Master thesis projects in plant pathology for 2022

Below are suggestions for master thesis projects in plant pathology for 2022. However, other ideas for thesis work may be discussed further with one of the following teachers:

- Dag-Ragnar Blystad (dag-ragnar.blystad@nmbu.no; dag-ragnar.blystad@nibio.no)
- Ingerd Skow Hofgaard (ingerd.hofgaard@nmbu.no; ingerd.hofgaard@nibio.no)
- May Bente Brurberg (may.brurberg@nmbu.no; may.brurberg@nibio.no)
- Arne Stensvand (arne.stensvand@nmbu.no; arne.stensvand@nibio.no)

1 Virus	**Tobamovirus i vektshuskulturer**
	Tobamoviruses in greenhouse crops

Viruses represent one of the most important threats to agriculture and horticulture. Several viral families include very stable and highly infective pathogens. Especially viruses in the genus *Tobamovirus* can cause unpredictable loss to tomato and cucumber production in Norway. In tomato, *Tomato brown rugose fruit virus* and other resistance breaking viruses and strains are posing a threat to the tomato industry. In cucumber, *Cucumber green mottle mosaic virus* has been a growing problem for the last decade.

Tobamovirus in tomato gives uneven ripening, necrosis and chlorosis.

The viral particles of tobamoviruses are extremely stable and infectious. They can be preserved in seeds for several years. In addition, tobamoviruses can survive outside of the host on inert and biological surfaces, as well as in water and soil for months or even years, without losing their virulence. Due to import of tomato and cucumber product as fresh vegetables and other products, we could be exposed to these viruses in our daily life, and infective virus particles may spread further into sewage, drainage and environmental waters which could be an unpredictable threat for plant health and food production in Norway.

Several aspects of tobamovirus identification, testing, epidemiology and control needs further investigations.

The master thesis project needs to be discussed and defined in cooperation with the student. Please contact Dag-Ragnar Blystad (dag-ragnar.blystad@nibio.no) or Zhibo Hamborg (zhibo.hamborg@nibio.no) for more information if you are interested in this topic.

2 Virus	Virus i jordbær – diagnostikk og kartlegging
	Strawberry viruses – diagnosis and survey

One of the causal agents, which can reduce fruit quality and quantity are viruses. Viral diseases can reduce strawberry yield by 20-40%, sometimes up to 70-80% or the plants can even die. The use of classical molecular biological methods (grafting of indicator clones, transmission electron microscopy, PCR) suggested that the most economically important strawberry viruses are strawberry mild yellow edge virus (SMYEV), strawberry crinkle virus (SCV), strawberry mottle virus (SMoV) and strawberry vein banding virus (SVBV), especially when it occurs in mixed infections. A new study showed that infection with only SMYEV caused a reduction in the number and size of fruits ranging from 28% to 63% compared to a healthy control.

Single virus infection usually does not show any specific symptoms on these plants, but only occurs when mixed viral infections are present. Therefore, often the most effective spreaders of viruses in strawberry are growers themselves, who unknowingly distribute diseased seedlings. Detection of viral pathogens and subsequent production of recovered seedlings is a very important prevention for the cultivation of healthy cultures.

We would like to develop a more cost-efficient and sensitive method to detect strawberry viruses with q-PCR in a new KAPPA (Norway and Czech Republic collaboration) project starting from 2021. In addition, survey of strawberry viruses in Norway and identification of unknown viruses that can infect strawberry cultures will also be studied in this project.

Strawberry crickle virus showing symptoms in a sensitive indcactor host.

