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Detection of *tobacco rattle tobravirus* and *potato mop-top pomovirus* in Swedish soil samples by the use of different bait plants and analysis by ELISA and biotest.

by

Patrick Sjöberg

Plant virology
Supervisor: Gunilla Åhman
ScanBi Diagnostics AB
P.O.Box 166
SE-230 53 Alnarp

Preface

The purpose of writing this bachelor thesis has been to evaluate if there is a possibility to analyse the presence of *tobacco rattle tobravirus* and *potato mop-top pomovirus* in soil by the use of ELISA and biotest. I would like to thank ScanBi Diagnostics that gave me the opportunity to test these techniques and especially my supervisor Gunilla Åhman who has been very helpful by proofreading my thesis, something that has been crucial if anyone should be able to understand the report. She has also supported me a lot during the experiment and with guidelines for the report. I would also like to thank Sanja Manduric at SLU for the nematode part and lending me literature for this bachelor. Finally I would like to thank Thilda Nilsson and Davida Johansson for their support during the writing of this report.

Alnarp, November 2006

Patrick Sjöberg

Summary

Tobacco rattle tobravirus (TRV) and *potato mop-top pomovirus* (PMTV) are two known viruses in Sweden which impair the quality of potato tubers by causing spraing. This experiment aimed at finding the best bait plants (plants that is susceptible to infection) for acquiring virus particles from soil, and to analyse the acquired viruses by both ELISA and biotest. The results were finished after 8 weeks with findings that indicate differences in the ability of acquiring virus particles between the bait plants. *Nicotiana clevelandii* and *N.debneyi* seem to be the best bait plants to use. These plants were able to acquire both plant viruses in high concentrations. ELISA results also verify that the vector for PMTV, *Spongospora subterranea f.sp. subterranea*, and the vector for TRV, *Trichodorus/Paratrichodorus* were present in the soil.

Another interesting discovery was that the only positive result of PMTV was found in soil samples that had been dried for at least two weeks. This could be confirmed by later tests that were made on a new batch of soil samples.

Five different indicator plants (plants that develop symptoms after inoculated with virus) were used for the biotest and out of these *Chenopodium amaranticolor* and *C.quinoa* seem to be the best to use because of the clearly visible local lesions that were easy to count. The biotest results were compared to the ELISA results and the plants that showed local lesions matched with positive ELISA values but the amount of lesions could not be correlated to high or low ELISA values. A combination of these techniques could be used in future work to increase the certainty of the diagnosis.

Sammanfattning

Tobaksrattelvirus (TRV) och potatismopptoppvirus (PMTV) är två välkända virus i Sverige som minskar kvaliteten på potatisknölar genom att orsaka rostringar. Målet med det här projektet var att hitta den bästa fångstplantan (växt som är mottaglig för virusinfektion) för att ta upp viruspartiklar från jordprover och att analysera virus med ELISA och biotest. Resultaten var klara efter 8 veckor, och det fanns en stor skillnad mellan fångstplantorna på att ta upp viruspartiklar. *Nicotiana clevelandii* och *N.debneyi* är de bästa plantorna att använda i och med att de kunde ta upp båda virus i höga koncentrationer. ELISA-resultaten kan också bekräfta att vektorn för PMTV, *Spongospora subterranea f.sp. subterranea*, och vektorn för TRV, nematoderna *Trichodorus/Paratrichodorus*, fanns i jordproverna.

En annan intressant upptäckt var att det enda positiva resultatet från ELISA på PMTV kom från torkade jordprover. Denna upptäckt kunde sedan bekräftas av ett andra test som gjordes på en ny omgång jordprover.

Fem olika indikatorplantor (växt som utvecklar lokala och systemiska symptom vid inokulering av virus) användes till biotestet, varav *Chenopodium amaranticolor* och *C.quinoa* fungerade bäst på grund av att det var lätt att räkna deras lokala lesioner (symptom). Biotestet jämfördes med ELISA-resultaten och det fanns ett samband mellan biotestsymptom och positiva ELISA värden. Det var däremot inte möjligt att se en korrelation mellan antalet lesioner och värde på ELISA, och för framtida bruk kan en kombination av biotest och ELISA vara en bra idé för att stärka säkerheten i analysen av jordprover.

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Hypothesis

It will be possible to discover both *tobacco rattle tobravirus* and *potato mop-top pomovirus* in soil samples by using bait plants and analysis by ELISA and biotest.

Introduction

Potato growers in Sweden have during many years had problems with spraing, which is not affecting yield but seriously impairs the quality of the tubers and makes them practically unsaleable in Sweden. Spraing has been considered to be caused by *tobacco rattle tobravirus* (TRV), which is spread by root nematodes in the soil. This is especially a problem in the southern and middle parts of Sweden, because of the warmer climate compared to the northern parts, and because the vector nematodes, *Trichodorus* and *Paratrichodorus* spp., are rare in northern Sweden.

TRV is difficult to test because of the many strains and the special characters of the virus. Biotest in form of sap inoculation to specific indicator plants is not a very sensitive method, and it does not detect all different strains equally well. Compared to biotest, ELISA can detect much lower virus concentrations, but strain differences make detection hazardous. Some strains are in fact impossible to discover with ELISA, since they lack a coat protein.

TRV is a bipartite virus with its genome divided in two pieces, RNA-1 and RNA-2, the coat protein being coded for by RNA-2. Since RNA-1 is able to multiply on its own, TRV strains without RNA-2, and, consequently, without coat protein, have evolved. These strains are undetectable by ELISA.

Another virus that is able to infect tubers is *potato mop-top pomovirus* (PMTV), which is known in other parts of the world as the major reason for spraing in potato. This virus is spread by the fungus *Spongospora subterranea f.sp. subterranea*, and since this fungus is found in almost all parts of Sweden it is possible that there is a greater risk of PMTV than TRV. PMTV is easier to detect than TRV because it does not have as many strains, and the character of the virus makes it suitable for detection by both ELISA and biotest.

PMTV is a tripartite virus with its genome divided in three pieces, RNA-1, RNA-2 and RNA-3, the coat protein being coded for by RNA-3. PMTV is not able to multiply without RNA-3, so ELISA is a reliable test method because the coat protein is always present.

In this project, conducted at ScanBi Diagnostics AB in Alnarp, an investigation of virus transmission and detection will be made. Transmission of TRV and PMTV by their vectors will be tested by five different bait plants. Along with virus detection by ELISA a biotest will be made with five different indicator plants.

Tobacco rattle tobravirus (TRV)

Tobacco rattle virus is transmitted by soil inhabiting ectoparasitic nematodes that belong to the genera *Trichodorus* and *Paratrichodorus* (Eriksson, 1974). TRV is a linear, positive single-stranded RNA-virus that consists of two RNA particles of different lengths (RNA-1 and RNA-2). TRV is rod shaped, 23 nm in diameter and tubular with a helical symmetry and a centrical canal with a diameter of about 5nm. The longer particle (RNA-1) is 185 to 196 nm and the shorter (RNA-2) is 50 to 115 nm, depending on the isolate (Robinson 2003).

TRV has a wide host range with more than 400 species in 50 different families, but systemic infection occurs only in a few of these (Robinson 2003). The host range contains many weeds and some examples are common chickweed (*Stellaria media* L.), shepherd's-purse (*Capsella bursa-pastoris* L.), field pansy (*Viola arvensis* Murr) and common couch (*Elytrigia repens* L. Desv. ex Nevski) which are all common weeds in Sweden (Svensson 1974). In rare cases the virus can be spread from mother plant by seed to progeny.

TRV is able to stay infective in the vector for many weeks, and there is evidence for transmission of the virus after storage of the nematodes in refrigerator for more than 9 months, which could be thought of as the winter period in Sweden.

RNA-1 of TRV (Fig 1) encodes four different proteins and the complete helicase/RNA polymerase gene occupies almost 75%. RNA-1 also encodes a movement protein which seems to be responsible for the cell to cell movement in the plant.

RNA-2 of TRV (Fig 1) encodes three different proteins where the coat protein is crucial for vector transmission by nematodes, but there are studies that suggest that more gene products in RNA-2 are involved in the transmission process (MacFarlane 1999, Vassilakos et al. 2000).

