Smart Disease Detection Seed Potatoes 2015 – 2018

Detection of virus and bacterial diseases using vision and sensor technology

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3 PhenoVation

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Samenvatting

Virus- en bacterieziekten vormen één van de grootste problemen in de pootaardappelteelt. Eenmaal aangetroffen in het veld kunnen viruszieke (Y-virus) en Erwinia zieke aardappelen leiden tot afkeuringen van partijen pootgoed met een financiële schade tot gevolg. De directe schade door Erwinia aandoeningen bedraagt voor de pootgoedtelers jaarlijks ca. € 12 miljoen.

De huidige detectie van ziek pootgoed vindt plaats door menselijke selecteurs. Jaarlijks wordt er in Nederland voor ongeveer € 6.5 miljoen aan kosten gemaakt voor handmatige selectie om de ziektedruk op het veld onder controle te houden. Veel schade ontstaat doordat de ziekte niet in een vroeg stadium wordt ontdekt. Daarom bestaat er de behoefte naar een snelle en adequate ziektedetectie. Een vroege detectie van zieke planten met moderne vision technieken kan de kosten voor selectie flink drukken. De nadruk ligt hierbij op de detectie van Erwinia, gezien de grote financiële schade.

Na een voorloopproject in 2010 en 2011 is het voorliggende onderzoek gestart als PPS van de Topsector Agro & Food als onderdeel van het onderzoeksprogramma "op naar precisielandbouw 2.0" met als partners BO-Akkerbouw / LTO Nederland, Kverneland, Agrico, HZPC, NAK en Phenovation BV met als looptijd 2015 – 2018.

Het doel van het project is het ontwikkelen van een smart ziekzoeker in pootaardappelen, een toepassing die in staat is om virus- en Erwinia zieke aardappelplanten te herkennen. Om tot een succesvol gebruik in de praktijk te komen, worden op voorhand hoge eisen gesteld aan de mate waarin deze zieke planten herkend worden. Immers, voorkomen moet worden dat bij controle door de NAK dient een veld pootaardappelen na "machinale" inspectie wordt afgekeurd.

De gedachte bij de start van het project was om achtereenvolgens:
1. onder lab omstandigheden diverse technieken te testen.
2. met de meest kansrijke toepassingen verder te gaan.
3. na 1-2 jaar in het lab de stap te maken naar het veld en de technologie te testen onder praktijkomstandigheden.
4. langs deze weg na 4 jaar een prototype te hebben dat als basis kan dienen voor het ontwikkelen van een commerciële toepassing door het bedrijfsleven.

Bij de uitvoering is deze lijn ook herkenbaar. Echter, de technische ontwikkeling van een voldoende goed werkend prototype vraagt een langere doorlooptijd.

In 2015 is gestart met een brede(re) verkenning van mogelijk geschikte technieken voor de herkenning van Erwinia en virus zieke planten. In het laboratorium metingen verricht aan in potten geteelde zieke en gezonde planten. Hierbij zijn 5 technieken benut:

1. Spectrale camera techniek

Voor het onderzoek is gebruikt gemaakt van een hyperspectraal camera (193 banden van 3 nm) in een opstelling, die scans maakt van de zijkant van de plant, om zodoende zowel de onderste als de bovenste delen van de plant te kunnen analyseren. Als onderdeel van het onderzoek is ook geanalyseerd of op eenvoudige wijze pixels van blad en stengel te onderscheiden zijn (voor Erwinia waarschijnlijk relevant). Dit blijkt eenvoudig te zijn door een zogenoemde lineaire classifier te trainen op het reflectiespectrum.

Viruszieke planten blijken goed vroegtijdig te herkennen te zijn. De resultaten zijn in de eerste meetweek gelijk aan de score van de gewas expert. Pixels uit de bladeren dragen iets meer bij aan het resultaat dan pixels uit de stengel. Bovendien blijken pixels uit het onderste deel van de plant beter resultaat te geven dan pixels die hoger in het gewas liggen.

Detectie van Erwinia blijkt met deze techniek heel moeilijk te detecteren. Belangrijkste reden is dat de symptomen heel lokaal zijn, zoals verwelking van top bladeren, zwartbenigheid in het onderste deel van de stengel. De analyse is gedaan op een random selectie van blad en stengel pixels van de hele plant.

Geconcludeerd is dat deze techniek tot goede resultaten leidt voor de detectie van viruszieke planten en daarom in het verdere onderzoek meegenomen is.