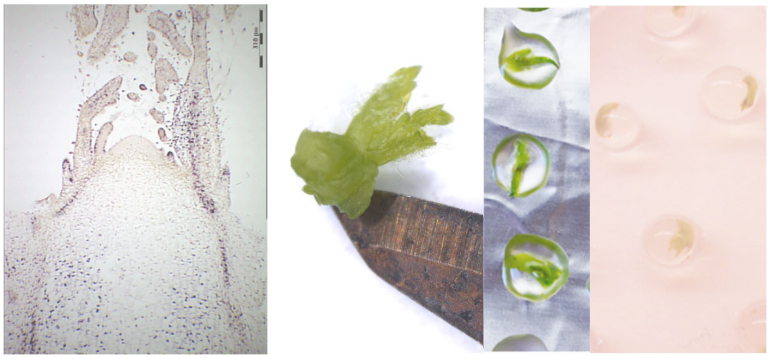
Please contact Dag-Ragnar Blystad (dag-ragnar.blystad@nibio.no) or Zhibo Hamborg (zhibo.hamborg@nibio.no) for more information if you want to study strawberry viruses in your master thesis.

3 Virus	**Kryopreservering av plantevirus**
	Cryopreservation of plant viruses

Plant viruses, positive controls for validation of diagnostic methods, require special preservation methods to ensure long-term viability/infectivity of the sample and to maintain the original characteristics. Preservation methods that are commonly used are: low temperature storage at -20°C or -80°C, freeze-drying, and virus maintenance ex situ (such as greenhouse). The latter is needed when virus can otherwise not be preserved, or virus cannot be transmitted by mechanical transmission, which many strawberry and raspberry viruses belong to. It is difficult to grow the viruses infected materials continually without any changes: free from other diseases and pests, or virus characterization can be lost. How to maintain these viruses has always been a challenge.

Successful preservation of apple stem grooving virus (ASGA), chrysanthemum stunt viroid (CSVd) and chrysanthemum chlorotic mottle virus (CChMVd) has been reported with cryopreservation without losing activity and infectivity afterwards. These data indicate that cryopreservation techniques have the potential to be applied for virus preservation.

In a new project (Norway and Czech Republic collaboration project) starting from 2021-Healthy berries in a changing climate, we would like to apply cryopreservation in conservation of strawberry and raspberry viruses, by cryopreservation of plant materials that are infected with known viruses. Strawberries and raspberries that are infected with certain/several viruses can easily be cryopreserved, afterwards taken up and grown as tissue culture and further on in a quarantine greenhouse when good positive controls are needed. Certainly, validation of cryopreserved virus genetic stability, viability and infectivity is needed to evaluate this technique for broad application.



Relevant molecular techniques such as localization and tissue culture will be included. Photo: Zhibo Hamborg.

Please contact Dag-Ragnar Blystad (dag-ragnar.blystad@nibio.no) or Zhibo Hamborg (zhibo.hamborg@nibio.no) for more information.

4 Virus	**Virus i bringebær – skadepotensiale, spredning og bekjempelse**
	Viruses of raspberry – loss, epidemiology and control

Det pekes ofte på at Norge har store muligheter for bærdyrking. Vi har entusiastiske og dyktige dyrkere som prøver å utnytt mulighetene. Det er imidlertid en bekymring at ikke plantehelse får nok fokus. Virussjukdommer i bringebær utgjør en flaskehals for å øke dyrkingssikkerheten. Det trengs for å forstå utfordringene med virus og hvordan virus best kan kontrolleres og bekjempes. Resultater fra prosjektet «*Diagnostikk, rensing og kryopreservering av bringebær, bjørnebær og sjalottløk*» (Rub&Al), 2016-2019, ga oss både ny kunnskap og nye diagnoseverktøy. I det nylig innvilgende prosjektet «*Healthy berries for a changing climate*» (KappaBerries), 2020-2024, får vi nye muligheter til å forske på virus i bringebær.

Mosaikk i bringebær – hvilke virus forårsaker slike symptomer?	Symptomer som skyldes *Black raspberry necrosis virus*, et av de mest utbredte og skadelige virus i bringebær i Norge.	Bringebær som smuldrer har vært undersøkt som et mulig virusproblem

Det er aktuelt med en oppgave som kan fokusere på kartleggingsarbeid og de forskjellige virus i bringebær sitt skadepotensiale, spredning og bekjempelse. Men det kan også være oppgaver som er mer molekylært rettet og med fokus på ett virus. Det er også aktuelt å studere bladlus-overføring av virus i bringebær.

