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POLLINATOR TREES AS A POTENTIAL INOCULUM SOURCES FOR FRUIT DECAY FUNGI ON APPLE.

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Preface.

This thesis was written at the Norwegian University of Life Sciences, Department of Plant sciences. Both Field and laboratory work was carried out both in NIBIO stations Lofthus and Ås in the spring and summer of 2017.

i. Acknowledgements.

I wish to pass special dedication to my family who have been there to provide strong support and inspiration during this project period

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ii. Abstract.

Over the recent years it has become common to plant cultivars of crab apple as pollinators at regular distance in the rows of the apple fields. Little or no specific knowledge exist about the susceptibility to diseases of the various crab apple cultivars used for pollination. The fruits are rarely picked or removed, and if still attached to the fruit trees the following growing season, they may thus become potential inoculum sources for fungi causing fruit decay on apple

Crab apple cultivars, 'Dolgo', 'Professor Sprenger', 'Kobenza', 'Golden Hornet', and 'Evereste', were evaluated as potential inoculum source for fruit decay fungi. These crab apple cultivars were used as pollinizers of apple cultivars grown in NIBIO experimental unit located in Ullensvang area: They were planted adjacent to apple cultivars 'NA 42-51', 'Elan', and 'Rubinola', to act as pollinizers.

To better understand if tree infections and mummified fruit could be contributing to apple fruit decay, sampling was conducted on three experimental orchards at NIBIO Ullensvang and Ås stations during the spring to summer growing season of 2017.

Asymptomatic leaves, fruits and symptomatic mummies were sampled from selected trees and cultivars from orchards, Olavshagen, Løeflaten and Kvitavoll. The samples were of similar developmental stage during each sampling. The samples were processed and incubated as described on materials and methods section. The resultant apple fruit decay fungi were then identified, quantified and recorded. Identification was done morphologically by eye or by use of stereo and/ or light microscope if need be. Those which could not be identified in this manner, pure isolates were made and re-assessed, identified and quantified. Quantification was done by counting the number of diseased samples relative to the total samples in each group (incidences).

A total of 11 apple fruit decay fungi were isolated, identified and quantified These apple decay fungal species were; Brown rot, Mucor, Fusarium, Bitter rot, Blue mold, Gray mold, Bull's eye rot, Cladosporium rot, Tricothecium, Sclerotinia and white rot. Incidences ranged between 0.33% to 66.7% on fruits. Leaves did not show much sporulation and incidences ranged between 40 to 80%. The frequency of incidences and number of apple fruit decay fungi was found to be higher on fruits than on leaves.

This study shows that both old and new fruits and leaves can be a source of inoculum to apple fruit decaying fungi. New fruits and leaves had quiescent infections while mummies were a host to a wide array of colonizing fungal species.

The main objectives of the study were;

1. Identification and quantification of putative pathogens present on fruits and leaves of 5 different cultivars at different times throughout the season
    a. Two crab apples and three apple cultivars-Løeflaten and Olavshagen orchards
    b. Five crab apple cultivars-Løeflaten orchard
2. Identification and quantification of putative pathogens present on mummified fruits at different times throughout the season
    a. Two crab apples cultivars -Kvitavoll orchard

Based on the results obtained, it is concluded that pollinator trees are a potential source of inoculum for fruit decay fungi on apples.

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1 Introduction

1.1 Apple

1.1.1 History of Apple Production in Norway

Fruit growing in Norway dates to the West-European middle age. Fruits were grown in monasteries over the period from 1100-1500. Hardanger is the main fruit growing area in Norway, with farms averaging around 2-5 hectares. The main fruit species grown are sweet cherries, plums, pears and apples. Fruit growing is seasonal due to weather constrains making production to be limited to only a few months of the year (Darnell 2003) which makes fruit growing an important economic activity. Ullensvang is the largest fruit producing area in Hardanger, which began in Opedal farm in Lofthus, over 800 years ago. (Sekse, L. (2007).

Currently, apple production is concentrated in southern Norway. According to data from Stat bank 2014, the leading counties are Hordaland, Telemark, Buskerud, Vestfold, Sogn, Fjordane and Rogaland (SSB 2014). The total cultivated area by 2017 is 13 791 decares(daa) (SSB 2017) a drop from 14 277 decares (daa), with a total production of 11.5 thousand tons apples in 2014 (SSB 2014b).

Norway benefits strongly from the mild Gulf stream in the Atlantic Ocean which without it, fruit production would not be possible at latitudes 58 – 62°N. The stream delays and reduces the intensity of winter frosts. Late flowering of fruit tree period makes blossom frost very rare. Most orchards are supplemented with irrigation during the summer periods. Fruit orchards are located mainly on landslip soils and moraines which are warm with good drainage. The fruit districts of western Norway are important tourist destinations, and the tourist industry has a turnover counting many times that of fruit production. The fruit growing is an important part of the total marketing of this region as a tourist destination. In this respect, fruit production in Norway is important beyond the value of the crop itself.

1.1.2 Apple production in Europe

Apple fruit production in the EU covers approx. 450 000 ha (Figure 1). Poland is the biggest apple producing country with nearly one third of the EU total apple tree area. Italy and Romania follow at 11 %. France (8 %), Germany (7 %), Spain (6 %) and Hungary (nearly 6 %). these countries represent important apple production sites covering more than 80 % of the total EU area under apple trees (Eurostat 2012).

1.2 Apple Pollination.

1.2.1 Crab Apples

Crab-apples belong to the family Rosacea. They are primarily used as ornamental trees mainly due to their attractive flowers and fruits (den Boer 1959). Crab-apples also are valuable as potential pollinizers in commercial apple orchards (Kwon *et al*., 2015) and in diseases management a good indicator plant for the detection of latent apple viruses (Cropley, 1968). Crab-apples can also be used as rootstocks, *Malus Robusta* 5, has been used as a rootstock due to its ability to withstand low temperatures (Tukey 1964).

The cultivated apple tree (*Malus domestica* Borkh.) shows gametophytic self-incompatibility (Sakurai *et al*., 2000) which necessitates pollen transfer from another pollinizer cultivar to set fruit. To enable the process of cross pollination, chosen cultivars need to bloom at approximately the same time, produce sufficient quantity of viable and compatible pollens. Crab apple cultivars serve this purpose and are currently used by most growers. Apple production and development follow a series of physiological events including fruit set (Sanzol and Herrero, 2001) which must be achieved to operate a successful orchard. Currently the most pressing issues facing apple production is decline in the quality and quantity of apples in Norway. Among the many issues affecting quality production is management of pest and diseases. it is important therefore, to select a pollinizer cultivar which can offer compatible pollen, and overlapping flowering period (Javid et al., 2017) and resistant to diseases.

When different cultivars are grown in one apple orchard, many aspects of cultivation become more challenging, including harvesting, pest and disease control and other cultural practises. This is mainly due to differences in cultivar characteristics. Crap apples have been planted as pollinizers in single-cultivar apple orchards worldwide since the 1960s (Church *et al*. 1983 & Gothard 1994). They have been also selected and bred (Ha and Shim 1995).

Bonn and Elfving, (1990) conducted an ongoing survey for over 20 years on crab-apples on resistance to common apple diseases and based on his study he concluded that the most outstanding performers include but not limited to M sargentii, M sieboldii var. zumi cv. Calocarpa and the hybrids Liset, White Angel and Mary Potter.

Apple fruit decay can be caused by several fungi. In Europe, the most common are, Bull's eye rot, caused by *Neofabrae* spp., bitter rot by *Colletotrichum* spp., brown rot by *Monilinia fructigena*, grey mould by *Botrytis cinerea* and Fusarium rot by several *Fusarium* species (Børve et al., 2013).

Incidence and severity of these causal agents may vary depending on cultivar, growing climate and agricultural practices. The main objective of the study was to identify and quantify apple fruit decay fungi on fruits and leaves of pollinator trees and apple cultivars.

1.2.2 Crab Apple fungal diseases

Apple rot is an economically significant disease on apple (*Malus domestica Borkh*) and is caused by several fungi which impact significantly on economic losses world over(Sutton et al.,2014).It has been recorded in northern Europe and Norway in particular that the most important fruit rot fungi are bitter rot C. *acutatum* ,Bulls eye rot *Neofabrae* ssp, Grey mold B. *cinerea*, Fusarium by several *fusarium* species, Brown rot caused by M. *fructigena* (Aderh &Ruhland) and Blue Mold caused by P. *expansum* (Børve et al., 2013).

Apple rot incidence usually vary depending on cultivar (Weber, 2011) and harvest time (Børve et al., 2013). Holb (2008) also documented that climatic conditions can significantly impact on the disease incidences.

To understand disease incidences, it's important to understand the mechanism behind infection in the orchard. Apple fruitlets and stems that remain attached to the twig as dead tissue overwinters over the season or beyond and usually are a source of primary inoculum for infection into the new season. These fruit mummies are formed at various developmental stages of the crop, mainly shortly after blossom, during the 'June drops' and decreases subsequently until harvest. The physiology behind retention of fruit mummies by the tree arises out of incomplete formation of the abscission layer at the base of the fruit stalk (Knoche et al. 2000). The tendency to retain fruit mummies is specific to certain apple cultivars and these mummies can be retained for up to three seasons (Quast and Weber 2008). By offering dead tissue near developing fruits, mummies are an attractive habitat for pathogenic fungi. Several fruit pathogens have been observed as colonisers These include *Diplodia seriata* De Not. (teleomorph: *Botryosphaeria obtusa* (Schwein.) as well as fungi associated with postharvest rots such as *Phacidiopycnis washingtonensis* Xiao & J.D and *Neofabrae* spp. which causes speck rot or Bull's eye rot (Weber 2012).

These mummies either produce conidia (Zhong *et al.,* 2008) or develop apothecia which release ascospores (Blakeman et al., 1982). Important requirements for mummies to provide primary inoculum are: (i) The pathogen must survive winter and (ii) the environmental conditions must be conducive for the pathogen to sporulate during the blooming period.

Plant pathogens also are vulnerable to antagonism from associated microflora. A small change on the association can cause a major shift on survival and activity of related pathogens. A survey of stone fruits infected by *M. fructicola* found that various fungi colonizing mummies included species of *Cladosporium, Penicillium* and *Tricothecium* and it is unknown how these fungi influence the survival of M. *fructicola* in mummies (Zhong *et al.*, 2008). Table 1, gives a summary of apple postharvest diseases

Table 1: Postharvest diseases in stored apples (modified from Jijakli and Lepoivre 2004)

Origin of infection	**Disease name**	**Causal agent**	**Source of inoculum**
Lenticel rot	Bitter rot	*Colletotrichum* spp.	Mummified fruit and cankers
	Bull`s eye rot	*Neofabraea perennans*	Mummified fruit and cankers
	Gloeosporium rot	*Neofabraea alba*	Mummified fruit and cankers and leaves
	Nectria rot	*Neonectria galligena*	Mummified fruit and cankers
	Speck rot or Rubbery rot	*Phacidiopycnis washingtonensis*	Mummified fruit
Core rot	Mouldy core rot and dry core rot	*Alternaria* spp.	Dry organs
	Wet apple core rot	*Fusarium* spp. *Fusarium avenaceum*	Various debris
Eye rot	Dry eye rot or blossom-end rot	*Botrytis cinerea* *Neonectria galligena*	Various debris and cankers
Wound pathogens	Blue mould	*Penicillium* spp.	Various debris, storage bins and walls or soil
	Grey mould	*Botrytis cinerea*	Mummified fruit and cankers
	Brown rot	*Monilinia fructigena*	Mummified fruit and cankers
	Mucor soft rot	*Mucor piriformis*	Organic matter
Other fruit rots	Phytophthora rot	*Phytophthora syringae* *P. cactorum*	Soil and cankers
	Fusarium rot	*Fusarium* spp.	Organic matter
	Rhizopus rot	*Rhizopus stolonifer*	Various debris

1.2.2.1 Brown Rot

Brown rot (Monilinia spp), causes blossom and twig blight and fruit rot in rosaceous fruit crops in most parts of the world. This includes M. fructicola (wint.) Honey, M. laxa (Aderh & Ruhl.) Honey and M. fructigena (ader. &Ruhl.) Honey.

1.2.2.1.1 History of taxonomy and nomenclature.

The first description of brown rot dates to 1796 (Persoon, 1796). The fungus was first named *Torula fructigena*, and later changed to *Monilinia fructigena*. (Wormald, (1919) later presented it by describing it as a fungus with buff-coloured pustules on fruits and named it *Oospora fructigena*, whilst the one with pustules as *Oospora laxa.* (Bonorden 1851) later changed it into *Monilinia cinerea.*

(Honey 1928) distinguished *Monilinia* from *Sclerotinia* spp based on its nature of stroma / sclerotial tissue and the type of conidial fruitification and declaring it as *Monilinia fructicola* (wint) honey

1.2.2.1.2 Geographical distribution

Monilinia spp. have been reported to occur across the world. *Monilinia fructicola* is common in North America, Australia, Asia, Africa, Central America, the Caribbean, South America, and New Zealand. *Monilinia fructicola* and M. *laxa* are present in Europe, South America, Asia, Australia, Africa and North America.