2. Chlorofyl Fluorescentie techniek

De meetresultaten met de chlorofylfluorescentie methode zijn op hoofdlijn vergelijkbaar met die van de spectrale techniek, zowel voor viruszieke als Erwinia zieke planten. Een voorwaarde voor dit resultaat is dat de planten geadapteerd moeten zijn aan een laag niveau van licht (bij voorkeur minimaal een half uur). Dit is een probleem voor meten in de praktijksituatie: alleen 's nachts meten

met een ziekzoekrobot is vooralsnog geen reële optie. Dit is de reden om deze techniek, die niet wezenlijk beter "presteert" dan (hyper)spectraal opnames. Daarom is deze techniek niet verder mee genomen in het onderzoek.

3. Thermische camera

De hypothese dat verwelkende Erwinia planten uit het veld een groter temperatuurverdeling laten zien binnen de plant blijkt juist. Bij een van de experimenten bleek dat planten die zijn opgegroeid in de proefruimte geen temperatuurverschil tussen Erwinia ziek en gezond laten zien. Bij jonge planten die met Erwinia geïnfecteerd zijn laten de onderste en oudere bladeren een verstoorde bladafkoeling / wateropname zien in vergelijking met de gezonde planten.
De verschillen zijn meetbaar in een geconditioneerde omgeving. In absolute zin zijn de verschillen in bladtemperatuur klein, zodat bij een vertaling naar een praktijktoepassing temperatuurreacties van een plant in buitencondities sterker gerelateerd zullen zijn aan zonstraling, temperatuurwisselingen, luchtvochtigheid en bodemvocht dan aan plant-infecties. Daarnaast vraagt de techniek real-time datalogging en zijn beeldcorrecties nodig. Dit maakt het minder kansrijk om deze techniek in te zetten in een praktijkomgeving, zeker niet als leidende (discriminerende) techniek, hoogstens als een ondersteunende. Deze techniek is daarom in het vervolgonderzoek niet verder meegenomen.

4. 3D techniek

Deze techniek is niet toegepast op virus besmette planten omdat het virusziek minder invloed heeft op de groei en groeisnelheid van een plant dan bij Erwinia zieke planten. De experimenten met de 3D camera tonen aan dat het plantoppervlak en de groeisnelheid bij Erwinia geïnfecteerde planten, bepaald uit het kleurenbeeld, significant kleiner is dan die van gezonde planten. Daarbij blijken de Erwinia geïnfecteerde planten minder compact zijn dan gezonde planten. Per saldo heeft een Erwinia geïnfecteerde plant een significant kleiner volume dan een gezonde plant.
Met een 3D camera met additionele kleurbeelden kan goed onderscheid gemaakt worden tussen Erwinia ziek en gezond, mits dit gebeurt over een tijdreeks. De techniek lijkt geschikt voor ziekzoeken, mits een groeiachterstand tijdig in het groeiseizoen kan worden vastgesteld. Deze techniek is daarom in het vervolgonderzoek verder meegenomen.

5. Force-A techniek

Force-A beschikt over een 2 typen handmeters, die veel in de druiventeelt worden ingezet voor de detectie van meeldauw aantasting en rijpheid van de druif. Uit een 1 op 1 vertaling naar Erwinia detectie komt naar voren dat deze technieken geen onderscheidend vermogen tussen Erwinia zieke en gezonde planten als het gaat om de chlorofyl index en de flavonolen. Een nadeel van de Force-A sensoren is dat zij zogenoemde spotmetingen doen en omdat ze niet beeldvormend (niet de hele plant wordt gemeten) worden mogelijk zieke plekken gemist. De huidige MX-330 en MX-375 zijn in de huidige uitvoering contactsensoren en dus minder geschikt voor veldmetingen.

In deze vorm zijn Force-A sensoren niet zinvol inzetbaar. De basis van de Force-A benadering is het zoeken naar specifieke afbraakproducten die te detecteren zijn. Dit vraagt om een nadere analyse van afbraakstoffen van de Erwinia bacterie en te verkennen of hiervoor een meetmethode te ontwikkelen is. Dit maakt deze techniek minder passend in het lopende project.

2016

In 2016 is het onderzoek voortgezet met de hyperspectraal techniek en de 3D techniek. Hiervoor is in navolging van 2015 opnieuw een pottenproef ingezet met zowel virus- als Erwinia zieke planten. Dit keer een grotere proef om tot statistisch betere resultaten te komen. Opvallend is wel dat de planten besmet met Ssp Brasiliensis nauwelijks opkwamen. De planten met Ssp atroseptica kwamen relatief goed op en die bemest met Dsolani duidelijk minder dan de Ssp atroseptica. 41,5%Verder viel op dat de planten in de meetperiode gedrongen waren: pas na afloop van de meetperiode kwamen de planten versneld in groei.