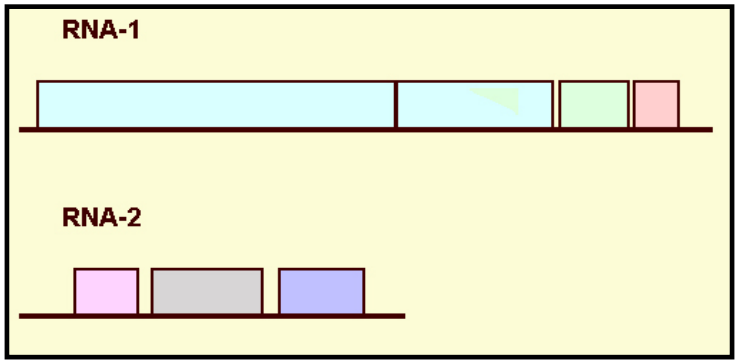

Fig 1: The two genomes of TRV showing RNA-1 with its four encoded proteins and RNA-2 with three encoded proteins (Robinson 2003)

The virus can cause two different types of infection, M and NM-type (MacFarlane et al. 1995). If host plants are infected with the NM-type of the virus it will multiply and spread in the plant independently of RNA-2 (Vassilakos et al. 2000), but does then only consist of the larger RNA-1 particles.

The M-type of the virus contains the whole viral genome and is thought to be the only type which is able to be transmitted by nematodes and produce both kinds of virus particles (MacFarlane et al. 1995, Robinson 2003, Hull 2004). Infection of TRV is difficult to detect and analyse by serological means such as ELISA because of the great serological variation (Brown et al. 1989). The NM-type is impossible to detect by ELISA because of the missing coat protein (Mumford et al. 2000). The NM-type can only be spread by infected material and by sap, which makes dispersal of the virus impossible in the field even if vector nematodes are present in the soil (Robinson 1992).

The symptoms that occur after infection of potatoes are dark stripes and spots within and on the outside of the tuber but most often near the skin (Svensson 1974) (Fig 2). The infection can also give rise to mosaic patterns on the leaves (Fig 2), deformed stalks and short petioles (Svensson 1974, Rydén et al. 1994). Spraing can sometimes be mistaken for physiological damages or infection with some strains of p*otato Y potyvirus* (Svensson 1974, Mumford et al. 2000).

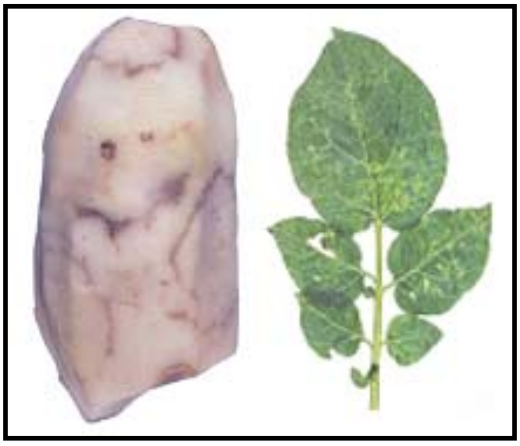

Fig 2: Potato tuber with symptoms of spraing from TRV and potato leaf with mosaic from infection of TRV (Robinson 2003)

Because of the long time that particles can stay infective and the problem with overwintering adult nematodes, infection and spread of TRV can start early in the growing season (Eriksson & Insunza 1986). When a field has been infected by TRV and there are vector nematodes present in the soil, eradication is almost impossible. The only options that might work are the use of transgenic plants and crop rotation. There are also large differences in susceptibility between different potato cultivars, and some such as Bintje, Magnum Bonum and King Edward seem to have a greater resistance to TRV than other commercially grown cultivars (Svensson 1974, Eriksson 1996).

Trichodorus & Paratrichodorus

Trichodorus & *Paratrichodorus* belong to the family *Trichodoridae* (Dijkstra & de Jager 1998). Nematodes in this family are 0.35 to 1.8 mm long and rounded at the ends (Boutsika et al. 2004). *Trichodorus* and *Paratrichodorus* are commonly known as stubby root nematodes (Harris 1992). They are migratory ectoparasitic nematodes that feed on both annual and perennial plants (Boutsika et al. 2004).

There is a large distribution of *Trichodorid* nematodes in the northern parts of the US and Europe. The species are most often found in lighter sandier soils and they seem to exist at all depths where roots are established (Eriksson 1974, Dijkstra & de Jager 1998). The highest amounts of nematodes are accounted for during spring and/or early summer (Eriksson & Insunza 1986). *Trichodorus and Paratrichodorus* are most common in the southern parts of Sweden but have been detected in almost all parts except the most northern region (Eriksson 1996) (Fig 3). The most common species in Sweden are *Paratrichodorus pachydermus*, *Trichodorus primitivus* and *Trichodorus similis* but findings of *Trichodorus sparsus* have been made (Eriksson 1996).

Soil inhabiting nematodes move between soil particles and the rate of movement depends on the thickness of the water film surrounding the soil particles (Harris 1992). Nematodes are very sensitive to drought, pressure or vibrations, so it is especially important to be careful when handling soil samples taken from the field (Eriksson 1974).

Feeding behaviour of *Trichodorid* nematodes can be divided into five phases; exploration, perforation of the cell wall, salivation, ingestion and withdrawal from the cell (Hull 2004). When the nematodes attack a plant the stylet is used to tear the wall of epidermal cells, and they are then able to feed on the cell content. After feeding on the epidermal cells the nematodes continue feeding on the deeper layers of the plant. The roots react by stopping cell elongation and start cell division below the attacked area, resulting in stunted roots. The nematodes can cause severe direct damage to plants, but the largest problem with these polyphagous nematodes is their ability to transmit viruses to the host plant (Harris 1992, Dijkstra & de Jager 1998).

Trichodorus and Paratrichodorus are able to transmit tobravirus particles which can stay infective for more than a year within the nematode (Dijkstra & de Jager 1998, Hull 2004). Ingested virus particles have been observed adsorbed to the cuticular lining of the oesophagus while the nematode feeds on an infected plant (Harris 1992, Hull 2004). Both adults and juveniles are able to transmit TRV but the juveniles loose this ability after moulting when the cuticular lining is shed and replaced. There is no replication of particles within the vector or any evidence for transmission through eggs (Robinson 2003, Hull 2004). The virus particles are supposed to be released because of a change in pH caused by the flow of saliva when the nematode starts feeding on a new plant host (Hull 2004). Due to the specificity in virus transmission, knowledge of the nematode species present makes it possible to assess which strain of the virus could be spread.

P.pachydermus can transmit several different strains of TRV and especially PPS1 which is known as the Swedish strain (Ploeg et al. 1992). PPS1 is also the most common strain found in Swedish soils. The other nematode species are able to transmit other strains of the virus but not PPS1 (Eriksson 1996).

It is difficult to decrease the amount of nematodes in a field. Chemical control by nematicides is not allowed in Sweden. Steaming has proven difficult to use because of the wide spread of nematodes in the soils down to depths of around 40 to 50 cm (Eriksson & Insunza 1986) while steaming only reaches depths of 30 cm (Harris 1992). A third method which could be used is crop rotation, but since the *Trichodoridae* are polyphagous a functional intermediate crop could be difficult to find. There is also a big risk that the nematodes will survive on weeds growing in or near the field.

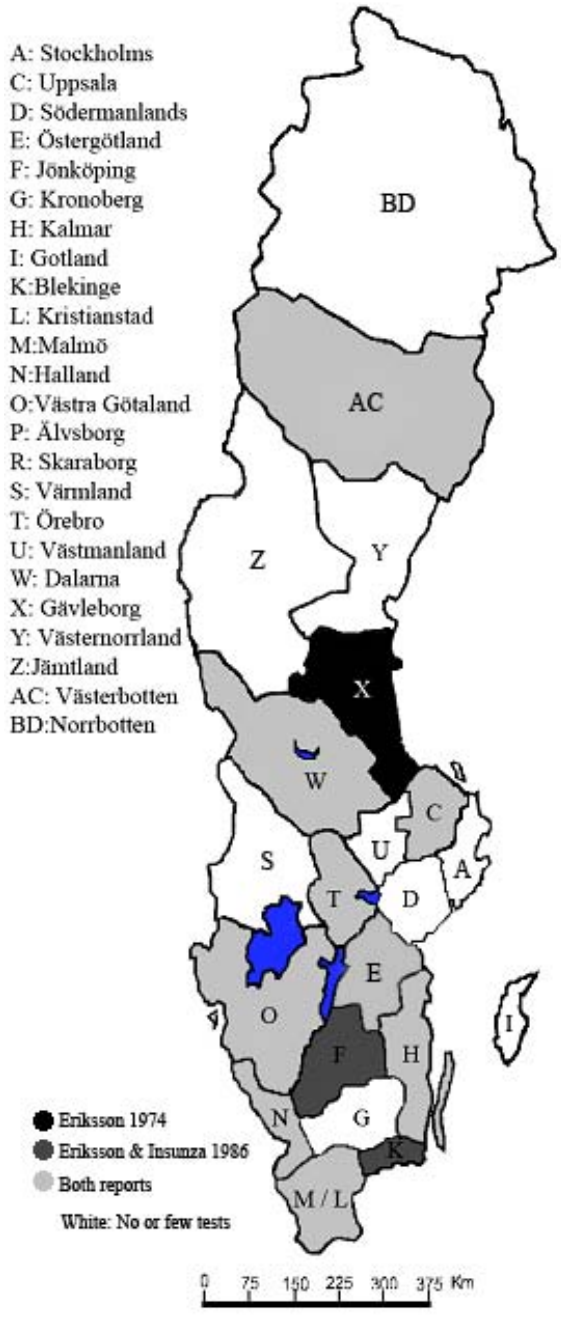

Fig 3: Distribution of *Trichodorus* and *Paratrichodorus* in Sweden according to Eriksson 1974 and Eriksson & Insunza 1986. There are more nematodes reported in the southern parts of Sweden, but this is much due to a higher rate of measurements at these locations. There are a few locations that seem to be free from nematodes and the reason for this is most probably that few tests have been conducted.

