1-4 generations depending on weather conditions. E.g., in Croatia *C.pomonella* can continue its lifecycle twice every year, and the second generation emerges between the middle of July and the middle of August. While in colder regions (like Norway), only one generation is reported (Pajač et al., 2011).

3.5.4. Damage and symptoms

The most common signs are the straight tunnels oriented toward the core, frass in the tunnels, consumed seeds, wide exit holes, and premature apple drop (CABI, Plantwise Knowledge Bank, n.d.). The entrance is usually at the calyx (the fruit end) or the more ripened side of the fruit (figure 6); however, it also can be elsewhere on the epidermis, Concerning the fact that covered fruits are preferable to codling moth's larva (Cranshaw & Hammon, n.d.). The entry hole is filled with frass (brown excretion) that larva leaves behind while consuming the flesh toward the core. In a successful attack, the larva locates and consumes the seeds in the fruits. Eventually, the larva leaves the fruit when they are fully developed, and the fruits tend to fall off prematurely due to seeds depletion. The larva usually attacks one fruit, especially in colder regions, when the codling moth has only one generation (CABI, Plantwise Knowledge Bank, n.d.).

Figure.6. Codling moth tunnels, entrance near the calyx (G. Kaisoon)

3.5.5. Climatic changes and the influence on the distribution

Climate conditions in Norway are very challenging for fruit production. The short growing season declines further toward the north. The low temperatures negatively affect sugar content in the fruit and considerably deplete the flower formation. However, the Norwegian climate is beneficial for a nice reddish colour on apples. The cold nights combined with sunny days contribute to better skin colour development (Tveito et al., 2007).

Global warming may ease the difficulties concerning apple production. It has been reported that the growing season in the past years extended, and the temperature enhanced (Tveito et al., 2007).

Reports from Hardanger disclose that the 2019 season was short but intense, and the production was sufficient. The season started with cold spring weather, but the summer was warmer with a new record of high temperatures and then wet autumn. Registrations by the local distributor show that he received 1600 tons of apples in four weeks, while last year, it took seven weeks to receive the same amount. Unfortunately, global warming can also be profitable for pests and diseases by extending their distribution in new regions (The local,2019).

Regarding *Cydia pomonella*, global warming has a critical impact on the Codling moth. Higher temperatures and a longer growing season will lead to more extensive spreading and more generations, and consequently, an extended damage period in the apple orchard (Pajač et al., 2011).

A previous study shows that the eastern part is more abundant with codling moths than the west. Pheromone traps and reports were used to investigate the codling moth distribution. Sogndal was the only place where the pest was observed in west Norway (until 1999). Codling moth has been observed over the eastern regions and further north to Lillehammer (except was the east side of lake

Byeren). The study also reports that there is a correlation between temperature and codling moth spreading (Sæthre & Edland, 2001).

The map below (figure 7) shows that the distribution altered by 2021. The distribution of *Cydia pomonella* is stretched further north up to Rana (2003), and more *C.pomonella* incidence registered in the west coast region.

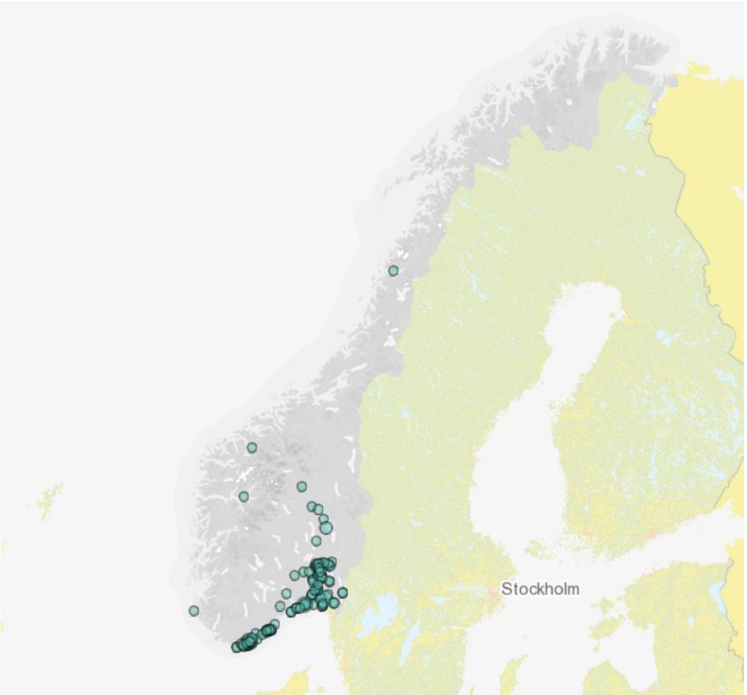

Figure.7. Codling moth distribution in 2021. Available at (Artskart.no)

Nevertheless, the temperature has a critical impact on oviposition. Usually, adult moths can deposit eggs within two days after emergence when the temperature is favourable. However, the preoviposition period becomes longer in colder weather. Consequently, eggs oviposition at lower temperatures (lower than 14) is insubstantial to induce a significant yield loss. Another suppressive condition is the rainy weather during oviposition. Rain will significantly enhance the mortality in the earlier phases of the codling moth. Therefore, Spraying should be applied only when the temperature is 14^{o}c or more during the flight period. Because at a lower temperature, egg deposition is insignificant (Sæthre & Hofsvang, 2002).

3.5.6. Codling moth traps

Pheromone traps: Using artificial pheromones that attract male moths, such lure helps study the moth activity, not suppressing the pest. We can develop the traps by adding pear ester to attract females as well, consequently traps gain slight suppression ability (Cranshaw & Hammon, n.d.).

Controlling traps: Such traps include water-thinned molasses (1:7) and yeast. We can also use beer or thinned fruit juice with two drops of soap. The mixture should be placed on or nearby the fruit tree to attract the codling moth ability (Cranshaw & Hammon, n.d.).

Light traps: Like many other insects, the codling moth can be attracted by light. Light traps can be effective; however, such traps are not widespread due to the relatively high cost (Cranshaw & Hammon, n.d.). Moreover, due to low selectivity, the traps eliminate beneficial insects as well (Ma & Ma, 2012).

3.5.7. Codling moth monitoring

Registration of codling moth by using pheromone traps display crucial information, such as flying activity, population number and the number of the generations.

Traps initially consist of artificial sex pheromones that attract males and a sticky base. Traps should be replaced every 3-4 weeks concerning declining pheromone efficacy. Pheromone traps should be placed when the trees start blooming and should be checked weekly. After emergence, the moth oviposits within a few days, depending on the temperature. Emergent and oviposition registrations are essential for effective management programs because the eggs are the most sensitive to pesticides.

Codling moth activities can also be predicted by combing the catches with the degree days model (table 2) because codling moth activities are highly dependent on temperature. The combination helps to estimate the optimal time for the management.

Degree days = (max temp – min temp) \2 – the base temperature (10^{o}c) (Cranshaw & Hammon, n.d.). However, another study reported that the base temperature of egg development is 8-9 ^{o}c in Norway (Sæthre & Hofsvang, 2002). Therefore, the table should be adjusted.

Table.2. Degree day model to predict codling moth activity (Cranshaw et al, n.d.)

Activity	Degree days
The emergence of the first generation	0
Eggs start hatching 1. st generation	220-250
The peak of eggs hatching 1. st generation	340-600
End of eggs hatching 1. st generation	920
Eggs start hatching 2. nd generation	1000-1100
The peak of eggs hatching 2. nd generation	1320-1720
End of eggs hatching 2. nd generation	2100

3.5.8. Management methods

- **Organic control implements**

Sanitation: The loose bark on the old trees, dropped apples and dead material on the ground provide a proper shelter for codling moth. Therefore, removing such materials from and around the tree is necessary to suppress the pest numbers. Regarding that first-generation larva start their invasion between May and June, it's very important to destroy the infested apples when some of them fall on the ground (Flint, 2018, s. 44).

Fruit thinning: Because fruit cluster is profitable to codling moth, thinning fruits can reduce the infestation. Furthermore, thinning provides more fruit coverage when applying pesticides. Moreover, removing infected apples (with entry holes) helps deplete the codling moth population (Cranshaw & Hammon, n.d.).

Bagging fruit: Using paper bags to enclose the fruits. The best time to do it is when the apple is between 13-25mm in its diameter. This method is very effective against codling moth, and doesn't inhibit the apple maturity, although the paper cover reduces the red colour development. (Flint, 2018, s. 44).

Truck banding: Providing an artificial place for pupation using cardboard, wavy cardboards stipes wrapped around the truck in the line between the smooth bark and the old rough one. The fake shelters must be checked weekly, especially during pupation, and collected larva should be eliminated (Flint, 2018, s. 44).

Cultivar's susceptibility: Cultivar choice by producers is an essential step. It is well known that late ripened cultivars are more vulnerable to codling moths, especially in the case of multiple generations (Cranshaw & Hammon, n.d.).