M. fructigena is widespread in Europe and some parts of Asia. In the 1970s it was identified in Maryland, but subsequently eradicated. It has also been reported in parts of South America. The recently described M. *polystroma* (formerly lumped into *M. fructigena*) causes brown rot in Japan and has also been reported in Hungary, China, Czech Republic, Poland, Serbia and Switzerland.

1.2.2.1.3 Symptoms on fruit.

Initial fruit lesions are brown, circular, and firm. As the infection progresses the whole fruit decays and turns brown. Tufts of mycelium and conidia (cream-white to buff coloured) sprout from the skin of the infected fruit, often arranged in concentric rings (Byrde and Willetts, 1977). When the relative humidity is low and/or when the fruits are not ripe, no mycelium and very few or no conidial tufts develop. Rotted fruits may either fall to the ground or dry out on the tree, leaving some hard, shrivelled mummies. Mummified fruit hang on branches of trees until spring or fall to the ground where they remain throughout the winter months, partly or completely buried beneath the soil or leaf litter (Byrde and Willetts, 1977). Infection of fruits usually take place at any point during fruit development and severity increases with ripening fruits.

1.2.2.1.4 Life cycle and Epidemiology

In spring, during the onset of rains, growth of the fungus is triggered by moisture and warmth and eventual production of asexual spores (conidia) on the surfaces of cankers and mummies from the previous season hanging in the apple trees. These mummies and shrivelled fruits that were infected and colonized by the brown rot fungus and other fungi over the previous growing season. M. fructicola produces ascospores which are sexual spores, they may also be produced from cup-shaped fruiting bodies called apothecia on mummies on the orchard floor or which are partially buried in soil of the orchard floor. Ascospores are forcibly ejected from the asci when the weather conditions are conducive. This later scenario is not common. Most infections begin from conidia.

Figure 1: Fruit infected with Brown rot. Photo: Titus Sigei

Development of infection starts during apple blossom, conidia and/or ascospores are splashed by water, blown by wind, or insects/ human vectors to flower surfaces and new twigs. These spores thereafter germinate infecting young flowers and twigs. This causes blights(browning) and death of affected plant tissue.

As harvest time approaches, cankers on twigs provide inoculum which starts infections on ripening fruit. Disease severity is progresses with fruit ripening. Factors which accelerate the spread of the disease include, rain, fruit maturity and clustering (Bryde & willets,1977 & Stensvand et al 2001). Harvesting operations and postharvest handling usually if not done carefully will create avenues for infection through wounds. In the field diseased fruits dry out, mummify and are often left hanging on the tree. These mummies and cankers will then provide overwintering structures for the fungus to survive through the harsh winter to the next growing season and the disease cycle starts again.

The rot surface of the fruit is usually covered with white fungal growth and black resting bodies (sclerotia).

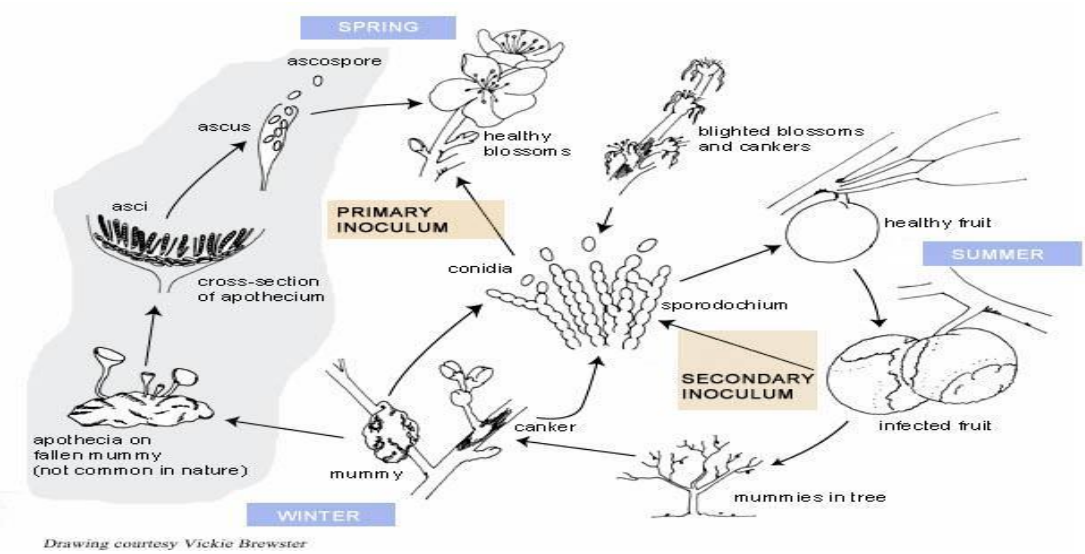

Figure 2. Diagram of Disease Cycle of Brown Rot of Stone Fruit **(**Ehlenfeldt, M. K., Stretch, A. W., & Brewster, V. (1995). Anthracnose Fruit-rot Resistance in Highbush Blueberry Cultivars. *HortScience*, *30*(4), 768-768.**)**

1.2.2.1.5 Control

Effective control is achieved through an integrated approach where risk assessment, cultural and chemical control measures are combined. During the growing season good control of scab and pests are critical to minimize entry points for brown rot.

At harvest due diligence is required to avoid creating wounds. Proper sorting and selection to ensure only uninfected fruits are stored. For organic growers control is only based on cultural methods as fungicide use is prohibited.

1.2.2.2 Speck Rot

Speck rot of apples is caused by *P. washingtonensis* This pathogen causes significant losses as described in a survey conducted in United States where the incidence of postharvest losses due to speck rot was found to between 17% to 26 % of all the orchards sampled (Xiao et al., 2006 ; Xiao et al ., 2005) and this was also found to be lower than *P. expansum* ,*B. cinerea* and *S.pyriputrescens* . This is a new pathogen which has not been reported widely (Xiao et al., 2006).

1.2.2.2.1 Geographical distribution.

P. washingtonensis has recently been reported in Washington state, United states (Xiao et al., 2006) and has consistently been isolated from decayed apple fruits. The colonies on PDA are first white and thereafter turn light gray with fluffy aerial mycelium with alternating rings of dark gray and light gray

mycelium particularly on the reverse side of the petri plates. Black pycnidia of the fungus formed on 5- to 7-day-old cultures starting from the centre of the plate. This pathogen has been reported from kiwi fruits under long storage conditions (Weber 2012). This pathogen has also been found on apples in northern Germany (Weber 2012), Italy in Torino province, Northern Italy (Garibaldi et al., 2010) and in Denmark in April 2010 (Maxin et al., 2011). In Northern Germany it was found to cause rubbery rot, a new storage disease of apples. Infected fruits had unusual firm texture which later became dark brown to black. As the fruit rots, the surface got covered with pycnidia oozing cream conidial exudates. Apple varieties infected included Germany's commercially important cultivars 'Jonagold' and 'Elstar'. Quantified losses during storage were below 1% but, in some cases, losses were up to 10%. 'Golden Hornet' crab apple cultivar used as pollinators in commercial orchards were reported to have heavy infections in October. Conidia acted as a source of inoculum into the following season from infected fruit mummies which were retained on the trees. (Weber 2011).

1.2.2.2.2 Life cycle and epidemiology

Speck rot (*P. washingtonensis*) is a postharvest disease of apples and fruit infection originates primarily from the orchard. The primary inoculum comes from dead or diseased plant tissue of the 'Manchurian' crab-apple pollinizers. The fungus produces pycnidia on crab apple twigs, branches and fruit mummies. The pycnidia contains the infective spores and usually spread by rain, irrigation water (overhead irrigation) to nearby apple trees and fruit.

Fruit rot symptoms develop during storage or at the market. In a recent study with apple twigs inoculated with *P. washingtonensis*, it was observed that the fungus survives all year-round and provide inoculum on diseased twigs to the next cropping season in the orchard (Xiao et al., 2006). Previous investigations revealed that stem-end rot and calyx-end rot are the primary symptoms of on diseased fruits (Xiao et al., 2006). As of now, no study has been successful in determining actual infection process of apple fruit by *P. washingtonensis*. In a study in northern Germany, mummified fruits of 'Golden Hornet' crab-apples planted as pollinators were identified as major sources of inoculum on apple varieties (Weber 2011).

1.2.2.2.3 Symptoms on fruits

Symptoms occur as stem-end rot, calyx-end rot or as both. The fruit tissue becomes either spongy to firm, and not easily distinguished from Gray mold. The affected tissue is not readily separable from healthy tissue. Decayed tissues vary from light brown to dark brown and sometimes takes on a black colour. The name Speck rot arose from the presentation of brown to black specks with white to light tan centres, which usually may appear around the lenticels. This is more seen on red apple cultivars

1.2.2.2.4 Control.

Orchard sanitation to reduce inoculum levels is critical, this can be done by removal of diseased plant tissues like mummified fruits, twig dieback and cankers both from apple and crab-apple cultivars. Highly susceptible crab-apples cultivars should be pruned before or right after bloom and if practical these cultivars can be replaced with resistant cultivars for long-term control and management. Postharvest treatment can be achieved through use of highly effective fungicides. Pre-harvest fungicidal sprays should also be applied when harvesting is near to reduce decay in storage.

1.2.2.3 Gray Mold

Gray mold (*Botrytis cinerea*) is the most common post-harvest fruit rot fungus on apples globally. The fungus spreads through fruit to fruit contact and during storage leading to significant losses as high as 20-60% with extended storage, and more so if the fruits were not treated with fungicides before storage. (Weber et al., 2017)

1.2.2.3.1 Symptoms

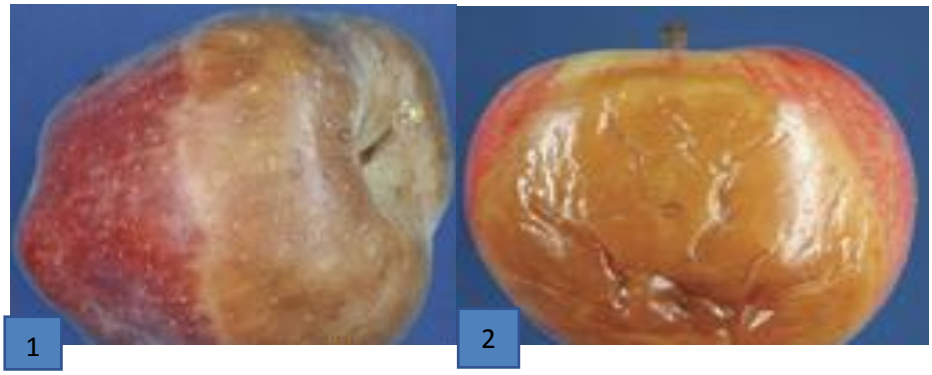

Figure 3. Pictures 1 & 2 showing Botrytis infection originating from different points on an apple fruit.

Pictures 1and 2: Courtesy of Washington state university-tree fruit research & extension centre

Picture 1 shows infection originating from stem; gray spore masses are usually visible at the diseased area under high humidity and **Picture 2** shows infection caused through wounded areas on the fruit; the decayed area turns brown, spongy to firm which later become soft as the rot advances.

1.2.2.4 Bitter rot

1.2.2.4.1 Geographical distribution

Heavy losses from bitter rot occur can occur in warm wet growing areas. This disease is of importance to all countries where commercial apples are grown (Weber 2012).

1.2.2.4.2 Symptoms.

Infections starts with small light brown spots which later become sunken as the infection progresses. From these arcevuli form on the lesions showing a concentric pattern. Under humid conditions the fruit skin raptures releasing salmon dark to dark brown spore masses. The rot beneath takes a cone shaped form when the fruit is excised to show internal infections. The fruit usually will have a bitter taste when tasted. (Sutton 1981).