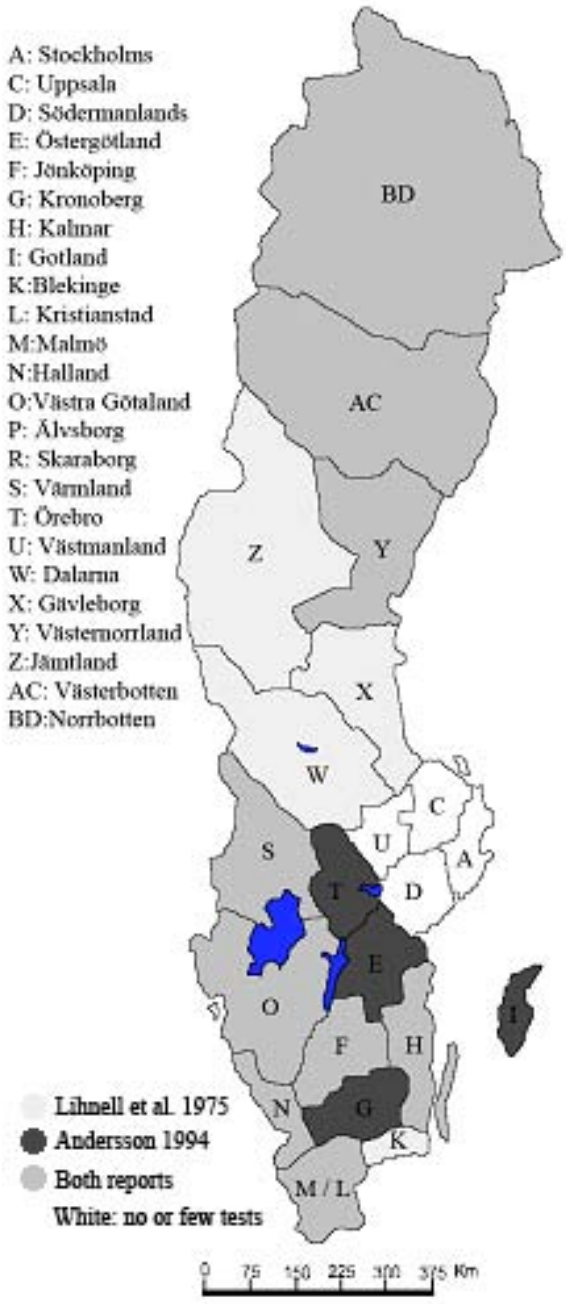

Fig 6: Distribution of *Spongospora subterranea* in Sweden according to Lihnell et al. 1975 and Andersson 1994. The fungus can be found in most parts of Sweden.

Potato mop top pomovirus (PMTV)

PMTV is a positive single stranded RNA virus consisting of three particles. It is a straight helically constructed virus with a hollow core. The particles are 100-150 nm and 250-300 nm with a width of 18-20nm. PMTV is transmitted by the algal fungus S*pongospora subterranea f.sp. subterranea* which is a soil borne pathogen (Rydén et al. 1986), and the virus is carried inside the resting spores where it can stay active for more than 10 years (Rydén et al. 1989, Sandgren 1996, Harrison & Reavy 2002).

The virus has a narrow natural host range compared to TRV but has been transmitted by sap inoculation to 26 species in the *Solanaceae* and *Chenopodiaceae* (Harrison & Reavy 2002). Among commercially grown plants only potato is infected by PMTV (Rydén et al. 1986). PMTV was first discovered in Northern Ireland and Scotland in 1966 (Rydén et al. 1989) but can now be found almost everywhere potato is grown (Harrison & Reavy 2002). PMTV has been detected in all types of soils and has therefore a potential of being spread to all parts of Sweden. It is unaffected by dry soil conditions but favoured by rain and irrigation.

Spraing as an effect of PMTV is considered a serious problem in Denmark and Finland (Rydén et al. 1986, Sandgren 1996), whereas spraing in Sweden has been known as a result of TRV. For a long time PMTV infection in Sweden was suspected, but it was not until 1985 when virus trials where conducted on several fields that this could be confirmed (Eriksson 1996). Then positive results were made on two varieties, Ukama and Vit Drottning from Halland and Ultuna. One factor that could explain this late discovery is that PMTV is difficult to transmit from infected material to indicator plants (Rydén et al. 1986).

PMTV is common in the southern and middle parts of Sweden but so far the northern parts seem to be free from infection, although *S. subterranea* is common also in northern Sweden, so there is a potential risk of spread to these parts (Sandgren 1996).

There are three known strains of PMTV. Strain T is more virulent than the others but is not transmitted by the fungal vector. The two other strains are PMTV-S from Scotland and PMTV-Sw from Sweden. Although the T strain is not vector transmitted, there is little strain variation in the coat protein (Harrison & Reavy 2002). A study was made with isolates from Denmark, Sweden and Finland, where it seems to be only small differences in serological characteristics (Eriksson 1996).

PMTV is a tripartite virus where RNA-1 encodes the replicase protein and a replicase read through protein, RNA-2 encodes the triple gene block protein and RNA-3 encodes the coat protein (Harrison & Reavy 2002) (Fig 4). The cell to cell movement is most probably controlled by the proteins which are encoded by the triple gene block in RNA-2.

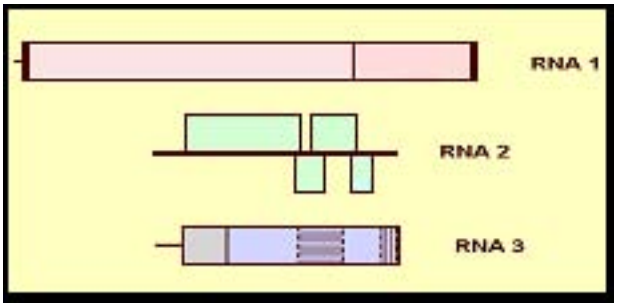

Fig 4: The three parts of the genome of PMTV showing the different coded proteins (Harrison & Reavy 2002)

Symptoms of PMTV vary between different potato cultivars and also with climate because symptoms are favoured by cold climate and rain. The most common symptoms on shoots (Fig 5) are yellow mottling and green mosaic patterns, especially on the lower leaves, V-shaped chlorotic spots and the extreme stunting of the shoots that is known as mop-top (Rydén et al. 1986, Harrison & Reavy 2002). Symptoms on the leaves are only shown in cool weather on plants from already infected seed potatoes (Rydén et al. 1989). The tubers of some cultivars develop concentric ring spots on the surface also known as spraing (Rydén et al. 1986, Harrison & Reavy 2002) (Fig 5). The symptoms of PMTV can sometimes be mistaken for those caused by TRV, but the latter virus causes production of corky tissue which PMTV does not (Harrison & Reavy 2002). Shallow ring spots are also more common with PMTV than with TRV (Rydén et al. 1986).

Some potato varieties are extremely sensitive to PMTV but most are more resistant, and for instance Bintje can be infected without showing any symptoms (Rydén et al. 1986, Sandgren 1996). In a Danish trial more than 40 cultivars were tested of which only two showed symptoms (Eriksson 1996). The difference in symptoms between cultivars could also depend on factors such as different races of *S. subterranea* or virus strain differences (Sandgren 1996).