Mating disruption: This mechanism is based on sex pheromones usage to mislead the males of codling moth. Such pheromones are naturally emitted from females, and covering the area with sex pheromones will impede males to locate and mate the females. Consequently, unfertilized females oviposit infertile eggs only that are nonviable. This technique is used frequently in fruit orchards, and it has higher efficiency in orchards with shorter trees because, by height, the pheromones lose their concentration gradually (Simon et al., 2007). However, it's not recommended in a smaller production. This method helps only with copulation disturbance and not eliminating the moth; in smaller fruit gardens, copulation can happen outside the garden; hence, mated females enter and lay fertile eggs. Therefore, other controlling methods should be considered (Flint, 2018, s. 44).

- **Codling moth resistance to pesticides**

During the 80s and 90s, pyrethroid and organophosphate were frequently used to control *Cydia pomonella*. Subsequently, the efficiency of these pesticides declined relatively fast as the pest gained resistance against those compounds. Many incidents of new pesticide groups resistance are reported. The rapid development of the resistance generated the cross-resistance phenomenon. This means that *C.pomonella* has the resistance to many pesticides group concurrently; therefore, the resistance multiplies and the management become more complicated (Pajač et al., 2011).

There is also a possibility of importing resistant moths from other countries, especially from warmer regions with a large population and many generations of codling moth (Agriculture and Horticulture Development Board, 2021). This risk could be higher in Norway, considering that the imported apple fruits and trees are Norway's major apple resource (Myren, 2020).

- **Efficient pesticides methods**

Pesticides application is not always the proper solution. Due to the flexibility and tolerance of codling moths, pesticides can cause a reverse effect by more resistant moths. Therefore, intensive pesticide implements are unadvised when the population is low (Pajač et al., 2011).

We can estimate the infestation level in the orchards visually or by using the traps. If the damaged apples exceed 5%, then we need advanced control implements, including pesticides.

There should be one trap in the centre of every 2.5 acres. The traps must be placed before 175-degree days (apple bud stage).

There are two threshold methods. The first method requires spraying pesticides if the catches exceed two moths per week in two consecutive weeks. The other approach combines the degree days with the number of catches. When the trap catches more than five adults, the spraying is needed at 425 degree days (the peak of eggs hatching). If the codling moth number doesn't exceed the threshold by 525 degree days, spraying is not recommended. But if the number exceeds the threshold, spraying is needed immediately (Brunner, 1993).

- **Biological control**

There are many natural enemies to the codling moth, including birds, spiders, nematodes, insects, and other microorganisms such as bacteria, fungi, and viruses. However, viruses, bacteria, and fungi are more viable regarding biological management.

It is insufficient to use biological control agents solely, even though it can slightly suppress the codling moth population. Along with mild pesticides (nontoxic to natural enemies) and mating disruption, applying bio-agent is essential in IPM programs (Brunner, 1993).

Natural enemies can be parasites, pathogens, and predators are most that target a specific life stage of the pest.

Table.3. Some of the codling moth natural enemies (CABI, 2021)

Natural enemy	**Type**	**Targeted stage**	**Applied as bioagent**
Ascogaster quadridentatus	Parasite	Larva	Austria; Baluchistan; Canada; Germany; Kazakhstan; New Zealand; South Africa; Sweden
Bacillus thuringiensis	Pathogen	Larva	France
Bassus rufipes	Parasite	Larva	Kazakhstan; Turkmenistan
Elodia morio	Parasite	Larva	Canada; Sweden
Paecilomyces farinosus	Pathogen		Sweden
Pimpla contemplator	Parasite		Germany; Kazakhstan; Switzerland
Trichogamma sp especially Trichogramma bezdencovii	Parasite	Eggs	Belarus; Bulgaria; France; Jammu and Kashmir; Romania; USSR; Kazakhstan; Moldova

- **CpGV**

CpGV or codling moth granulosis virus is well studied as a biological control agent. The virus was discovered in Mexico in 1963, but the high costs of this agent limited its production. Recently, after restrictions on pesticide use and reports about codling moths' resistance to pesticides, the CpGT production became more acceptable.

It has been reported that the efficiency of the granulovirus can vary between 70-90%. Pesticides are more efficient, but the problem is that codling moth can adapt and develop resistance against pesticides. Additionally, the virus can continue suppressing the pest population throughout winter. According to Charmillot, granulovirus combined with pesticides or mating disruption can be the ideal method (CABI, Plantwise Knowledge Bank, n.d.).

Another study states that yearly application of the codling moth granulovirus has led to a significant reduction in the damage caused by *C. pomonella*. The level of damaged fruit was lowered by approximately 57.4% (Danelske et al., 2017).

3.6. Other common pests and diseases in the apple tree

There are plenty of pests and diseases that attack apple orchards. The causal agent can be fungus (e.g., apple scab, powdery mildew, and monilia), bacterial (e.g., fire blight), oomycete (e.g., phytophthora crown and root rot), arthropods (e.g., codling moth, leafrollers, round-headed apple tree borer, aphids, apple fruit moth, and fruitlet mining tortrix) (Plantvillage, n.d.). Moreover, many diseases can also be triggered by mineral deficiency or toxicity.

The following is pests and diseases are either observed in the apple orchard (Åsbakken) or has some similarity with *Cydia pomonella* (can induce internal tunnels).

3.6.1. Apple scab

Class: Dothideomycetes
Order: Pleosporales
Family: Venturiaceae
Genus: Venturia
Species: Venturia inaequalis (CABI, 2019)

Venturia inaequalis is globally spread, and it attacks apple trees targeting shoots, foliage and fruits.

Symptoms: Discoloration or dark stained spots on the targeted leaves and fruits. The young leaves are more vulnerable to the fungus, and the heavily attacked leaves are deformed and fall off eventually. Furthermore, defoliation may occur due to very heavy infection. The symptoms on the leaves and the fruits are easy to define. The attack on the shoots causes cracks, thus, more exposure to other pathogens (Plantix, n.d.).

Lifecycle: *Venturia inaequalis* survives the winter as spores on the falling leaves or hypha in the shoot's wounds. The fungus resumes growth and forms spores that will eventually be dispersed by wind. Spores land on new leaves and fruits to start the infection. Rainy and wet weather is essential for a successful infection. Primary infection by ascospores is completed in June, while secondary infection happens during the summer and autumn by the conidia spores. I.e., the primary infection is triggered from last year's old leaves, which is the primary source of infestation. All the apple cultivars, especially Gala, are vulnerable to apple scab (Plantix, n.d.).

It's imperative to use resistant apple varieties to apple scab in organic production. One infected tree can cause a serious problem the following season because it becomes a source of new spores.

The fungi can adapt to the less vulnerable verities and break their resistance. Fortunately, chemical control is very effective against apple scab (Plantix, n.d.).

3.6.2. Oystershell scale

Class: Insecta

Order: Hemiptera

Family: Diaspididae

Genus: Lepidosaphes

Species: Lepidosaphes ulmi (CABI, 2019)

Distribution: Asia, Africa, Europe, North and south America and Oceania (CABI, 2019).

Oystershell scale can attack more than 130 plants, mostly Rosaceae and Leguminosae family (Johnson & Lyon, 1976), and attack mainly aerial parts, mostly the bark on shoots and stems and the fruits, and less on the leaves (Naturalis Biodiversity Center, n.d.).

Description: This pest belongs to a group called the armoured scale. It has a tube-shaped cover 1-3.5 mm long. This cover is silvery grey in female become coppery brown through ageing. The cover in males is light brown, smaller, and parallel-sided (Johnson & Lyon, 1976).

Under the cover is the insect that has a sac look, no eyes, no legs, and no antennae. The insect has long mouthparts to penetrate the plant tissue.

Life cycle: There is some confusion about the generation number, either one or two every year. However, females lay eggs in the late summer, approximately 40- 150 eggs per female. The eggs overwinter under the female cover while females die just after the egg deposition. Eggs hatch at the end of May and the beginning of June and continue for 14 days, but it can be altered due to different weather conditions (Karren & Roe, 2019).

Light yellow nymphs with legs emerge after hatching. They move over the bark, settle down, and their long mouthparts penetrate the plant tissue and begin to conceal a protecting waxy coating after feeding the female remain at the same place permanently. The young shells will look like adults gradually throughout the development (Karren & Roe, 2019).

Damage: Oystershell scale is a globally important pest in fruit trees and is one of the most scale pests invading European orchards (Kozar, 1990).

Foliage discolouration and early fall, branches coated with this scale look crusty. Infested fruits will have bleached spots in the injured area, causing aesthetic damage and premature apple drop. The tree will be covered with scale and looks unsightly (Gill, 1997). The heavy infestation can be severe, branches die, shoots and leaves deform (Kosztarab, 1990), and it can seriously weaken a mature tree.

3.6.3. Apple blossom weevil

Class: Insecta
Order: Coleoptera
Family: Curculionidae
Genus: Anthonomus
Species: Anthonomus pomorum (CABI, 2020)

Distribution: Mainly in the Northern Hemisphere (CABI, 2020).

The host: apple primarily, and it can attack pear.

Pest description: the adult is a 3.5 to 6 mm long body, dark brown or black, covered with pubescence that forms a light V-shaped patch on the elytra. The mature larva can reach 8 mm body length. The body of *Anthonomus pomorum* is white, with a dark brown head and no legs (Jaastad, 2013).

