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Department of Plant Science

Virus infection in seed potatoes used by farmers in the county of Vestfold, Norway.

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M. Sc. Plant Science

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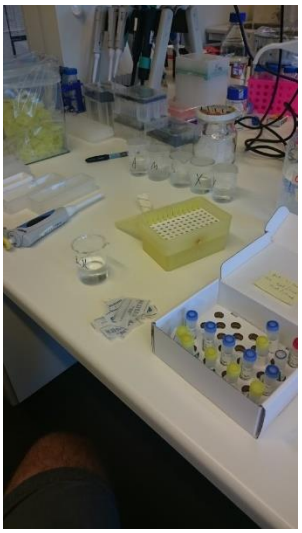

Pictures from my work. Mong, 2016

Norwegian University of Life Science

Ås, December 15, 2016

Bjørn Andreas Mong

Foreword

Any master degree at the Norwegian University of Life Science should be ended with a thesis building on an independent study conducted by a student. I will now end my studies in Plant Sciences with this 30 credits thesis in plant pathology with emphasis on plant virus diseases. It has been a very interesting topic and I feel that I have learn a lot. Only a few years ago, I would not have seen myself writing a thesis like this. I intended to do something in technology, innovation or production of food. So, when I chose this topic many people were surprised. I had no experience in potatoes or doing work in open fields.

For choosing the topic for my thesis it was important for me to combine many different subjects. Plants, production, law, economics, technology, and plant pathology. So, after talking with different supervisors I ended up with Dag-Ragnar Blystad at NIBIO. Today I'm glad that he wanted to be my supervisor, and he have been very helpful during this study. He has given me many advices and together we have been meeting often to discuss the thesis. The whole way, he has guided me on the right track when I sometimes lost the momentum.

For me, the summer 2016 will always be remembered for the analysis of viruses in potatoes. I used more hours this summer in the field, than I have been doing for my whole life. So, this has been a good experience for me.

Thanks to Siri Abrahamsen at Norsk Landbruksrådgiving and Kari Ørstad and Torfinn Torp at NIBIO, for their help. Siri for helping me in the beginning by providing me with information about the farmers and other useful knowledge on potato cultivation. Kari Ørstad has been very helpful teaching me how to do the work at the laboratory and to run the ELISA. Torfinn Torp, the statistician, have from the start of this work contributed with the knowledge on how to do the calculations that was needed for this fieldwork.

I will also thank all my friends and family that have been helping me with feedbacks and good comments under the writing process.

Ås, 15 December 2016

Summary

During the summer 2016, 3 potato cultivars at 15 different farms located in the county of Vestfold were tested for 6 different potato viruses. This is potato virus A (PVA), M (PVM), S (PVS), X (PVX), Y (PVY) and potato leaf roll (PLRV). A serological method (ELISA) was used at the laboratory to analyze the results. The cultivars surveyed were Asterix, Innovator and Saturna.

In short, the results from this survey of virus infection in seed potatoes used by farmers in county Vestfold showed very little virus infection in the seed potatoes, except for PVS. On average, it was 0.15 % PVY, 0.45 % PVA, 0.06 % PVM, 12.3 % PVS, 0.09 % PVX and 0 % PLRV.

Counting the farmers' fields PVY was found in 4 out of 15 fields, whereas PVA was found in 6 out of 15 fields. PVS was found in 15 out of 15 fields. For PVM there were infections in 3 out of 15 farms. PVX was found in 6 out of 15 fields. For PLRV there was no infection in any out of 15 fields.

The highest incident of PVY was found in Saturna with 1.6 %, for PVA in Innovator with 1.8 %, for PVS in Asterix with 43.9 %, for PVM in Innovator with 0.4 %, and for PVX 0.2 % was highest incident in all 3 cultivars. It was not found any incident of PLRV.

The low incidence in the investigated fields is probably due to a systematic, long-term work on potato virus control, where relatively frequent replacement of seed-potatoes must be regarded as the most important factor.

Sammendrag

I løpet av sommeren 2016, ble 3 potet sorter fra 15 ulike gårder i Vestfold testet for 6 forskjellige potetvirus. Potet virusene som det ble testet for var potet virus A (PVA), M (PVM), S (PVS), X (PVX), Y (PVY) og potet bladrullevirus (PLRV). En serologisk fremgangsmåte (ELISA) ble anvendt på laboratoriet for å analysere resultatene. De tre brukte potet sortene som ble valgt var Asterix, Innovator og Saturna.

Resultatene fra denne undersøkelsen viser virusinfeksjon i settepoteter som blir brukt av bønder i Vestfold Det var svært lite virusinfeksjon i disse feltene, med unntak av fra PVS. I gjennomsnitt var det 0,15 % PVY, 0,45 % PVA, 0,06 % PVM, 12,3 % PVS, 0,09 % PVX og 0 % PLRV.

I bøndenes åkre ble det funnet PVY i 4 av 15 felt, mens PVA ble funnet i 6 av 15 felt. For PVS ble det funnet i 15 av 15 felt. For PVM ble det funnet i 3 av 15 gårder. For PVX ble det funnet i 6 av 15 felt. For PLRV ble det funnet i 0 av 15 felt.

Den høyeste forekomsten av PVY ble funnet i Saturna med 1,6 %. For PVA var det i Innovator med 1,8 %. For PVS i Asterix med 43,9 %. For PVM i Innovator med 0,4 %. Den høyest forekomsten var av PVX var 0,2 % og denne var lik i alle tre sortene. Det ble ikke funnet noen tilfeller av PLRV.

Den lave forekomsten virus i de undersøkte feltene skyldes sannsynligvis et systematisk og langsiktig arbeid med virusbekjempelse, hvor relativt hyppig utskiftning av sette-poteter må ansees som den viktigste faktoren.

Content

1. Introduction

Vegetative plant propagation plays a large role in food production worldwide today. Fruits and berries, potatoes and other starchy crops are vegetative propagated. Vegetative propagation means that the plant is not propagated by true seeds, but by rhizomes or tubers, or by making nodal stem cuttings, runners, graft wood, cuttings or tissue culture. Applied research and development regarding vegetative propagation has been very important and had a huge impact on the food security the last century. One of the main goals in plant certification has always been to limit plant diseases so that the production is kept on a level were the farmer does not lose money. Once a virus infects a plant it will easily spread from plant to plant. Over many years, the yield and quality will be reduced. To meet the problems of viruses in vegetative crops like potatoes, there has been made systems for controlling the level of virus in Norway.

In this thesis, I have tested three potato cultivars Asterix, Saturna and Innovator for six different potato viruses (A, M, S, X, Y and LR) from 15 different farms in the county of Vestfold, Norway 2016. The aim of this thesis is to say something about the percentage of viruses in Norwegian seed potatoes, as well as facilitating increased proportion of healthy, certified seed potatoes in the Norwegian potato production. This will hopefully lead to increased value throughout the Norwegian potato production.

2. Literature

2.1. Introduction to potato

Potato is one of the world's favorite root vegetable, a member of the nightshade family *Solanaceae*, it originated in South America and has been grown in Europe since the 16th century. They are the swollen portion of the underground stem which is called a tuber and is designed to provide food for the green leafy portion of the plant. If allowed to flower and fruit, the potato plant will bear an inedible fruit resembling a tomato (USDA, unknown).

Whether potato is cooked, mashed, baked or made into French fries, many people often think of the potato as a comfort food. This sentiment probably inspired the potato's scientific name, *Solanum tuberosum*, since *solanum* is derived from a Latin word meaning "soothing" (Grubb & William, 1912). The potato's name also reflects that it belongs to the *Solanaceae* family whose other members include tomatoes, eggplants, peppers, and tobacco.

There are many varieties of edible potatoes. They range in size, shape, color, starch content and flavor. The skin of potatoes is generally brown, red, or yellow, and may be smooth or rough, while the flesh is yellow or white. There are also other varieties available that feature purple-grey skin and a beautiful deep violet flesh. As potatoes have a neutral starchy flavor, they serve as a good complement to many meals. There are not that many types of food that can claim so many possible variations as the potatoes. By the early 19th century, potatoes were being grown extensively throughout Northern Europe, and potatoes were almost solely relied upon as a foodstuff in Ireland owing to this vegetable's inexpensive production and the poor economy of this country. Yet, in 1845 and 1846, a potato blight (*Phytophthora infestans*) ruined most of the potato crop in Ireland and caused major devastation, this event is known as the Irish Potato Famine.

2.2. Introduction to plant virus and diagnosis

Plant viruses are among the smallest plant pathogens, but can cause loss of yield and quality in a lot of important crops. There has been described more than 1000 different plant viruses. Viruses are known to be unique pathogens, very different from other parasitic microorganisms. Viruses is small obligated intracellular parasites, that means they are capable of reproducing themselves inside the cells of a host. Viruses consist of a nucleic acid and coat of protein, the nuclear acid is where the genetic code is, this is available as DNA or RNA. Most of the virus contains RNA. Some virus also has outer membrane of carbohydrates and/or lipids (Agrios, 2011).

Viruses differs from other microorganisms since as they have no metabolism. Most microorganisms can be grown *in vitro*, that means on agar in a petri dish on a cell free substrate. However, viruses get inactive outside living cells, and are therefore only able to multiple within living cells (Bos, 1999).

Plant virus name are based on the host plant and the symptoms that the virus caused. One example is tobacco mosaic virus, this virus was first described in tobacco that showed symptoms of mosaic pattern in the leaves. It does not exclude that a virus can have multiple hosts or cause different symptoms. Latin terms are not used for virus nomenclature, like it does for plant, algae, fungal and animals nomenclature. The virus name is usually shorted to an acronym (Rose & Dolja, 2006). An example is tobacco mosaic virus, the acronym is TMV, and Potato leaf roll virus become PLRV. The family name *Potyviridae* is derived from the PVY.

Plant viruses are classified in the following classes according to The International Committee for Taxonomy of Viruses (ICTV, 2016)

Order (*virales*)
Family (*viridae*)
Subfamily (*virinae*)
Genus (*virus*)
Species

These criterions are important for the classification of virus:

- Nucleic acid type
  - dsDNA
  - ssDNA
  - dsRNA
  - ssRNA (-)
  - ssRNA (+)
- Virus protein
- Symptoms
- Serology
- Morphology
- How the virus is spread

2.2.1 Symptoms of virus

Symptoms can be useful for discovering infections and helpful in diagnosis of the virus. However, virus can very much look like symptoms caused by abiotic stress such as draught, cold, toxins, pesticides, and nutrient deficiency. Therefore, symptoms are often not good enough for drawing a conclusion on the virus diagnostic. However, it can help you get an idea of what type of virus you may have present in the plant. The virus is transported in the phloem following the source-to-sink for photo assimilates. This is important to understand how the virus is moved around in the plant. Sink and source is explained on which plant organ that are importing or exporting photosynthetic products. Sink is where it is imported, like roots, tubers, developing fruits and immature leaves. Source is an exporting organ that can produce photosynthetic products, made in leaves (Taiz & Zeiger, 2003). Virus are often accumulated in the younger upper leaves on the plant and often were the symptoms are strongest. Local symptoms are when you can see symptoms around the place where the inoculation found place. When the virus spreads from the inoculation and causes symptoms in other parts in the plant, then it is called systemic symptoms (Bos, 1999).

However, a certain virus often make specific combinations of only a few symptoms in a specific crop. This will enable us to discern viruses just by visual inspection. Some symptoms of virus are mottle, mosaic, chlorosis, vein clearing, leaf spots, leaf rolling, leaf distortion, rugose, enation. One other critical symptom of virus is reduced yield abnormal flowers, fruits, and seed, stunting, stem pitting and necrotic browning on tubers.