1.2.2.4.3 Biology

The fungus produces the sexual stage (perithecia)which gives rise to ascospores. The asexual stage produces conidia. These two stages are important for infection to occur. The spore bearing bodies develop and survive on cankers on the fruit tree and with the onset of rains the ascospores are released and are disseminated on air currents. Conidia on the other side are spread by rain water and irrigation water (Kim et al.,1986)

The disease is associated with ripening fruits, but studies have established that apples are susceptible at all stages of their development. This is primarily due to the ability of the fungus to penetrate intact skin (Drake ,1971)

The optimal infection temperature is at 26˚C but epidemics are common with wet growing season. Infected fruits may completely rot on the trees or shrivel into mummies which survives through winter and will serve as inoculum source in the new growing season. Cankers also are inoculum source (Sutton ,1981) Post-harvest infections occur through injuries from handling and transport (Combrink, 1984)

1.2.2.5 White Rot

1.2.2.5.1 Occurrence

Botryosphaeria spp occur on many hosts and have a wide geographical distribution. It is more common in areas with moist, temperate apple-and pear-growing regions of the world (Brown and Britton 1986; Marsberg et al. 2017)

1.2.2.5.2 Symptoms

Initial symptoms are small brown spots on the fruit which are slightly sunken. The flesh becomes soft and the fruit takes a bleached or cooked appearance with drops of liquid visible on the rotted area. Higher temperatures of over 23˚ C encourages faster rotting of fruits (Marsberg et al. 2017)

1.2.2.5.3 Biology

White rot is caused by the fungus *Botryosphaeria dothidea*. The fungus overwinters as black pycnidia and perithecia on cankers and mummified fruits. The fungal spores are also found on fire-blighted twigs or cankers. The fungus is unable to penetrate the cell wall and hence wounding of the fruits is necessary for infection to occur. (Brown and Britton 1986; Marsberg et al. 2017). Fruits are also infected through lenticels. spread from fruit to fruit is not usually (Brown and Britton, 1986)

Spores are released from perithecia during spring rains. Conidia on the other hand are produced from pycnidia and are then spread by rain water or splashed to other parts of the tree throughout the summer. Apple fruits may have infection but rotting progresses as the fruit matures. At conducive temperatures (24 ˚ C) for disease development, mature fruit rot progresses quickly within a few days. The development of infection and fruit rot is favoured by conditions that reduces tree vigour (Marsberg et al. 2017; Xu et al. 2015)

Apples are susceptible from several weeks after petal fall until harvest. Most early season infections are latent and manifest when fruits begin to ripen. Twig and limb infections are associated with hot dry weather, during summer months, during freeze injury and when the fruit trees are not properly fed (Brown and Britton, 1986)

Cankers develop during periods of moisture stress and as soon as moisture stress is over, wound periderm restricts growth. (Brown and Britton, 1986)

Isolates of *B. dothidea* vary in colony morphology and pigmentation when grown on PDA. Colonies range from white to yellow, green, brown or olive, with the most intense colour in the oldest part of the culture. (Brown and Britton, 1986)

1.2.2.5.4 Control

Strict orchard hygiene and effective crop husbandry to improve tree vigour should be implemented. Timed removal and destruction of mummies and dead wood / branches affected by fire blight is critical to reduce initial inoculum. (Brown and Britton, 1986)

Preventative fungicides are also used in areas with epidemics. Examples are Captan and thiophanate - methyl. (Brown and Britton, 1986).

1.2.2.6 Blue Mold

1.2.2.6.1 Occurrence.

Blue mold caused by *Penicillium spp* is the most destructive rot in stored apples. It is important in all producer countries. Examples are the USA, The UK, Poland, Italy, Israel, India and Australia (Baker & Heald ,1934)

1.2.2.6.2 Symptoms.

Initial symptoms are soft watery brown spots which progresses faster at temperatures between 20˚C and 25˚C.Infected fruits show direct and visible margin between the decayed and healthy tissues. Blue green spore masses can be seen on the surface of the lesion under humid conditions. (Baker & Heald ,1934)

1.2.2.6.3 Biology

Blue mold usually infects through wounds on the fruit caused primarily through post-harvest handling and processing. Hail just before harvest can also initiate wounding of fruits which provides avenues for infection. Rapid development occurs at warm and humid storage conditions (Baker & Heald ,1934) The susceptibility of the lenticels to infection are conditioned by delayed picking, bruises, and storage of the apples. Other diseases infection sites may favour its development. These diseases include *Gloeosporium, Mucor or Phytophthora* (Baker & Heald ,1934)

1.2.2.6.4 Control

Primary control is to ensure careful handling of fruits during harvest, transport and post-harvest handling to minimize wounding. Strict hygiene both in the orchard, packing and processing areas is critical for control. Effective control of *Gloeosporium, Mucor* or *Phytophthora* will lower infection. Calcium sprays helps to boost resistance against the fungus. (Baker & Heald ,1934)

1.2.2.7 Calyx-End Rot

This is a sporadic and a minor disease of apple. (Jones and Aldwinckle, 1990)

1.2.2.7.1 Occurrence

Calyx-end rot occurs all over the world in apple growing regions. (Jones and Aldwinckle, 1990).

1.2.2.7.2 Symptoms

The disease starts one month after bloom and starts in the calyx end of the apple fruit. The lesions are slightly sunken, 0.5 to 1.0 mm in diameter. The lesions are grayish tan with a dry rot which later form noticeable red borders. Infected fruits usually drop immaturely. (Jones and Aldwinckle, 1990)

1.2.2.7.3 Biology

Calyx end rot is caused by *Sclerotinia sclerotiorum* (Lib.) de Bary (Syn. Whetzelinia sclerotiorum (Lib.) Korf & Dumont). The fungus produces hard irregular and black sclerotia (0.5-0.5 mm in diameter) that germinate by producing white fluffy mycelium or one to many apothecia (0.5-8.0 mm in diameter. The apothecia are funnel shaped to flat and varies in colour from white to light tan, pink or pale orange with slender stalks. The asci are hyaline, cylindrical and contain unicellular ascospores (2-15× 6-28µm) which are singly aligned, elliptical to ovoid, narrow at the base and slightly broader at the apex (Becker 1983)

Infections are commonly spread and carried by wind. These ascospores are produced from apothecia from infected weed hosts like dandelion and wild clover. For apothecia to develop and produce ascospores the soil needs to be wet for several days at temperatures between 15-25˚ C and first infect through senescent flower parts adhering to fruit (Becker 1983)

In New York (USA) *S. Sclerotiorum* has been recovered from fruit mummies that results from retention of killed fruitlet mummies after application of thinning sprays. The relevance of this scenario to the disease epidemiology is unknown (Becker 1970).

1.2.2.7.4 Control

Successful disease management depends on weather, reduction of alternate hosts and manipulating the environmental conditions that favour sclerotia germination. It takes several days of wet soil conditions and temperatures above 11-15^{o}C for sclerotia to germinate and produce apothecia. Any fungicide application must be done prior for best results. Infected fruit should be thinned, removed from the orchard and destroyed. Throwing of diseased fruits onto the orchard floor will result to build up of inoculum into subsequent growing seasons (Becker, (1970).

1.2.2.8 Rhizopus Rot

1.2.2.8.1 Occurrence

Rhizopus is one of the most destructive post-harvest diseases on stone fruits and is prevalent worldwide. Heavy losses especially in peaches, nectarines and apricots have been recorded. Infection

on apples is rare. Fruits weakened by over maturity or freezing injury are usually prone to this disease. Affected tissues become soft and watery with a sour smell (Kwon et al., 2011)

1.2.2.8.2 Symptoms

Fruits have circular water-soaked areas and can easily be displaced from healthy tissue. Profuse course white mould strands, with globular white spore-heads (sporangia) which turn black and visible with naked eye. The affected tissue thereafter becomes soft and watery, releasing acidic and fermented odour. (Kwon et al., 2011)

1.2.2.8.3 Biology

The fungus exists on dead plant material and spore commonly found on the atmosphere. Immature fruits are usually resistant to infection and become susceptible as they mature and are prone to injuries. Injuries from harvesting and handling increases infection and once established, infection spreads to adjacent fruits causing extensive nesting. Infection is favoured by warm and moist environment. Rhizopus rot usually is not likely to occur on fruits handled carefully during picking, packing, and transportation (Kwon et al., 2011)

1.2.2.8.4 Control

Fungicides are effective if sprayed shortly before maturity. Prevention of wounding arising from harvesting, handling and transportation is also critical. Pre-harvest fungicidal sprays also are effective to reduce post-harvest rotting. Hygiene at the orchard level is very important to reduce inoculum load. (Kwon et al., 2011)

1.2.2.9 Cladosporium Rot

1.2.2.9.1 Occurrence

Cladosporium is a weak parasite and its occurrence is widespread on all stone fruits producer countries. Cladosporium rot frequently follows chilling injury. (Jones and Aldwinckle, 1990).

1.2.2.9.2 Symptoms

Infected lesions are associated with injuries and covered with white mold which give rise to velvety dark green spores. Rotting is limited at the surface, but decay extends deeply. (Sholberg & Haag, 1996).

1.2.2.9.3 Biology

The fungus survives on dead plant material in the soil and spores are spread by air and cause infection to fruits which have been damaged by rain or by rough handling. Decay can also occur during cold storage (Jones and Aldwinckle, 1990)

1.2.2.9.4 Control

The most effective control is prevention of fruit skin damage during harvest and post-harvest handling. Fungicides are also effective to prevent spore germination. Refrigeration storage also helps to minimize the rate of mould development. (Sholberg & Haag, 1996).

1.2.2.10 Fusarium

1.2.2.10.1 Occurrence

Fusarium rot incidences occur on apples while under storage. It is a brown, soft, and watery circular necrosis, that gradually spreads over infected tissue, which becomes slightly sunken, sometimes with dense whitish mycelium (Jones and Aldwinckle, 1990).

1.2.2.10.2 Symptoms

Infected fruits present circular, brown, watery necrosis with visible whitish, yellowish or pink mycelia covering the lesions which initially develops from the core and subsequently spread into the surrounding cortex, often with a white to rose-reddish mycelium. Cultures on PDA formed abundant white aerial mycelium with yellow to rose pigment and a dark pink to red reverse (Snowdon ,1990)

1.2.2.10.3 Biology

Wet growing conditions during bloom, enables spore-contaminated rain water to enter the flower, and initiate infections (Sørensen *et al*., 2009). Disease incidence is generally below 1%, although in some cases more than 5% have been reported (Weber, 2009). Fusarium infections often begin as wet apple core rot, later spreading outwards to colonize the entire fruit. Wet growing conditions in the early season, enable spore contaminated water to enter the flower, thereby initiating infections. Depending on the variety, this early infection of the apple core leads to an increased incidence of Fusarium rot in storage (Sorensen et al., 2009).

1.2.2.10.4 Control

This can be achieved by use of resistant cultivars. Cultural activities such as crop rotation, use of clean planting material and minimizing or initiating wound healing prior to storage can also assist to control post-harvest incidences. Biological control agents, ultraviolet radiation and chemical control interventions have been used lately (Ranganna et al.,1997).

2 Material and Methods

2.1 Description of the study site

This study was performed in South Western Norway, Hardanger county. The experimental orchards were in Ullensvang area at NIBIO experimental fields Løeflaten, Olavshagen and Kvitavoll. Løeflaten was planted in the year 2010 with a plant to plant spacing of 1×3.5, Kvitavoll in the year 2012, with a plant to plant spacing of 1m×4m and Olavshagen in the year 2010 with a plant to plant spacing of 1m×4.5m. All planting material was sourced from Fjeld Hagebruk.

The apple cultivars were grown in rows with crab apples interplanted at regular intervals. The orchards were supplemented with irrigation and grown on soil media. Convectional pest and diseases management practises were adopted.

Figure 4. Crab apple cultivars ‘Golden Hornet’, ‘Evereste’, ‘Dolgo’, ‘Prof Sprenger’ and ‘Kobenza’ in that order from left to right. Photos by Jorunn Børve NIBIO Ullensvang.

2.2 Sample Collection (Young fruits, mummies, stems and leaves.)

2.2.1 Kvitavoll Mummies.

Sampling was done on selected crab apple trees from cultivars “Dolgo” and “Evereste” which were inter planted within rows of “Summered” apple cultivars at regular intervals. The sampled trees were different each time in location. Plant to plant spacing was 4m× 1m with an average row length of 450m. Crap apple cultivar “Evereste” were planted on the eastern edge of the orchard at the end of each row while cultivar “Dolgo” planted on the remaining portion of the orchard to the western end. There total trees per cultivar were as follows “Dolgo” (48), “Evereste” (10) and the main apple cultivar “summered” were 459 trees. The orchard was planted in the year 2012 and all planting material sourced from Fjeld Hagebruk-a Norwegian nursery.

Sampling was done only on mummies. The mummy age and sizes varied with some from the immediate season while others were from 2016 production season. It was necessary to standardize the samples into 10 per tree because of its wide variation and availability of experimental space and equipments. For the first month all picked samples were run through the experiment, and through the challenges faced with incubation, a decision to standardize was reached. All were symptomatic but needed further laboratory work to correctly identify the colonizing pathogens.

Figure 5. Overwintered fruit mummies. Photo by Jorunn Børve NIBIO Ullensvang.

2.2.2 Løeflaten and Olavshagen new fruits and leaves.

In this experiment sampling was done at two experimental orchards Løeflaten and Olavshagen. The two fields were located adjacent to one another approximately 150 metres apart. Løeflaten was planted in the year 2010. The plants were planted in rows with crab apples cultivars "Dolgo", "Everest", "Professor Sprenger", "Golden hornet" and "Kobenza" interplanted between apple cultivars "Na 42-51" ,"Collina" ,"NA 46-49", "BC-9P-15-30", "Appache", "Machiels", "Rubinola", "Elan", "Rubinstep" and "Creston" at regular intervals. Plant to plant spacing was at 3.5m × 1 m with a total plant population of 428 trees. The rows were approximately 428 m. From these cultivars Apple cultivars 'Rubinola', 'Elan' and 'NA 42-51'were investigated.