Symptoms: Larva target buds and flower parts, making holes on the buds, and the flowers eventually turn brown. Adults feed on the underside of the leaf while the upper side remains intact. Adults can also attack fruits, leaving holes in the epidermis. The infestation is higher in eastern Norway than in the western regions (Jaastad, 2013).

Surprisingly, a slight infestation may have a beneficial thinning effect, while the heavy attack can destroy the whole crops owing to blossoms depletion (Agriculture and Horticulture Development Board, 2021).

Lifecycle: The adult overwinters in hiding places in the wood cracks. Beetles favour shaded sites, hence more likely to be at the borders of the orchard near forests. The adult emerges simultaneously with the bud's formation, and the abundance of buds is the most attractive target for this pest (David'yan, n.d.).

The adult lays one egg in each bud, then ten days later, the eggs hatch and the larva consume the stigma and the filaments of the flower. They destroy the petals to ensure that the flower won't open, and eventually, they eat the flower pedicel. After one month, the larva reaches the final developmental stage. Then it pupates under the flower. Adults emerge again after three weeks. This emergence happens after fruit formation. Hence, adults target the fruits (figure 8) and then overwinter to the next season (Jaastad, 2013).

Figure.8. Damage by the second-generation adult weevil (G. Kaisoon)

Apple blossom weevil is very destructive to the apple yield. It was unnoticeable in the conventional orchards due to the high efficacy of the utilized pesticides. Contrarily in organic production, this pest is widespread (Agriculture and Horticulture Development Board, 2021).

3.6.4. Apple fruit moth

Class: Insecta

Order: Lepidoptera

Family: Yponomeutidae

Genus: Argyresthia

Species: Argyresthia conjugella (CABI, 2019)

Argyresthia conjugella is considered one of the most critical pests in Scandinavia. Rowan, *Sorbus aucuparia,* is the preferred host. However, when rowan is insufficient, female moths invade apple trees committing massive damage to the crop or destroying it completely.

The attack can be predicted by measuring flowering and fruits formation in rowan; the significant fruit-setting correlates with no attack on apples. Flowering in rowan is cyclic. Thus, the plant has less fruit every second or fourth year. This defensive technique forces the moth to find another host to attack (Jaastad et al., 2002).

Argyresthia conjugella is observed in Asia (Himachal Pradesh in India), Fennoscandia (Scandinavia and some parts of Russia), Hungary, Lithuania and the Netherlands (CABI, 2019) (figure 9). The species has also been observed in North America

Natural enemies: The bacteria *Bacillus thuringiensis galleriae* and the anthropod *Microgaster polita*. Both enemies attack the larval stage (CABI, 2019).

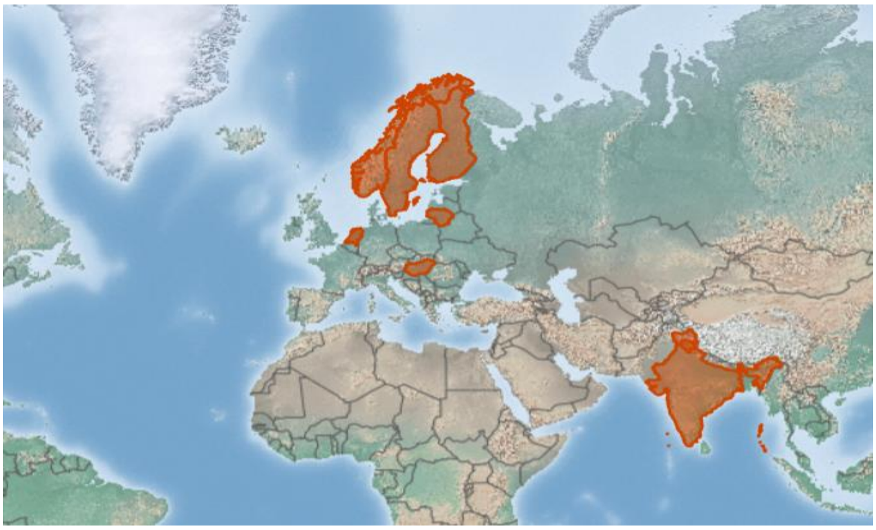

Figure.9. Apple fruit moth distribution (CABI, 2019)

Description: The adult is 10-14 mm long (figure 10). The forewings have a brown-grey colour, a white stripe on the rear edges of the wings disturbed in the middle by a dark brown band, and two white patches on the front. The hindwings are brown-grey with few long hairs. The mature larva is about 7 mm long with a faded yellow-reddish body. The eggs are round, light red with dark spots (International pheromone systems, n.d.).

Figure.10. Apple fruit moth (F. Falkenberg, 2015)

Life cycle: The adult deposits eggs on the rowan in the middle of June. The eggs hatch after approximately two weeks. The emerged larva penetrate the fruit establishing tunnels toward the seeds. The larva lives inside the fruit throughout its final maturity. When the larva is fully matured, it will drop on the ground and enter the pupal stage. The pupa overwinters in the soil. New emergence starts early June the following year (Kobro et al., 2010).

Damage: The larva (the harmful stage) attack the fruit and consume the flesh and seeds. Annual attack on rowan exhausts the plant. Therefore, the plant will not form fruits eventually. Fruit-setting in rowan is synchronized as a defence mechanism to stop the moth from attacking the fruits. When the fruits of rowan are insufficient for fruit moths, they are forced to find another host for oviposition (Kobro et al., 2010).

White powder on the apple skin is a sign of a fruit moth attack. The larva bores in the flesh and searches for the seeds. Regarding rowan, moths find their way to the seeds easily. On the contrary, larva struggle to locate the seeds in apples (due to differences in fruits sizes); thus, many tunnels across the flesh are established, forming a network that reflects their confusion (Kobro et al., 2010).

3.6.5. Fruitlet mining tortrix

Class: Insecta

Order: Lepidoptera

Family: Tortricidae

Genus: Pammene

Species: Pammene rhediella (CABI, 2020)

Pammene rhediella is distributed mainly in Europe, and the bacteria *Bacillus thuringiensis* is the natural enemy of this pest (CABI, 2020).

Host plants: Apple, plum, hawthorn, pear, sour cherry (Lepidoptera, n.d.).

The larva of fruitlet mining attacks the fruit trees in June and July. The majority of tortrix species feed on the foliage of fruit trees, but unfortunately, the fruits are more favoured for the larva of this tortrix. Usually, moths are active at night, but *P.rhediella* is an exception. The adults can be observed during the daylight, primarily on sunny days (Gratwick,1992, s.137).

Description: The larva is light green, thin, and 12-25 mm long. The larva can be identified by their wiggling backwards when disturbed. They can be very challenging to observe since they tend to hide in covered and protective places, especially the tips of the shoots where there are many leaves wrapped together (Gratwick,1992, s.137).

The adult has a dark colour on the wings 9-12 mm long (figure 11). The colour of the forewing shifts from dark to reddish yellow toward termens (outer edges). The hindwings are dark green (NBN atlas, n.d.).

Figure.11. Fruitlet mining tortrix (E. Mjaaland, 2015)

Damage: the apple cluster is more likely to be attacked, and a silky webbing can be noticed around the cluster. The larva makes a few millimetres deep hole in the apple. Usually, the entry hole is circled with a dark ring. The larva can bore the flesh profoundly, and it is very unlikely to reach the core; hence the seeds will remain intact. Consequently, apples don't fall prematurely. The larva can attack a single fruit as well. In that case, the entrance is shaded by a leaf, slight punctures on the fruit and winding tunnels (Gratwick,1992, s.137).

We can distinguish between the damage initiated by the fruitlet tortrix and the one done by the codling moth. The tunnels caused by the codling moth are straight and direct toward the core, while fruitlet induces winding tunnels and slight punctures on the apple skin.

Injury initiated by tortrix is more likely to be noticed at harvest. By this time, the damaged area becomes harder, has a corky surface, and is deformed, concerning less growth in and around the injured area (Gratwick,1992, s.137).

Lifecycle: The adult lays eggs in early May, then the hatched larva feeds on fruits and blossoms. After the final development, the larva pupates to survive the winter. The emergence of new adults occurs in April, the flowing year (Lind et al.,2003, s. 158).

Early May is the deadline for the pest control interventions. The direct control of this pest is very challenging because of the low efficacy of insecticides to suppress the pest population. Ryania can be effective against younger larva, but applying this compound has many restrictions, especially in organic production. Applying pyrethrum-rotenone or *Bacillus thuringiensis* may have a good suppressive effect against tortrix (Lind et al.,2003, s. 158).

4. Materials and methods

4.1. Location

The assay carried out in Åsbaken orchard (59°40'07.9"N 10°46'02.7"E), trees are planted in rows, and the distance between rows is approximately 2,5 m. The orchard has a rectangle shape (figure 12) and follows IPS (integrated production system). The untreated Aroma row is located along the northwest side of the orchard. The trees in this row are old, untrimmed, and without pest management or fertilization practices.

Due to aphids' outbreak in the orchard, Calypso was applied on the trees (except for the untreated Aroma). Spraying took place at 08. 01.2021, and the dosage was 0,2 ml\L.