2.2.2 Virus identifications

When a virus attacks a crop, it is important to know how the virus is transmitted to choose the best strategy for future control. Identification is therefore an important part of the disease control.

There are five main groups of methods for detecting viruses, this are:

- Serology (ELISA)
- Biological (test plants)
- Molecular (PCR)
- Electron microscopy
- Symptoms (eyes)

One of the most widely used diagnostic test that is used for testing plant virus is Enzyme-Linked Immunosorbent Assay (ELISA) (Regenmortel & Mahy, 2009). This test was also used under the work for this thesis and is explained more in the methods chapter. When it comes to identification of virus in plants, the ELISA test is the most widely used immunological technique (Rose & Dolja, 2006).

2.2.3 How to identify or detect plant viruses

There are two ways to identify or detect plant virus. These are diagnosis and detection. Diagnosis is, how to find out which virus might be present. Detection is the methods to use for testing if a certain virus is present or not.

The picture in *Figure 1* is showing the two ways and how it could lead to a conclusion. Diagnosis is shown above the line, detection is shown under the line

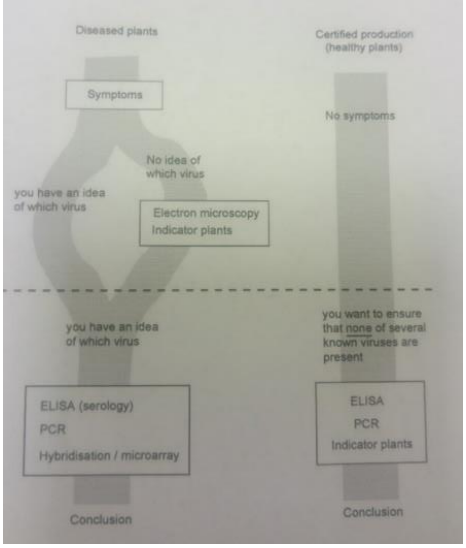

Figure 1 Lund, 2004

2.3. Epidemiology and economy

2.3.1 Epidemiology

Epidemiology is the study about development and spread of diseases and factors that influence this spread (Campbell & Madden, 1990). An epidemic is the increase of diseases in time and space. There are two concept that are important to understand, and they are the disease triangle and tetrahedron.

The disease triangle was first drawn in 1960 (Stevens, 1960), but the theory was described already, by Duggar in 1909: ". . . *the abundance of a very large number of fungous diseases is directly connected with or conditioned by climatological factors . . . factors may affect independently host and parasite, and they may affect the interrelations of these organisms.*" (Duggar, 1909). The triangle explains the interaction between plant, pathogen, and environment (Francl, 2001). Over time plant pathologists added one more factor, time. Then they came up with the tetrahedron. The tetrahedron is the triangle, but were time effects the pathogen, host and environment. Unlike in the triangle where they affect each other.

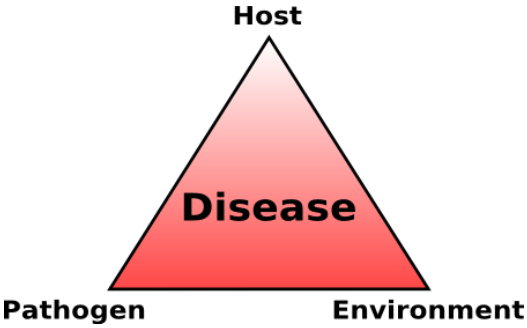

Figure 2 Illustration of the "disease triangle" Figure credit: Ed Zaborski, University of Illinois

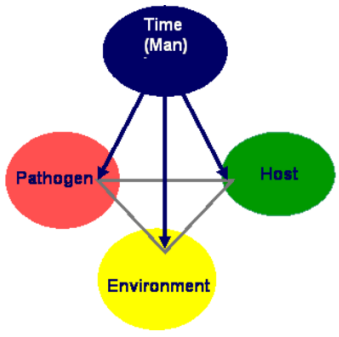

Figure 3 Illustration of the diseases tetrahedron, waternut.org

Plant viruses are often spread from plant to plant by organisms, known as vectors. These are normally insects, nematodes, and some fungi. Potato viruses can be spread on three ways that is relevant; mechanically, by insects and by organisms in soil (Munthe, 2003). When it comes to potato

virus (PVA, M, Y and PLRV) aphids is the only known vector in Norway for spreading these viruses. PVX is spread only by mechanical means. PVS can be spread both by aphids and mechanical.

Aphids transmit virus in two different ways, persistent and non-persistent.

By persistent transmission the aphids take up the plant sap trough the body and the virus has time to recycle itself (Bos, 1999). This means that a virus is taken up by a vector and the virus enter the alimentary canal, pass the gut wall, distribute in to the body fluid and enter the salivary glands. After this the vector is latent with the virus for days, months or years (Munthe, 2003). Vectors that spread virus this way, often have long feeding time on leaves before the plant get infected. The time can often be 15 minutes or more, this is because it takes time for the virus to reach the vascular parts in the plant. Viruses like this are often associated with high specificity and have very intimate biological relationship between vector and virus. The virus is often spread only by a certain type of single vector species. The potato virus that is spread this way is PLRV, and some known examples of aphids is: *Aphis nasturtii, Aulacorthum solani and Myxus persicae* (Munthe, 1996).

Non-persistent transfer means that the virus is transmitted by aphids into the plant, but the virus is not present in the aphid over long time. The inoculation time is quite short, and it is from a few seconds to minutes. In detail the aphids transmit the virus into the parenchyma, that is a metabolically active plant tissue consisting of thin wall cells, within air-filled spaces at the cell corner (Taiz & Zeiger 2010). Most likely is it the epidermis, the outer layer of the plant cell were the infection happened. For non-persistent transmission, there is no latency period, but the virus can be transmitted immediately. The virus is often lost after the first probe on a virus free plant, therefore is it called non-persistent transmitted (Radcliffe & Ragsdale, 1980). The potato viruses that is non-persistent transmitted is PVA, PVS, PVM and PVY.

Pathogens spreads through vector insects represents a complexity that is hard to explain by the classic diseases triangle. It is hardly possible to give advice to farmers on how to protect potato plants against virus, that is spread through vectors. It is a topic that include susceptible host, viable inoculum, and favorable environment (Ragsdale et al. 1994). Aphids are among the most important pests, especially true for potato in temperate agricultural zones (Minks & Harrewijn, 1987).

2.3.2 Economy

Most virus infection causes yield reductions. The level of reduction is highly variable dependent on the virus strain, time of infection and the environmental factors. Yield loss can be reduced directly due to the virus infection or by making the plant more susceptible to abiotic stress (cold, draught, nutrition deficiency). The yield reduction is 0,5% per percentage virus, this is for PVA and PVY (Aspeslåen et al., 2016).

Data for the world's yield loss due to plant viruses do not exist. The yield loss due to all plant diseases is estimated at 60 billion US $ per year worldwide. It is believed that plant viruses come after fungi that cause the highest yield loss. If the virus is acting alone there is possible to use this numbers under to estimate the yield loss. But if one or more virus is combined, it is hard to say something about how the virus will affect each other and later how much the virus will apply of damage. (Matthews, 1991).

Estimated yield loss caused by potato viruses, will vary by the strains of the virus one example is *Table 1* that is showing yield loss from different strains of PVY.

- Potato virus A up to 40 % (Wale, 2008)
- Potato virus S 20 % (Burrows & Zitter, 2005)
- Potato virus M 5-20 % (Bjørling & Lindsten 1973)
- Potato virus X 15-20 % (Wale, 2008)
- Potato virus Y 70 % (Aspeslåen, 2016)
- Potato leaf roll virus 100 % (Munthe, 1996)

Table 1 Yield loss caused by different varieties PVY (Aspeslåen, 2016)

Strain of PVY	Y^0	Y^N	Y^{NTN}
Reduction in yield	50-70%	15-30%	15-30%
Importance	Big	Big	Low

2.4. Potato viruses

The hierarchy of recognized viral taxa is therefor:

Order
Family
Sub-family
Genus
Species

2.4.1.1. Potato virus A

Potato virus A (PVA) is ssRNA virus with filamentous particles, normal length c. 730nm, in the genus *Potyvirus*. The only known host are in the Solanaceae (Bartels. R, 1971). PVA is transmitted by aphids in a non-persistent manner and experimentally by sap-inoculation. Sap-transmission without abrasive is usually difficult because of low virus concentration. PVA is widely distributed were potato is grown. It is hard to see symptoms caused by PVA in test plants, therefor electron microscopy is often used. The most used method for detect PVA is serology and ELISA, PVA is also winter-tested in Norway along with PVY.

Unassigned
Potyviridae
Unassigned
Potyvirus
PVA

Host range, symptoms and main diseases

Leaves of infected potatoes may show a mild mosaic, roughness of the surface, waviness of the leaf margin, or no symptoms at all, depending on the variety and on the weather. Some hypersensitive varieties develop top necrosis. Potatoes infected with PVA in combination with PVX and PVY show crinkle symptoms.

PVA is transmissible by aphids, and some of them are; Aphis frangulae, A. nasturtii and Myzus persicae (Klingen et al. 2012).

2.4.1.2. Potato virus M

Potato virus M (PVM) is a ssRNA virus with straight to slightly curved filamentous particles c. 650x12nm, in the genus *Carlavirus.* It is sap-transmissible by a few species of aphids in the non-persistent manner (Wetter, 1972). PVM is a virus distributed worldwide. Most potato varieties infected with PVM also contain PVS. In mixed infections, symptom expression in potato depends on the virulence of the PVM isolate (Howard & Wainwright, 1960).

Tymovirales
Betaflexiviridae
Quinvirinae
Carlavirus
PVM

Host range, symptoms and main diseases

PVM can be separated by inoculation to tomato which is immune to potato virus S. The potato variety Saco from USA and the Varetta from Germany is cultivars which is highly resistant to PVS and PVX, can also be used for separation of PVM (Loebenstein & Carr, 2007). PVM causes various symptoms like mottle, mosaic, crinkling and rolling symptoms in leaves, and stunting of shoots symptoms range from very slight to severe that's depending on virus strain and potato variety. PVM is transmissible by aphids *Myzus persicae*, *Aphis frangulae, A. nasturtii,* and *Macrosiphum euphorbia* (Klingen et al. 2012). PVM present in the cultivar King Edward is not transmitted by aphids (Kasanis, 1960).

2.4.1.3. Potato virus S

Potato virus S (PVS) is a ssRNA virus with straight to slightly curved filamentous particles c. 650 x 12 nm, in the genus *Carlavirus.* It is sap-transmissible to a limited range of species (Wetter, 1971). Some isolates are aphid-transmissible. World-wide distribution. PVS causes few or no symptoms and is carried by many cultivated potato varieties (Burrows & Zitter, 2005).

Tymovirales
Betaflexiviridae
Quinvirinae
Carlavirus
PVS

Host range, symptoms and main diseases

Often there is no symptoms of PVS, but some symptoms can be found on early season cultivars. Serological tests are the best way of diagnosing the virus, since there are few symptoms. The symptoms may be amplified with other viruses like some strains of PVA and PVX. Symptoms that PVS causes could be mild mottling, bronzing, necrotic spots on leaves and slight deeding of the veins.

PVS is transmitted by aphids in a non-persistent manner by *Acyrthosiphon pisum, Aphis nasturtii* and *Myzus persicae* (Klingen et al. 2012). But by mechanically transmissible and though tubers. Plants seems to be resistant later in the season against PVS (Burrows & Zitter, 2005). It is important to prevent mechanical spread within the fields by sanitizing the tools and movement on the field.