Crab apples ''Dolgo'' and ''Kobenza'' were planted at Olavshagen orchard. The orchard was planted in the year 2010.The plants were in regular rows with crab apple cultivars "Dolgo" and "Kobenza' interplanted at regular intervals. ''Dolgo'' was interplanted on 3 rows of apple cultivar "Summered" on the north side of the orchard and "Kobenza" on the remaining three rows with apple cultivar "Aroma" on the southern part of the orchard. There were 6 rows in total. Plant to plant spacing was 4.5m× 1m with a row length of 358m. The total plant population was 358 plants. Apple cultivar "Aroma" was selectively thinned while "Summered" was not thinned. Crab apples 'Dolgo' and 'Kobenza' were investigated. M9 was used as a rootstock and was sourced from the Norwegian Elite plant station.

Sampling was done biweekly for the months of June, July and August of 2017 on 6th June 20th June 4th July 27th July and 15th August. A total of 1250 fruits and 1250 leaf samples were collected during this study period. In each sampling 10 new fruits and 10 new leaves were randomly sampled from five trees of the five selected cultivars into different sampling bags. The sample bags were clean. The trees sampled each time were different but always from the five cultivars. The sample bags were then labelled with sampling date, cultivar and orchard and taken to the lab for further experimental work. All samples were asymptomatic at the time of sampling.

2.2.3 Løeflaten new fruits.

Sampling was done on new fruits from crab apple cultivars "Golden Hornet", "Prof Sprenger"," Kobenza", "Dolgo" and "Evereste". Løeflaten was planted in the year 2010. The plants were planted in rows with crab apples cultivars "Dolgo", "Everest", "Professor Sprenger", "Golden hornet" and "Kobenza" interplanted between apple cultivars "Na 42-51" ,"Collina" ,"NA 46-49", "BC-9P-15-30", "Appache", "Machiels", "Rubinola", "Elan", "Rubinstep" and "Creston" at regular intervals. Plant to plant spacing was at 3.5m × 1 m with a total plant population of 428 trees. The rows were

approximately 428 m. A total of 1200 fruits were sampled during this study period. Sampling was done once weekly on 2nd,16th, 23rd and 30th on the month of August 2017.No sampling was done on 9th of August 2017.Sampling was replicated into two and in each sampling 10 fruitlets were sampled randomly from three trees of the five selected cultivars and stored on different clean sample collection bags. The same procedure was used for the second group of samples. Fruits were picked with stems attached. The samples represented the same developmental stage in the orchard. The sample bags were then labelled with sampling date, cultivar and orchard name and taken to the lab for further experimental work. All samples were asymptomatic at the time of sampling.

2.2.4 Incubation

Where sporulation (Young fruits, leaves and stems) was lacking, samples were incubated on a custom-made incubation chamber, making sure no sample were in contact with one another on top of metal grids with wet paper towels underneath to maintain high humidity. The sealed boxes were then frozen at -18°C for 5 hrs and then incubated at 20°C for 6 days in 24 hr continuous fluorescent light. For mummies which could not be identified by sporulation by eye and stereo and / or electron microscope, small segments (2 mm -5mm length) from diseased tissue were excised with a sterile scalpel and placed on PDA augmented by penicillin G and streptomycin sulphate and incubated on the laboratory bench for 6 days to allow for fungal sporulation.

Figure 6. Newly Incubated samples.

Figure 7. Incubated samples showing sporulation after 6 days at 20 °C. Photo by: Titus Sigei.

Figure 8. Apple leaves showing sporulation of Bitter rot fungi (*Colletotrichum* spp*)* after 6 days incubation at 20 ° C. Photo: Titus Sigei.

2.2.5 Laboratory work

Equipment's and reagents for lab work were provided by NIBIO Ås and Ullensvang stations.

The samples were treated separately throughout the study period.

2.2.5.1 Kvitavoll mummies

In the laboratory, physical examination was done to separate those mummies that could be assessed and those which needed further laboratory work to initiate sporulation. Those which showed sporulation were subjected to light microscopy, identified and scored for disease incidences (Crous et al.,2019) Sutton et al.,2014). Incidence was calculated as the number of fruits/stems /stems/ mummies which showed sporulation of identified disease relative to the total samples on the sample lot. For that which could not be identified, small segments (2 mm side length) from diseased tissue were excised with a sterile scalpel and placed on PDA augmented with penicillin G and streptomycin sulphate and incubated on the lab bench at 20˚C at 24 hrs continuous fluorescent light for 6 days to allow for fungal sporulation. Identification was then done using the same procedure above. Identification was done using relevant taxonomic keys up to the species level where applicable.

Figure 9. Fruit mummies on PDA plates ready for incubation. Photo: Titus Sigei

Figure 10. Sample preparation for isolation and incubation on a laminar hood chamber at NIBIO Ås Laboratory. Photo: Titus Sigei.

2.2.5.2 Løeflaten and Olavshagen new fruits and leaves.

In the lab the samples were surface sterilized using 0.5NaOCL for 30 seconds,70% EtOH for 1 min and then rinsed on autoclaved water for 2 mins and left to airdry on a laminar hood and later frozen at -18 ° C for 5 hrs. Fruits and leaves were treated separately and independently throughout the study period. The samples were incubated on sterile plastic boxes on top of metal grids with wet paper towels underneath to maintain high humidity (10 samples per box) at 20°C on incubation laboratory room with 24 hrs continuous fluorescent light for 6 days to initiate sporulation Those that could not be identified, diseased tissues from margins were excised using a sterile flamed scalpel and using a pair of forceps plated on PDA and incubated as previously mentioned. Assessment and identification was then done using relevant taxonomic keys to species level. The plated petri dishes represented the tree and cultivar where samples were taken from.

2.2.5.3 Løeflaten new fruits

In the lab, the two subgroups of samples were treated separately with each sampling period. In one subgroup the samples were surface sterilized using 0.5NaOCL for 30 seconds, 70% EtOH for 1 min and then rinsed on autoclaved water for 2 mins and left to airdry on a laminar hood and later frozen at -18 ° C for 5 hrs. They were then incubated at 20 ˚C, 24 hr continuous artificial light for 6 days on sterile plastic boxes on top of metal grids with wet paper towels underneath to maintain high humidity. The other subgroup was incubated directly without surface sterilization and freezing following the same incubation chambers and conditions.10 fruits were incubated per box (Fig 13) and thereafter the fungi were identified by eye or microscopy using culture characteristics and conidial morphology and scored for disease incidences. Those that could not be identified were further re-isolated, pure cultures made and thereafter assessed in the same manner.

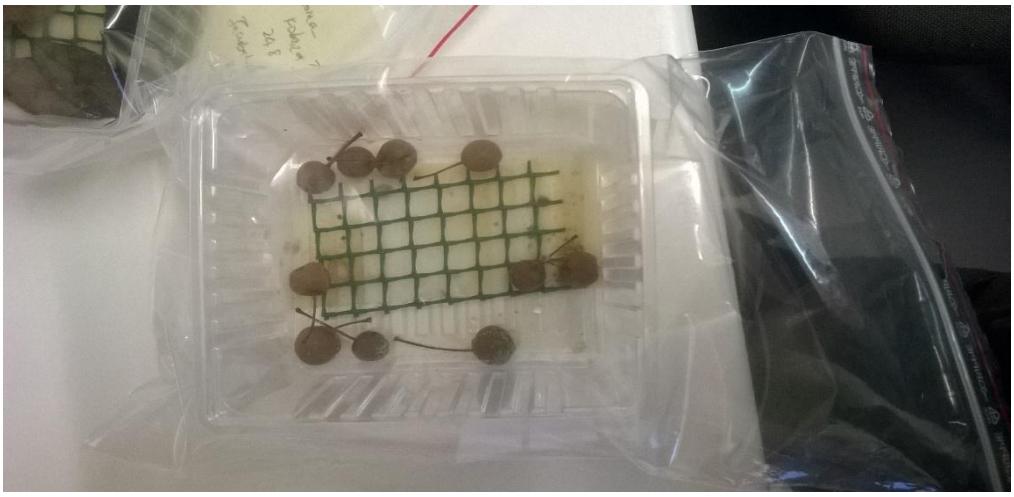

Figure 11. Incubated fruit mummies on custom made incubation chamber. Photo: Titus Sigei

2.2.6 Evaluation

Disease incidence was assessed by counting the number of samples showing sporulation of identified fungal disease relative to the total samples in the lot. This was calculated as the number of samples in each replicate showing sporulation relative to the total number of samples in the replicate. A replicate was a tree in this case. (**Disease incidence was calculated as: P = n/N*100. (P – disease incidence, n – number of attacked samples, N – total number of investigated samples)**. A sample was treated per tree, cultivar and orchard.

Examination of young, actively growing fungal colony margin was used for identification and preparation of slides. These were the points where spores were actively produced. Preparation of slides for microscopy from a fungal colony, was done by putting a small drop of water on a microscope slide,

and then by use of a sterilized inoculating needle, a small (no more than 2 mm square) portion of the colony margin was removed. The piece of colony was then placed in the mounting medium, and using a second sterile needle, filaments were spread evenly. A cover slip was the placed over the mount, lowering one edge to the slide before the other so that air bubbles can escape. The remaining air bubbles were removed from the mount by gently heating it over an alcohol flame.

Colonies were identified to species level based on culture characteristics and conidial morphology (Barnett and Hunter, 1998), (Crous et al.,2019; Sutton et al.,2014) and by use of stereo and light microscope if need be. Below is the summary of fungal colony morphology descriptions;

Gray mold -Fruits showed abundant grey to brown masses of conidia. Conidia were borne in grape like clusters which started only with mycelium, then followed by erect fasciculate conidiophores which were olive coloured. Black sclerotia were flat, loaf shaped or hemispherical. These started out as colourless to white, either fluffy or oppressed and later turned gray brown.

Brown rot- Sporulation on samples was brown orange, with white gray mycelium. Conidia were pink gray or tan when seen singly but when in mass they were lemon shaped and in one celled chains.

Mucor rot- Colonies were fast growing, cottony to fluffy, white to yellow, becoming dark-grey, with the development of sporangia. Sporangiophores were erect, simple or branched, with large globose to spherical, multispored sporangia. Sporangiospores were hyaline, grey brownish, globose to ellipsoidal, and smooth-walled.

Rhizopus rot- Colonies grew rapidly, which appeared cottony and darkened with age to become gray yellow brown. The reverse of the culture was white. Mycelia had numerous stolons connecting to long sporangiophores. Sporangiophores were unbranched, long, and terminate. Columella and sporangium easily collapsed after discharging spores

Bitter rot- Conidia were light brown and were produced as a sticky mass which was pink to salmon in colour. Colonies produced white mycelia after 6 days of incubation with abundant conidia which were hyaline, one celled, straight, cylindrical, and on average 10.45 to 15.78 × 3.56 to 5.89 μm in size.

Fusarium-Spores are colourless, canoe-shaped and with a distinct "foot cell" at the lower end and divided by several cross-walls. Conidiophores clustered to form sporodochia which produced large masses of spores. Cultures were brightly coloured

Blue mold- Spores were in unbranched chains, borne from clusters of cylindrical to bottle-shaped phialides. Old colonies were usually green in colour.

Cladosporium- Colonies were dark greenish black. The reverse of the isolate was black. The fungus was relatively a slow grower. Spores are 1- or 2-celled and which occurred in long, branching chains. The spores are easily disrupted by any slightest movement and this made it difficult to make microscope slides to observe the whole structure. The fungus was easily identified by eye.