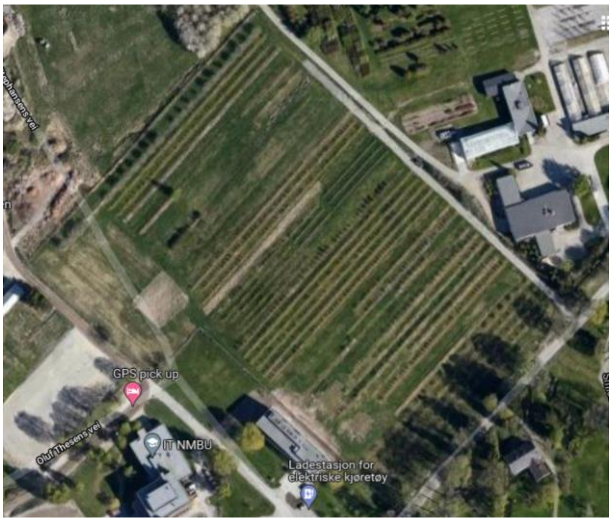

Figure.12. Fuit orchard location

4.2. The selected cultivars

The cultivars that are investigated in this study can be harvested from early September to the middle of October. Cultivars according to harvesting time from early to late are Discovery, Sunrise, Summerred, Aroma, Gravenstein, and Elstar, respectively.

4.3. Experiment design

Five replicates (trees) for each cultivar (five replicates from untreated Aroma trees were also selected), the whole yield of each tree is harvested at the appropriate time for each cultivar (table below). The harvesting time were estimated based on theoretical references (Nilsen, 2020; Oplysningskontoret forfrukt og grønt, n.d.) and recommendations based on maturity analysis (S.Hansen & K.Grønnerød, faculty of biosciences at NMBU) (table 4). Principally, trees were randomly selected (lots), but some trees were overlooked because of insufficient apple quantity (less than ten apples). Dropped apples under the tree crown were also harvested in separate boxes. Regarding untreated Aroma trees, only 30 apples were harvested from randomly selected branches; because the trees are very high, and harvesting the whole yield of these trees is challenging and time-consuming. Fruits are then stored in refrigerating chamber at 4^{o}C until evaluation. Apples with excessive damage (mostly rotten and cause inability to identify the causal factor) were excluded.

Apples were primarily be evaluated for infestation by codling moth. Subsidiarily, the symptoms of fruitlet mining tortrix and apple fruit moth are registered, owing to the similarity between fruitlet mining tortrix and codling moth and the importance of apple fruit moth in Norway. Significant infestation by other pests or diseases is also noted.

Table.4. The harvesting date of the selected cultivars (Nilsen, 2020; Oplysningskontoret forfrukt og grønt, n.d.).

Cultivar	Recommended harvesting date	Harvesting date in the experiment
Discovery	Beginning of Sep.	06.sep
Sunrise	Between Sep. and Oct.	12.sep
Untreated Aroma	Late Sep. to early Oct.	13.sep
Summerred	15. to late Sep.	22.sep
Aroma	Late Sep. to early Oct.	22.sep
Gravenstein	15. to late Sep.	30.sep
Elstar	Beginning to mid Oct.	06.oct

4.4. Assay 1

Visual assessments are used to investigate infestation symptoms using the NLR pest recognition guide and theoretical references describing the infestation symptoms (Joshi et al., 2015; CABI, Plantwise Knowledge Bank, n.d.).

Apples were evaluated individually. Fruits with stings (potential larval attack) or entry holes were selected for further investigation. The larval attack is categorized into three levels, slight or short stings (entry hole < 5mm), long stings (entry hole > 5mm), and successful entry with established tunnels toward the core.

The destructive method was carried out for internal damage monitoring by cutting the suspected apples to reveal the inner tunnels and craves initiated by the larva. The cut should be on the entrance or stings and toward the core, and by twisting the knife when it's deep enough (about 5 cm), the apple open at the weakened and craved area (tunnels).

4.5. Pest identification

Codling moth: Entry hole usually observed at the calyx with brown excretion that is pushed out. Tunnels are wide and straight to the core; seeds are consumed if the tunnels reach the core. Usually, the codling moth has a single attack on the fruit, especially in regions with only one generation per year of this pest (CABI, Plantwise Knowledge Bank, n.d.).

Fruitlet mining tortrix: The entry hole is usually shallow, the tunnels are winding and avoid the core, and the seeds remain untouched. The entry area has a faded colour (a leaf used as a cover). Through time, tunnels become corky, and the injured area deforms (Gratwick,1992, s.137).

Apple fruit moth: The external symptoms are white powder and multi-entry holes on the skin. The interior damage is a network of tunnels in the fruit flesh around the core (Kobro et al., 2010).

Identification can be challenging when the inner area is excessively rotted.

Some of the figures that are used from the NLR leaflet (The Norwegian Agricultural Extension Service) (Rein, 1996)

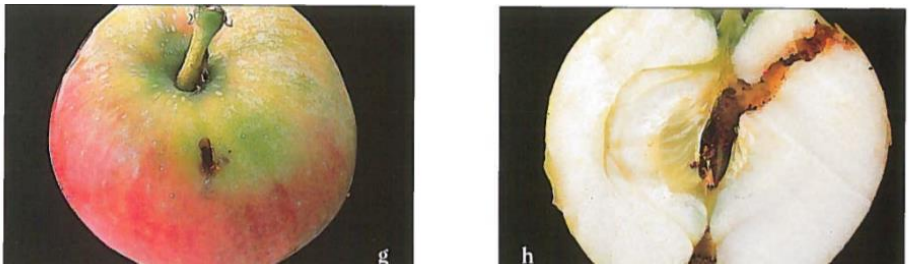

Figure.13. Damage caused by codling moth (Rein, 1996)

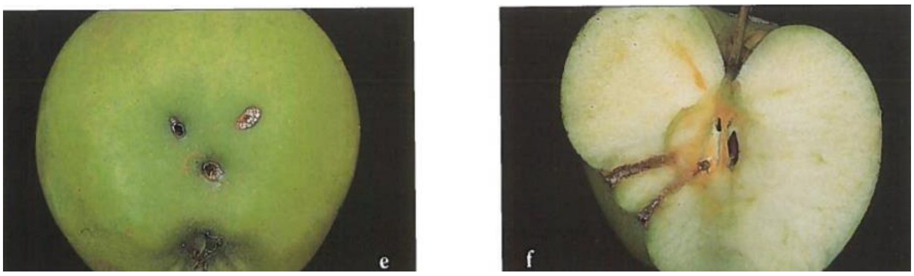

Figure.14. Damage caused by Fruitlet tortrix (Rein, 1996)

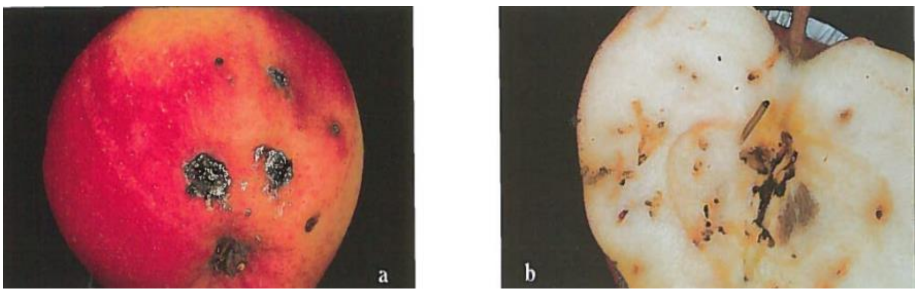

Figure.15. Damage caused by Apple fruit moth (Rein, 1996)

4.6. Assay 2

The infestation ratio was evaluated of the same cultivar in different farming methods. The infestation was evaluated of both conventional and untreated Aroma the same way as in assay 1.

4.7. Data analysis

Data is entered in an excel sheet and analyzed in RStudio R-4.1.1. ANOVA statistical analysis was carried out to study the differences between the cultivars regarding the infestation level.

Tukey test with a 95% confidence interval (p-value = 0.05) was also performed to display the differences between the groups. Additionally, a ggplot was conducted to demonstrate the results (the influence of cultivar, harvesting time and total apple on the infestation level).

4.8. Meteorological data

The temperature and precipitation data obtained from Åsbakken meteorological station.