2.4.1.4. Potato virus X

Potato virus X (PVX) is a ssRNA virus with filamentous particles ca. 513 x 13nm in the genus *Potexvirus*. PVX infect species in the *Solanaceae* and its sap transmittable, mainly by mechanical contact. (Kerlan & Lesemann, 1989)

Tymovirales
Alphaflexiviridae
Unassigned
Potexvirus
PVX

Host range, symptoms and main diseases

The symptoms of PVX is chlorosis, stunting, decreased leaf size, mosaic, and necrotic lesion tubers in combination with PVY (Burrows & Zitter, 2005). Often there is no symptoms from PVX alone. The symptoms increase with coinfection with other viruses like PVA and PVY, this can cause more severe symptoms and yield loss than either virus alone. The source of this virus is infected tubers. Certified seed-potato is the most important way to control PVX, but also important measures to avoid mechanical spread to neighbor plants, and the movement to new fields. Spread occurs using tools and machinery. Disinfect all tools, rogue infected plants, and limit within-field movement.

2.4.1.5. Potato virus Y

Potato virus Y (PVY) is a ssRNA virus in the genus *Potyvirus*, one of six in the family *Potyviridae*. PVY is aphid transmitted in the non-persistent manner by *Myzus persicae* that is the most important vector. It is worldwide distributed and is one of the most economical important plant pathogens. PVY is responsible for diseases in many crops, most of them belonging to the Solanaceae and some flowers. There are many strains of PVY and some of them is PVY^0, PVY^N and PVY^{NTN}, were PVY^0 and PVY^N is the most common strains in potato (Blystad & Munthe, 1997). In potato crops, the PVY^O strain occurs worldwide.

Unassigned
Potyviridae
Unassigned
Potyvirus
PVY

Host range, symptoms and main diseases

In potato, PVY causes a severe disease called mosaic or rugose mosaic. Symptoms are variable depending on viral strain, host cultivar, climatic conditions, and whether it is a primary infection (inoculation by aphid vectors) or secondary infection (when mother tuber is infected) (Wale et al., 2008). Symptoms for PVY^O could be yellowing and later necrotic leaves for the primary infection from aphids. The secondary symptoms of PVY^O could be mosaic, but also includes collapse and leaf drop, which remain clinging to the stem. Secondary infected plants often also have a dwarfed growth.

PVY^N have many of the same symptoms that PVY^O has, but not that strong. When it comes to dwarfed growth PVY^O and PVY^N are equal (Blystad & Munthe, 1997). Symptoms like foliage and growth reduction are more severe when PVY occurs in combination with other viruses, especially PVX and PVA

2.4.1.6. Potato virus leaf roll

Potato leaf roll virus (PLRV) is a virus with ssRNA in the genus *Luteovirus* that containing isometric particles c. 24nm (Munthe, 1996). PLRV is mainly limited to species in the *Solanaceae* and then potato, but also some species of tomato (Harrison, 1984). PLRV is today eradicated in Norway since around the 1950, and have not been detected the last 20 years (Munthe, 1994). PLVR is the most economical and devastating virus in potato in most of the areas were potato is grown (Burrows & Zitter, 2005).

Mononegavirales
Unassigned
Unassigned
Luteovirus
PLRV

Host range, symptoms and main diseases

Symptoms of primary infections is on younger leaves which are yellow, this can usually be seen at the top of the plant. Other symptoms are leaves rolling. Secondary symptoms are smaller plants, older leaves are rolled and could also be chlorotic (Munthe, 1996). PLVR is transmitted on a persistent manner by aphids (explained in more details in chapter 2.3.1).

Test plants are not good to use for testing for PLRV since it not transmittable with sap but by aphids or by grafting.

2.5. Production of certified Seed-potato in Norway

From 1939 Norway, have had their own state-controlled seed-potato production. The goal has been to reduce the diseases and increase the quality of potatoes. Through this control, the level of virus in seed-potatoes is low. Since potato is a vegetative propagated crop it demands much work to get it diseases free, and sometimes very difficult. It is important to have a disease-free mother plant from the start. If vegetative propagation is compared with other methods, we can see that virus and other viroid's and phytoplasma is problematic. Virus infect through cells and phloem, this means that if the mother plant is infected it would affect all tubers, seedlings, or plant from tissue culture. It is important to work on the nuclear stock program in Norway to secure crops with good quality. It is important in the future that the methods for preparing fresh material is continued so there can be established, produced, and maintained healthy virus free seed potatoes of new potato cultivars.

Tabell 2 Table Various pests spread by vegetative propagation compared with seed propagation (Blasted & Munthe, 2016)

Pest	Vegetavitve propagation	Seeds
Virus	+++++	+
Viroid's	+++++	+
Bacteria	++	+
Phytoplasma	+++++	-
Fungi	+/+++*	+++
Insects	+	-
Nematods	+/+++*	-

2.5.1 Certified seed-potato production

The nuclear stock program for potatoes demands collaboration between many different actors. In the table below the different partners and institutions and the roles they have in the seed potato production are briefly mentioned.

Tabell 3 Nuclear stock program for potato in Norway

Class	Year	Main certification step	Partner/ institution
Treatment or quarantine	Testing and propagation of tissue culture	Tissue culture bank	NIBIO
P1	Mini tubers in greenhouses	Prebasis	Overhalla
P2	1 year, outdoor production		Overhalla
P3	2 year		Overhalla (farmers)
P4	3 year		Overhalla (farmers)
B1	4 year	Basis	Seed potato companies /farmers
B2	5 year		Seed potato companies/ farmers
B3	6 year		Seed potato companies/ farmers
C	7 year	Certified	Seed potato companies / Farmer

NIBIO plant health

For many years, NIBIO plant health has worked for diagnostics and production of diseases free plants in order to fight plant pest in vegetative propagation. It is very important to have the best plants available for a good quality of the crop. NIBIO have the overall responsibility to maintain the Potato gene bank, that contain all the old cultivars and the breeding lines from Graminor. These plants are kept virus free at the Potato gene bank. NIBIO has been an active actor in the work to produce healthy seed potatoes. Its NIBIO's official role to grow and test potato tissue culture plants for quarantine diseases that are imported from other countries for future production in Norway.

NIBIOS main tasks in the production of certified plants:

- Guidance and testing for diseases on potato
- Quarantine for imported potato plants
- Potato gene bank

- Development and establishment of certified production of plant material that are vegetative propagated.
    - International projects
    - Maintain the work on seed-potato and improvement on the system
- Development of new diagnostics methods

Graminor and Overhalla

Graminor have the responsibility for breeding of new cultivars of potato and horticultural crops in Norway. They work for higher accessibility and variations in diseases free material that is custom-made for using in Norway. Graminor is responsible for all processing and production of prebasis seed potatoes P1-3 to be used in Norway, but this work is done at Overhalla klonavlsenter AS

Graminor are breeding for:

- Better qualities such as: Storage capacity, Short growth time and high crop yield.
- Higher resistance against major potato diseases like blight and cancer
- Quality for the consumers like: Cooking quality, shape, color, texture, size and more
- The industry requires a certain level of starch and solids in the potato

Overhalla Klonavlsenter AS

Overhalla is the only authorized prebasis center for seed potato in Norway, and the authorization is given by The Norwegian food safety authority. Overhalla is producing the first two generations of prebasis P1 and P2. P3 and P4 can also be produced at Overhalla and sold direct to other seed potato growers.

The production agreement of P1 and P2 from Graminor is:

- Propagation of P1 is in laboratory and minitubers in greenhouses
- Disease test of all P1 and P2
- Propagation of P2 on their own fields in Overhalla
- Maintenance and storage of potato cultivars used in the production
- Follow the seed potato market, and try to serve it as well as possible.

The Norwegian food safety authority

The Norwegian food safety authority (Mattilsynet) is the supreme authority when it comes to seed-production in Norway. They manage regulations and control productions, and they have responsibility for knowledge and advises to LMD (Ministry of Agriculture and Food) about import, production, risk-taking and quarantine assessment.

2.5.2 Seed-potato law in Norway.

Norway and most of the countries in Europe got their own national program for controlling pest that is a problem in vegetative propagation. In Norway is this what we call nuclear stock program. The basis for this is set in the law "*Lov om matproduksjon og matvaretrygghet mv. (matloven*)". The details regarding certified production of potato are described in the regulation "*Forskrift om settepoteter*". Law for seed-potato production also includes regulations concerning grants for certified seed-potato. (Legal LOV-1995-05-12-23-§18) It is forbidden to import potatoes for further propagation in Norway. All potatoes must be imported as tissue-culture for quarantine growth, before it is sent for propagation and further testing.

This is the most important

- Import of seed potatoes may only be authorized by the Mattilsynet.
- There must not be cultivated potatoes on areas for seed potatoes cultivation in the three preceding years.
- There are regulations about distance. (Prebasis (P1-3) 1.5m from other prebasis areas, and 25m away from Basis(B1-3). Prebasis must be grown at least 100m away from uncontrolled cultivation of potatoes.)
- One public growth control each season.
- All classes are tested for infection levels of PVA and PVY in the winter test

It is important to mention that there is performed test for other important diseases like Potato cyst nematode (PCN) each year on fields used for seed potato production. This has nothing to do with virus, but may have a huge impact on production of seed potatoes.

3. Material and methods

3.1. Introduction

This study has been conducted from May to December 2016. A work plan was made in March about how the samples should be collected during field work and other details on sampling. This was done to secure a representative coverage of the potatoes in the field. The samples were collected from three different potato cultivars, Saturna, Asterix and Innovator. The sampling was done on 15 farms in Vestfold. Prior to the sampling, an email was sent to all the farmers. The samples were collected between 21st and 25st of June. The samples were stored cold or frozen all the time during the fieldwork and transportation. The ELISA testing was done between June 28th and August 15th at the laboratory at The Norwegian Institute of Bioeconomy Research (NIBIO).

Choosing the farms and the potatoes cultivars
Vestfold was chosen as a good location for doing fieldwork, because of the importance of Vestfold for potato production, the relative closeness to NMBU and because the good relationship to the agricultural extension service (Norsk Landbruksrådgivning, NLR) in Vestfold. It was important to get the help from NLR to choose relevant farms. It was decided to collect samples from five farms for each cultivar. Samples were collected from a total of 15 farms, five for, each cultivar: Asterix, Saturna and Innovator.

3.2. Choosing the methods

3.2.1. Statistics

To estimate the proportion, *p*, of infected plants in a field I used the estimator. This is explained in more detail in *attachment 3.*

$$\hat{p} = 1 - \left(1 - \frac{Y}{m}\right)^{\frac{1}{n}}$$

where *m* is the number of samples, each consisting of *n* plants. *Y* is the number of samples infected among the *m*. A sample is infected if at least one of its *n* plants are infected. I use *m* = 90 and *n* = 5.

In addition to calculate the estimator $\hat{p}$, I calculated the approximate confidence interval for *p*, with confidence coefficient 1 – α, given by

$$\left[\hat{p} - z_{\alpha/2} \cdot \frac{\sqrt{Y \cdot \left(1 - \frac{Y}{m}\right)^{\frac{2}{n}-1}}}{n \cdot m}, \hat{p} + z_{\alpha/2} \cdot \frac{\sqrt{Y \cdot \left(1 - \frac{Y}{m}\right)^{\frac{2}{n}-1}}}{n \cdot m}\right]$$

I used α = 0.05, and then $z_{\alpha/2} = z_{0.025} \approx 1.96$.