De resultaten liggen in lijn met die van 2015 voor herkenning van viruszieke planten. Voor de verbetering van de Erwinia herkenning zijn in dit jaar een serie technieken binnen de 3D omgeving verkend (zogenoemde features, zoals de convex hull benadering, volume bepaling) die vervolgens in een machine learning algoritme zijn meegenomen. In dit jaar is ook getest of de zogenoemde 2,5D methode (combinatie van 2D met diepte informatie) een vergelijkbaar resultaat geeft dan een echte 3D informatie. De correlatie blijkt hoog te zijn, waardoor snellere en goedkopere methoden binnen bereik komen. Het aantal goed geclassificeerde planten lag in 2016 op 87,3%. Echter het percentage goed gedetecteerde Erwinia planten lag op slechts 41,5%.

2017

In 2017 is de stap gezet naar metingen in het veld. Dit vond plaats op een proeflocatie van de NAK in Emmeloord waar een zevental rijen van 110 en 66m zijn geplant met een relatief hoge besmettingsgraad van Erwinia en virus. In de aanloop naar het seizoen is een speciale meetunit ontworpen die 2 rijen tegelijk kan meten d.w.z. 1 rij met een 3D camera (Ensenso) en 1 rij met een hyperspectrale camera (Specim FX10). Deze camera's zijn afgeschermd van daglicht en voorzien led- resp. halogeen verlichting.
Bij de analyse van de datasets is gefocust op de data van rij 6 en 7 (resp. ras Vermont met PVY en PCR/11 met PVY). De data zijn geanalyseerd met behulp van zogenoemde Deep Learning methodiek. Het Deep Learning systeem is getraind met data van rij 2 en 3 en vervolgens zijn de planten van rij 6 en 7 met het algoritme beoordeeld. De resultaten uitgedrukt in het percentage goed gescoorde

planten (zowel ziek als gezond) ligt in de verschillende meetweken tussen de 89 en 93%. De resultaten uitgedrukt in het nauwkeurigheid van alleen goed gescoorde zieke planten ("goed gescoord" / "goed gescoord + fout gescoord") ligt aanmerkelijk lager nl. 40-53% in de eerste 3 meetweken (slechts 23% in de 4e meetweek). Ten behoeve van het artikel Potato Virus Y detection in Seed potatoes Using Deep Learning on Hyperspectral Images (Polder et all, 2019) is ook een analyse gemaakt waarin gecorrigeerd is voor vervuiling van de data door beeldmateriaal van buur-planten. Na correctie stijgt de nauwkeurigheid van de eerste meetweken naar 78 – 92% (slechts 30% in de 4e meetweek). De eerste stijging is spectaculair en kan verklaard worden door de effecten van door elkaar heen groeiende planten en kleine onnauwkeurigheden in GPS locaties van elke plant). Een verklaring voor de lage nauwkeurigheid in de 4e week is op dit moment niet te geven, maar verdient wel aandacht in een vervolgproject.

De analyse van de Erwinia zieke planten vond in 2017 ook plaats met machine learning technieken. De best werkende techniek (multi-layered perceptron) liet een percentage van goed gescoorde planten zien van 87%. Echter, de nauwkeurigheid van uitsluitend besmette planten laat te wensen over: slechts 41% van de zieke planten wordt goed geclassificeerd. Het is duidelijk dat deze resultaten moeten verbeteren om tot een praktijkwaardige toepassing te komen.

2018

In 2018 zijn de metingen in het proefveld van NAK herhaald, nu met meer planten en een iets grotere plantafstand (50cm). Dit laatste is gedaan om gedurende een groter deel van het teeltseizoen beeld van separaat groeiende planten kunt meten. Opnieuw is gemeten met dezelfde meetunit met zowel de hyperspectrale meettechniek als de 3D techniek. Afwijkend ten opzichte van 2017 is de gekozen analyse techniek voor Erwinia. Gestimuleerd door de goede resultaten van de Deep Learning techniek voor virusziekte planten is deze in 2018 ook gebruikt voor de analyse van de Erwinia planten.

Voor virusdetectie zijn in 2018 vergelijkbare scores gevonden als in 2017. Het percentage van goed geclassificeerde planten ligt tussen 89 en 93%. Ook de nauwkeurigheid van de beoordeling van zieke planten ligt in de eerste 3 meetweken op een goed niveau van 55-63%, terwijl de nauwkeurigheid in de 4e week tegenvalt (34%). Het lijkt erop dat de slijtage van het bladpakket in de latere weken leidt tot een slechtere detecteerbaarheid van viruszieke planten.