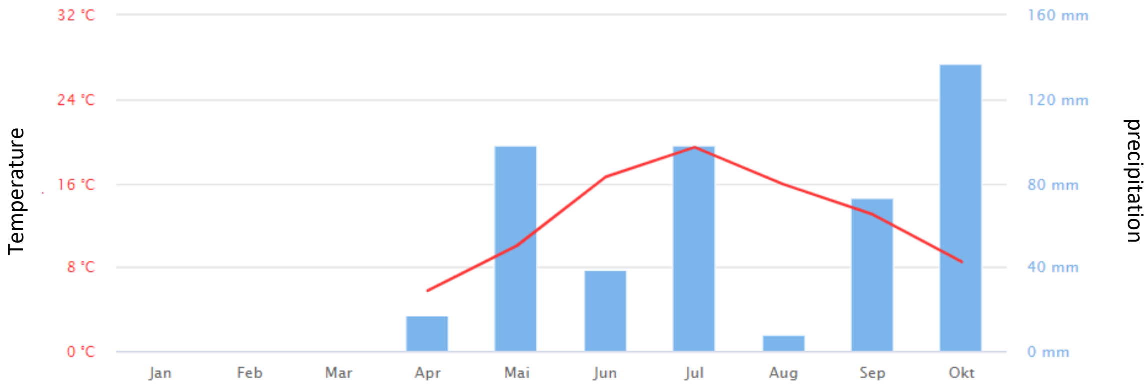

Figure.16. The average temperature and rainfall in the orchard in 2021 (Landbruks Meterelogisk tjeneste, NIBIO)

5. Results

The main results

Table 5 displays the mean values of the main results of the different cultivars. The harvesting was carried out between 06.09 (experiment day 1) and 06. 10 (experiment day 31). Cultivars were evaluated within a few days after each harvest. Stings is an uncompleted larva attack (codling moth, fruitlet mining tortrix and apple fruit moth); stings may also indicate larval mortality.

Table.5. The results mean value of different apple cultivars group, the infestation by codling moth (CM%), stings done by larva (stings %) and apple without damage was estimated in %

Cultivar	Experiment day (Harvesting)	Total yield (apples quantity per tree)	Apples with no-damage %	Stings %	CM%	Total larval attack (stings + CM) %
Discovery	1	74	82,38	14,54	3,08	17,62
Sunrise	7	96	90,66	6,57	2,77	9,34
Aroma untreated	8	44	61,08	22,32	16,60	38,92
Summerred	17	109	87,76	11,18	1,06	12,24
Aroma	17	48	84,62	15,38	0,00	15,38
Gravenstein	25	28	73,93	16,53	9,54	26,07
Elstar	31	109	81,22	8,60	10,18	18,78

The damage rate in pendent and dropped apples

The infected apples are most likely to drop prematurely; therefore, the infestation ratio is higher in the fallen apples than the apples on the tree (table 6).

Table.6. The damage ratio in dropped apples and the pendent apples in the different apple varieties

Cultivar	Discovery	Sunrise	Aroma untreated	Summerred	Aroma	Gravenstein	Elstar
CM in pendent apples %	1,45	2,79	12,67	0,84	0,00	8,62	9,04
CM in fallen apples%	27,78	15,00	25,22	1,98	0,00	7,40	19,72

ANOVA plot

Apple cultivars differentiate in their vulnerability to codling moth (Untreated Aroma is not included)

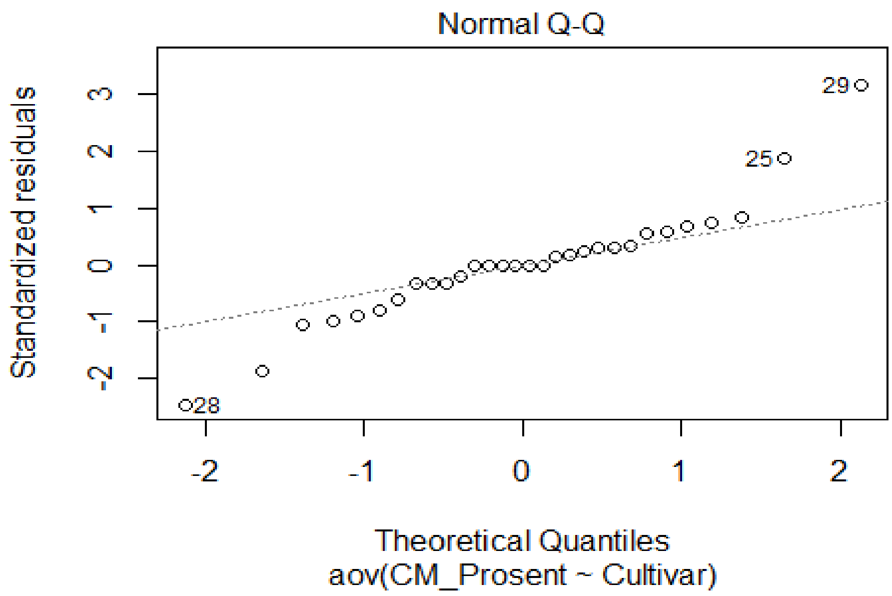

Figure.17. ANOVA test plot shows that the different cultivars vary in their vulnerability to *Cydia pomonella*.

Tukey test (Cultivars and the infestation by codling moth)

Table.7. Tukey test. The difference between the cultivars is significant when P-value < 0,05

Cultivars	Difference of Means	P-Value
Discovery - Aroma	3.082	0.728
Elastar - Aroma	10.180	0.001
Gravenstein - Aroma	9.540	0.003
Summerred - Aroma	1.060	0.996
Sunrise - Aroma	2.780	0.803
Elastar - Discovery	7.098	0.038
Gravenstein - Discovery	6.458	0.071
Summerred - Discovery	-2.022	0.938
Sunrise - Discovery	-0.302	1.000
Gravenstein - Elastar	-0.640	1.000
Summerred - Elastar	-9.120	0.004
Sunrise - Elastar	-7.400	0.028
Summerred - Gravenstein	-8.480	0.009
Sunrise - Gravenstein	-6.760	0.053
Sunrise - Summerred	1.720	0.968

Grouping the cultivars

Depending on the previous table (Tukey, table 7), we can group the cultivars according to the significant differences in their susceptibility. Cultivars with no significant difference share the same group letter (A, B, C) (table 8).

Table.8. The variation in codling moth infection in the studied cultivars

Cultivar	Mean value of codling moth damage	Group regarding their vulnerability to codling moth
Aroma	0.00	A
Summerred	1.060	A
Sunrise	2.780	A B
Discovery	3.082	A B
Gravenstein	9.540	B C
Elastar	10.180	C

There are overlapping between the groups. However, the most substantial difference is between group C and the other groups.

Tukey test (Harvesting date and the infestation by codling moth)

Table.9. Differences in damage rate regarding harvesting time. There is a significant difference when P > 0,05

Cultivars	Difference of Means	P-Value
7-1	-0.302	0.9999094
17-1	-2.552	0.6594005
25-1	6.458	0.0461101
31-1	7.098	0.0238055
17-7	-2.250	0.7532834
25-7	6.760	0.0338958
31-7	7.400	0.0172386
25-17	9.010	0.0005640
31-17	9.650	0.0002376
31-25	0.640	0.9982378

From the table, cultivars harvested at day 25 and 31 (Gravenstein and Elstar) differ significantly from cultivars harvested at earlier dates day 1, 7 and 17.

Boxplot (The infestation rate in the studied cultivars)

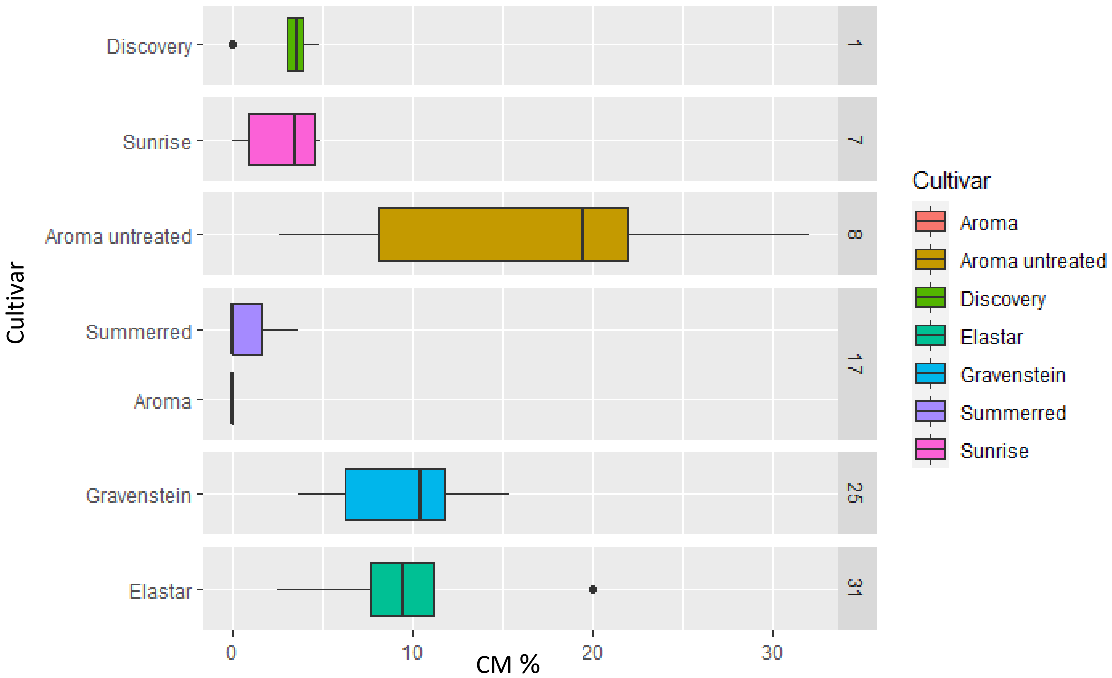

Figure.18. Boxplot shows the codling moth preferences among the cultivars. The selected cultivars are grouped according to the harvesting day (1, 7, 8, 17, 25, 31). Except for untreated Aroma, cultivars harvested in closer dates (about six days) had almost similar infestation value.