Occasionally samples with symptoms and signs of more than one fungal pathogen were found, in such situation classification was done presumably on the oldest and more severe disease

Figure 12. Observation of fungal spores using a stereo microscope. Photo by Titus Sigei

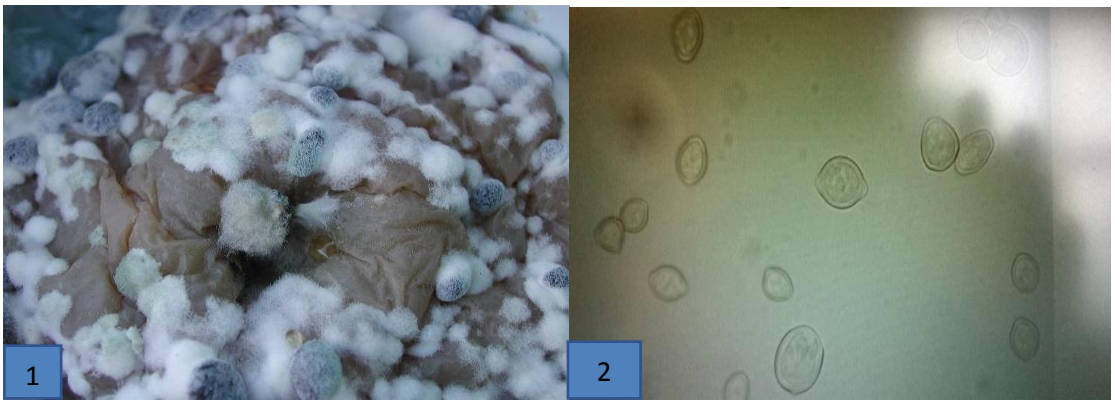

Figure 13. Picture 1 & 2 in that order showing mycelium and conidia of *Monilinia* spp seen under × 20 magnification. Photo: Titus Sigei

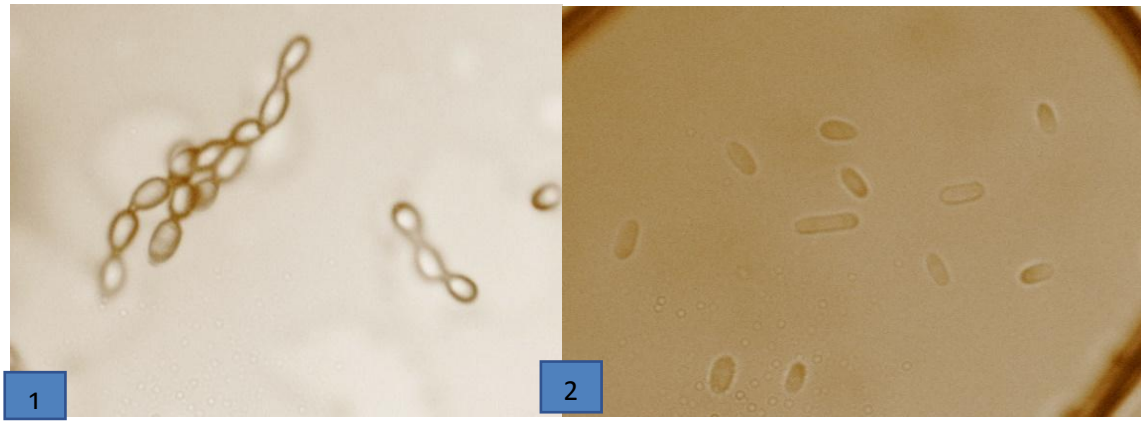

Figure 14. Picture 1 and 2 in that order showing branched chained conidial spores of *Monilinia* spp and *Botryosphaeria* spp seen under × 20 magnification. Photo: Titus Sigei

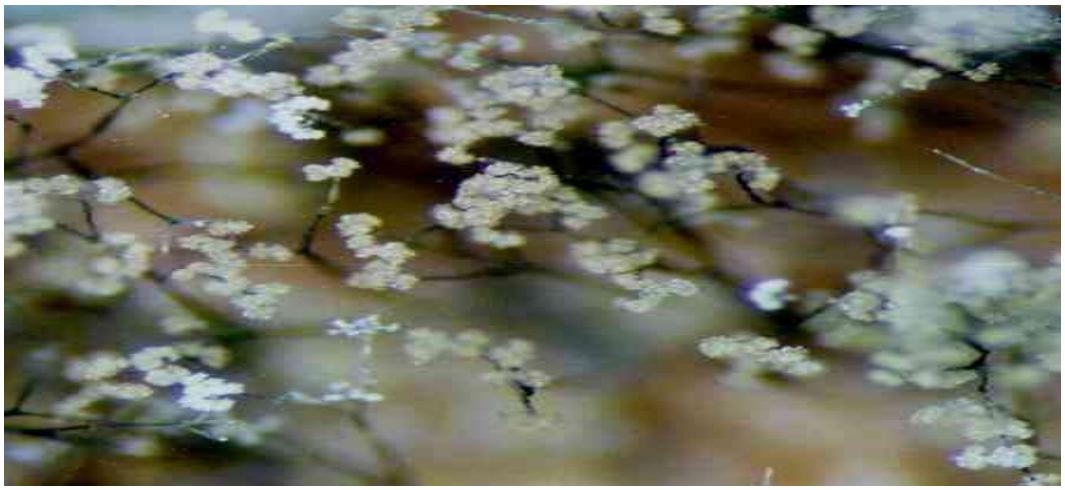

Figure 15. Spores of *Botrytis* spp seen under × 20 magnification. Photo: Titus Sigei

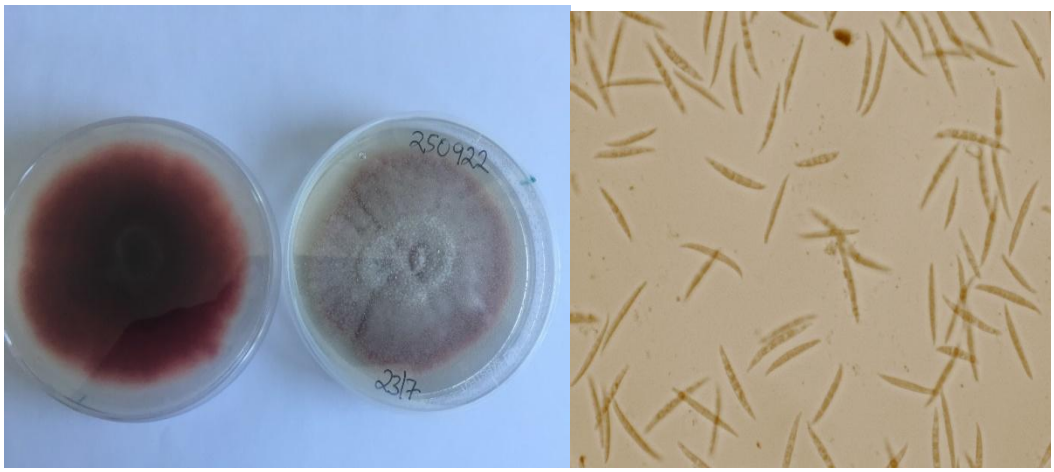

Figure 16. Fusarium spp isolate raised on PDA. **Figure 17.** Fusarium conidia seen under × 20 magnification. Photos by Titus Sigei.

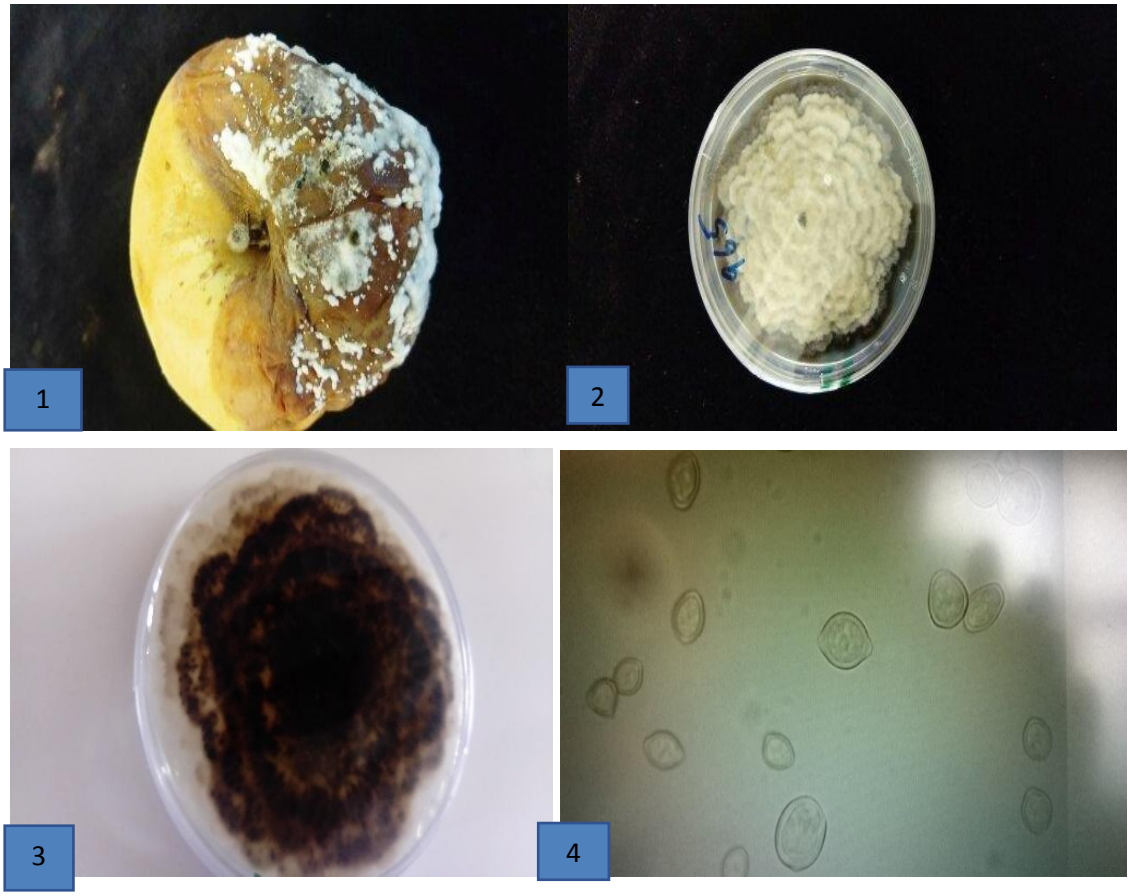

Figure 18. Picture 1) showing apple fruit infected with *Monilinia* spp, Picture 2) Showing *Monilinia* spp isolate on the upper side of the petri dish and Picture 3) showing the reverse side and Picture 4) showing lemon shaped *Monilinia* spp conidia seen under × 20 magnification. Photo Titus Sigei.

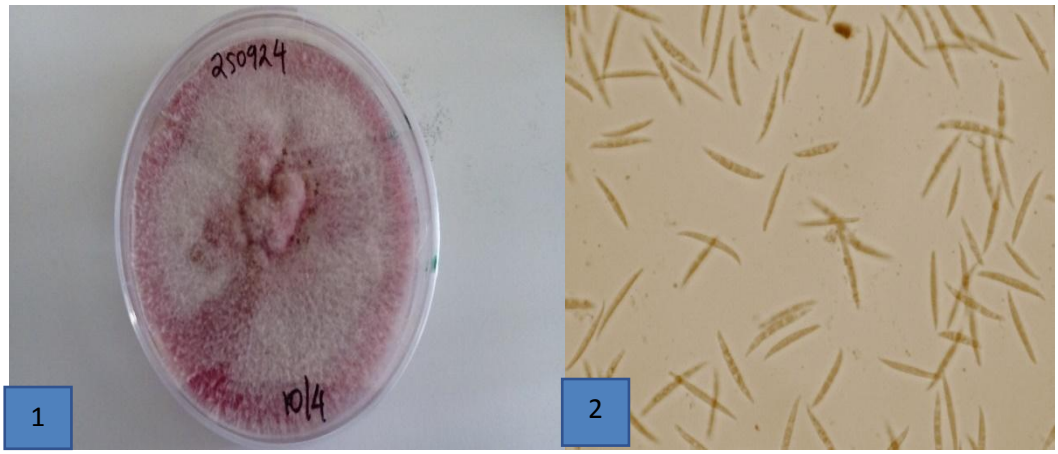

Figure 19. *Fusarium* spp single spore isolate and conidia (× 20 magnification). Photo: Titus Sigei.

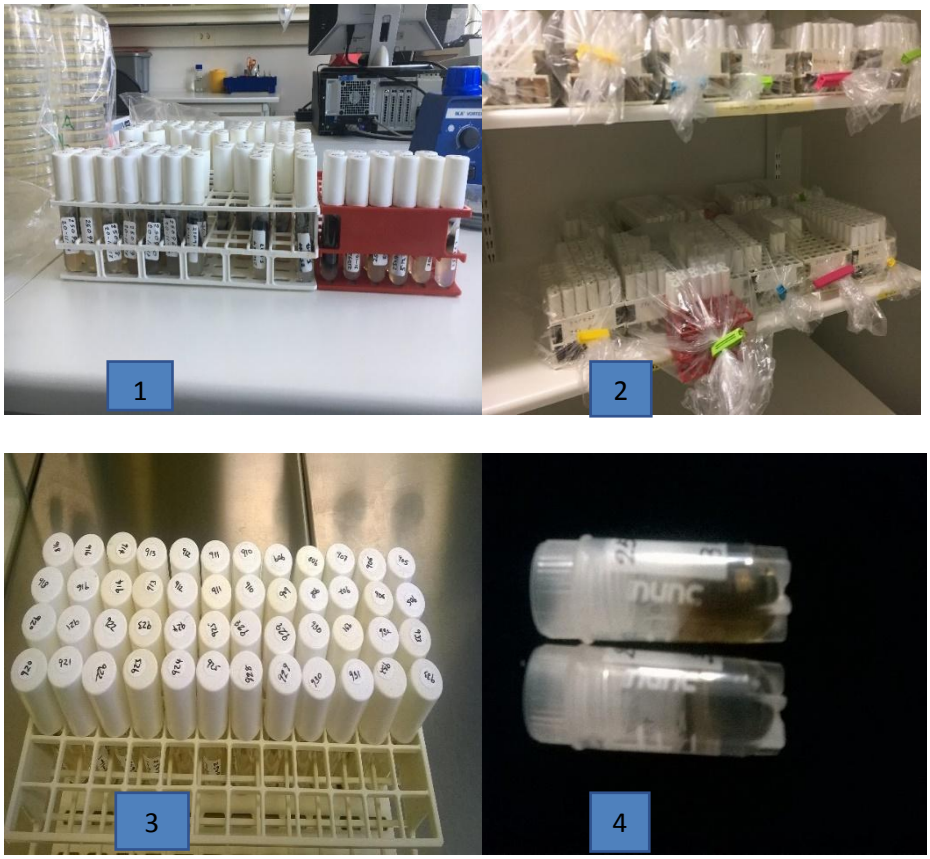

Figure 20. Pictures 1,2,3 and 4 showing single spore pure culture isolates storage in NIBIO Ås. Photo Titus Sigei.