Tubers that do not show any symptoms during harvest can develop these during storage, especially if the temperature is fluctuating (Rydén et al. 1986). Infection by PMTV is seen as a qualitative problem because consumers are able to recognize poor tuber quality already when only 4% of the tubers are showing symptoms (Sandgren 1996).

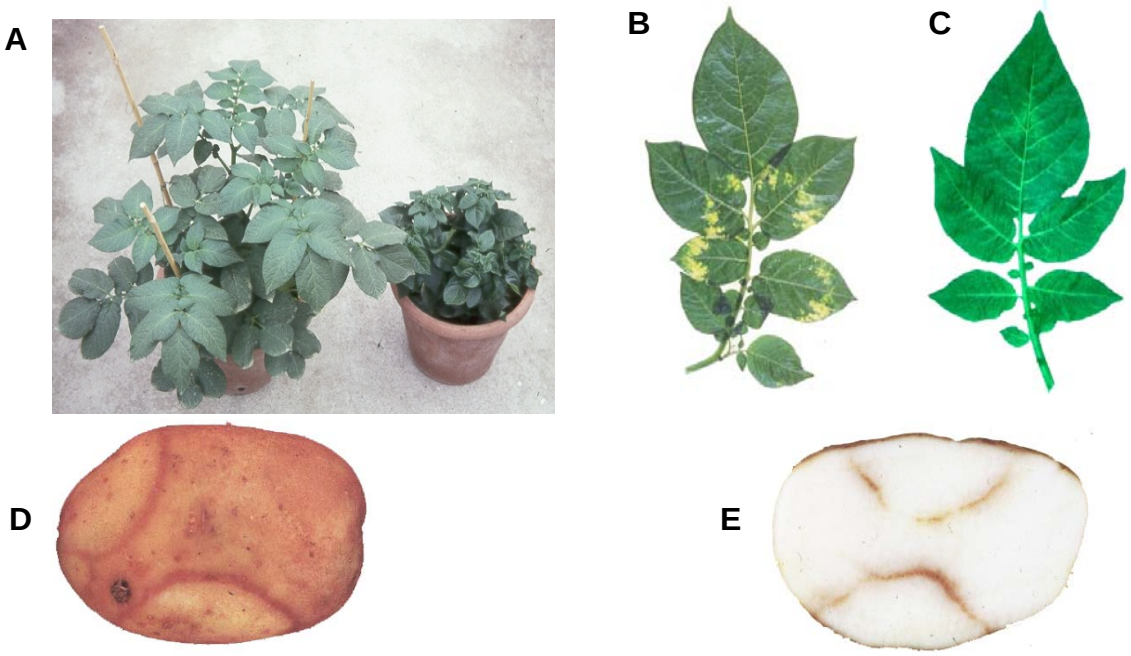

Fig 5: A: Healthy plant and plant with symptoms of mop-top, B: Mottling on potato leaf after infection of PMTV, C: V-shaped spots on the leaves of potato infected with PMTV, D: Symptoms of spraing on the skin of a potato tuber infected with PMTV, E: Symptoms of spraing on the inside of a potato tuber infected with PMTV (Harrison & Reavy 2002)

PMTV can be spread by soil, machinery, tools and seed potato (Rydén et al. 1986, Sandgren 1996). Infection of PMTV can be greatly reduced by treating the soil with fungicides or by using sulphur which will bring the pH down below 5, but the treatments are expensive and will not give long lasting protection since an increase of virus particles can be measured when pH returns to normal (Rydén et al. 1986).

Spongospora subterranea f.sp. subterranea

Spongospora subterranea is the causing agent of potato powdery scab disease (Merz 1989) and is also responsible for the spread of *potato mop top pomovirus* (Agrios 1997).
S. subterranea is found all over the world (Harris 1992) but is thought to originate from South America (Andersson 1994). It is established in most regions of Sweden but the spread of spores in the northern parts is uninvestigated (Fig 6). *S. subterranea* has a wide host range and can infect most members of the *Solanaceae* family (Andersson 1994).

S. subterranea is an obligate parasite meaning that it needs living host cells for survival (Andersson 1994). The plasmodium lives of the host cell it invades but does not kill the host, on the contrary, many times the invaded cells are stimulated by the pathogen to abnormal growth and division so that more nutrients can be taken up by the pathogen. Some cells are later killed as the plasmodium spreads out (Kunkel 1915). The abnormal cells are often five to ten times as large as normal and most of the growth is outwards towards the skin of the tuber (Kunkel 1915).

S. subterranea moves from plant to plant by zoospores in the soil (Harris 1992) and is transmitted between fields by water, machinery or infected transplants (Agrios 1997). The spores find their way into the tuber through lenticels, sprouts and wounds (Kunkel 1915).When zoospores come in contact with a root they withdraw the flagella and produce a cyst. The cyst then forms an infection tube that penetrates the root cell wall (Dijkstra & de Jager 1998). A zoosporangium with secondary zoospores is then formed and matures within 4-5 days (Merz 1989) (Fig 7). The secondary zoospores continue to infect root hairs, epidermal cells or the cortex in stolons, roots or tubers and form resting spores (Andersson 1994). Then depending on the environmental factors the resting spores will either continue in a resting stage or germinate and release new zoospores. After harvest there is a great risk of spore germination during storage which will lead to secondary infections and by this cause dry rot (Kunkel 1915).

S. subterranea is distinguished by its zoospores that have two flagella of different lengths by which it moves forward (Andersson 1994, Dijkstra & de Jager 1998). The zoospores are oval or sphaerical, and vary between 2.5 and 4.5 µm in diameter. The resting spores (cysts) are 3.5 to 4.5 µm in diameter. They are often gathered in clusters (cystosori) which are between 19 and 85µm in diameter, depending on the amount of spores.

Resting spores need water to release the zoospores and according to Hims (1976) the largest risk for infection is when young seedlings are planted during a rainy season, because this will lead to a lot of free zoospores in the soil (Andersson 1994).

The optimum temperature for S. subterranea is 14°C to 20°C. The fungus is easily reproduced and spread under wet conditions and is therefore depending on rainfall or irrigation. It is not especially sensitive to fluctuations in pH (Andersson 1994). *S. subterranea* survives during long periods of drought as resting spores (Kunkel 1915), and both PMTV and *S. subterranea* have been found in fields that have not been used for potato growing for more than ten years (Rydén et al. 1989, Harris 1992).

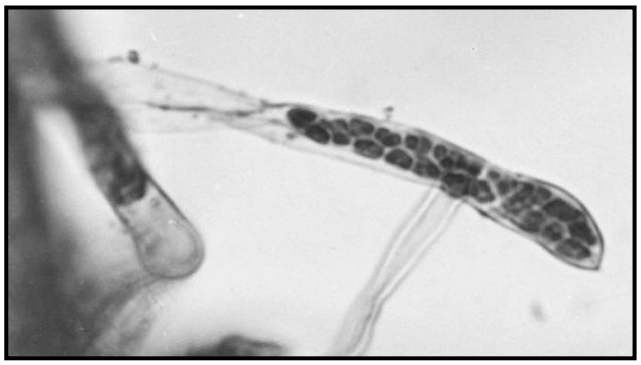

Fig 7: Zoosporangia in the root hairs of a potato plant (Harrison & Reavy 2002)

Symptoms from the infection are found on tubers, stolons and roots as blemishes that will decrease the market value. Zoospores are sensitive to environmental changes and fungicides, but the resting spores are resistant to all pesticides (Merz 1989).

A number of plant viruses are known to be transmitted by fungi, and the genus *Spongospora* is able to transmit more than 15 different viruses (Dijkstra & de Jager 1998). The fungus is infected by virus particles during its development in the plant. PMTV is encapsulated in resting spores and transmitted to the roots by zoospores (Harrison & Reavy 2002).

Materials & Methods

Soil samples

Soil samples were collected from localities in Östergötland with known problems of root nematodes and spraing. The soil samples gathered for the project were taken from fields where soils are known to contain populations of *Paratrichodorus* and *Trichodorus.* A project was started in 2005 with the aim of decreasing nematode populations by the use of intermediate crops. The intermediate crops used were *Raphanus sativus* ssp. *oleiformis* (Oil radish), *Sinapsis alba* x *Brassica juncea* (Caliente mustard) and *Eruca vesicaria* L. (Garden rocket), and they were compared to stubble field fallow. The sample size was approximately 0. 5 kg of soil from each field. In total, 22 samples were gathered and sent by post. They were stored in a cold chamber for one day before each of them were divided into 5 pots were the bait plants were planted for virus acquisition. Bait plants are plants that are susceptible to virus infections.