3.2.2. Elisa

Why chose ELISA

ELISA was chosen because it is reliable and effective established this test for detection of plant virus (Clark & Adams, 1977). Today ELISA is an essential diagnostic method for potato plants and certification of seed potatoes. Even if the PCR is a newer method, ELISA still has a place in routine testing of large-scale for detection of potatoes viruses like A, M, S, X, Y and LR. ELISA works so good because it is an efficient and sensitive method (Volker et al., 2011). When using the ELISA, two antigen-specific antibodies are applied. The first antibodies will attach to the well on the microtiter plate. If the material is contained with the specific virus, the virus will bind to the antibody. The second virus-specific antibody will bind the virus if an antigen was captured. In the end of ELISA there is added a chemical reaction. If the test is positive, the colour will change to yellow.

3.3. Preparation of the work

To prepare myself for the work, I had training in doing ELISA-testing, statistical calculations, and coordination for the fieldwork including sampling, interviews and labeling of the samples.

The ELISA training was done at the lab of NIBIO in April 2016 using infected potato samples harvested in the greenhouses of NIBIO. A work plan for ELISA was set up *attachment 2*.

To secure a representative, randomized sampling, I made a pattern to follow during the sampling. The idea was to collect five leaves, one leaf from each plant and then go up one row, some steps to the right and collect five leaves again. With this method, I would be able to cover a large area of the fields.

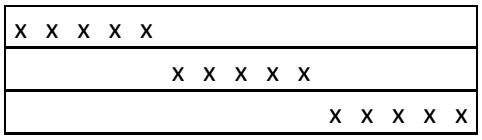

Figure 4 Collection pattern

I marked the ELISA bags with a farm-number 1-15 and sample-number 1-90. These 90 ELISA bags were put in a plastic bag ready for use. The contact information on the farmers, was given NLR, and every farmer were called two weeks before the fieldwork started. When calling the 15 farmers I found out how the driving schedule should be, and were then able, to make a plan for collecting all the samples effectively. I also made a paper with questions for the farmers, that I visited (Attachment 1).

Making the interviews for the farmers. It was important to get as much information as possible from the farmers, about the potato production. The interviews were important for evaluation of the results later. (Attachment)

3.4. Fieldwork

The fieldwork was done in the county Vestfold, between 21st and24th of June. Under my fieldwork, I had access to a private house, and had a – 20 °C freezer where I could store the samples during the period of fieldwork. I also had a cooler and freezing element for keeping the leaf samples cold during transport. I used a small shoulder cooler with two elements, that was possible to carry in the field. All samples were stored cooled or frozen since the minute they were collected. When arriving at a specific farm the interview with the farmer started and the location of the field was inspected. Then the sampling pattern was followed (Figure 4). The 90 ELISA bags were put into a plastic bag and into the cooler. This procedure was followed on every farm. When the work was finished for the day, all the samples were put in the freezer to stay frozen. After all the samples were collected they were transported directly to a -20 °C freezer at NIBIO. The samples were kept frozen all the time under the work, except when they were analyzed in the laboratory. After the laboratory work the samples were returned to the freezer, so they could be re-analyzed if needed.

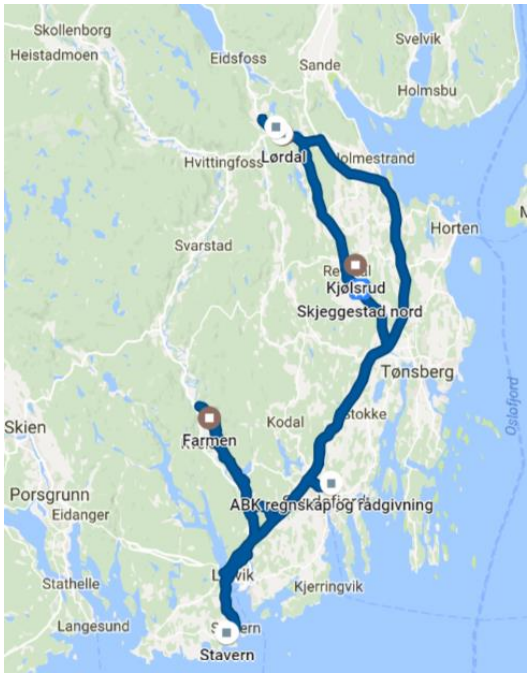

Figure 5 Map of were the samples was collect in Vestfold

3.5. Laboratory work

The work was done from June to August 2016. When following the ELISA protocol, one test normally took four days as described below:

1. Coating:

One ELISA-plate (96 wells microtiter plate) was coated with 100 µl solutions of 1:1000 µl IgG with coating buffer of each potato virus (A, M, S, X, Y and LR). The plate was added two negative control and two/four positive control well. Each plate belonging to specific virus was added the IgG solution. After this the plate had to stay cold and was coved in a plastic bag and placed at +4 °C overnight.

2. Adding the plant material:

The plate was washed in the ELISA washing machine, 3x3 times. Then, the plate was added 100 µl of plant material from the ELISA bag in each of the well on the plate. After this the plate was put into a plastic bag and put at +4 °C overnight for incubation.

3. Conjugated:

The plate was washed 3 x 3 times and IgG-conjugate, diluted 1:1000, was added to each well. One conjugate for each virus (A,M,S,X,Y and LR) was diluted in sample buffer. After this the plate was put into a plastic bag and put at +4 °C overnight for incubation.

4. Adding substrate:

This step started by measuring the reaction buffer with a pill, one pill for 40lm buffer. I normally made 200ml for covering 18 plates (3 virus series). The buffer was putted in a lightproof closet, since the solution react in light. I had to wait 15-20 minutes for the pill to get dissolved. When the tablet was dissolved, the plates was washed 3 x 3 and 100µl of the solution were added to each well. The incubation of the plate was in room temperature in dark, for around 30-120 minutes to see a color changing. After the control-well was starting to get yellow the plates were scanned. This was done by ELISA scanner (Spectra max 190).

Figure 6 Work at the laboratory. (Mong, 2016)

3.6. Materials

3.6.1. Material for fieldwork

- 15x 90 ELISA bags
- Plastic-foot cover
- Cooler with freezing elements
- Small cooler for carrying in the field that could hold 90 ELISA bags
- Driving schedule

3.6.2. Material for laboratory work

- Virus laboratory at NIBIO
- Pipettes
- ELISA plates 6x15
- Buffers
- Substrate buffer and tablets (4-Nitrophenyl phosphate disodium salt hexahydrate, SIGMA) Light sensitive and store at -20 degrees
- ELISA wash-machine (Skatron Skran washer 400)
- ELISA homogenizer (Homex 5)
- ELISA scanner (Spectra max 190)
- Software on the computer (SoftMax Pro 6)
- ELISA protocol
- Control samples for the viruses were provided from NIBIO
- ELISA plates (microtitre plates The ELISA was performed in 96-well microtitre plates with medium binding capacity (Art. No. 655080, Greiner Bio-One GmbH, Maybachstr. 2, D-72636 Frickenhausen, Germany). All lots of microtitre plates are certified)
- Potato virus kit (ELISA reagent for laboratory use, Bioreba), this contain conjugate and coating for the six potato viruses A, M, S, X, Y and PLRV (Table 5 and 6).

Tabell 4 Conjugate

Antigen	Conjugate
Potato virus A	PVA IgG conjugated w/AP, Bioreba –Nr. 270627
Potato virus M	PVM IgG conjugated w/AP, Bioreba-Nr. 221342
Potato virus S	PVS IgG conjugated w/AP, Bioreba- Nr. 120650
Potato virus X	PVX IgG conjugated w/AP, Bioreba-Nr. 130664
Potato virus Y	PVY IgG, (mono cock) conjugated w/AP, Bioreba -Nr. 211279
Potato virus LR	PLRV IgG conjugated w/AP, Bioreba -Nr. 080570 (1ml)

Tabell 5 Coating

Antigen	Coating
Potato virus A	IgG, Bioreba- Nr. 260627 (1ml)
Potato virus M	IgG, Bioreba- Nr. 211342 (1ml)
Potato virus S	IgG, Bioreba- Nr. 110650 (1ml)
Potato virus X	IgG, Bioreba- Nr. 120664 (1ml)
Potato virus Y	IgG, Bioreba- Nr. 201279 (1ml) mono cock
Potato virus LR	IgG, Bioreba- Nr. 070570 (1ml)

Buffer recipes:

- Washing buffer; (pH 7.4; for 1000 ml) 3L in distilled water,240g NaCl, 6g KH_2PO_4, 34.5g Na_2HPO_4, 6g KCl and15g Tween 20
- Sample buffer; pH 7.4 Buffer: 8 g NaCl, 0.2 g KH_2PO_4, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g KCl, 0.2 g sodium azide, 20 g polyvinyl pyrrolidone, 0.5 g Tween, made up to 1000 mL with distilled water.
- Substrate buffer; 800 mL distilled water, 97 mL diethanolamine, 0.1 g magnesium chloride, 0.2g sodium azide, made up to 1000 mL with distilled water.
- Coating buffer; pH 9.6 1.59 g Na_2CO_3, 2.93 g $NaHCO_3$, 0.2 g sodium azide, ingredients made up to 1000 mL with distilled water.

3.7. Reliability and validation

The sampling was discussed with the statistician at NIBIO before the field work started. We decide to collect 90 x 5 samples in total of 450 plants form each farm, and all together on 15 different farms. Then it would get easy to do the laboratory work and the analysis after. A statistical formula was made so it was possible to collect 5 leaf samples and later calculate it to say something of the percentage of virus in the specific field. We chose this amount of plants also because of the space on ELISA plates. 1 ELISA plate has 96 spaces, and since we used 2 spaces for positive control and 2 for blank, there was 92 places left.

Fieldwork

The fieldwork was selected to be done in June, because of the germination of the potatoes. It was important to have small plants, just the upper leaves, since these that are the leaves I was going to collect. With help from NLR, Vestfold, we decide the dates for the fieldwork when the plant had the approximately right size.

3.8. Error sources

Extra testing

Some of the test turned out wrong and had to been redone, especially some of the PLRV samples, since this is a quarantine virus. From 5-9 September I was testing 7 PLRV samples that was found to be positive under the first ELISA. This was tested again just to be sure that it was negative. All the test turn out to be negative. There was also some test on some PVY and PVA, were the control was not working, this was done again just to be sure of the results.

4. Results

The results are showing the estimates of the proportion of each virus that was found under the ELISA-testing, for each field. The estimates were multiplied by 100, and therefore expressed in percent. In addition, I calculated approximate 95 % confidence intervals for the proportions, also given in percent. The estimates and confidence intervals are shown in the graphs for each virus and cultivar.

Field number	Cultivar	A%	M%	S%	X%	Y%	LR%	Age of seed-potatoes
1	Asterix	1,1	0,2	19,2	0,0	0,0	0,0	3
2	Asterix	0,0	0,0	2,1	0,0	0,0	0,0	1
3	Asterix	0,0	0,0	43,9	0,0	0,0	0,0	0
8	Asterix	0,7	0,0	15,4	0,2	0,0	0,0	2
13	Asterix	0,0	0,0	3,3	0,2	0,0	0,0	1
4	Innovator	1,8	0,0	13,3	0,0	0,2	0,0	1
9	Innovator	1,1	0,0	6,0	0,2	0,2	0,0	1
10	Innovator	0,0	0,4	26,7	0,2	0,2	0,0	1
11	Innovator	0,0	0,2	16,7	0,2	0,0	0,0	1
6	Innovator	1,4	0,0	23,9	0,0	0,0	0,0	2
14	Saturna	0,7	0,0	0,4	0,0	0,0	0,0	1
15	Saturna	0,0	0,0	0,4	0,2	0,0	0,0	1
12	Saturna	0,0	0,0	11,1	0,0	0,0	0,0	1
7	Saturna	0,0	0,0	1,1	0,0	1,6	0,0	1
5	Saturna	0,0	0,0	0,9	0,0	0,0	0,0	2

Tabell 6 Results from virus testing of leaf samples from 15 potato fields in county Vestfold.