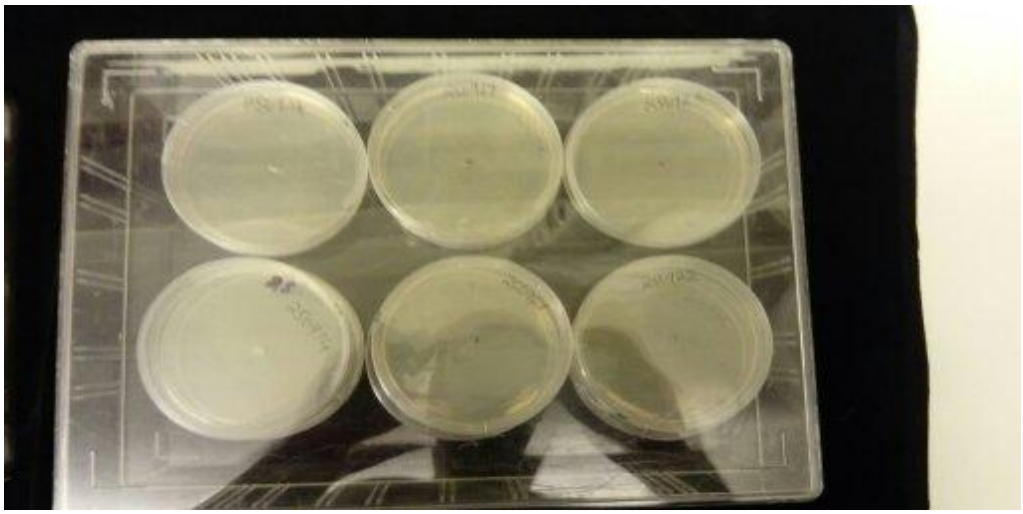

Figure 21. isolates ready for incubation. Photo: Titus Sigei

Figure 22. Isolates requiring cleaning to obtain pure cultures. Photo: Titus Sigei

2.2.7 Raw Data Capture.

Pictures and notes were taken on data capture book. In the lab spore pictures were taken and recorded for further use.

2.2.8 Analysis of data

All statistical analysis was performed using Generalized Linear Model (GLM) procedure of SAS (SAS institute, Cary, NC, USA). Statistical analysis was performed to determine relationship between cultivars, sampling period and effect of non-sterilized and unfrozen samples on apple decay fungal disease incidences.

Difference between and within samples were separated using a one-way analysis of variance (ANOVA and to satisfy the assumptions of ANOVA, Student-Newman-Keuls Test method was used to separate means at α=0.05.

Performing a statistical analysis of variance on the means over the study period resulted in measures of significant difference, in this case represented by the superscripted letters associated with each mean. Means not connected by the same letter are significantly different from each other.

As was expected of the study identified and quantified apple decay fungi did not show much variations with previous studies (Sholberg & Haag, 1996). The data trends agreed with our hypothesis that pollinator trees contribute inoculum which causes apple fruit decay.

3 Results

From this investigation a total of 11 species of apple decay fungi were identified from all the investigated samples. Table 2 below outlines the results as per each experiment.

Table 2: Summary of isolated and identified fruit decay fungi from mummies, new fruits and leaves from three experimental orchards in Ullensvang, South western of Norway.

Study Site	**Plant part sampled and investigated**	**Fungi identified**
Experimental orchard Kvitavoll	Mummies	Brown rot, Mucor, Fusarium, Bitter rot, Blue mold, Gray mold, Bulls eye rot and Cladosporium rot.
Experimental orchard Løeflaten (5 Crab Apple Cultivars) *	New fruits	Gray mold, Blue mold, Tricothecium, Brown rot, Fusarium, Mucor
Experimental orchard Løeflaten and Olavshagen (Two Crab Apples & three Apple Cultivars)	New fruits and leaves	Blue mold, Fusarium, White rot, Mucor, Bitter rot, Sclerotinia, Brown rot and Cladosporium rot

* The samples were directly incubated. Surface sterilization and freezing at -18 ˚ C not done**.**

3.1 Kvitavoll Mummies.

A total of 8 species of apple decay fungi were isolated, identified and quantified namely Brown rot, Mucor, Fusarium, Blue mold, Bitter rot, Gray mold, Cladosporium rot, Bulls eye rot and White rot (Table 3 & 4). The crab apple cultivars under investigation were ‘Dolgo’ and ‘Evereste’

3.1.1 Crab apple cultivar 'Dolgo'

Table 3. Percentage mean incidences of fruit decay fungi identified and quantified from crap apple cultivar "Dolgo" for the study period June to September 2017.

Month	Brown rot	Mucor	Fusarium	Bitter rot	Blue mold	Gray mold	Bull's eye rot	Cladosporium rot
June	15	31a	9	5	4	0b	0b	0b
July	18.8	0	3.8	10	5	0b	10a	8.75a
August	20	0	4.2	1.6	8.3	19.2a	8.3b	5.8a, b
September	21.7	0.8b	10	1.6	0	15a	1.67b	0b
P-Value	**0.7845**	**0.0001**	**0.2879**	**0.1596**	**0.1644**	**0.0001**	**0.0224**	**0.0305**

Different letters indicate significant differences using Tukey`s test (α =0.05).

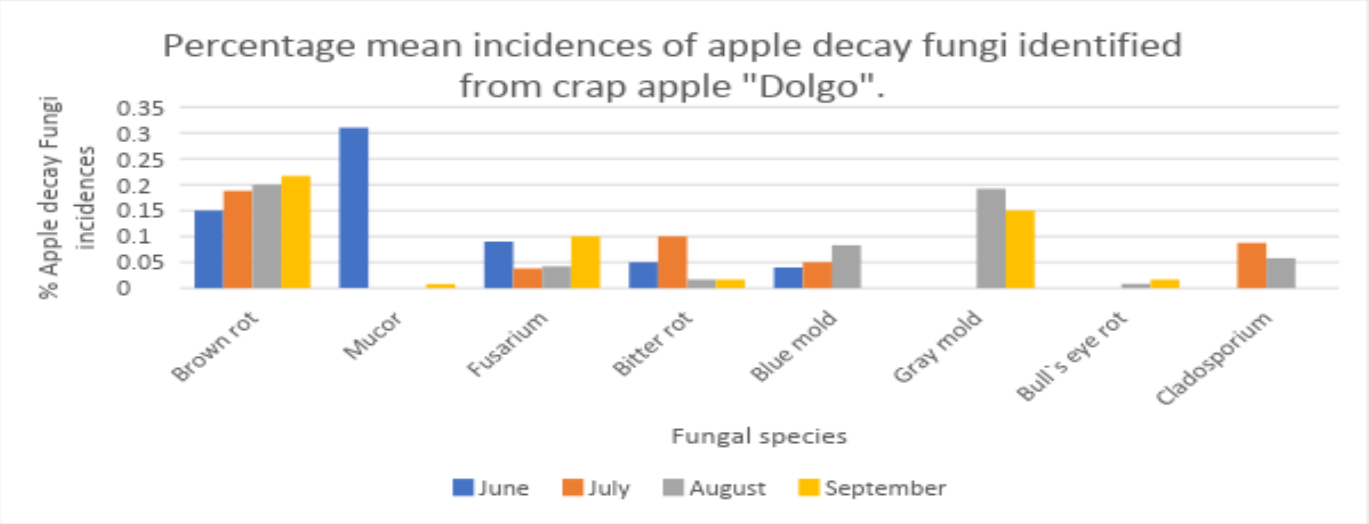

Figure 23. Percentage mean incidences of fruit decay fungi identified and quantified from crap apple cultivar "Dolgo" for the study period June to September 2017.

The results (**Table 3)** indicates a positive correlation between disease incidences and the sampling period for Mucor (P=0.001), Gray mold (P=0.0001), Bull's eye rot (P=0.0224) and Cladosporium rot (P=0.0305). Mucor was dominant in June with a percentage mean incidence of 31% and drastically dropped to 0.8% in September. Gray mold did not record any incidence in June and July and a marked increase was recorded in August (19.2%) and September (15%). Bull's eye rot had zero incidence in June, then markedly increased to 10% in July 8.3 in August and dropped markedly to 1.67% in

September. Cladosporium rot on the other hand started with 0 % incidence in June and rapidly increased to 8.75 % in July, dropped a little in August to 5.8% and zero incidence in September (**Table 3).**

There were big variations between the percentage mean incidences and this could be the reason why the level of significance was low. Brown rot ranged between 15-21.7%, Mucor 0.8-31%, Fusarium 3.8% to 10%, Bitter rot 1.6-10%, Blue mold 4-8.3 %, Gray mold 15-19.2%, Bull's eye rot 1.67-10% and Cladosporium 5.8 - 8.75% during the four months of study (Table 3). Only brown rot showed a clear trend of increase with fruit development towards maturity (**Fig .23**).

3.1.2 Crab Apple cultivar 'Evereste'.

Table 4. Percentage mean incidences of fruit decay fungi identified and quantified from crap apple cultivar "Evereste" for the study period June to September 2017.

Month	**Brown rot**	**Mucor**	**Fusarium rot**	**Bitter rot**	**Blue mold**	**Gray mold**	**Bull's eye rot**	**Cladosporium rot**
June	2	5	22.5	0	27.5	0	0	0
July	2	0	10	66.7	13.3	0	13.3	3.3
August	23	0	3.3	0	0	6.7	13.3	16.7
P-Value	**0.64**	**0.444**	**0.367**	**0.111**	**0.217**	**0.111**	**0.265**	**0.346**

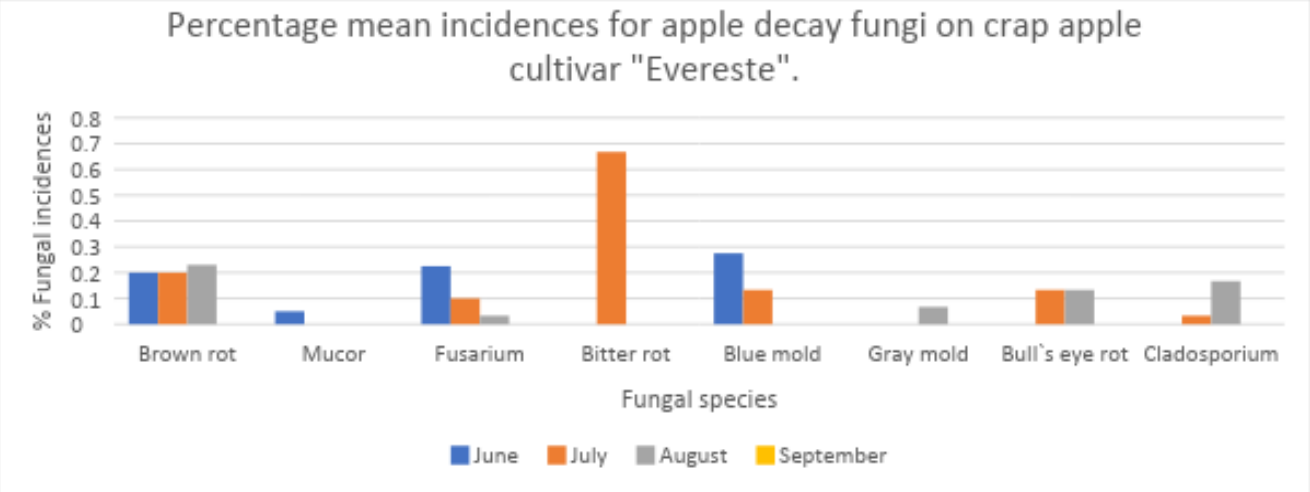

Figure 24. Percentage mean incidences of fruit decay fungi identified from crap apple cultivar "Evereste" for the study period June to September 2017

There was no correlation between the sampling periods and percentage mean disease incidences for crab apple cultivar 'Evereste'(P=0.05) **(Table 4).** It is important to note that no sampling on this cultivar was done in the month of September 2017 because there were less trees of 'Evereste'. Sampling was only done on Cultivar 'Dolgo' on the month of September. This could have altered the results achieved. The dominating fungi was Bitter rot with a mean percentage incidence of 66.7 %. Percentage mean incidence variations were as follows ; Bitter rot (0 %- 66.7%), Brown rot (2-23%), Blue mold (0-27.5%), Fusarium rot (3.3 -22.5%), Cladosporium rot (0-16.7%), Bull's eye rot at(0-13%)and Gray mold at(0- 6.7%) .Only Brown rot, Bull's eye rot and Cladosporium rot showed an increasing trend with fruit development towards maturity (**Fig. 24**). Mucor, Blue mold, Fusarium rot and Gray mold recorded decreasing incidences with fruit development. **(Fig .24**).