Bij de analyse van de Erwinia zieke planten is zoals aangegeven gekozen voor een analyse op basis van het Deep Learning algoritme. Hiervoor zijn 2 systemen gebruikt, de zogenoemde ResNet 18 en Resnet 50. Met de eerste wordt een hele hoog percentage van de planten goed gescoord (95%) tegenover 82% met Resnet 50. Deze percentages zijn overigens verrassend goed. Ook de nauwkeurigheid van ziek geclassificeerde planten ligt hoog (92% bij Resnet 18 en 80% bij Resnet 50).

Samenvatting

Samenvattend kan gesteld worden dat de afgelopen 4 jaar flinke vorderingen zijn gemaakt in de detectie van virus en Erwinia zieke planten. Dankzij de benutting van Deep Learning technologie zijn de percentages goed gescoorde planten op een hoog niveau beland. Vooral op het terrein van Erwinia detectie is vooruitgang geboekt. De nauwkeurigheid van de detectie van Erwinia zieke planten is sterk gestegen.
Opvallend is de goede score op viruszieke planten in het begin van het seizoen terwijl die later in het seizoen sterk wegzakt. Dit vraagt nader onderzoek.

Voor de vertaling naar een werkend prototype is meer tijd nodig. Het detectiesysteem moet robuuster worden. Deep learning vraagt om een grote(re) dataset waarin allerlei rassen, de verschillende relevante Erwinia (3) en virussoorten worden meegenomen. Daarnaast zijn er vragen ten aanzien van het vereiste kwaliteitsniveau: wanneer voldoet het systeem aan de gangbare NAK eisen?

De cameratechnologie zal ook de komende jaren snel veranderen. In het project is gewerkt met relatief dure en langzame hyperspectraal techniek. Voor Deep Learning wordt doorgaans gebruik gemaakt van RGB camera-beelden. Het is de vraag of dit ook voor de toekomst de geëigende techniek zal zijn, die een grote(re) werksnelheid mogelijk maakt.

Het verdient aanbeveling om nader onderzoek te starten waarin bovenstaande vragen centraal staan.

Summary

Virus and bacterial diseases are one of the biggest problems in seed potato cultivation. Once found in the field, virus-sick (Y-virus) and Erwinia-sick potatoes can lead to rejections of batches of seed potatoes resulting in financial damage. The direct damage caused by Erwinia disorders is approximately € 12 million annually for seed potato growers.

The current detection of diseased seed is done by human selectors. Annually, about € 6.5 million in costs is incurred in the Netherlands for manual selection to keep disease pressure on the field under control. A lot of damage occurs because the disease is not detected at an early stage. That is why there is a need for rapid and adequate disease detection. Early detection of diseased plants with modern vision techniques can considerably reduce the costs of selection. The emphasis is on the detection of Erwinia, given the major financial damage.

The project's objective is to develop a smart disease detection system in seed potatoes, an application that is able to recognize potato and Erwinia disease potato plants. In order to be successful in practice, high quality demands must be met. After all, when inspected by the NAK, a field of seed potatoes should not be rejected after "machine" inspection.

The basic idea of the project was to start with testing various techniques under lab conditions and to continue with the most promising ones. After 1-2 years in the lab to make the step to the field and to test the technology under practical conditions. And by the end of a project aiming at having available a prototype that can serve as a basis for the development of a commercial application by the business community.

In the first year 5 techniques were explored: hyperspectral imaging, chlorophyll fluorescence, 3D, thermal camera's and finally an existing technology developed by Force-A (a French company active in the vineyards). After analyzing the potential and limitations of the techniques, both the hyperspectral imaging and 3D were selected as the most promising technologies. In the next 3 years, first another year with lab tests was performed in order to optimize the data capturing and analysis. Potato plants in pots inoculated with viruses and Erwinia were used for this purpose.
In year 3 and 4 more practical field trials were conducted with a bigger number of plants. For this purpose a measuring device was developed capturing hyperspectral data (Specim FX10 camera) and 3D data (Ensenso).

In all four years good results were obtained for detecting virus. Small improvements were obtained in year 3 and 4 by using Deep Learning technology. At the end the percentage of well-classified plants varied between 89 and 93%. The accuracy of detection of sick plants is better in the early stage of the selection season and at a good level of 55-63%, while the accuracy in a later stage is disappointing (34%). It seems that the wear of the leaf package in the later weeks leads to a poorer detectability of virus-sick plants.