4.1 Results PVY

Only two cultivars were found to be infected by PVY, those are Innovator and Saturna, not Asterix. The highest infection found was 1,6 % in Innovator. PVY was found in altogether 6 out of the 15 investigated fields.

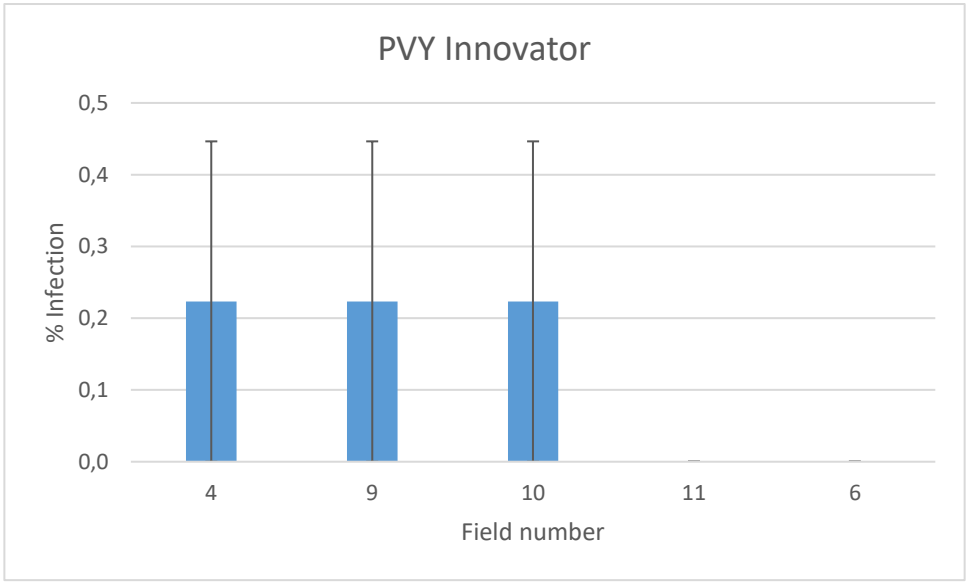

Figure 7 Results of PVY in Innovator

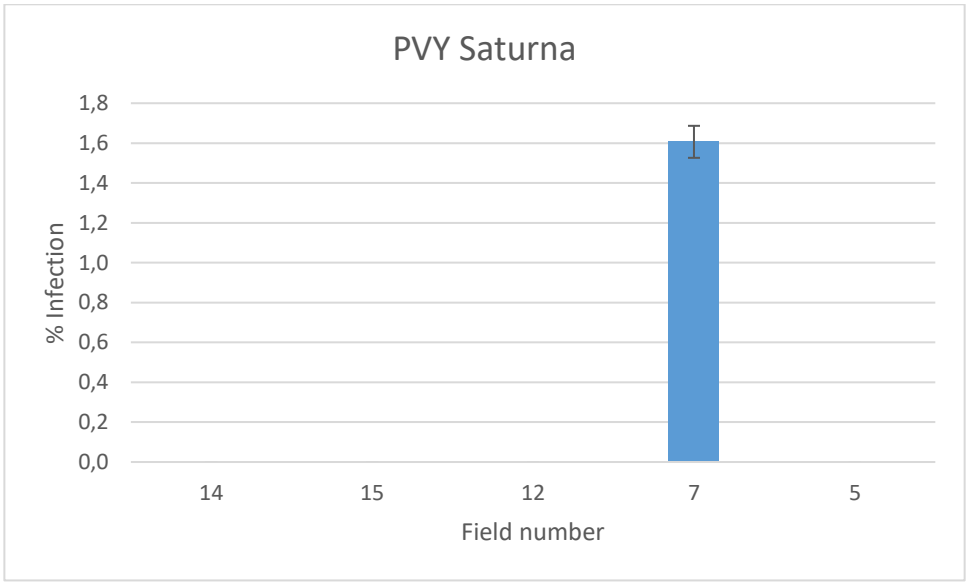

Figure 8 Results of PVY in Saturna

4.1. Results PVA

Each of the cultivars were found to be infected by PVA. The highest infection was 1,8 % in Innovator. PVA was found in altogether 6 of 15 investigated fields.

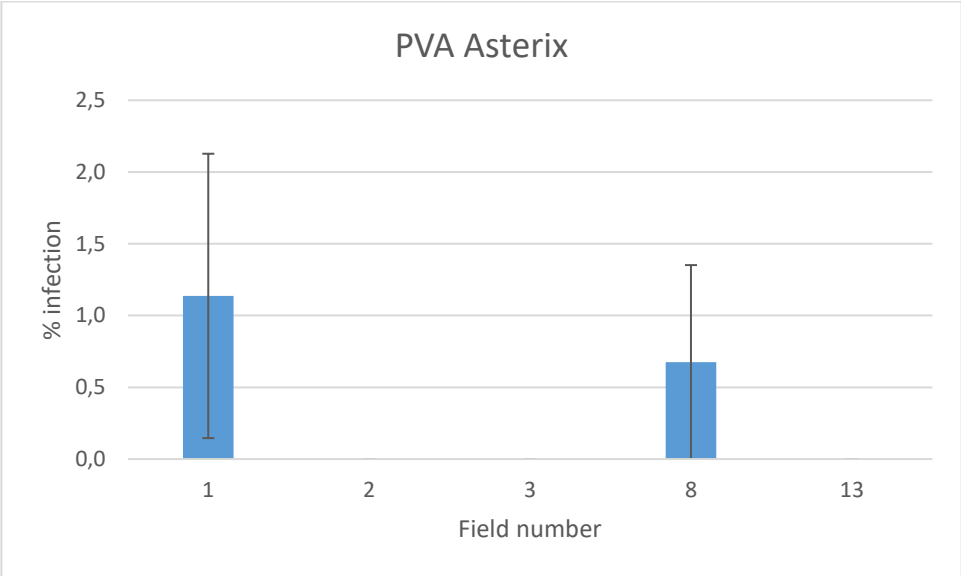

Figure 9 Results of PVA in Asterix

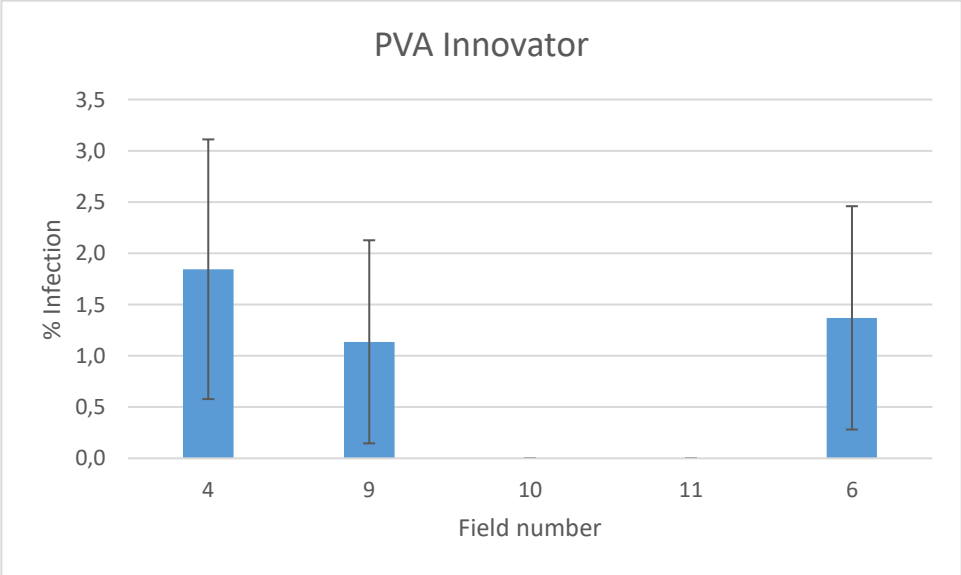

Figure 10 Results of PVA in Asterix

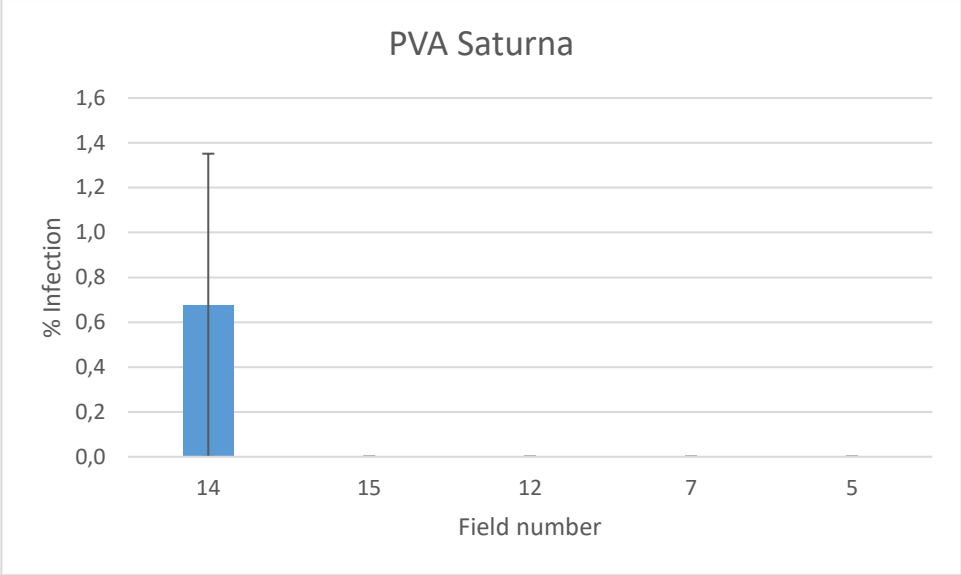

Figure 11 Results of PVA in Saturna

4.2. Results PVS

Each of the cultivars were found to be infected by PVS. The highest infection was 43,9 % in Asterix. PVS was found in altogether 15 of 15 investigated fields.