3.2 Løeflaten Crab Apples (New Fruits)

The study was done on crab apple cultivars 'Dolgo', 'Evereste', 'Professor Sprenger', 'Kobenza' and 'Golden Hornet'. Sampling was done on only new fruits. This investigation involved two different treatments; Surface sterilized and frozen samples and unsterilized and unfrozen samples.

3.2.1 Unsterilized and unfrozen new fruits.

From this experiment, a total of 6 species of fungi were isolated, identified and quantified (Table 5)

Table 5: Percentage mean incidences of apple decay fungi identified and quantified from unsterilized and unfrozen new fruits of crab apple cultivars 'Kobenza', 'Evereste', 'Prof. Sprenger', 'Golden Hornet' and 'Dolgo'.

Sampling Date	**Gray mold**	**Blue mold**	**Tricothecium**	**Brown rot**	**Fusarium rot**	**Mucor**
2.8.2017	3	0	0	0c	0.33	2
16.8.2017	1.67	5.33a	0	3c	1.33	3
23.8.2017	7.67a	8a	1.97a	12b	1.33	2.33
30.8.2017	6.67a	5.67a	0	33a	0.67	4.33
P-value	**0.0016**	**0.0007**	**0.0076**	**0.0001**	**0.5503**	**0.6793**

Different letters indicate significant differences using Tukey`s test (α =0.05).

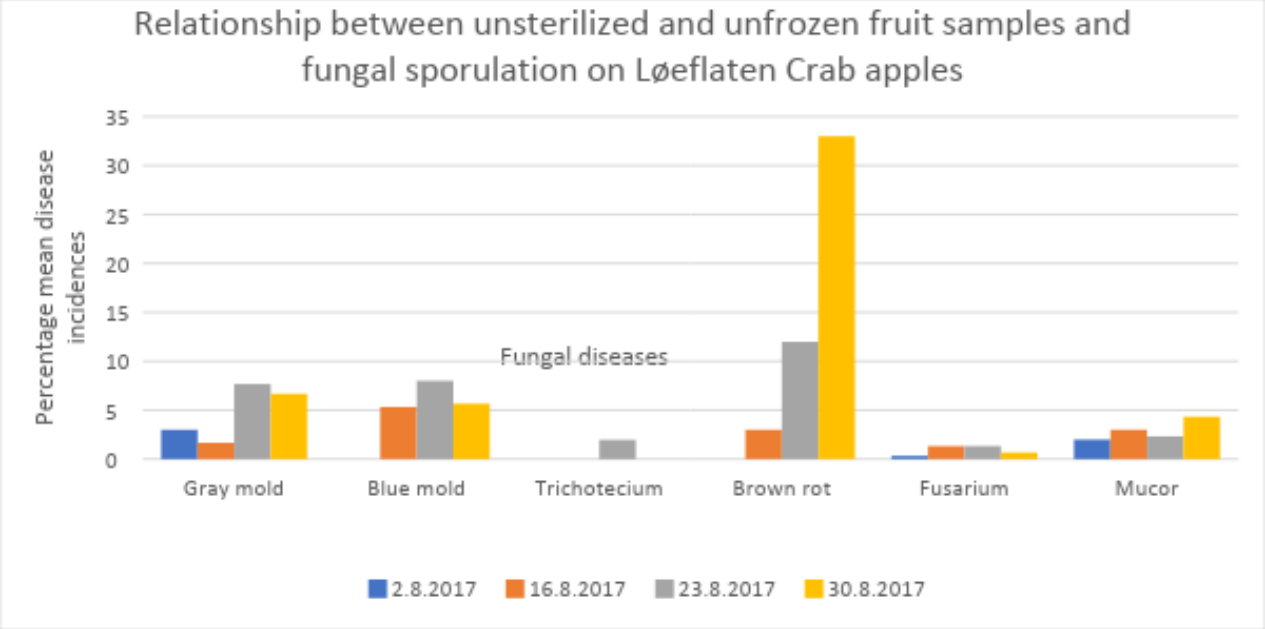

Figure 25. Effect of unsterilized and unfrozen samples on apple fruit decay fungi incidences.

From the results obtained, incubated samples without surface sterilization and freeze treatment had less number of fungal species than sterilized and frozen samples **(Table 5 & 6).** This means that the inoculum load on fruit surface was lower than inside the fruit tissue. 6 fungal species were identified on unsterilized against 9 from sterilized and frozen fruit samples. It is important to note the effect of freezing fruit samples on fungal sporulation. By freezing the samples at -18˚ C the plant defence system was weakened by destabilizing the physical and chemical barriers and when these obstacles were overcomed, pathogens inside the tissue could sporulate (Jones & Dangl., 2006). Freezing also stimulated fungal sporulation (Børve et al., 2010).

Surface sterilization was meant to clear all microbial load on fruit surface and ensure only what sporulated was within the plant tissue. This study cannot conclude 100% that what sporulated from the unsterilized and unfrozen samples was present on the fruit surface. It is assumed that the 6-day incubation period was too short to have meaningful sporulation from within the fruit tissue and so it is assumed that all that sporulated was out of inoculum on the fruit surface.

To support these findings a previous study, found that the main source of inoculum for microflora of apples was found to originate from the orchard itself. This microflora can be categorized into two; natural occurring microflora and contaminating microflora. Natural microflora is usually found adhering to the surface of the plant tissue and they tend to be harmless and stable. Most of these are beneficial and but can influence on development of disease causing microflora. Contaminating

microflora in the other hand arise from external vectors which are agents of inoculum transfer which include but not limited to crop residues including mummies, twigs and leaves, rain, soil, dust, irrigation water and equipments, insects, birds and rodents and through human spread (Vismer et al., 1996). It was also demonstrated that such microorganisms are not able to penetrate plant tissues but needed avenues of entry. In the orchard wounding caused by handling, insect pests damage and hail usually facilitate entry and thereafter initiate disease development (Vismer et al., 1996).

To be sure that these results were not by chance percentage mean disease incidences were compared between the sampling dates. Significant **(**Table 5**)** correlation was recorded on Gray mold (P=0.0016), Blue mold (P= 0.0007), Trichotecium (P=0.0076) and Brown rot (P=0.0001). Gray mold was highest on 23rd and 30th of August 2017, Blue mold on 16th,23rd and 30th, Trichoteceum on 23rd and Brown rot on 23rd of August 2017**.** From (Figure 25) we can see that the highest and significant (P=0.05) incidences occurred on 23rd of August 2017 and the lowest on 2nd of August 2017.Only Brown rot showed a clear increase of disease incidences against fruit development. (Figure 25)

Figure 26. Unsterilized and unfrozen samples before **(**Picture 1) and after **(**Picture 2) incubation for 6 days at 20˚C.Photo: Titus Sigei.

3.2.2 Sterilized and frozen new fruits.

Crab apple cultivars ‘Dolgo’, ‘Kobenza’, ‘Golden Hornet’, ‘Prof Sprenger’ and ‘Evereste’ were investigated.

The results are summarized in **(**Table 6)

From this investigation results recorded indicated there were more apple decay fungal species on surface sterilized and frozen fruit samples than from unsterilized and unfrozen fruit samples. This means that inoculum load differed between the fruit’s surface and the inner tissue. In the orchard it is

difficult to determine latent infections unless tissue pathological test is done. These results have shown that for better management decisions on disease control it is important to know the microbial load both on the fruit surface and inside the fruit tissue through pathological tests.

To test cultivar resistance, percentage mean disease incidences were compared between the five crab apple cultivars (Table 6). Dolgo was susceptible to Gray mold (P=0.0257) Prof Sprenger to Blue mold(P=0.0046) and Sclerotinia (P=0.0012), Golden Hornet to Fusarium rot (0.8821) and White rot (P=0.0048). Kobenza was resistant to all the identified apple decay fungi according to the results obtained.

Table 6. Relationship between crap apple cultivars and susceptibility to apple decay fungal diseases -Løeflaten experimental orchard.

Cultivar	Gray mold	Blue mold	Fusarium rot	White rot	Mucor	Bitter rot	Sclerotinia	Brown rot	Cladosporium rot
Dolgo	17a	2b	3.6b	0b	0	0	0b	0	0
Everest	8.6a, b	2b	1.6b	0b	0	0	0b	0	0
Golden Hornet	14.6a, b	4b	13.6a	8.4a	1.6	2.8	4.8a	0	0
Kobenza	4.2b	6ab	2.8b	2b	1.6	0	0.4b	4.4	4.4
Prof. Sprenger	13.8a, b	9.2a	6.4b	4.8b	0.8	0.8	5.2a	4	4
P-Value	**0.0257**	**0.0046**	**0.8821**	**0.0048**	**0.1956**	**0.0308**	**0.0012**	**0.0143**	**0.1584**

Different letters indicate significant differences using Tukey`s test (α =0.05).

The fruit samples in this study were surface sterilized and frozen as shown on materials and methods.

3.3 Olavshagen 2 crab Apples and Løeflaten 3 Apple cultivars (New fruits and leaves)

The study was done on crab apple cultivars Dolgo and Kobenza and apple cultivars Rubinola, NA 42 51 and Elan. Sampling was done on new fruits and leaves. The crab apples were in experimental field Olavshagen and the apple cultivars in experimental field Løeflaten. The two fields were at approximately 150 meters apart.

3.3.1 New leaves.

A total of 7 fungal species were identified and quantified. On overall disease incidences were found to be less on leaves than on fruits. The data was not subjected to data analysis and not much could be interpreted. It is assumed that if more incubation time was given meaningful results would have been achieved.

Table 7. Comparison between percentage mean disease incidences on new leaves and sampling period for Løeflaten and Olavshagen samples.

Sampling period	Rep	Blue mold	Fusarium rot	Mucor	Bitter rot	Sclerotinia	Cladosporium rot	Rhizopus
6th June 2017	crab apple	0	0	0	0	0	0	0
20th June 2017	crab apple	0	0	0	0	40	0	0
4th July 2017	Apple cultivars	40	40	40	80	0	0	0
27th July 2017	Apple cultivars	0	80	0	0	40	40	0
15th August 2017	Apple cultivars	40	0	0	0	0	0	80

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3.3.2 New fruits Olavshagen and Løeflaten.

In this study 2 crab apple and 3 apple cultivars were investigated. The number of apple decay fungal species identified varied across the cultivars- Dolgo had 8 species of fungi, Elan 7 species, Kobenza 7 species, NA 42 51 6 species and Rubinola 5 species. Significant correlation between sampling dates and disease incidences were noted on Crab apple cultivar Dolgo and apple cultivar Elan (P=0.05). On Crab apple ‘Dolgo’, Gray mold (P=0.0277) and Brown rot (P=0.0158) showed significant correlation while for ‘Elan’ White rot was the only one with significant correlation (Table 8, 9, 10,11 and 12).

Table .8. Relationship between percentage mean disease incidence and sampling period on crab apple cultivar 'Dolgo'-Olavshagen experimental orchard.

Sampling period	**Gray mold**	**Blue mold**	**Fusarium rot**	**white rot**	**Mucor**	**Bitter rot**	**Sclerotinia**	**Brown rot**
6th June 2017	48a	0	0	0	0	0	0	0b
20th June 2017	2b	0	0	0	0	0	0	0b
4th July 2017	36a	6	6	8	0	4	2	0b
27th July 2017	0b	0	2	4	4	2	0	18a
15th August 2017	26a	8	14	0	0	10	0	12a, b
0.0005	**0.0277**	**0.0465**	**0.5652**	**0.0705**	**0.436**	**0.4362**	**0.1467**	**0.0158**

Different letters indicate significant differences using Tukey`s test (α =0.05).

Table .9. Relationship between percentage mean disease incidence and sampling period on Apple cultivar 'Elan'-Løeflaten experimental orchard

Sampling period	Gray mold	Blue mold	Fusarium	white rot	Mucor	Sclerotinia	Brown rot
6th June 2017	18	0	0	0b	0	0	0
20th June 2017	2	0	0	0b	0	0	0
4th July 2017	10	2	2	14a, b	2	4	0
27th July 2017	6	6	6	2b	0	0	2
15th August 2017	12	6	10	22a	0	4	0
P -Values	**0.5197**	**0.0166**	**0.1075**	**0.0134**	**0.436**	**0.0705**	**0.4362**

Different letters indicate significant differences using Tukey`s test (α =0.05).

Table .10. Relationship between percentage mean disease incidence and sampling period on crab Apple cultivar 'Kobenza'-Olavshagen experimental orchard.

Sampling period	Gray mold	Blue mold	Fusarium	White rot	Sclerotinia	Brown rot	Cladosporium rot
6th June 2017	0	0	0	0	0	0	0
20th June 2017	22	0	0	0	0	0	0
4th July 2017	18	2	8	4	16	0	0
27th July 2017	2	0	4	0	0	2	8
15th August 2017	26	2	4	2	12	8	0
P-Values	**0.1229**	**0.4362**	**0.1487**	**0.4362**	**0.0705**	**0.5102**	**0.1628**

Table .11. Relationship between percentage mean disease incidence and sampling period on crab Apple cultivar 'NA 42 51'-Løeflaten experimental orchard

Sampling period	**Gray mold**	**Blue mold**	**Fusarium**	**white rot**	**Mucor**	**Bitter rot**
6th June 2017	3	10	18	0	0	0
20th June 2017	3	10	8	0	0	0
4th July 2017	3	10	20	0	6	12
27th July 2017	3	10	6	4	4	0
15th August 2017	3	10	4	0	2	0
P-values	**1**	**1**	**0.1981**	**0.0623**	**0.459**	**0.0587**

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Table .12. Relationship between percentage mean disease incidence and sampling period on Apple cultivar 'Rubinola'-Løeflaten experimental orchard.