Ta kontakt med Dag-Ragnar Blystad (dag-ragnar.blystad@nibio.no) eller Zhibo Hamborg (zhibo.hamborg@nibio.no) dersom du er interessert i en oppgave innen dette temaet. Oppgaven blir definert nærmere i samtale med studenten og vil involvere flere veiledere / medveiledere innen virus og entomologi etter hva som naturlig ut fra vinklingen som defineres i samtale med studenten.

5 Virus	**Potetbanken og genressurser**
	The potato tissue culture bank and genetic resources

Potetbanken hos NIBIO er grunnleggende for et friskt utgangsmateriale av potet i Norge.

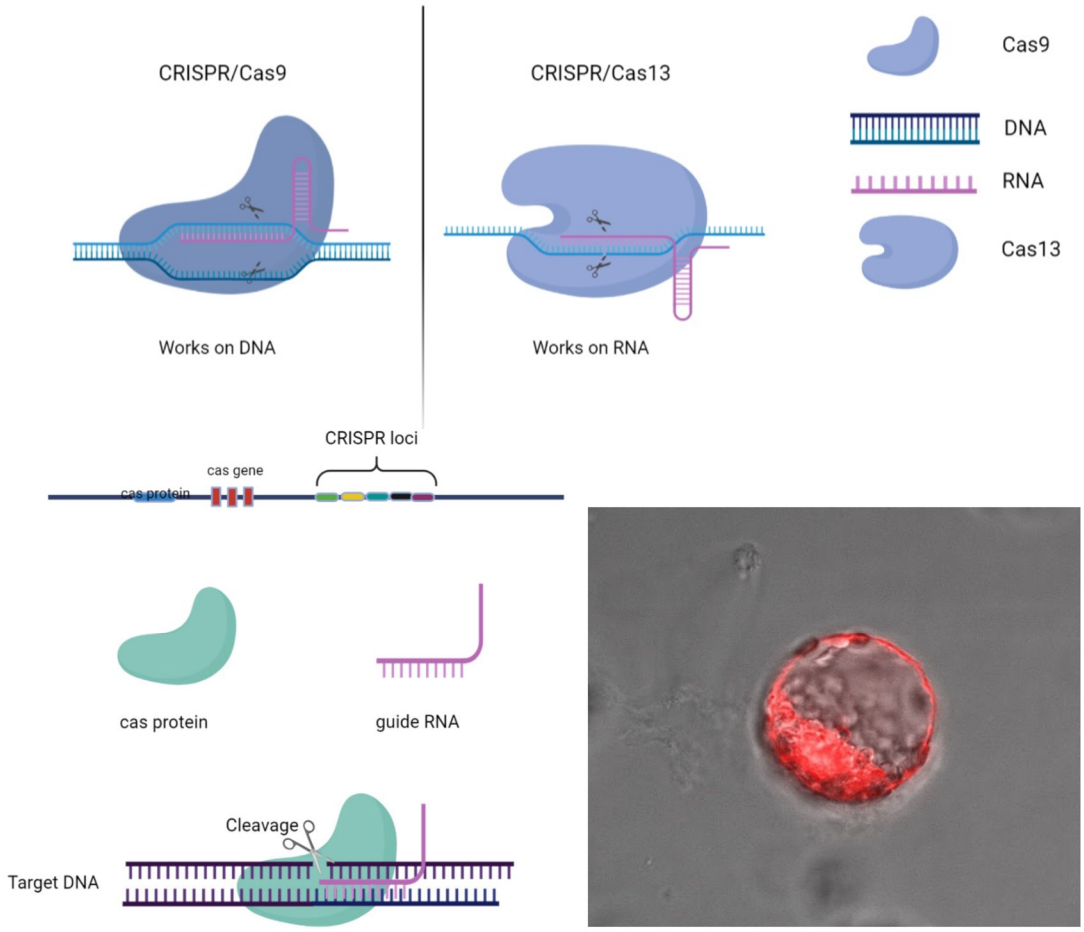
NIBIO Divisjon for bioteknologi og plantehelse har en samling av friskt, virusfritt kjernemateriale av mandatsorter av potet (Potetbanken) og vegetativt formert løk. Kompetansen til å lage og vedlikeholde et slikt friskt utgangsmateriale er av avgjørende betydning for plantehelsen til klonfomerte vekster. NIBIO har gjennom dette arbeidet viktige oppgaver knyttet til mat- og dyrkingssikkerhet, bevaring av genressurser og innovasjon.