Boxplot (The stings rate in the studied cultivars)

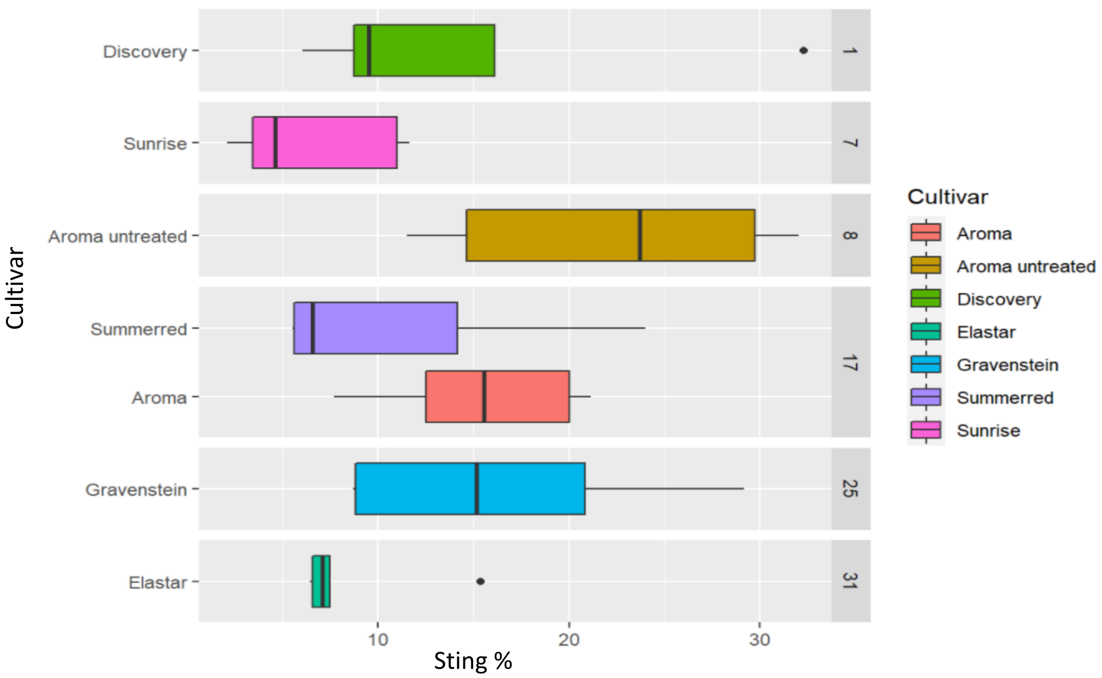

Figure.19. Possible codling moth attack (stings). Regarding assay 1, Aroma and Gravenstein have more stings than the other cultivars. Different harvesting dates didn't affect the stings ratio.

The influence of the total yield on the susceptibility to codling moth

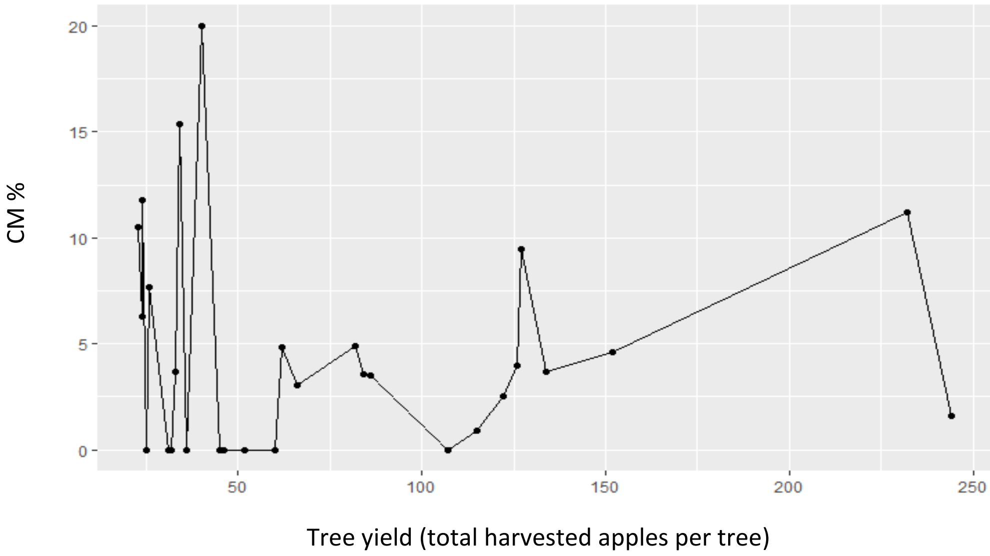

Figure.20. The relation between damage rate by codling moth and the tree yield. There is no correlation between the infestation level and the total number of apples. CM does not discriminate between the trees regarding their bearing.

Codling moth monitoring

Monitoring *Cydia pomonella* was performed by N.Trandem (Research scientist at NIBIO) using a pheromone trap placed on an untreated Aroma tree to monitor the codling moth. According to N.Trandem, the first catch of codling moth (6 adults) was between 28.05 and 03.06. There were eight moths in the trap during the following week, and then the number declined to 2-3 moths in the consecutive weeks. No catches were registered between 03.07 to 09.07 due to no or very few active adults. Surprisingly, new catches of codling moth occurred at 23.08, 01.09, and 15.09 late in the season, by two, four, and one adult, respectively. Results provide a strong indication of second-generation`s emergence in the same season.

In addition, five other pheromone traps were placed in the orchard to investigate apple fruit moth. However, there were codling moth adults trapped in the lure, maybe due to contamination by pheromones that attract codling moth somehow. The results by S.Markusson (internship student at NIBIO) correspond with the results of the codling moth trap. The first flight was registered at 04.06, and then the catches declined.

These results correlate with my registration of codling moths symptoms on apples. During the evaluation, living larva were found inside some apples on 15.09, 20.09, and 21.09; the infested apples were harvested on 12.09 and 13.09. The larva differentiated in size and damage level, the ones found on 21.09 was bigger, and the initiated damage was greater.

6. Discussion

6.1. Assay 1

The susceptibility index of apple cultivars for *Cydia pomonella* is derived from three major factors: codling moth behaviour (Jackson, 1982; Wildbolz, 1958), cultivar properties, and timing (Joshi et al., 2015). Therefore, we must understand the interaction between these factors to clarify the differences in susceptibility of the different cultivars.

The statistical analysis of the results shows significant differences between the selected cultivars regarding their susceptibility to codling moth. The infestations ratio ranged between 0% (Aroma) and 10.18% (Elstar) and was highest in Elstar and Gravenstein (group C) (table 8).

The results also indicate that harvesting time has the dominant impact on susceptibility (table 9 and figure 18). Late cultivars are more prone to attack by *C.pomonella* than early and moderate early cultivars. The results correspond with a previous study in USA, where the study reported that late ripened cultivars have a higher vulnerability to codling moth (Joshi et al., 2015).

The infestation by codling moth triggers after consecutive procedures, starting with choosing the proper place for oviposition, egg hatching and larva activity. Therefore, dysfunction in one of these incidents will terminate the infestation.

We categorized the damage into three components in the experiment: small stings, long stings, and codling moth successful attack. The stings indicate an unsuccessful attack by the larva. Some larva dies a short time after feeding on apples (Grotte et al., 1999). Economically, both damages are aesthetic defects, but the long stings enhance bacteria and fungi attack. Thus, the fruit is inedible (Brunner, 1993).

6.1.1. The adult preferences

It`s undoubtedly that the adult codling moth discriminates between the different apple cultivars. The results in this assay indicate that *C.pominella* presence in late ripened cultivars is substantially higher than in earlier varieties (table 9). Apple cultivars differ in their chemical and physical properties, which may influence such preferences (Joshi et al., 2015).

Codling moths' affinity to sweet bait is well documented, and feeding the codling moth on different sweet baits has been investigated. The study reports that sucrose and honey water treatments resulted in higher fertility and longevity for codling moths than water, apple juice, apple flesh, or starvation treatments (Wenninger & Landolt, 2011). Therefore, higher sugar content is profitable for *C.pomonella*.

Consequently, we can hypothesize that apples with higher sugar contents are more likely to be attacked by codling moths. Hence, higher susceptibility. Furthermore, it has been published that, in general, the early cultivars have lower sugar content and higher acidity compared with later cultivars (Onik et al., 2019). Therefore, late cultivars are more vulnerable than early cultivars due to their variation in sugar content. The hypothesis correlates with the results of this experiment. However, I didn’t measure the sugar content of the different cultivars. Moreover, sugar content varies through time due to starch degradation and sugar accumulation during fruit`s ripening (Johnston et al., 2009). Accordingly, more data is needed for more accurate investigation.

Many studies have proved that some of the chemical compounds in the fruits entice adult codling moth. E.g., Acetic acid, ester, dibutyl sulfide. Fermentation and some microbes are also good attractants. Furthermore, some of these compounds can be used to enhance the efficacy of the controlling traps. E.g., the use of acetic acid combined with N-butyl sulfide and pear ester in traps increased the catches (Landolt et al., 2014). Therefore, a higher accumulation of these compounds in the plant may correlate with higher vulnerability to codling moth.