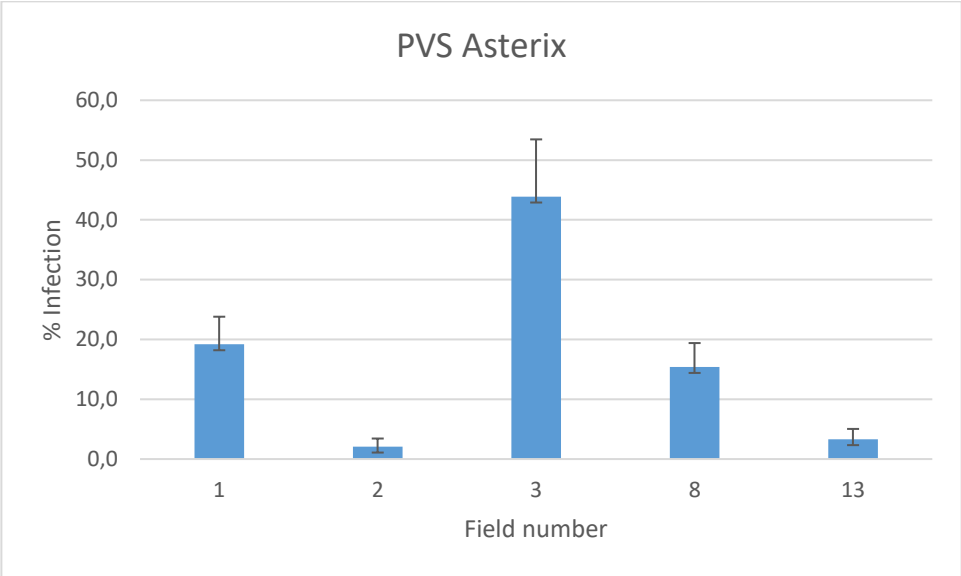

Figure 13 Results of PVA in Asterix

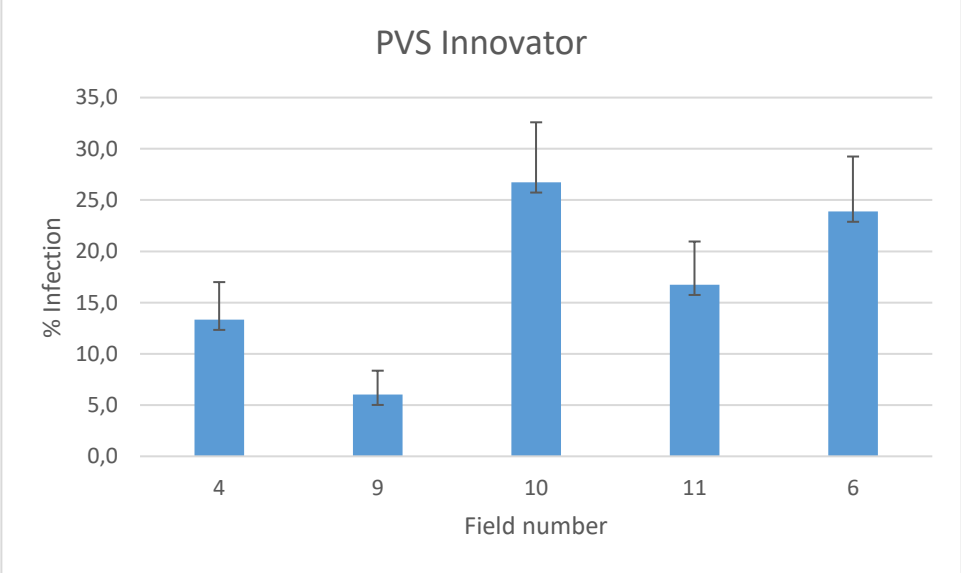

Figure 14 Results of PVA in Innovator

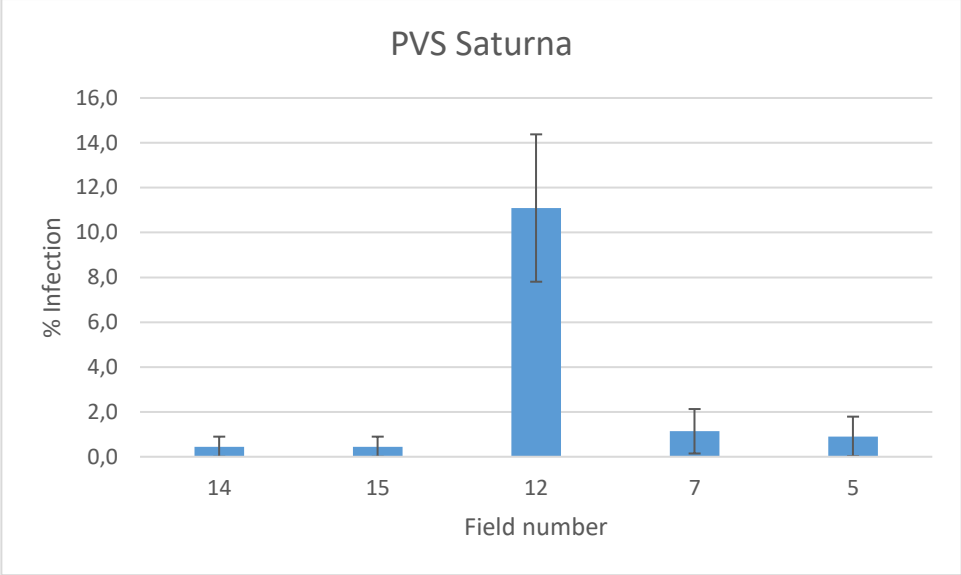

Figure 15 Results of PVS in Saturna

4.4 Results PVM

Only two cultivars were found to be infected by PVM, those are Asterix and Innovator not Saturna. The highest infection found was 0,4 % in Innovator. PVM was found in altogether 3 out of 15 investigated fields.

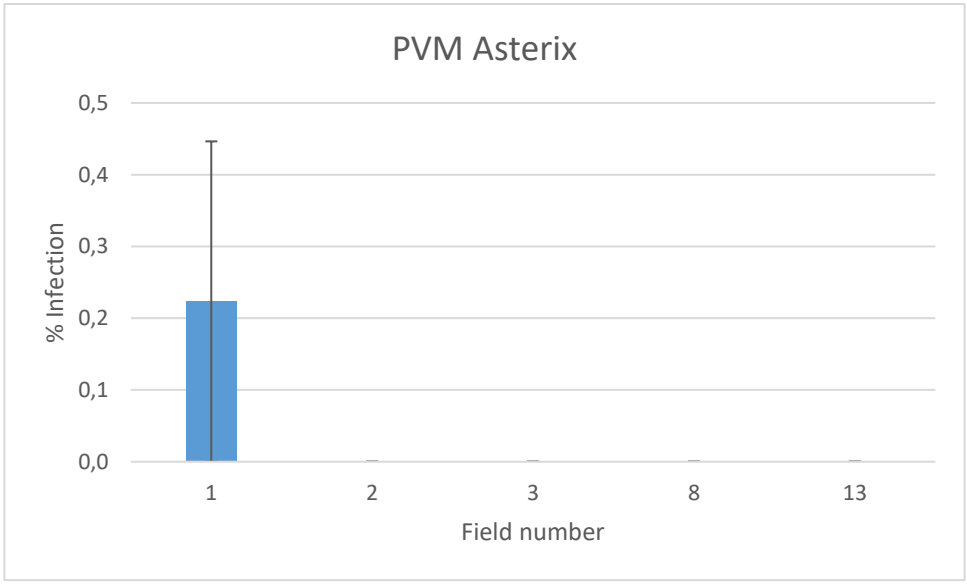

Figure 16 Results of PVM in Asterix

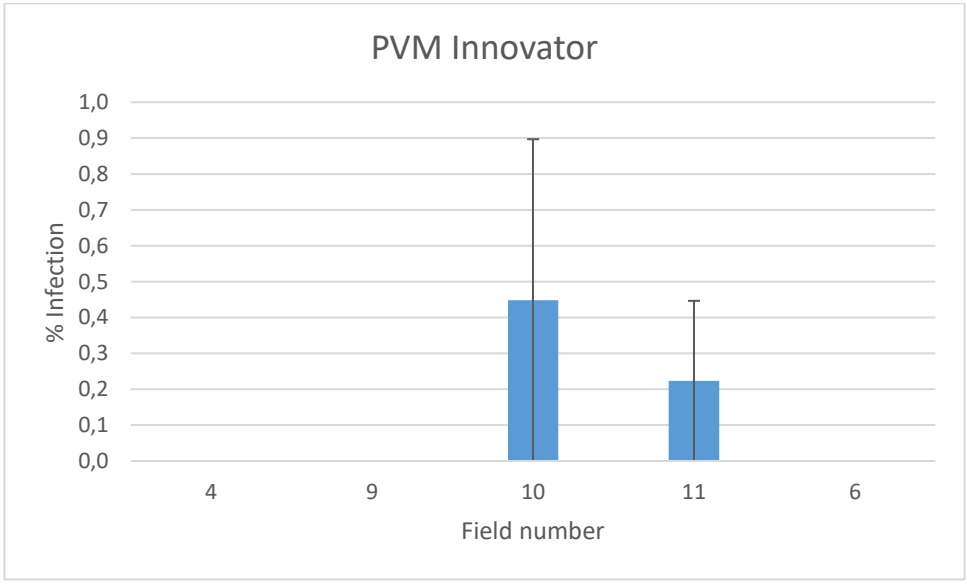

Figure 17 Results of PVS in Innovator

4.5 Results PVX

Each of the cultivars were found to be infected by PVX. The highest infection was 0,2 % in all three cultivars. PVX was found in altogether 6 of 15 investigated fields.