En masteroppgave innenfor dette området vil kunne defineres i flere mulige retninger:

- Rensing av «nye», gamle sorter som bør inn i Potetbanken / klonarkivet. Dette vil være en oppgave som vil fokusere på vevskultur og virustesting med ELISA-metoden, PCR og testplanter
- Beskrive og sammenligne gamle sorter som vi har i banken for om mulig å avsløre dubletter som egentlig er samme sort, men med forskjellige navn. Dette arbeidet kan ha flere hovedretninger:
    - Genetisk analyse som utføres i samarbeid med andre kompetansemiljøer i NIBIO.
    - Dyrkingsforsøk for å studere og beskrive utvikling og utseende med beskrivelser og fotografering. Dette kan innbefatte studier av utvalgte sorters dyrkingsverdi og matkvalitet.
    - Sammenstilling av gamle data om sortene, dyrkingsverdi og bruk.

Ta kontakt med Dag-Ragnar Blystad (dag-ragnar.blystad@nibio.no) dersom du er interessert i en oppgave innen dette temaet. Zhibo Hamborg (zhibo.hamborg@nibio.no) og Morten Rasmussen (morten.rasmussen@nibio.no) vil være i veilederteamet. Oppgaven blir definert nærmere i samtale med studenten og kan involvere flere medveiledere etter hva som er naturlig ut fra vinklingen som defineres i samtale med studenten.

6 Virus	**Funksjonelle studier av CRISPR-Cas-systemene for viruseliminering**
	Functional Studies of the CRISPR-Cas Systems for Virus Elimination



Summary

CRISPR Cas9 has caught the attention of many scientists during the last 5 years. There are many groups doing research on CRISPR Cas9 in plant biology. The focus of these groups is mainly on introducing new trains to plants by DNA modifications and to diversity the CRISPR toolbox for enhanced gene editing. My lab is focusing on CRISPR in a very different way. In my lab we are exploring a different Cas protein (Cas13a and the orthologues Cas13b and Cas13d) which does not target DNA, but rather RNA. In addition, my lab is not focusing on introducing traits or modifying the plant genome, but rather on using Cas13 as a method to localize plant viruses within the cells and to kill them. The thesis will be focused on characterizing the activities, cellular localization and ability to cleave viral RNA of Cas13 proteins. This type of study involves good amount of gene cloning and molecular techniques, the use of delivery vectors, plant cell transfections and cellular localization studies. The student will gain competence in the following areas and techniques: Molecular biology, virology, gene technology, recombinant DNA techniques, plant genetics and plant pathology. This study is part of a CRISPR project in collaboration with China and might provide you the opportunity to spend some time in my colleague's lab in Beijing. If you are interested drop me a line.

Subject area

Molecular Biology, Virology, Cell Biology, Plant physiology, Plant-pathogen interactions

Language thesis (Norwegian and/or English)

Bachelor or Master thesis

Credits

60 credits

Project/company

NIBIO

Please contact

Carl.spetz@nibio.no

Dag-ragnar.blystad@nibio.no

Our publications on CRISPR:

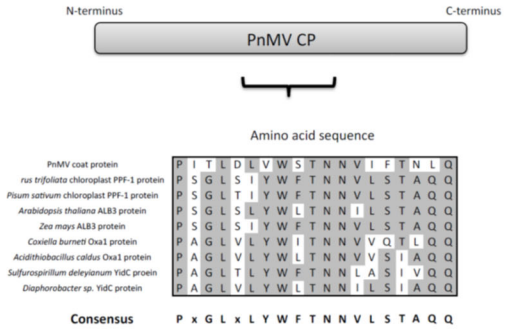
- Xu Z, Kuang Y, Ren B, Yan D, Yan F, **Spetz C**, Sun W, Wang G, Zhou X, Zhou H (2021). SpRY greatly expands the genome editing scope in rice with highly flexible PAM recognition. ***Genome Biol***. Jan 4;22(1):6. doi: 10.1186/s13059-020-02231-9. PMID: 33397431; PMCID: PMC7780387.

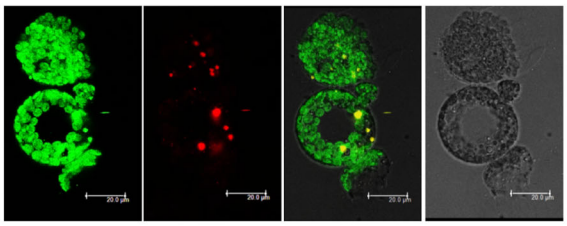
- Liu, L., Kuang, Y., Yan, F., Li, S., Ren, B., Gosavi, G., **Spetz, C**., Li, X., Wang, X., Zhou, X., & Zhou, H. (2020). Developing a novel artificial rice germplasm for dinitroaniline herbicide resistance by base editing of OsTubA2. ***Plant biotechnology journal***, 10.1111/pbi.13430. Advance online publication. https://doi.org/10.1111/pbi.13430

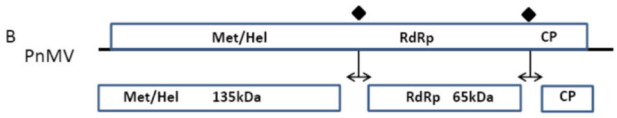
- Wang, M., Xu, Z., Gosavi, G., Ren, B., Cao, Y., Kuang, Y., Zhou, **C., Spetz**, C., Yan, F., Zhou, X., & Zhou, H. (2020). Targeted base editing in rice with CRISPR/ScCas9 system. ***Plant biotechnology journal***, 10.1111/pbi.13330. Advance online publication. https://doi.org/10.1111/pbi.13330

- Kuang, Y., Li, S., Ren, B., Yan, F., **Spetz, C**., Li, X., Zhou, X., & Zhou, H. (2020). Base-Editing-Mediated Artificial Evolution of OsALS1 In Planta to Develop Novel Herbicide-Tolerant Rice Germplasms. ***Molecular plant***, *13*(4), 565–572. https://doi.org/10.1016/j.molp.2020.01.010

7 Virus	**Dissekere virale replikasjonsmekanismer**
	Dissecting viral replication mechanisms







Summary

Replication of all positive-sense single-stranded RNA viruses occurs in specific structures in close association with cellular membranes. Targeting of the viral replication complex (RC) to the site of replication is mediated by the interaction of viral-encoded proteins and host factors. Electron microscope studies have shown that *Poinsettia mosaic virus* (PnMV, family *Tymoviridae*) infection is associated with the presence of vesicular structures in the chloroplasts, which indicates that the replication of PnMV might occur in association with chloroplast-derived membranes. Using computer assisted homology search, we have identified that the coat protein (CP) of PnMV shows similarity to membrane bound proteins and contains a conserved amino acid sequence motif found in members the Alb3/Oxa1/YidC protein family. This protein family is involved in the insertion of proteins into intracellular membranes. We hypothesize that the targeting of the PnMV RC to the chloroplast is mediated by viral-encoded CP. We plan to test this hypothesis carrying out co-localization studies using transient and stable expression of GFP-tagged viral proteins and confocal laser scanning microscopy. This type of study involves good amount of gene cloning and molecular techniques, the use of delivery vectors, plant cell transfections and cellular localization studies. The student will gain competence in the following areas and techniques: Molecular biology, virology, gene technology, recombinant DNA techniques, plant genetics and plant pathology.