Cydia pomonella is highly attracted to some volatiles emitted from foliage and fruits of apple trees. The volatiles attract codling moth and stimulate the oviposition. The most efficient attractant is kairomones (e.g., *E, E* α-farnesene and *Z, E* α-farnesene, which are natural compounds in the fruit skin tissue (Sutherland et al., 1977)). Releasing those compounds (especially α-farnesene) stimulate the moth to oviposit on the fruits (Joshi et al., 2015). Furthermore, α-farnesene not only attract the gravid moth to oviposit but can entice the newly hatched larva as well (Wearing & Hutchins, 1977). Volatiles are highly synthesized during the later maturating stages (Johnston et al., 2009); therefore, the fully matured fruits are the most attractive for *C.pomonella*.

The same conclusions were published in an experiment investigating codling moth preferences in apple cultivars. The experiment was carried out by Joshi et al. (2015) in Pennsylvania, USA, where ten cultivars (only Sunrise is common with my experiment) were inspected for their vulnerability to codling moths. Results showed that that oviposition was more frequent in late varieties. The authors concluded that volatiles could have some impact on the selection of apple cultivar by the moth (Joshi et al., 2015).

Moreover, early in the season, the codling moth prefers to oviposit around the fruit, while it deposits eggs directly on the fruit later in the season (Wildbolz, 1958). Such preferences can indicate that matured fruits are most preferred for oviposition. Ripened fruits provide a successful larval entry, which can be due to starch degradation and flesh softening (Joshi et al., 2015). Starch degradation is an early ripening process, while volatiles biosynthesis happens later in the season (Johnston et al., 2009). Therefore, the emitted volatiles indicate fruit softness for *C.pomonella*, while the absence of such compounds indicates harder fruits.

Moreover, volatiles are also emitted from foliage (Wearing & Hutchins, 1977). Furthermore, we can assume that the foliage emits more volatiles than fruits early in the season, it`s because the volatiles synthesis in fruits is ethylene dependent and happens later in the season (Wearing & Hutchins, 1977). Therefore, codling moth deposits more eggs on the leaves early in the season than directly on the fruits.

Additionally, studying the phenology of codling moths in other hosts may reveal some facts related to apples. It has been published that the codling moth discriminates between walnut cultivars and that chemical compounds and fruit size can influence such selection (Bezemer and Mills, 2001). However, in our results, the size of the fruit didn't affect the infestation level. Aroma and Gravenstein apples are recognized as large-sized fruits (Nilsen, 2020). Codling moth infestation was lowest in Aroma 0% and next highest in Gravenstein 9.54%. Nevertheless, the stings rate was almost equivalent 15,38% and 16,53%, respectively, and are the two highest among the studied cultivars with a good margin (table 5). Stings are indicators of a failed larval attempt to penetrate the fruit; the causal larva can be codling moth, fruitlet tortrix or apple fruit moth. The contradictory differences between infestation and stings may reflect that size can be an attractant for codling moth. Still, the failure to penetrate the fruit is high, maybe due to the early harvesting of Aroma (harvested at the earliest recommended date) and the late harvesting of Gravenstein.

There is no correlation between tree yield and codling moth attack (figure 20). The highest yield was in Sunrise, Summerred and Elstar, and the infestation rate varied massively between these cultivars, the same observation in cultivars with low yield (Aroma and Gravenstein) (table 5).

6.1.2. Oviposition site

Oviposition site selection by codling moth is crucial. The deposited eggs must adequately attach to the surface to survive. Unfixed eggs could easily be removed by wind or rain, and therefore the mortality enhances (AL Bitar et al., 2012). It has been reported that rough surfaces (like the surface on the lower leaf side) give stronger egg adhesion. However, females are more likely to oviposit on smooth surfaces (the upper side of the leaves and the fruits). This selection is because the smooth surfaces provide a better binding for the adult and neonates, although the lower side provides better egg attachment (AL Bitar et al., 2012).

Moreover, the hairy surfaces can hinder the female from ovipositing, and it has been published that codling moth does not lay eggs on peach fruits where pubescence is denser (Hagley et al., 1980). Therefore, we can assume that cultivars with smooth skin are preferable. However, our study has no precise results that profoundly describe the skin texture; hence, further investigation should be carried out.

Additionally, adult moths prefer depositing eggs on neighbouring leaves early in the season, while oviposition occurs directly on the fruit later (Wildbolz, 1958). Moreover, oviposition on leaves extends the time larva need to reach and eventually penetrate the fruit. Consequently, hatched larva on leaves is more prone to elimination due to unfavourable conditions, like starvation and predators (Jackson, 1982). Therefore, larval mortality early in the season is higher due to oviposition on leaves. The result of the experiment shows that earlier collected cultivars (Discovery, Sunrise, Aroma and Summerred) have a lower infestation ratio than the late-harvested cultivars (Gravenstein and Elstar) (table 8). That can be either owing to higher larva mortality or less preferred oviposition site on early harvested varieties.

6.1.3. Larval behaviour

Eggs survivals are high during the season; on the contrary, only 33% of the hatched larva can induce long damage inside the fruit (successful attack). Unexpectedly, most larval deaths happen under proper weather conditions (Geier, 1963).

After hatching, the larva must undergo four steps before entering the maturating phase. Hatched larva start with searching for the fruit, studying the surface, entering the fruit, and finally feeding interiorly. For the first generation, searching for a suitable place for pupation is an extra step (Jackson, 1982). After reaching the fruit surface, some larva don`t start boring the fruit immediately, but they stall instead. This wondering time increases larval mortality due to prolonged exposure to fatal factors. Consequently, the first instars are the most prone to elimination (Jackson, 1982).

Larval movements are oriented and have a developed thigmotactic sense (Coutin, 1959). Furthermore, there are observations of no larval activity after hatching. Stalling occurs to escape unfavourable conditions or due to the inability of locating the fruit (Jackson, 1982). Moreover, Jackson and Harwood (1980) observed that the larva is more likely to emerge during the morning on warm days, where the temperature is lower than in the afternoon.

Larvae are photopositive, and their movement toward the light in vitro is reported. This movement could reflect their activity on trees, where the majority of the fruits are on the end of branches where the light is stronger (Jackson, 1982). The larva can also locate the fruits by olfaction (Mclndoo, 1929); an equivalent study showed the movement of newly hatched larva toward apple skin in vitro (Sutherland, 1972). Moreover, similar attraction occurred when replacing the apple skin with α-farnesene compounds (Jackson, 1982). Therefore, we can summarise that the larval movements are organized and guided by visual and smell senses.

The population depletes the most during the first instars movements and before entering the apple. Depletion could be due to predators, starvation and inappropriate weather conditions (Jackson, 1982). Therefore, the longer time outside the fruit results in higher mortality.

Plant surface is very crucial in understanding the pest's interaction with the plant, such as oviposition, egg adhesion and larval locomotion (Albitar, 2014).

The plant surface is chemically and physically defined by the cuticle covering the epidermis on aerial parts. The cuticle varies among the plants and consist of wax and cutin (Barthlott et al., 1998). Many plant parts also have trichomes consisting of an epidermis cell extension. The trichomes have a critical role in plant resistance by providing a mechanical hindering (Peter et al., 1995). Epicuticular wax and trichomes are recognized as direct defence strategies against insects (Schoonhoven et al., 2005; Southwood, 1986). The high density of trichomes and wax on the plant surface significantly reduces the attachment ability of many herbivorous insects (AL Bitar et al.,2012). It also provides a mechanical impediment that limits fruit penetration by the larva (Southwood 1986).

Searching time is affected by many factors, like distance, path complexity, and weather condition. The high temperature and the rain are fatal for the larva (Jackson, 1982). Moreover, larval mortality enhances when oviposition occurs on the leaves (Wildbolz, 1958), especially when combined with hairy surfaces that hinder the larval movement (Hagley, Bronskill & Ford, 1980). Consequently, because of the long path to the fruit and surface hindrance, larval mortality is higher in earlier cultivars due to longer exposure to predators, unfavored weather conditions and energy loss. Moreover, even if the larva reaches the fruit, they may not have enough energy to penetrate the fruit due to starvation after the long searching journey (Jackson and Harwood 1980).

Additionally, flesh firmness and skin texture can influence penetration ability (Grotte et al., 1999). Moreover, differences in susceptibility in apple cultivars can be due to wax toxicity to neonates (Putman, 1962; Hagley et al., 1980). The thickness does not only vary among the different cultivars (Homutová & Blažek, 2006), but it also differs within the same cultivar (Knuth & Stösser, 1987). The skin thickness in apples is not equal on the fruit. The skin is denser at the stem end than the calyx (stylar end) and medium-thick where the diameter of the fruit is biggest (Homutová & Blažek, 2006). Usually, firmness tests didn't include the skin contribution to the overall firmness. A published paper shows that skin thickness is essential regarding firmness (Grotte et al., 1999).

Pear has shown another defending mechanism against codling moth. Some pear cultivars have developed stone cells around the calyx (favoured spot by larva), and these cultivars were less vulnerable to codling moth (Westigard et al., 1976). However, such cells are not found in apples, but it indicates that harder flesh is undesirable regarding codling moth.