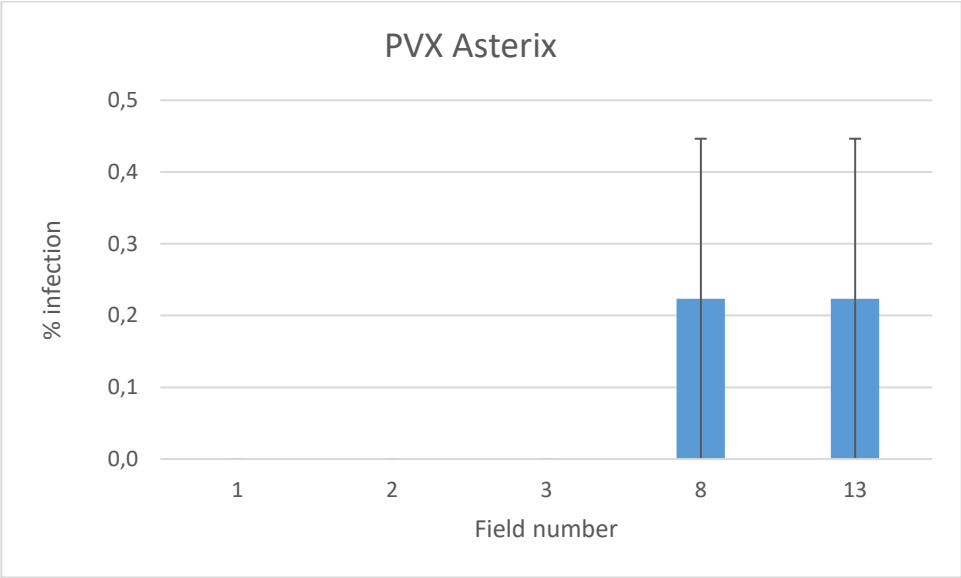

Figure 18 Results of PVX in Asterix

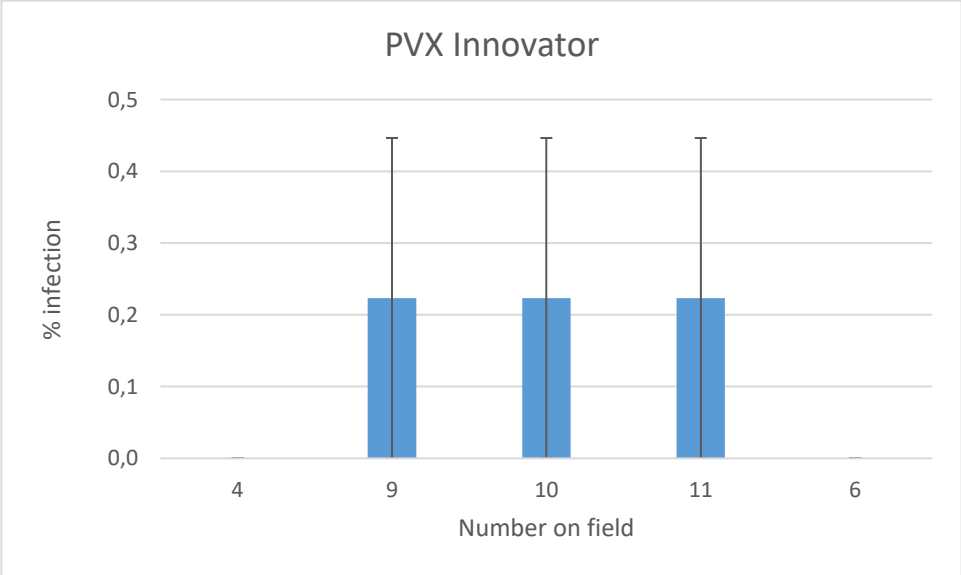

Figure 19 Results of PVX in Innovator

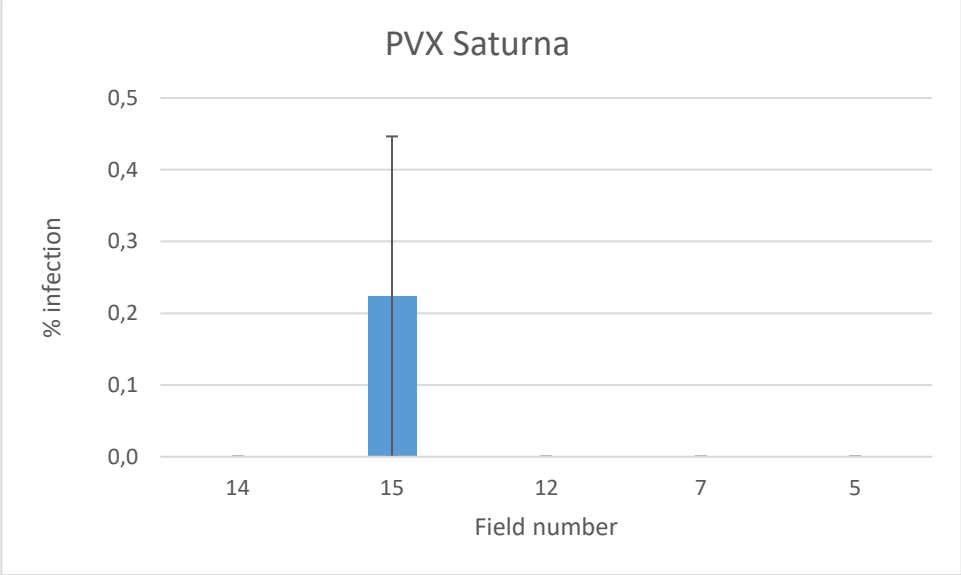

Figure 20 Results of PVX in Saturna

4.6 Results PLRV

Non of the cultivars were found to be infectd by PLRV.

5 Discussion

I have made a survey of virus infection in seed potatoes used by farmers in county Vestfold. In short, the results showed very little virus infection in the seed potatoes, except for PVS. On average, it was 0.15 % PVY, 0.45 % PVA, 0.06 % PVM, 12.3 % PVS, 0.09 % PVX and no PLRV.

Compared to other counties, Vestfold looks all over to have low values of PVA and PVY. A winter-test on PVA and PVY was done in 2011.This test is showing that some counties have higher values of PVY, as is the case in Hedmark and Toten. These 2011-results showed 0% PVA in Vestfold, also for PVA Hedmark had higher values than Vestfold (Steinsholt, 2012). Even if the test is four years old, it seems that my results show the same picture.

Results of PVY

In total, there was very little of PVY found. Many of the farmers used insecticides early against aphids. This in combination with young seed potatoes is probably the main reason that there were almost no PVY to be found. PVY is economical important so a high incidence would have affected the economic result. In total of 15 farms there was only found PVY at 4 of them. Winter-test of PVY from 2011 in Vestfold also showed low values of PVY. The results from that test was that there was found 0 % PVY in 4 farms, there was found 1-4 % PVY in 2 farms, 5-10 % in 3 farms and 11-20 % in 1 farm (Steinsholt, 2012). These results are not so far from mine, just a little higher.

Results of PVA

There was also found few PVA infected samples in total. Only at 6 of the 15 farm there were found PVA. PVA is also a virus that is economical important. Winter-test of PVA from 2011 in Vestfold also showed low values of PVA 0 % (Steinsholt, 2012). These results are almost identical to the results that I found.

Results of PVM

There was also found little of PVM, only at 3 farms, and the values was only 0,2 % and 0,4 %.

Since PVA and PVY is winter tested and PVM is not, I have no data to compare with.

Results of PVS

In PVS I expected high values, and the values were between 0,4-43 % so it is much higher than the rest of the viruses. PVS was also found at all 15 farms. This values can be explained with there has not been test for PVS since prebasis, and PVS is also a virus that spreads fast between the plants

Since PVA and PVY is winter tested and PVS is not, I have no data to compare with.

Results of PVX

There was also found little of PVX. PVX was found at 6 farms and the value was 0,2 % at each of them. PVX was a common potato virus in potato in Norway earlier, but now that there is control of seed-potato PVX is not that common any more. And I expect low values from PVX.

Since PVA and PVY is winter tested and PVX is not it is hard to say something about the values I got.

Results of PLRV

I did not expect to find this virus, since this virus is eradicated in Norway (Munthe, 1996). It is important to test against PLRV, just to exclude that there are no positive samples, since it's a quarantine virus.

There was not much viruses to find, PVS was the only virus that was found with infection over 1.8 %. I was surprised that the values were so low, and expected higher values. I was informed by NLR (Siri Abrahamsen, personal information) that the farmers in Vestfold was renewing their seed potatoes often. This was also confirmed by many of the farmers I visited. This may explain the results I got, since the seed potato was new or just up to 2 year in use, only 1 of 15 famers had seed potato older than 2 years.

When interviewing the farmers on "How old the seed potatoes was", I immediately realized that the question was wrong (attachment 1). I should rather have asked, from which class the seed potato came from or if the farmer had propagated the potatoes by themselves.

After going into the methods, I used, if there was something wrong, I found out that I had been very careful in the lab and it should be no doubt about the results. If I discovered that something was wrong, I just started the test again, just to be sure that it was correctly done. There was used aluminum foil to cover the samples all the time when handling the substrate buffer, this was to cover for potential light, that could affect the results. The samples were also kept in a light-prof closet all the time, this to get as clear result as possible and no infiltration from light to influence the result.

Problem with positive control in PLRV

There were some problems with the PLRV control under the ELISA tests, many of the samples had to be redone. It was ordered new control samples and many of the samples were tested again. The new test showed no positive results. 7 out of 15 farms were tested again for PLRV. The test had to be retested because of the control from PLRV I used did not work. The first time.

Problem with PVA and PVY controls

There were also some problems with some of the PVA and PVY controls. I don't know why the control didn't work, probably it was something I did wrong with the buffers, since it was only PVA and PVY from the same series, there was nothing wrong in the four other viruses PVM, PVS, PVX and PLRV. This was also a check point for me to see that the ELISA was working. But in this case, the whole ELISA plate had the same value, and therefore I had to be test it again.

Collection of samples in the field

Al the samples were collected late spring/ early summer. This period was selected because of non-or low activity of aphids. The goal of this fieldwork was to find the infection rate in the seed potatoes used by the farmers. Later in the season virus spread by aphids would maybe affected the results. The plants I selected was also few rows into the fields, this because of if there were some aphids they would probably be in the outer row. When I was asking the farmers, they were also saying there was no aphids. Virus spread by aphids within the 2016-season did not affect my results, but it was important for me to take precautions.

6 Conclusion

The results showed very little virus infection in the seed potatoes, except for PVS. On average, it was 0.15 % PVY, 0.45 % PVA, 0.06 % PVM, 12.3 % PVS, 0.09 % PVX and no PLRV. I expected 0 % PLRV and some high values from PVS. This is also what the results is showing, 0 % PLRV and 0.94-44% PVS. There were found almost nothing of PVY, PVA, PVM and PVX.

It is important to work on the nuclear stock program in Norway to secure crops with good quality. It is important in the future that the methods for preparing healthy plant material is continued so there can be established, produced, and maintained healthy virus free seed potatoes of new potato cultivars.

Control of aphid-transmitted virus in potato is complex and different tactics are required to minimize virus spread. At a minimum, the degree of isolation, the source of inoculum, the timing, and the intensity of aphid activity along with identifying the predominant aphid species, are needed to develop an integrated control program.
Culture control is often the most effective control measures, but this again requires knowledge of the vector, biology, and the ecology.

Suggestions for further work is to understand the complexity of potato viruses and how they interact with each other.

My work illustrates that a long term focus on virus control by renewing seed-potatoes frequently, combined with aphid control, can give an effective management of virus diseases in potato.

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Attachment 1 Interviews

Spørreskjema for besøk på gårder i Vestfold

Navn på gård og dyrker:

Dato for besøk:

Lokalitet og planteverntiltak:

Potetsort?

Dekar og antall planter?

Når var settepotetene nye? Hvilket år?

Når ble potetene satt?

BBCH skala. (Spiredato, plantehøyde og størrelse)

Attachment 2 ELISA

Hensikt:

Generelt ELISA- skjema

Plate nr: _____

Behandling:	Detaljer:	Dato:	Sign:
Coating:	..... µl pr brønn av antiserum i fortynning ..... i coatingbuffer, inkubert ..... t ved .....°C	.....	.....
Vask:	3x ..3.. min med ..... vaskebuffer ☐ ☐ ☐	.....	.....
Prøve:	.....µl pr brønn av .....g bladmateriale i ..... ml ferdig samplebuffer inkubert .....t ved .....°C.	.....	.....
Vask:	3x ..3.. min med .....kit- vaskebuffer ☐ ☐ ☐	.....	.....
Konjugat:	.....µl pr brønn av konjugat (alkalisk fosfatase, AP) fort. ..... i samplebuffer inkubert .....t ved ....°C. .	.....	.....
Vask:	3x ..3.. min med .....kit- vaskebuffer ☐ ☐ ☐	.....	.....
Substrat:	.....µl pr brønn av .1/2 tablett til 50 ml substratbuffer inkubert .....t. ved .....°C.	.....	.....

Plateoversikt

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Konklusjon:

Attachment 3 Statistics

Vi ser på et felt med poteter. Et felt er et jorde med poteter på en gård. Fra feltet tas det m prøver [typisk $m = 90$]. Hver prøve stammer fra n planter [typisk $n = 4$ eller $n = 5$]. Hver plante kan være smittet av et virus [V] eller ikke [ikke V]. Plantene blandes og en prøve er smittet hvis minst en av de n plantene i prøven er smittet. Hvis ingen av de n plantene i prøven er smittet er prøven ikke smittet.

Vi antar at totalt antall potetplanter i feltet [N] er stort i forhold til de $m \cdot n$ plantene som inngår i vårt prøvemateriale. Vi lar p være sannsynligheten for at en tilfeldig valgt plante i feltet er smittet av viruset. p uttrykker andelen smittede planter i feltet og $100 \cdot p$ uttrykker hvor mange prosent av plantene i feltet som er smittet. Vi skal estimere p. I tillegg skal vi si noe om usikkerheten i estimatet for p.

Vi starter med en bestemt prøve bestående av n planter. For hver av disse plantene har vi [tilnærmet]

$$P(V) = p \tag{1}$$

Vi lar X være antall planter blant de n som er smittet. Da er X tilnærmet binomisk fordelt med parametre n og p, skrevet

$$X \sim bin(n, p) \tag{2}$$

Sannsynligheten for at minst en av de n plantene i prøven er smittet er da gitt ved

$$q = P(X \geq 1) = 1 - P(X = 0) = 1 - \binom{n}{0} p^0 (1-p)^{n-0} = 1 - (1-p)^n \tag{3}$$

Uttrykket i (3) er sannsynligheten for at vår bestemte prøve er smittet.