Subject area

Molecular Biology, Virology, Cell Biology, Plant physiology, Plant-pathogen interactions

Language thesis

Norwegian and/or English

Bachelor or Master thesis

Credits

60 Credits

Project/company

NIBIO

Please contact

Carl.spetz@nibio.no

Dag-ragnar.blystad@nibio.no

Our publications on Poinsettia mosaic virus

- Spetz C & Blystad D-R (2015) A Membrane-Binding Conserved Motif in the Coat Protein of PnMV Seems to Mediate Chloroplast Targeting. *Acta Horticulturae* 1072, 97-104.
- Spetz C, Clarke JL, Dees MW, Haugslien S, Moe R, & Blystad D-R. (2012) RNA silencing-based resistance in Nicotiana benthamiana Is transiently broken by graft inoculation of Poinsettia mosaic virus January 2012. *Acta horticulturae* 941(941):91-108
- Clarke, J.L., Spetz, C.J., Haugslien, S., Dees, M.W., Moe, R. & Blystad, D.-R. (2011). Production of transgenic Poinsettia with Resistance against Poinsettia mosaic virus (PNMV) using Agrobacterium- Mediated Transformation. Acta Horticulturae 901:87-93.
- Dees, M.W., Spetz, C.J.J. & Blystad, D.-R. (2011). First report of Tulip virus X (TVX) in Norway. Acta Horticulturae 901:215-222.
- Fløistad, E., Spetz, C. J.J. & Blystad, D.-R. (2011). Virus elimination and retention of free - branching in Poinsettia. Acta Horticulturae 901:207-213.
- Spetz, C., Moe, R. and Blystad, D-R. (2008). Symtomless infectious cDNA clone of Norwegian isolate of *Poinsettia mosaic virus*. *Archives of Virology* 153, 1347-1351
- Clarke, J.L., Spetz, C., Haugslien, S., Xing, S., Dees, M.D., Moe, R. and Blystad, D- R. (2008). Agrobacterium tumefaciens-mediated transformation of poinsettia, *Euphorbia pulcherrima*, with virus-derived hairpin RNA constructs confers resistance to Poinsettia mosaic virus. *Plant Cell Reports* 27, 1027-1038

8 Sjukdoms-resistens	**Bruk av CRISPR-teknologi for å utvikle sjukdomsresistent salat**
	Using CRISPR technology to develop pathogen resistant lettuce

Each year 30-40 % of global crop production is lost to pathogens like fungi, bacteria, insects, nematodes and oomycetes, and is thus a major threat to the farmer's economy and global food security. Successful pathogen infection involve targeting plant factors encoded by susceptibility (S) genes to manipulate host processes to their advantage. Some of the processes pathogens use to promote infection, are suppression of plant defenses, nutrient acquisition, and transport of pathogenic proteins in the host cell.

We have done RNA sequencing of lettuce (*L. sativa*) plants infected *S. sclerotiorum* and identified several highly up regulated genes that are candidate genes for susceptibility. In this project, we will transform different cultivars of lettuce with CRISPR-Cas9-constructs targeting genes potentially involved in susceptibility for *S. sclerotiorum*. Several different transformation methods will be used and modified to obtain optimized transformation frequency.

You will learn: Standard molecular techniques such, PCR, real-time qPCR as well as plant and pathogen specific techniques, CRISPR/Cas9 gene editing and bioinformatics. Optimization of methods for Agrobacterium-mediated transformation af plant tissue.