Sampling period	**Gray mold**	**Blue mold**	**Fusarium**	**white rot**	**Mucor**
6th June 2017	16	0	0	0	0
20th June 2017	14	0	0	0	0
4th July 2017	6	0	28	16	0
27th July 2017	10	14	0	0	0
15th August 2017	2	20	0	0	2
P-values	**0.0869**	**0.1201**	**0.0357**	**0.1628**	**0.436**

4 Discussion

The present study provides direct evidence that pollinator trees are a source of inoculum for fruit decay fungi on apples. In this study apple decay fungi were isolated, identified and quantified from mummies, new fruits and leaves. In summary a total of 11 apple decay fungal species were identified namely Brown rot, Gray mold, Blue mold, Cladosporium rot, White rot, Mucor, Rhizopus rot, Fusarium rot, Sclerotinia, Bitter rot and Trichotecium.

Though the study was done under laboratory-controlled conditions suitable for fungal sporulation, these results predicts similar occurrence under similar conditions in the orchard. The hypothesis that only mummified (old fruits) would contribute inoculum was not true. This study has demonstrated that both old, new fruits and leaves were able to sporulate. According to (Teixidó et al., 1999), developing plant tissue presents a rich environment of nutrients and water, which is conducive to support significant development of microbial activity. These results were seen to agree with previous studies by (Teixidó et al., 1999) which suggested that as nutrients levels change on ripening apples, the balance and ecological succession of bacteria, yeasts and filamentous fungi changed both on fruits and leaves.

4.1 Kvitavoll mummies.

Mummies plays an important role as the main survival structures of most fungal pathogens in the orchard, it is only through understanding of the host plant, the virulent pathogen and environmental conditions that we can initiate control by either modifying, reducing or eliminating one of these favourable conditions. In this study the results obtained proved that mummies are a source of primary inoculum for apple decay fungi. 8 species of apple decay fungi were isolated and identified (Table 3 & 4).

The variations (Table 3 & 4) on disease incidences clearly indicated that cultivars varied on susceptibility to various apple decay fungi (Sholberg and Hagg, 1966). These variations could be attributed to inherent cultivar resistance and the ability of the cultivar to retain mummies after the production season. According to (Willets, 1968) mummified fruits develop due to dehydration after infection in the previous season and consists of a mixture of fungal and plant tissue. On some apple varieties these aborted fruits remain on position and mummify, instead of falling onto the ground. This mummy can be formed at various times during the growing season, most frequently during the month of June. These mummies may become colonized by several storage-rot fungi throughout the season, providing a source of inoculum for apples prior to harvest (Sutton 1981; Schulte 1997; Weber 2011). Another study done by (Quast and Weber 2008) in Northern Germany revealed that apple cultivars differed on their tendency to retain mummies and by having these mummies in close proximity to

developing fruits, mummies provided an attractive habitat for pathogenic fungi and eventual source of inoculum into the new season , mummy-retaining varieties such as ‘Elstar’ are known to be at an elevated risk from post-harvest diseases, especially in fruit from orchards under reduced-fungicide or organic management (Knoche et al. 2000; Holb 2008).A combination of humid weather followed by temperatures of 15-20˚C in spring, will induce formation of a fresh crop of inoculum on the surface of mummified fruits (Byrde, 1954).

According to results obtained from the laboratory-controlled experiments (Table 3 & 4) there were wide variations making it difficult to show relationship between disease incidences and sampling periods. The different sampling periods represent variations on weather conditions which would initiate response on the fungi colonizing the mummies conferring a seasonal pattern of inoculum in spore content. Significant correlation between percentage mean disease incidences and sampling months were recorded for Mucor (P=0.0001), Gray mold (P=0.0001), Bull’s eye rot (P=0.0224) and Cladosporium (P=0.0305) on cultivar ‘Dolgo’(Fig.3). This expressed the effect of climatological conditions on subsequent re-sporulation of colonizing fungal pathogens on mummies after the overwintering period. Investigated mummies were from 2016 and 2017 production seasons, indicating that cultivars infected early as well as late infected fruits contribute to production of primary inoculum into the next season.

No significant results were recorded from crab apple cultivar ‘Evereste’ (Fig.4). No sampling was done on this cultivar on the month of September 2017.There were less trees of ‘Evereste’ to sample from and this could have influenced on results recorded.

This scenario can be interrupted by the early removal of diseased mummies on trees and together with what was mechanically or naturally thinned and other debri from the orchard floor destroyed. This will reduce on the inoculum load. Thinning of fruits to reduce fruit to fruit infections through rain flash is important, a study by (Leeuwen et al., 2000) showed that fruit to fruit contact is one of the mechanism for spread of disease within tree, though the final infection depends on presence of injuries or wounds as avenues of infection.

Variations on percentage mean incidences noted on both cultivars is believed to be due to differences in cultivar susceptibility, growing practises like fruit thinning, removal of mummies and the ability of different cultivars to retain mummies and thereafter the ability of the fungi to survive through winter. More research focussed on this is needed.

4.2 Study on new fruits and leaves-Løeflaten and Olavshagen experimental fields.

In this experiment new fruits and leaves were investigated. The samples represented similar developmental stages on the orchard. All were asymptomatic. The percentage mean incidences for the various apple decay fungi identified are summarized on (Tables 8, 9, 10, 11 and 12).

From investigated leaves samples 7 fungal species were isolated, identified and disease incidences scored. The identified apple decay fungal diseases were Blue mold, Fusarium, Mucor, Bitter rot, Sclerotinia, Cladosporium and Rhizopus rot. The dominant fungi were Bitter rot ((80%), Rhizopus (80%) and Fusarium (80%).The data obtained from leaves was erratic and with minimal incidences. Data analysis was not done (Table 7). Possible causes for deviation from my expectation was assumed to be related to the 6-day incubation period provided. It is assumed that if more days were provided then sporulation incidences would have increased.

A study done by (Van leeuwen et al., 2000) showed that infections of pome fruits appear approximately 5-6 weeks after full bloom and subsequently infection of healthy fruits occur continuously up to harvest time. These infections occur via cracks and wounds on the fruit skin and via fruit to fruit contact (Michailides & Morgan, 1997). In this study involving new fruits on orchards Løeflaten and Olavshagen, The number of apple decay fungal species isolated and identified varied across the cultivars- 'Dolgo' had 8 species, 'Elan' 7 species, 'Kobenza' 7 species, 'NA 42 51' 6 species and 'Rubinola' 5 species. Significant correlation between sampling dates and disease incidences was positive on crab apple cultivar 'Dolgo' and apple cultivar 'Elan' (P=0.05). On Crab apple 'Dolgo', Gray mold (P=0.0277) and Brown rot (P=0.0158) showed positive correlation while for 'Elan' only White rot (P=0.0137) had positive correlation.

White rot recorded highest percentage mean incidence (22%) on 15th of August on apple cultivar 'Elan', while Gray mold was highest on 6th June, 4th July and 26th of August 2017 on crab apple cultivar 'Dolgo'. Brown rot was highest on 27th July 2017 on apple cultivar 'Elan'. These variations indicate seasonal variations on inoculum load on the orchard.

(Dennis, 1983; Arthey and Ashurst, 1996) observed that as apples developed to maturity they became susceptible to attack by a variety of fungi to which they maintained resistance during their early periods of development. The results obtained clearly shows that developing fruits and leaves harbour latent infections in the orchard which in a normal situation are not easily seen (Table 8, 9,10,11, and 12).

4.3 Løeflaten Five Crab Apples.

In this study five crab apple cultivars were investigated. All were asymptomatic. Samples were in two subgroups; one subgroup was incubated without surface sterilization and freeze treatment at -18˚ C for 5hrs and the other sub group sample surface sterilized and frozen before incubation as outlined in materials and method section.

4.3.1 Unsterilized and unfrozen fruit samples

A total of 6 species of apple decay fungi were identified and quantified namely; Gray mold, Blue mold, Trichotecium, Brown rot, Fusarium rot and Mucor. Significant differences between the sampling periods and percentage mean disease incidence were noted on Gray mold, Blue mold, Trichotecium and Brown rot with P values of (0.0016,0.0007,0.0076 and 0.0001) respectively (Table 5).

These results (Table 5) indicates presence of inoculum on the fruit surface. It also indicates varying levels of inoculum load over the production season. In a study on olive anthracnose epidemics progression was to be influenced by several factors, including weather conditions (Kaul., et al 1985; Moral., et al 2012 & Talhinhas., et al, 2011), cultivar susceptibility (Moral *et al*., 2009; Moral *et al*., 2012 & Mateo-Sagasta, 1968) and fruit maturity (Moral et al., 2008 & Mateo-sagasta, 1968).The controlled environment studies have shown what would happen in the orchard if conditions favourable for disease development were present.

In orchard conditions during spring, conidia are dispersed by rain splash from mummified fruit to developing fruits, causing quiescent infections. During autumn, as fruits ripen to maturity, these pathogens become a major causes of fruit rot, producing large amounts of conidia initiating secondary disease cycles (Moral *et al*., 2009; Tapero, 2008). At the end of winter, the cycle starts again, with infected rotting fruits mummifying as humidity decreases, while temperature increases. The mummies more so those left in the tree canopy, will contribute conidia for new infections on developing fruits during the following spring (Trapero & Moral, 2008).

4.3.2 Surface sterilized and frozen samples.

The same scenario was noted for the fruits which were surface sterilized and frozen. A total of 9 apple decay fungi were isolated, identified and quantified. In comparison sterilized and frozen samples (Table 5) had more fungal species than unsterilized and unfrozen samples. It was found that Trichotecium only occurred on unsterilized and unfrozen samples while White rot, Bitter rot, Sclerotinia and Cladosporium occurred on sterilized and frozen samples.

By freezing the samples at -18˚ C the plant defence system was weakened (physical and chemical barriers such as the cell wall, waxes, hairs, antimicrobial enzymes, and secondary metabolites) and when these obstacles are overcomed, pathogens inside the tissue could sporulate (Jones & Dangl., 2006). Freezing also stimulates sporulation of fungal pathogens (Børve et al., 2010). This may be the reason for variance between the two treatments on the number of fungal diseases identified. (Table 5 & 6).

The source of inoculum for these latent infections are likely to be due sporodochia produced on blighted blossoms and mummies retained on the crab apple trees from the previous season and are spread from fruit to fruit through contact, rain splash ,wind, insects ,birds and man (Byrde & Williams , 1977).Splash is important for short range dispersal within the tree (Abonyi et al., 2015) while vector borne and airborne conidia (Kable , 1965) ensure dispersal within the orchard.

These results also demonstrated that crop developmental stage, optimal environmental conditions and presence of right amounts of inoculum are required for disease development. Optimal environmental conditions for fungal growth, sporulation, release of spores to initiate infection is high humidity (> 90% RH) and cool weather with a temperature range of 18 to 26°C (Agrios, 2005)

5 Conclusion.

In fine, it can be concluded that the presence of a wide array of fungal pathogens in the different samples is a matter of great concern. For these disease-causing fungi to infect a combination of many factors must be present at one point in time. These include; primary inoculum, source of nourishment, entry points into the plant tissue, perfect climatic conditions - a combination of temperature and humidity and varietal susceptibility. Removing or altering any these variables will stop fungal infection from starting.

This study has demonstrated that both symptomatic (mummies) and asymptomatic fruits and leaves are hosts to a variety of fungal species and inoculum load varied by season due to climate and crop phenology. The variation on mummies was mainly due to climatic conditions while that of new fruits and leaves was due to both climatic conditions and crop phenology. This is the reason why variations were recorded across the sampling periods on number of fungal species and incidences of apple decay fungi between the different samples. (Table 3,4 ,6, 8,9,10,11,12).

Inoculum may also come from the host itself and is mainly dispersed by water splash, fruit to fruit contact and between old and new leaves and fruits. This should also be considered when making control decisions

Cultivars also vary widely with respect to apple decay fungal disease resistance and cultivar resistance alone cannot adequately provide control. There is a need to incorporate other control measures within reach and which are cost effective to the grower. These actions include cultural activities like the clearing of debris, mummy removal both on the orchard floor and on trees (if applicable and cost-effective) which will reduce on the inoculum load. However, to the best of my knowledge, this approach has not been rigorously tested under commercial conditions. Further studies and work need to be done to see the feasibility of mummy removal.

Further research is also needed to identify ways of lowering the amount of primary inoculum and one of the possible areas would be to understand the mechanism behind (re) -sporulation after overwintering and together with other control measures adequately lower the inoculum load.

More breeding efforts are needed for development of crab apple cultivars which are resistant, and which do not retain mummies without compromising on their major role as pollinizers.

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