Vi tar m prøver. For hver av disse prøvene har vi at sannsynligheten for at prøven er smittet er [tilnærmet] lik q. Vi lar Y være antall prøver blant de m som er smittet. Da er Y tilnærmet binomisk fordelt med parametre m og q, skrevet

$$Y \sim bin(m, q) \tag{4}$$

q kan estimeres med

$$\hat{q} = \frac{Y}{m} \tag{5}$$

Forventning og varians til $\hat{q}$ er gitt ved henholdsvis

$$E(\hat{q}) = q \tag{6}$$

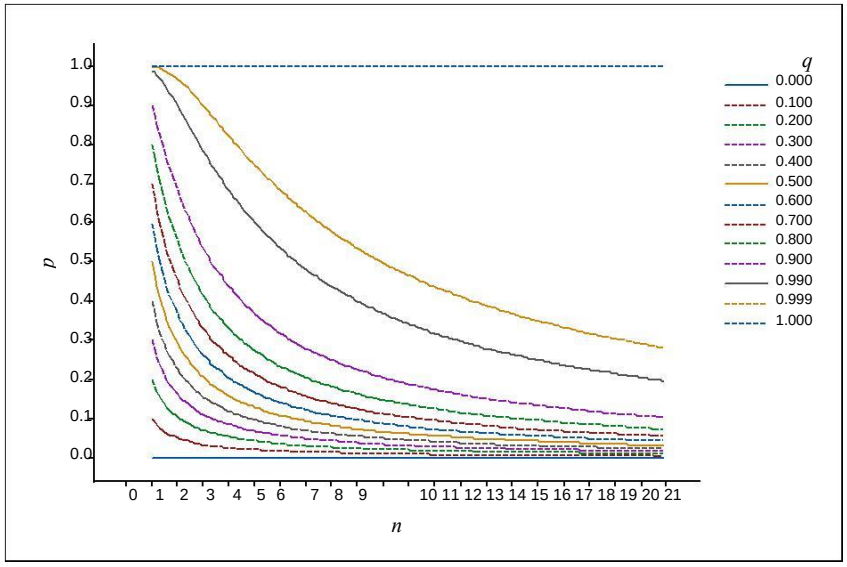
og

$$Var(\hat{q}) = \frac{q(1-q)}{m} \tag{7}$$

En estimator, $\hat{p}$ for p, får vi nå ved å erstatte q med $\hat{q}$ og p med $\hat{p}$ i (3) og å løse den likningen som da framkommer med hensyn på $\hat{p}$. Det gir

$$\hat{p} = 1 - (1 - \hat{q})^{\frac{1}{n}} \tag{8}$$

Figur 1 skisserer hvordan $\hat{p}$ avhenger av $\hat{q}$ og n. n kan bare ha heltallige verdier, 1, 2, …



Figur 1. $\hat{p} = 1 - (1 - \hat{q})^{\frac{1}{n}}$

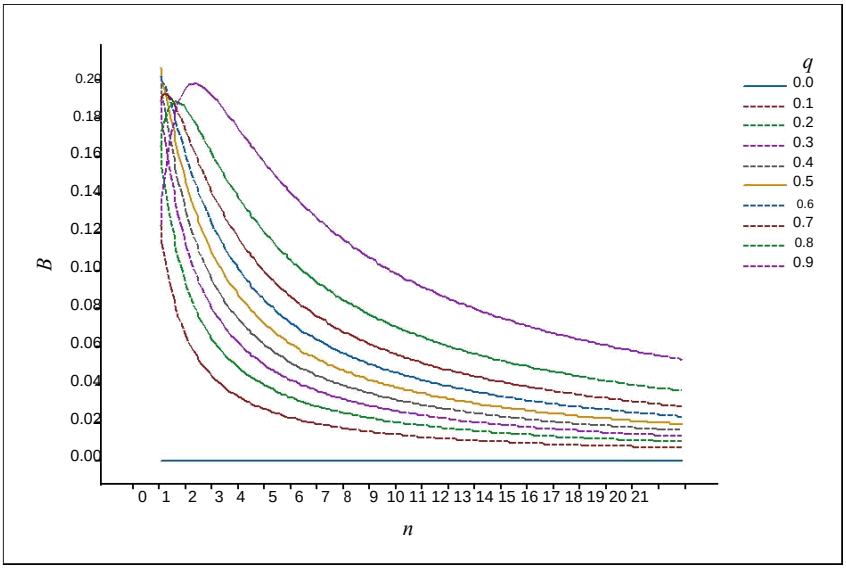
Det kan vises at estimatoren $\hat{p}$ i (8) er tilnærmet normalfordelt med forventning p og varians $\frac{\hat{q}(1-\hat{q})^{\frac{2}{n}-1}}{n^2 \cdot m}$. Fra dette følger at et konfidensintervall for p med konfidenskoeffisient tilnærmet lik $1 - \alpha$ er gitt ved

$$\hat{p} \quad - z_{\alpha/2} \cdot \sqrt{\frac{\hat{q}(1-\hat{q})^{\frac{2}{n}-1}}{n^2 \cdot m}}, \hat{p} + z_{\alpha/2} \cdot \sqrt{\frac{\hat{q}(1-\hat{q})^{\frac{2}{n}-1}}{n^2 \cdot m}} \tag{9}$$

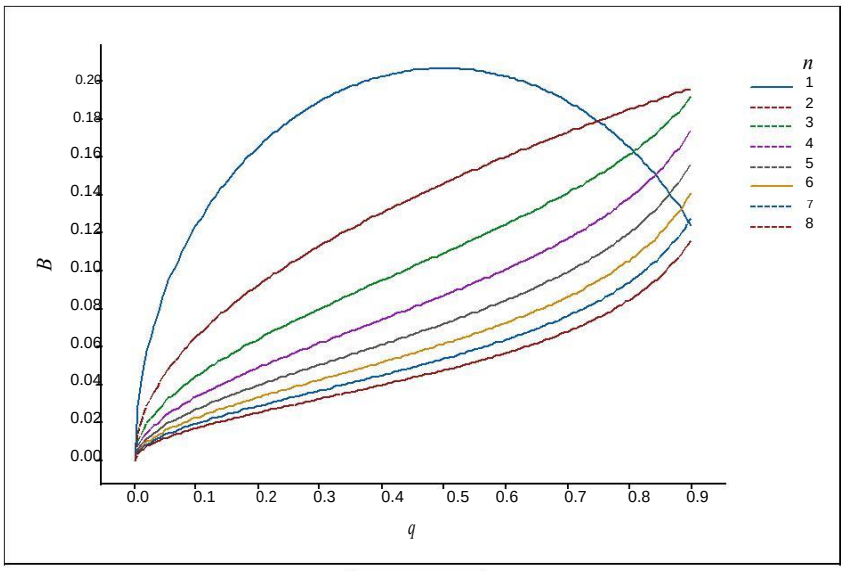
der $z_{\alpha/2}$ er øvre $\alpha/2$ – fraktilen i standard normalfordelingen. Bredden på konfidensintervallet i (9) er

$$B = 2 \cdot z_{\alpha/2} \cdot \sqrt{\frac{\hat{q}(1-\hat{q})^{\frac{2}{n}-1}}{n^2 \cdot m}} \tag{10}$$

For $\alpha = 0.05$ [$z_{\alpha/2} = z_{0.025} = 1.96$] og $m = 90$ skisserer figurene 2 og 3 hvordan B avhenger av $\hat{q}$ og n. n kan igjen bare ha heltallige verdier, 1, 2, …



Figur 2. $B = 2 \cdot z_{\alpha/2} \cdot \sqrt{\frac{\hat{q}(1-\hat{q})^{\bar{n}2-1}}{n^2 \cdot m}}$, $\alpha = 0.05$ og $m = 90$.



Figur 3. $B = 2 \cdot z_{\alpha/2} \cdot \sqrt{\frac{\hat{q}(1-\hat{q})^{\bar{n}2-1}}{n^2 \cdot m}}$, $\alpha = 0.05$ og $m = 90$.

Oppsummering

p som uttrykker andelen smittede planter i feltet kan estimeres med

$$\hat{p} = 1 - 1 - \frac{Y}{m}^{\frac{1}{n}} \quad (11)$$

der m er antall prøver som tas [typisk $m = 90$ og hver prøve stammer fra n planter] og Y er antall prøver blant de m som er smittet.

Et tilnærmet $1 - \alpha$ konfidensintervall for p er

$$\hat{p} - z_{\alpha/2} \cdot \frac{\sqrt{Y \cdot \left(1 - \frac{Y}{m}\right)_{n-1}^{2}}}{n \cdot m}, \hat{p} + z_{\alpha/2} \cdot \frac{\sqrt{Y \cdot \left(1 - \frac{Y}{m}\right)_{n-1}^{2}}}{n \cdot m} \qquad (12)$$

Desto smalere dette intervallet er desto mer nøyaktig estimeres p. Desto bredere intervallet er desto mer usikkert er estimatet i (11) for p. Dette kan brukes for å vurdere krav til antall planter i hver prøve, n, se figurene 2 og 3.

$z_{\alpha/2}$ er øvre $\alpha/2$ – fraktilen i standard normalfordelingen, typisk gir $\alpha = 0.05$ at $z_{\alpha/2} = z_{0.025} = 1.96$.

Merknad

For spesialtilfellet $n = 1$ [en plante i hver av de m prøvene] gir (3) at $q = p$ og (4) at $Y \sim bin(m, p)$. Estimatoren i (11) blir

$$\hat{p} = \frac{Y}{m} \qquad (13)$$

og konfidensintervallet i (12) blir

$$\frac{Y}{m} - z_{\alpha/2} \sqrt{\frac{\frac{Y}{m} \cdot \left(1 - \frac{Y}{m}\right)}{m}}, \frac{Y}{m} + z_{\alpha/2} \sqrt{\frac{\frac{Y}{m} \cdot \left(1 - \frac{Y}{m}\right)}{m}} \qquad (14)$$

Estimatoren i (13) er den vanlige estimatoren for den binomiske sannsynligheten p og intervallet i (14) er det vanlige tilnærmede $1 - \alpha$ konfidensintervallet for p.

Eksempel

Anta at vi tar $m = 90$ som hver stammer fra $n = 4$ planter. Vi finner at $Y = 9$ av de 90 prøvene er smittet. Da har vi:

$$\hat{p} = 1 - \left(1 - \frac{Y}{m}\right)^{\frac{1}{n}} = 1 - \left(1 - \frac{9}{90}\right)^{\frac{1}{4}} = 0.025996$$

Vi anslår altså at om lag 2.6 % [2.6 ≈ 100·0.025996] av plantene i feltet er smittet.

Videre

$$\hat{p} - z_{\alpha/2} \cdot \frac{\sqrt{Y \cdot \left(1 - \frac{Y}{m}\right)^{\frac{2}{n-1}}}}{n \cdot m}, \hat{p} + z_{\alpha/2} \cdot \frac{\sqrt{Y \cdot \left(1 - \frac{Y}{m}\right)^{\frac{2}{n-1}}}}{n \cdot m} =$$

$$0.025996 - 1.96 \cdot \frac{\sqrt{9 \cdot \left(1 - \frac{9}{90}\right)^{\frac{2}{4-1}}}}{4 \cdot 90}, 0.025996 + 1.96 \cdot \frac{\sqrt{9 \cdot \left(1 - \frac{9}{90}\right)^{\frac{2}{4-1}}}}{4 \cdot 90} = [0.00923, 0.04277]$$

Det betyr at vårt tilnærmede 95% konfidensintervall for andelen smittede planter i feltet, p, er [0.00923, 0.04277].

Med $n = 6$ og $Y = 27$ [og $m = 90$] får vi fra tilsvarende resultater som ovenfor at vi anslår at om lag 5.8 % [$5.8 \approx 100 \cdot 0.057713 = 100 \cdot \hat{p}$] av plantene i feltet er smittet. Det tilnærmede 95 % konfidensintervallet for andelen smittede planter i feltet, p, er [0.03647, 0.07895]. Om vi vil uttrykke også konfidensintervallet i prosent så er det tilnærmede 95 % konfidensintervallet for antall prosent smittede planter i feltet, $100 \cdot p$, lik [3.65 %, 7.90 %].

Nibio, 3. juni 2016
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