Bait plants

The bait plant species used for the experiment are known from earlier experiments to be susceptible to infection with TRV and PMTV. The bait plants were *Nicotiana benthamiana, N.clevelandii, N.debneyi, N.tabacum* 'White Burley' and *Petunia hybrida*. The plants had been sown 6 weeks before they were planted in the collected soil samples. The pots used for the experiment were 4 * 4 * 4 cm and put 10 cm over the table on aluminium net to minimize the risk of contamination from the surrounding pots (Fig 8). A total of 110 pots with one bait plant in each were placed in the greenhouse at a temperature of 20-25ºC with a day length of 16 hours.

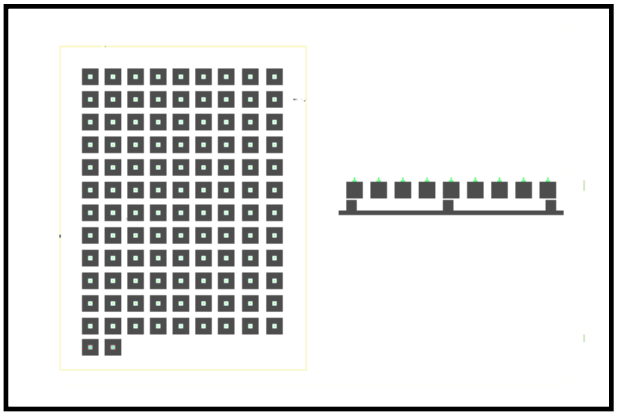

Fig 8: Bait plant setup during virus acquisition. A total of 110 plants were put in pots and elevated 10 cm over the table to minimize contamination between the samples.

After cultivation for three weeks, the roots of each bait plant were taken out of the pot, rinsed, cut into pieces and mashed in a mortar with 0.02M phosphate buffer pH 7.4, containing 0.15M NaCl and 2% PVP (polyvinylpyrrolidone). The content of each mortar was either used directly for ELISA, or divided in two parts of which one was taken to ELISA and the other to biotest.

ELISA

ELISA (enzyme-linked immunosorbent assay) is a common method used for analysing plant viruses (Van Regenmortel 1982). It is a serological method which is useful because of the economical benefits compared to other test methods and because it is easy to use in routine testing of a large number of samples.

ELISA is a sensitive method which is able to detect virus particles in concentrations as low as 1-10 ng/ml. The antibodies used for serological tests are produced in vertebrates, often rabbits, by injecting virus antigens (Hull 2004). The ELISA technique uses microtitre plates with 96 wells, and antibodies to the specific virus that is to be tested are added to these plates. Then the plant sap is added, and if the virus to be tested is present in the sap, the virus particles will adhere to the antibodies. To make it possible to detect the virus, a second batch of antibodies, this time enzyme-linked, is added to the wells. If the virus is present, the enzyme-linked antibodies will adhere to the particles, and the enzyme will cause a change of colour in the finally added chromogenic substance. The coloration can be detected and measured by a spectrophotometer, most often at an absorbance of 405nm. The intensity of colour is proportional to the virus concentration in the sample (Fig 9).

ELISA was performed according to Clark & Adams (1977). The antibodies to TRV were bought from Loewe Biochemica in Germany and the antibodies to PMTV from Bioreba AG in Switzerland.

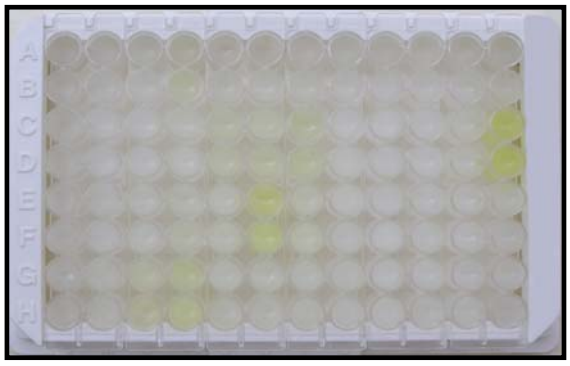

Fig 9: ELISA plate with positive values (yellow wells) from PMTV tests

Biotest

Another method of detecting plant viruses is by inoculating plant sap to herbaceous indicator plants (Rydén 1977). Indicator plants are plants that show clear local and systemic symptoms when inoculated with plant viruses. Extracts from plants to be tested can contain substances which are able to inactivate the virus or have an inhibitory effect on virus replication when the pH value falls as it does when the cells are crushed. To prevent this it is important to keep a high pH of the extract and to dilute this to lower the effects of inhibition. The most common substance to use during extraction is a phosphate buffer with a pH of 7 to 8. The indicator plants are often placed in a dark chamber 1-2 days before inoculation to make them more susceptible to infection. To facilitate the inoculation of virus particles, an abrasive is added to the extract. The abrasive will cause small wounds in epidermis which will function as entry holes for the virus particles. The inoculum is applied by gently rubbing it onto the leaves of the indicator plants. After inoculation the leaves are washed with tap water to remove excess sap and abrasive (Németh, 1986).

The biotest plant species chosen are known from previous tests to develop both local and systemic symptoms of TRV and PMTV. The plants were *Chenopodium amaranticolor, C.quinoa, Nicotiana debneyi, N.tabacum* cv. Samsun and *N.tabacum* cv. Xanthi. They were sown four weeks before inoculation and planted in pots, 4 * 4 * 4 cm, which were put 10 cm over the table on aluminium net to minimize the risk of contamination from the surrounding pots. The inoculum was prepared as described above, mixed with Celite as an abrasive and then inoculated to two plants of each indicator species. They were placed in a colder area in the greenhouse to increase the probability of symptom development. A total of 95 plants was inoculated and studied for two weeks.

Results

The virus acquisition tests confirm that there were both *Trichodorid* nematodes and *S.subterranea* in the soil samples. It was also verified that the bait plants used for the experiment were able to get infected by both TRV and PMTV. The ELISA detected the presence of both viruses in the bait plant roots. The biotest results were compared with the corresponding ELISA values and the plants with local lesions also show higher ELISA values but there was no correlation between the amount of lesions and ELISA values (Appendix).

During the transmission tests 16 of the 110 bait plants died because some of the samples came from heavy clay soils which were not suitable for plant growth. From the remaining 94 plants it was possible to calculate mean values of the ELISA results (Appendix) to establish which of the plant species that was most efficient as bait plant (Table 1). The best plant for acquisition of TRV was *Nicotiana benthamiana,* which however is difficult to use because of weak growth and small root systems. *N.debneyi* and *N.clevelandii* also gave high ELISA values but they had larger root systems and good growth during the trial. *N.tabacum* and *Petunia* had the lowest values in the ELISA tests (Table 1). The results for PMTV were a bit different with high values for *N.clevelandii*, *N.debneyi* and *Petunia*. *N.benthamiana* and *N.tabacum* caused low PMTV values in ELISA (Table 1). The highest loss of bait plants happened to *N.benthamiana* and *N.tabacum* (Appendix).
A statistical comparison test was made in Minitab to establish if the bait plants gave equal ELISA means. In the TRV tests a relation was found between *N.debneyi*, *N.clevelandii* and *N.benthamiana* aswell as between *N.tabacum* and petunia. In the PMTV tests a relation was found between *N.debneyi* and Petunia aswell as between *N.benthamiana* and *N.tabacum* (Appendix I).

Bait plant	Mean TRV values from ELISA	Mean PMTV values from ELISA
Nicotiana debneyi	0.62	0.13
Nicotiana clevelandii	0.56	0.20
Nicotiana tabacum	0.42	0.07
Nicotiana benthamiana	0.68	0.08
Petunia	0.38	0.16

Table 1: Mean ELISA values from each bait plant and virus.

Of the five plant species used for biotest only two were used to compare results with ELISA because it was only *Chenopodium amaranticolor* and *C. quinoa* that had local lesions which could be counted (Table 2). These plants showed local lesions within a few days from inoculation (Fig 10). The other indicator plants also got symptoms but not as clearly as *C.amaranticolor* and *C.quinoa*.

Fig 10: Local lesions of TRV on *Chenopodium amaranticolor* (left) and *C.quinoa* (right)

Sample	*Chenopodium amaranticolor (local lesions)*	*C.quinoa (local lesions)*	ELISA
81	0	0	0.218
82	0	1	0.221
83	0	1	0.199
84	0	0	0.194
85	0	3	0.378
86	0	0	0.291
87	10	19	2.000
88	2	2	0.211
89	11	2	0.247
90	0	0	0.175
91	3	0	0.851
92	16	21	1.340
93	22	10	1.012
94	24	48	1.292
95	9	13	0.468
96	20	12	1.318
97	34	11	0.747
98	18	15	0.538
100	2	0	0.183

Table 2: TRV detection results in biotest and ELISA

From the ELISA results a table of all values from each field were combined to compare the results after the different intermediate crops (Table 3).