The theoretical information from NIBIO mentioned that the skin of early cultivars (Discovery and Summered) was denser (table 1). Moreover, the flesh firmness was higher in Discovery, Sunrise and Summered (table 1). Furthermore, except for Aroma, cultivars with thinner skin and less flesh firmness were more infected by codling moth (table 5). However, skin properties are not stable within the same cultivars (Knuth & Stösser, 1987), and therefore such hypothesis should be investigated using more precise data.

Assuming that stings are codling moth unsuccessful attack, we can measure the larval mortality by estimating stings\total penetrating attempts (stings + codling moth successful damage) ratio. According table 5, we find that larval mortality is 82,52%, 70,34%, 91,34% 0,00% 63,41% and 45,79% in Discovery, Sunrise, Summerred, Aroma, Gravenstein and Elstar respectively, the results indicate that larval mortality is lowest in Gravenstein and Elstar. Still, stings can also be induced by fruitlet mining tortrix (no registration of apple fruit moth in the orchard) or by conditions unrelated to the cultivar`s properties.

6.1.4. Timing

The relative low vulnerability of earlier cultivars to codling moth could be because of timing issues (Joshi et al., 2015). Larval emergence and fruit maturity may not be simultaneous.

Furthermore, oviposition is weather dependent, and the temperature should be above 14 c˚ degrees in the evening for sufficient oviposition (Sæthre & Hofsvang, 2002).

Results report that Aroma and Summered were less infected by codling moth (table 5). Both cultivars were harvested at 22.09; therefore, the timing issue may be the reason for the low damage rate. Codling moths prefer to oviposit on late cultivars, and they are attracted by the volatiles emitted from ripened fruits (Joshi et al., 2015). Therefore, the oviposition period maybe occurs when Aroma and Summerred fruits emitted less volatiles than the other cultivars.

Furthermore, the larval fully maturating requires approximately four weeks to be completed. Then larva pupates in a covered place (Welter,2009, s. 174), and the new adults emerge when the weather condition is favourable. In countries with two generations of *C.pomonella*, the second generation emerges between the middle of July and the middle of august the same year (Pajač et al., 2011).

Moreover, According to N.Trandem, the first catch in this season was registered between 28.05 and 03.06. New catches we registered the following weeks until it stopped on 03.07. Then later in the season, new catches were registered on 23.08, 1.09, and 15.09 by two, four, and one adult, respectively. Additionally, during apple evaluation, living larva were found inside some apples on 15.09, 20.09, and 21.09 (apples were harvested on 12.09 and 13.09).

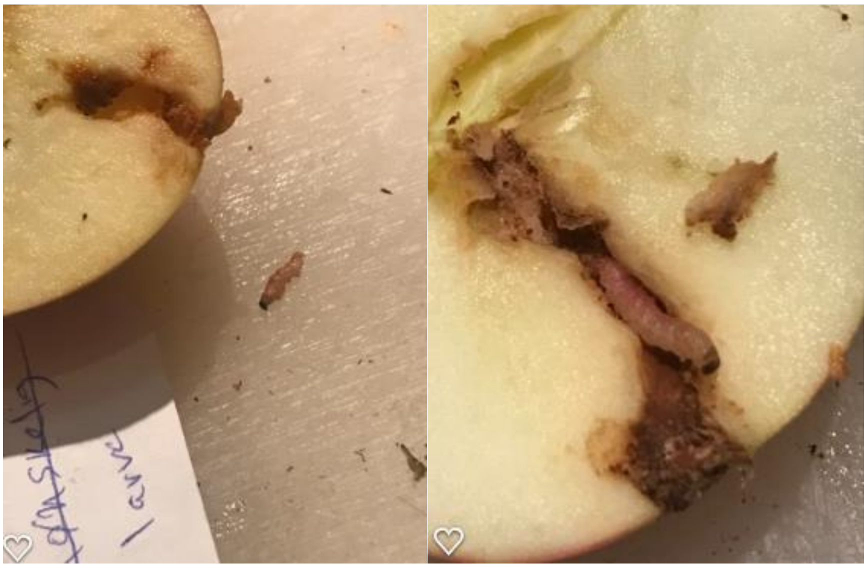

Figure.21. The living larva found during evaluation at 15.09 on the left and 21.09 on the right (G.Kaisoon)

The monitoring data and the experiment`s observation strongly indicate the emergence of second-generation in this season. Corresponding reports declare that some second-generation adults may emerge in the same season due to the high temperature earlier this season (Jaastad & Trandem, 2021).

Assuming that the emergence of the second generation was on 23.08 (monitoring data), the larva hatch approximately 90 degree-days after adult emergence, and by using a degree-day calculator (Varsling innen planteskadegjørere, NIBIO), we find that the new larva hatched on 07.09. This is represented by finding the living larva inside the fruits during September (figure 21).

The early harvested cultivars in the season may escape the second-generation attack. Contrarily, the infestation becomes more significant in late cultivars because of the exposure to second-generation moths (Cranshaw & Hammon, n.d.).

6.2. Assay 2

The different tree architecture influences the tree's attractiveness as a host for insects (Simon et al., 2007). Moreover, in a previous study, it has been reported that winter pruning significantly reduced the egg deposition on the plant (approximately 50%) in two arthropods (Simon et al., 2007).

Concerning the fact that covered fruits are preferable to codling moth (Cranshaw & Hammon, n.d.), we can assume that untreated trees are more likely to provide a possible place for oviposition. The untreated Aroma trees in the orchard (Åsbakken) were neither trimmed nor sprayed with pesticides. The high density of branches and leaves provide proper shelter for many insects, including codling moths. Moreover, untreated Aroma trees are old and can serve many opportunities for pupation (Jaastad & Trandem, 2021) due to the tree's loosen bark and the cracks in the wood (CABI, Plantwise Knowledge Bank, n.d.). In our results, Aroma and untreated Aroma differed considerably in infestation ratio (0.00% and 16,60%), respectively. The results correspond with the previous reports. However, the infestation variation can also be due to management practices in the orchard. Furthermore, the location of the untreated Aroma trees (orchard`s edge) may affect the infestation rate because trees at the orchard`s edge are more exposed to wind, thus less efficient pest management (mating disruption and pesticides spraying) (University of California, 2017). Still, no pest control implements were applied on untreated Aroma trees.

The infected untreated trees significantly enhance the codling moth population by serving proper places to feed on and pupate. After overwintering, a new generation emerges the next year, and a new attack begins (Welter,2009, s. 174). Consequently, the moth numbers increase and may eventually overwhelm the whole orchard.

6.3. Other observation

- Only apples of untreated Aroma had a heavy attack of blossom weevil. Due to the efficient suppression of pesticides, blossom weevil is hardly noticed in conventional orchards. On the contrary, it is very destructive in organic production (Agriculture and Horticulture Development Board, 2021). The infestation was excessive (34% of the apples) in the untreated Aroma number 4 (number 10 raw 41 in the orchard).
- Many Aroma fruits developed water core in the flesh, a physiological disorder presented by water-soaked areas in the apple flesh. Water core formation is cultivar dependent (Beaudry, 2014). Aroma's fruits also had symptoms of Calcium deficiency (about 37% of the fruits). Maybe these diseases have some impact on the absence of codling moth attacks (only stings are found).
- Aroma number 3 (tree number 6 in the raw 41) had a noticeably lower damage ratio, 2,60%, than the damage ratio in the group 16,60%. It could be due to the pheromone trap located on the same tree. The pheromone trap maybe had some local disruption effect.
- No symptoms of apple fruit moths attack because when the rowan fruits are sufficient, apple fruit moth does not attack apple orchard (Jaastad et al., 2002).
- Premature fruit dropping due to seeds depletion (CABI, Plantwise Knowledge Bank, n.d.). The infestation ratio in dropped fruits was significantly higher than in pendent apples (table 6). The more infected dropped apples may reflect higher infestation in the tree.
- Summerred was the most targeted cultivar by birds (13,77%). It could be that birds also have their preferences.

6.4. Conclusion

Unlike pear, there is no reported resistance mechanism against *Cydia pomonella* in apple cultivars. However, in this study, the results show that *C.pomonella* preferred the late-harvested cultivars for oviposition. Therefore, late cultivars are unsuitable for organic production. On the contrary, the early harvested cultivars may suit the organic production. However, their vulnerability to other pests and diseases should be evaluated.

Some of the cultivar`s properties like sugar content, volatiles, skin texture and flesh firmness appear to impact such preferences. Still, such hypotheses must be investigated. The older trees seem to have a higher infestation ratio because of their abundance of proper shelters for pupation. Additionally, the high temperature in this season was preferable for *C.pomonella* and resulted in the emergence of the second generation in the same year (2021).

6.5. Further studies

- Studying the influence of the early and late harvesting on codling moth`s attack. We can harvest the same cultivar in two different periods and measure the variation in the infestation ratio.
- Investigating the impact of the skin texture and flesh firmness on the codling moth larva`s ability to penetrate the fruit. More measurements and information about the skin and flesh properties of the various cultivars are needed.

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