For further information: Tage Thorstensen, phone: 40200909, e.mail: tage.thorstensen@nibio.no, May Bente Brurberg, phone: 92609364, e-mail: may.brurberg@nibio.no

9 Sopp-sjukdommer	**Sjokoladeflekk i bønne – sjukdomsorganismer og risiko for fungicidresistens**
	Chocolate spot in faba bean – disease organisms and risk for fungicide resistance

Photo: Heidi U. Aamot and Chloè Grieu

Increased cultivation of protein crops such as faba bean for food and feed in Norway is desirable many reasons. Chocolate spot, caused by *Botrytis cinerea* and *B. fabae,* is one of the most destructive diseases in faba bean. Since integrated pest management (IPM) based on a long crop rotation and ploughing after harvest does not effectively control the disease, fungicide application during flowering is commonly used. However, the effect of spraying is highly variable, and the reasons for this are not fully understood. Moreover, the occurrence and importance of seed infection is unclear. **Therefore, we need an updated survey on which species of *Botrytis* that are responsible for the development of chocolate spot of faba bean and their response to different fungicides**.

A master thesis will include systematic collection of faba bean plants during the growing season and isolation of *Botrytis*. Isolates will be identified to species using DNA based methods (barcoding). Also seed samples will be studied and possible isolates will be identified using the same methods. Studies of effect of fungicides will be performed in laboratory experiments, with opportunity for development of screening methods and bioassays.

Supervisors: Ingerd Skow Hofgaard (NIBIO, NMBU) ingerd.hofgaard@nibio.no, Guro Brodal (NIBIO) guro.brodal@nibio.no, and Heidi Udnes Aamot (NIBIO) heidi.udnes.aamot@nibio.no.

10 Sopp-sjukdommer	**Frukttrekreft (*Neonectria ditissima*) - sporespreiing**
	European canker (Neonectria ditissima)- spore dispersal

Background

European canker caused by *Neonectria ditissima* is an important disease on apple in northwestern Europe. Development of fruiting bodies (perithecia) might be closely related to climate as well as dispersal of spores in different times during the year. Spore trap samples have been collected in Finland, Germany and Norway (in project KreftKamp 2017-2021). Modelling and final assessing will be performed during 2022-2024.

European canker inoculum can be sexual (from red perithecia, left) or asexual (condia in yellow-white sporulation, right) on apple.

Thesis

The master thesis will be carried out with existing and possibly also new data (collected by the student) from spore traps. The student will do microscopic spore counts, data analysis and modeling. Much of the practical work is flexible and can be done when preferable for the student.

Supervisors: Arne Stensvand (NMBU/NIBIO) arne.stensvand@nmbu.no/ arne.stensvand@nibio.no and Jorunn Børve (NIBIO), jorunn.borve@nibio.no.

11 Sopp-sjukdommer	**Lagersjukdomar på eple, inokulerings- og lagerforsøk**
	Postharvest diseases on apple, inoculation and storage experiments

Background
Apple is the most important fruit crop in Norway. There is a lack of knowledge about the biology and risk for latent infections of the pathogen in young trees.

Apple fruit can have several different diseases and decays.

Thesis
The master thesis work has several options: 1. Storage experiments of inoculated fruit to gain basic knowledge about the relationship between fruit, storage condition and pathogen. The work will start in summer/early autumn and finish in December/January. 2: Sample pathogens in field during summer and autumn, do isolations and do identification with molecular methods in autumn/winter in order to gain new pathogen biology knowledge. 3. Storage experiments with focus on physiological decay in order to gain more knowledge about the mechanism and influencing factors. Can be focused on metabolism study at Ås, storage of fruit at Ås or at NIBIO Ullensvang.
NIBIO Ullensvang may host the student and offer a summer job/internship.

Supervisors:
If pathology focus: Arne Stensvand (NMBU/NIBIO) arne.stensvand@nmbu.no/ arne.stensvand@nibio.no, May Bente Brurberg (NIBIO/NMBU), may.brurberg@nmbu.no/ may.brurberg@nibio.no and Jorunn Børve (NIBIO) jorunn.borve@nibio.no

If storage/fruit quality/postharvest physiology focus: Siv Fagertun Remberg (NMBU) siv.remberg@nmbu.no and Jorunn Børve (NIBIO) jorunn.borve@nibio.no