Sample site	Sample no	Intermediate Crop	TRV	PMTV
Sample site 1	(66-70)	Caliente mustard	0	0
	(61-65)	Garden rocket	+	0
	(76-80)	Oil radish	+++	0
	(71-75)	Stubble field fallow	0	0
Sample site 2	(96-100)	Caliente mustard	++	0
	(86-90)	Garden rocket	+	0
	(91-95)	Oil radish	+++	0
	(81-85)	Stubble field fallow	0	0
Sample site 3	(56-60)	Caliente mustard	+++	0
	(46-50)	Garden rocket	++	0
	(51-55)	Oil radish	++	0
	(41-45)	Stubble field fallow	+++	0
Sample site 4	(16-20)	Caliente mustard	0	0
	(6-10)	Garden Rocket	0	0
	(11-15)	Oil radish	+	0
	(1-5)	Stubble field fallow	0	0
Sample site 5	(21-25)	Oil radish P1	0	++
	(26-30)	Oil radish P2	0	+
	(31-35)	Oil radish P3	0	+++
	(36-40)	Oil radish P4	0	0
Sample site 6	(101-105)	Oil radish	0	0
	(106-110)	Stubble field fallow	+	0

Table 3: Detected amounts of TRV and PMTV after different intermediate crops

+ = 0.3 – 0.6 OD-value in ELISA (TRV)

++ = 0.6 – 0.9 OD-value in ELISA (TRV)

+++ = 0.9 - 1.2 OD-value in ELISA (TRV)

+ = 0.15 – 0.3 OD-value in ELISA (PMTV)

++ = 0.3 – 0.45 OD-value in ELISA (PMTV)

+++ = 0.45 - 0.6 OD-value in ELISA (PMTV)

Discussion

It was possible to detect virus particles in the soil samples by both ELISA and biotest. The chosen bait plants were all able to get infected by the viruses although *Nicotiana clevelandii* and *N.debneyi* were among the best when root systems were compared. The ELISA values for these two plants were also relatively high for both PMTV and TRV. Unfortunately more than half of the soil samples were from heavy clay soils which had a negative effect on the growth of the bait plants. *Trichodorid* nematodes and subsequently TRV are not commonly found in heavy clay soils due to the dense soil properties which make it difficult for the nematodes to move.

The results from sample sample site 4 could be misguiding because these were the most dense soil samples and many of the bait plants in them were not able to survive. In the lighter soils almost all of the bait plants survived and *N.clevelandii, N.debneyi* and *Petunia* had nice root systems and grew better than *N.benthamiana* and *N.tabacum*. In general *N.benthamiana* and *N.tabacum* were difficult to use since they were weak and their root systems were not very well developed. An important factor for these types of experiments is to have plants that are able to grow in most soil types such as *N.clevelandii, N.debneyi* and *Petunia*.

An interesting coincidence was that samples 21 to 40 turned out to be old soil samples which had been left to dry before they were used in the experiment. These samples were the only that recorded presence of PMTV, and this indicates that soil samples that are analysed for presence of PMTV need to be dried for one or two weeks before planting bait plants in them. This is also supported by information from Harrison & Reavy (2002) that soil samples should be dried and then moistened again before testing PMTV. The same soil samples also showed a very low presence of TRV compared to the other samples, which could be explained by the fact that *Trichodorid* nematodes are not able to survive in dry soil conditions.

After this experiment another batch of soil samples were sent from Halland. These were divided in two batches of which one was dried for two weeks before use and the other was used directly. The results from this trial gave further evidence for the theory above, because the dried samples showed presence of PMTV but not of TRV. The other batch which was not dried did show presence of TRV but not PMTV.

In future analysis of soil samples of these two viruses it is recommended that the samples should be divided in dried/not dried parts for accurate measurements on viral presence.

The indicator plants that were used for sap inoculation showed symptoms after only three to four days. Of the species used the best were *Chenopodium amaranticolor* and *C.quinoa*, which got clear local lesions. It was decided to use *C.amaranticolor* and *C.quinoa* in comparison to ELISA because it was only possible to count lesions on those plants as the other plants did not have as well defined lesions (Fig 11). Biotest is not a secure method of determining the presence of a specific virus, but together with ELISA it is a helpful tool of determining virus concentration and at the same time it acts as a control to ELISA, since it has a broader strain spectrum.

Fig 11: Difference between *Chenopodium amaranticolor* (left) and *Nicotiana debneyi* (right) in biotest. *C.amaranticolor* has very clear and easy countable lesions.

No virus analysis was made before the intermediate crops were sown; hence it is impossible to tell if any of them had an impact on the virus amount in the soil. They were used (among other purposes) in the hope of bringing down the amount of spraing, primarily by restriction of the vector nematode populations. Their depressive effect on the spraing itself remains to be proved. If, however such an effect would be found, the question is: What is depressed? Is it one or both of the viruses, one or both of the vector organisms or maybe all four factors? Further work must be done before this question can be answered.

Conclusions

It was possible to transmit both TRV and PMTV particles from the soil samples to all of the bait plants. It was also possible to analyse the virus concentration by ELISA and biotest. Of the five bait plants used in this trial, *Nicotiana clevelandii* and *N.debneyi* had the overall best characteristics by giving both high ELISA values for both viruses and at the same time being easy to grow in both light and fairly heavy soils. In the biotest, two indicator plant species had the best properties, *Chenopodium amaranticolor* and *C.quinoa*. On these two plants it is easy to count the local lesions and by that have a possibility to compare the results of ELISA and biotest. The lesions appeared already after a few days and an analysis can then be carried out over a short time period.

A very important fact is that for test of PMTV the soil samples have to be left to dry for 2 weeks before bait planting, while for TRV the soil samples must be kept moist all the time.

From the time a soil sample is received it will take approximately seven weeks before an analysis can be made (Table 4). First of all a four week period from sowing to planting of bait plants is needed and meanwhile it is possible to divide the collected samples in two batches were one is kept moist for TRV testing and the other is left to dry for test of PMTV. Then the bait plants are planted in the collected samples and left to acquire virus for three weeks, and during this time the indicator plants, sown 1-2 weeks before bait planting, should be planted at least two weeks before the bait plant roots are harvested. After this period the ELISA and biotests are made, and during the time the ELISA results are analysed it is possible to study the upcome of local lesions on the biotest plants. If sowing of bait plants and indicator plants can be made in advance, the time can be shortened to 3 weeks for TRV and 5 weeks for PMTV tests.

Time Schedule	
Sowing of bait plants	4 weeks before planting
Soil drying	2 weeks before planting
Virus acquisition	3 weeks
Indicator sowing	4 weeks before use
Biotest - ELISA	1 week - 2 days
Analysis of results	Analysis of ELISA is done parallell to biotest

Table 4: Time schedule for virus tests of PMTV and TRV by both ELISA and biotest

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APPENDIX I

Results from ELISA for both TRV and PMTV. The x points out dead plants.

TRV Plate 1		TRV Plate 2		TRV Plate 3		PMTV Plate 1		PMTV Plate 2		PMTV Plate 3	
1	X	56	1,003	101	0,195	1	X	56	0,126	101	0,076
2	X	57	1,432	102	X	2	X	57	0,082	102	X
3	0,216	58	0,899	103	X	3	0,040	58	0,116	103	X
4	X	59	2.000	104	X	4	X	59	0,054	104	X
5	X	60	1,056	105	0,308	5	X	60	0,095	105	0,138
6	0,209	61	1,267	106	0,363	6	0,090	61	0,054	106	0,183
7	0,207	62	0,197	107	0,281	7	0,060	62	0,089	107	0,132
8	X	63	0,187	108	X	8	X	63	0,055	108	X
9	X	64	0,238	109	X	9	X	64	0,089	109	X
10	X	65	0,222	110	0,409	10	X	65	0,057	110	0,184
11	0,284	66	0,202	PK	1,567	11	0,130	66	0,099	PK	0,522
12	X	67	0,195	NK	0,329	12	0,040	67	0,047	NK	0,251
13	0,540	68	0,225	B	0,338	13	0,100	68	0,094	B	0,167
14	X	69	0,240			14	X	69	0,057		
15	0,228	70	0,213			15	0,050	70	0,102		
16	X	71	0,160			16	X	71	0,054		
17	0,210	72	0,227			17	0,090	72	0,089		
18	0,378	73	0,188			18	0,041	73	0,056		
19	0,226	74	X			19	0,076	74	X		
20	X	75	0,205			20	0,050	75	0,104		
21	0,312	76	1,392			21	0,456	76	0,050		
22	0,214	77	1,445			22	0,332	77	0,113		
23	0,330	78	1,211			23	0,092	78	0,055		
24	0,197	79	1,473			24	0,192	79	0,095		
25	0,279	80	0,958			25	1,042	80	0,055		
26	0,170	81	0,218			26	0,044	81	0,080		
27	X	82	0,221			27	0,352	82	0,056		
28	0,194	83	0,199			28	0,054	83	0,108		
29	0,216	84	0,194			29	0,118	84	0,047		
30	0,171	85	0,378			30	0,059	85	0,088		
31	0,200	86	0,291			31	0,349	86	0,124		
32	0,192	87	2.000			32	FULL	87	0,133		
33	0,225	88	0,211			33	0,090	88	0,055		
34	0,173	89	0,247			34	0,039	89	0,083		
35	0,190	90	0,175			35	0,613	90	0,060		
36	0,169	91	0,851			36	0,245	91	0,098		
37	0,189	92	1,340			37	0,144	92	0,062		
38	0,141	93	1,012			38	0,036	93	0,102		
39	0,229	94	1,292			39	0,082	94	0,055		
40	0,176	95	0,468			40	0,049	95	0,108		
41	0,713	96	1,318			41	0,110	96	0,045		
42	1,295	97	0,747			42	0,045	97	0,088		
43	0,450	98	0,538			43	0,098	98	0,061		
44	1,579	99	X			44	0,057	99	X		
45	0,767	100	0,183			45	0,103	100	0,104		
46	1,340	PK	2.000			46	0,040	PK	0,574		
47	0,220	NK	0,149			47	0,082	NK	0,105		
48	0,647	B	0,203			48	0,053	B	0,098		
49	0,197					49	0,100				
50	0,694					50	0,040				
51	1,689					51	0,081				
52	0,208					52	0,055				
53	0,217					53	0,098				
54	1,636					54	0,039				
55	0,332					55	0,089				
PK	2.000					PK	2.000				
NK	0,206					NK	0,067				
B	0,270					B	0,093				

Results from ELISA for both TRV and PMTV on each individual bait plant

N.Debneyi	TRV	PMTV	N.Clevelandii	TRV	PMTV	N.Tabacum	TRV	PMTV	N.Benthamiana	TRV	PMTV	Petunia	TRV	PMTV
6	0,209	0,090	7	0,207	0,060	3	0,216	0,040	19	0,226	0,076	15	0,228	0,050
11	0,284	0,130	12	0,146	0,040	13	0,540	0,100	24	0,197	0,192	20	0,176	0,050
21	0,312	0,456	17	0,210	0,090	18	0,378	0,041	29	0,216	0,118	25	0,279	1,042
26	0,170	0,044	22	0,214	0,332	23	0,330	0,092	34	0,173	0,039	30	0,171	0,059
31	0,200	0,349	27	0,205	0,352	28	0,194	0,054	39	0,229	0,082	35	0,190	0,613
36	0,169	0,245	32	0,192	2,000	33	0,225	0,090	44	1,579	0,057	40	0,176	0,049
41	0,713	0,110	37	0,189	0,144	38	0,141	0,036	49	0,197	0,100	45	0,767	0,103
46	1,340	0,040	42	1,295	0,045	43	0,450	0,098	54	1,636	0,039	50	0,694	0,040
51	1,689	0,081	47	0,220	0,082	48	0,647	0,053	59	2,000	0,054	55	0,332	0,089
56	1,003	0,126	52	0,208	0,055	53	0,217	0,098	64	0,238	0,089	60	1,056	0,095
61	1,267	0,054	57	1,432	0,082	58	0,899	0,116	69	0,240	0,057	65	0,222	0,057
66	0,202	0,099	62	0,197	0,089	63	0,187	0,055	79	1,473	0,095	70	0,213	0,102
71	0,160	0,054	67	0,195	0,047	68	0,225	0,094	84	0,194	0,047	75	0,205	0,104
76	1,392	0,050	72	0,227	0,089	73	0,188	0,056	89	0,247	0,083	80	0,958	0,055
81	0,218	0,080	77	1,445	0,113	78	1,211	0,055	94	1,292	0,055	85	0,378	0,088
86	0,291	0,124	82	0,221	0,056	83	0,199	0,108				90	0,175	0,060
91	0,851	0,098	87	2,000	0,133	88	0,211	0,055				95	0,468	0,108
96	1,318	0,045	92	1,340	0,062	93	1,012	0,102				100	0,183	0,104
101	0,195	0,076	97	0,747	0,088	98	0,538	0,061				105	0,308	0,138
106	0,363	0,183	107	0,281	0,132							110	0,409	0,184
Total	12,34	2,53		11,17	4,09		8,00	1,40		10,14	1,18		7,58	3,19
Mean	0,62	0,13		0,56	0,20		0,42	0,07		0,68	0,08		0,38	0,16

Results from ELISA for both TRV and PMTV on each individual bait plant

N.Debneyi	PMTV	PMTV/Mean	N.Clevelandii	PMTV	PMTV/Mean	N.Tabacum	PMTV	PMTV/Mean	N.Benthamiana	PMTV	PMTV/Mean	Petunia	PMTV	PMTV/Mean
6	0,090	0,71	7	0,060	0,29	3	0,040	0,54	19	0,076	0,97	15	0,050	0,31
11	0,130	1,03	12	0,040	0,20	13	0,100	1,36	24	0,192	2,43	20	0,050	0,31
21	0,456	3,60	17	0,090	0,44	18	0,041	0,56	29	0,118	1,50	25	1,042	6,54
26	0,044	0,34	22	0,332	1,62	23	0,092	1,24	34	0,039	0,49	30	0,059	0,37
31	0,349	2,76	27	0,352	1,72	28	0,054	0,73	39	0,082	1,04	35	0,613	3,85
36	0,245	1,94	32	2,000	9,79	33	0,090	1,22	44	0,057	0,72	40	0,049	0,31
41	0,110	0,87	37	0,144	0,70	38	0,036	0,49	49	0,100	1,27	45	0,103	0,65
46	0,040	0,32	42	0,045	0,22	43	0,098	1,32	54	0,039	0,50	50	0,040	0,25
51	0,081	0,64	47	0,082	0,40	48	0,053	0,72	59	0,054	0,69	55	0,089	0,56
56	0,126	0,99	52	0,055	0,27	53	0,098	1,32	64	0,089	1,13	60	0,095	0,59
61	0,054	0,42	57	0,082	0,40	58	0,116	1,57	69	0,057	0,72	65	0,057	0,35
66	0,099	0,78	62	0,089	0,43	63	0,055	0,74	79	0,095	1,20	70	0,102	0,64
71	0,054	0,43	67	0,047	0,23	68	0,094	1,28	84	0,047	0,59	75	0,104	0,65
76	0,050	0,40	72	0,089	0,44	73	0,056	0,76	89	0,083	1,06	80	0,055	0,34
81	0,080	0,63	77	0,113	0,55	78	0,055	0,75	94	0,055	0,70	85	0,088	0,55
86	0,124	0,98	82	0,056	0,27	83	0,108	1,46				90	0,060	0,38
91	0,098	0,78	87	0,133	0,65	88	0,055	0,74				95	0,108	0,68
96	0,045	0,35	92	0,062	0,30	93	0,102	1,38				100	0,104	0,65
101	0,076	0,60	97	0,088	0,43	98	0,061	0,82				105	0,138	0,86
106	0,183	1,45	107	0,132	0,64							110	0,184	1,15
Total	2,53			4,09			1,40			1,18			3,19	
Mean	0,13			0,20			0,07			0,08			0,16	

Results from ELISA for both TRV and PMTV on each individual bait plant

N.Debneyii	TRV	TRV/Mean	N.Clevelandii	TRV	TRV/Mean	N.Tabacum	TRV	TRV/Mean	N.Benthamiana	TRV	TRV/Mean	Petunia	TRV	TRV/Mean
6	0,209	0,338	7	0,207	0,371	3	0,216	0,512	19	0,226	0,334	15	0,228	0,601
11	0,284	0,459	12	0,146	0,261	13	0,540	1,282	24	0,197	0,292	20	0,176	0,464
21	0,312	0,505	17	0,210	0,375	18	0,378	0,897	29	0,216	0,320	25	0,279	0,736
26	0,170	0,275	22	0,214	0,382	23	0,330	0,782	34	0,173	0,256	30	0,171	0,451
31	0,200	0,324	27	0,205	0,366	28	0,194	0,459	39	0,229	0,339	35	0,190	0,500
36	0,169	0,274	32	0,192	0,344	33	0,225	0,533	44	1,579	2,337	40	0,176	0,463
41	0,713	1,155	37	0,189	0,338	38	0,141	0,334	49	0,197	0,291	45	0,767	2,023
46	1,340	2,172	42	1,295	2,319	43	0,450	1,068	54	1,636	2,421	50	0,694	1,829
51	1,689	2,737	47	0,220	0,393	48	0,647	1,535	59	2,000	2,960	55	0,332	0,876
56	1,003	1,625	52	0,208	0,372	53	0,217	0,515	64	0,238	0,352	60	1,056	2,783
61	1,267	2,053	57	1,432	2,563	58	0,899	2,133	69	0,240	0,354	65	0,222	0,584
66	0,202	0,327	62	0,197	0,352	63	0,187	0,443	79	1,473	2,179	70	0,213	0,562
71	0,160	0,259	67	0,195	0,348	68	0,225	0,533	84	0,194	0,286	75	0,205	0,541
76	1,392	2,255	72	0,227	0,406	73	0,188	0,446	89	0,247	0,366	80	0,958	2,526
81	0,218	0,353	77	1,445	2,587	78	1,211	2,875	94	1,292	1,912	85	0,378	0,997
86	0,291	0,471	82	0,221	0,395	83	0,199	0,471				90	0,175	0,461
91	0,851	1,378	87	2,000	3,581	88	0,211	0,501				95	0,468	1,233
96	1,318	2,135	92	1,340	2,399	93	1,012	2,403				100	0,183	0,481
101	0,195	0,316	97	0,747	1,338	98	0,538	1,277				105	0,308	0,811
106	0,363	0,588	107	0,281	0,503							110	0,409	1,079
Total	12,34			11,17			8,00			10,14			7,58	
Mean	0,62			0,56			0,42			0,68			0,38	

Correlations: Quinoa; ELISA

```
Pearson correlation of Quinoa and ELISA = 0,696
P-Value = 0,001
```

Regression Analysis: Quinoa versus ELISA

```
The regression equation is
Quinoa = - 1,403 + 15,54 ELISA


S = 8,82894   R-Sq = 48,4%   R-Sq(adj) = 45,4%


Analysis of Variance

Source      DF       SS       MS      F      P
Regression   1  1244,95  1244,95  15,97  0,001
Error       17  1325,15    77,95
Total       18  2570,11
```

Fitted Line: Quinoa versus ELISA

Correlations: Amaranticolor; ELISA

```
Pearson correlation of Amaranticolor and ELISA = 0,696
P-Value = 0,001
```

Regression Analysis: Amaranticolor versus ELISA

```
The regression equation is
Amaranticolor = - 1,403 + 15,54 ELISA


S = 8,82894   R-Sq = 48,4%   R-Sq(adj) = 45,4%


Analysis of Variance

Source      DF       SS       MS      F      P
Regression   1  1244,95  1244,95  15,97  0,001
Error       17  1325,15    77,95
Total       18  2570,11


Fitted Line: Amaranticolor versus ELISA
```

One-way ANOVA TRV: *N.debneyi; N.clevelandii; N.benthamiana; N.tabacum*; Petunia

```
Source  DF      SS     MS     F      P
Factor   4   1,162  0,290  1,20  0,318
Error   89  21,609  0,243
Total   93  22,771

S = 0,4927   R-Sq = 5,10%   R-Sq(adj) = 0,84%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level           N    Mean   StDev  --+---------+---------+---------+-------
N.debneyi      20  0,6173  0,5248             (----------*----------)
N.clevelandii  20  0,5586  0,5870          (----------*----------)
N.benthamiana  15  0,6758  0,6881              (------------*-----------)
N.tabacum      19  0,4215  0,3149    (----------*----------)
Petunia        20  0,3794  0,2730  (----------*----------)
                                  --+---------+---------+---------+-------
                                  0,20      0,40      0,60      0,80

Pooled StDev = 0,4927


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99,35%


N.debneyi subtracted from:

                Lower   Center   Upper
N.clevelandii  -0,4929  -0,0588  0,3754
N.benthamiana  -0,4104   0,0585  0,5274
N.tabacum      -0,6356  -0,1958  0,2440
Petunia        -0,6720  -0,2379  0,1962

               ---------+---------+---------+---------+
N.clevelandii         (----------*---------)
N.benthamiana           (----------*-----------)
N.tabacum         (----------*----------)
Petunia         (----------*----------)
               ---------+---------+---------+---------+
                     -0,40      0,00      0,40      0,80


N.clevelandii subtracted from:

                Lower   Center   Upper
N.benthamiana  -0,3516   0,1173  0,5861
N.tabacum      -0,5769  -0,1371  0,3027
Petunia        -0,6133  -0,1791  0,2550

               ---------+---------+---------+---------+
N.benthamiana           (-----------*-----------)
N.tabacum          (----------*----------)
Petunia           (----------*---------)
               ---------+---------+---------+---------+
                     -0,40      0,00      0,40      0,80
```

```
N.benthamiana subtracted from:

             Lower   Center   Upper  ---------+---------+---------+---------+
N.tabacum  -0,7285  -0,2543  0,2198   (-----------*----------)
Petunia    -0,7653  -0,2964  0,1725  (-----------*----------)
                                     ---------+---------+---------+---------+
                                           -0,40      0,00      0,40      0,80


N.tabacum subtracted from:

           Lower   Center   Upper  ---------+---------+---------+---------+
Petunia  -0,4819  -0,0421  0,3977         (----------*----------)
                                     ---------+---------+---------+---------+
                                          -0,40      0,00      0,40      0,80
```

One-way ANOVA PMTV: *N.debneyi; N.clevelandii; N.benthamiana; N.tabacum*; Petunia

```
Source  DF      SS      MS     F      P
Factor   4  0,2275  0,0569  1,03  0,394
Error   89  4,8942  0,0550
Total   93  5,1217

S = 0,2345   R-Sq = 4,44%   R-Sq(adj) = 0,15%


                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level           N    Mean   StDev  ----+---------+---------+---------+-----
N.debneyi      20  0,1267  0,1082       (----------*---------)
N.clevelandii  20  0,2046  0,4311               (---------*----------)
N.benthamiana  15  0,0789  0,0392  (-----------*-----------)
N.tabacum      19  0,0739  0,0265   (---------*----------)
Petunia        20  0,1595  0,2414           (---------*---------)
                                  ----+---------+---------+---------+-----
                                    0,00      0,10      0,20      0,30

Pooled StDev = 0,2345


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons

Individual confidence level = 99,35%


N.debneyi subtracted from:

                Lower   Center   Upper
N.clevelandii  -0,1287   0,0779  0,2844
N.benthamiana  -0,2710  -0,0478  0,1753
N.tabacum      -0,2621  -0,0528  0,1565
Petunia        -0,1738   0,0328  0,2394

               -------+---------+---------+---------+--
N.clevelandii             (---------*---------)
N.benthamiana     (-----------*----------)
N.tabacum          (---------*----------)
Petunia                (----------*---------)
               -------+---------+---------+---------+--
                   -0,20      0,00      0,20      0,40
```

```
N.clevelandii subtracted from:

                 Lower   Center   Upper
N.benthamiana  -0,3488  -0,1257  0,0975
N.tabacum      -0,3400  -0,1307  0,0786
Petunia        -0,2516  -0,0451  0,1615

               -------+---------+---------+---------+--
N.benthamiana  (----------*----------)
N.tabacum      (---------*----------)
Petunia            (----------*---------)
               -------+---------+---------+---------+--
                   -0,20      0,00      0,20      0,40


N.benthamiana subtracted from:

             Lower   Center   Upper  -------+---------+---------+---------+--
N.tabacum  -0,2306  -0,0050  0,2207       (-----------*----------)
Petunia    -0,1425   0,0806  0,3038            (----------*----------)
                                    -------+---------+---------+---------+--
                                        -0,20      0,00      0,20      0,40


N.tabacum subtracted from:

           Lower  Center   Upper  -------+---------+---------+---------+--
Petunia  -0,1237  0,0856  0,2949            (---------*----------)
                                  -------+---------+---------+---------+--
                                      -0,20      0,00      0,20      0,40
```