For Erwinia the results in the first years were suboptimal. It took until the fourth year that a significant improvement was achieved by using the Deep Learning approach for data analysis. Two systems were used for this, the so-called ResNet 18 and Resnet 50. With the first, a very high percentage of the plants was scored well (95%) compared to 82% with Resnet 50. These percentages are surprisingly good. The accuracy of sick classified plants is also high (92% with Resnet 18 and 80% with Resnet 50).

Resume
In summary, it can be said that considerable progress has been made in the past 4 years in the detection of virus and Erwinia diseased plants. Thanks to the use of Deep Learning technology, the percentages of well-scored plants have reached a high level. Progress has been made especially in the area of Erwinia detection. The accuracy of the detection of Erwinia diseased plants strongly improved.

The good score on virus-sick plants at the start of the season is striking, while it sinks sharply later in the season. This requires more attention in the future.

More time is needed for the translation to a working prototype. The detection system must become more robust. Deep learning requires a large(r) data set that includes different potato varieties, the various relevant Erwinia (3) and virus types. In addition, there are questions about the required quality level: when does the system meet the NAK requirements?

The camera technology will also change rapidly in the coming years. The project has worked with relatively expensive and slow hyperspectral technology. For Deep Learning, RGB camera images are often used. The question is whether this will also be the appropriate technology for the future, which makes a large(r) working speed possible.

It is recommended to start further research on the above mentioned questions.

1 Introduction

In temperate regions, the main diseases of the seed potato crop are caused by viruses and bacterial infections (Dickeya and Pectobacterium). In the Netherlands, the world's major supplier of certified seed potatoes, these two diseases are responsible for an average 14.5% declassification of seed lots (over the period 2009-2016) and an average 2.3% rejection (source: Dutch General Inspection Service NAK). This results in a total value decrease of almost 20 million euros per year for all Dutch producers.

A potato crop can be challenged by various viral pathogens resulting in a broad spectrum of different symptoms. Potato virus Y (PVY, genus Potyvirus, family Potyviridae), is one of the most prevalent and important viruses in potatoes globally (Valkonen, 2007) and is in the top–ten of most damaging plant viruses (Scholthof, *et. al.*, 2011). Different strains of PVY have been identified that vary in symptom expression, including mosaic leaf discolorations caused by PVY^{O}, stipple streak caused by PVY^{C}, necrotic leaf spots caused by PVY^{N} and PVY^{NTN} and necrotic spots on tubers caused by PVY^{NTN}. (Verma, *et. al.*, 2016). In the Netherlands, PVY^{O}, and the recombinant strains PVY^{NTN} and PVY^{N-Wi} prevail (Verbeek, *et. al.*, 2009).

There is a lack of efficient resistance in cultivated varieties, but symptom expression is variety dependent. Cultivars such as Russet Norkotah and Shepody rarely show symptoms and if so, only very mild symptoms. Nevertheless, infections of these cultivars with PVY often result in a decrease in marketable yield (Hane and Hamm 1999). The symptomless infected plants can also be a reservoir for transmission by aphids (Draper, Pasche and Gudmestad, 2002).

Management of PVY is predominantly based on the use of certified, pathogen free seed, the exclusion of virus infections by roguing of symptomatic plants that can serve as inoculum source, and an early harvest, before winged aphids occur that spread the virus (Woodford, 1992, Robert, *et. al.*, 2000). In addition, sanitizing tools, planters and cultivators, weed control, in particular of solanaceous species, removal of volunteer potato plants and the use of mineral oils to reduce spread of aphids, are used in management practices. Insecticides have a low effect on the transmission of the virus, as the aphids often transmit the PVY before they are killed (Shanks and Chapman 1965, Boquel, *et. al.*, 2013, Boquel, *et. al.*, 2014, Gibson, *et. al.*, 1982, Boiteau and Singh, 1999, Lowery and Boiteau, 1988, Boiteau, *et al.*, 1985).

In order to prevent declassification, farmers put in a lot of effort to detect diseased plants and remove them before inspection by the Dutch General Inspection Service (NAK). The average input of manpower is estimated to be 6,2 hrs/ha (KWIN akkerbouw, 2015). Manual selection by visual observations, is labor intensive and cumbersome, in particular late in the growing season when the crop is fully developed. The cost related to plant selection by farmers is about 8 Million euros per year (40.000ha, 6,2 hrs/ha, av. labor cost: €32,50/hr). In addition, the availability of skilled selection workers is getting more and more a problem. Specialized farmers that are unable to do the selection work themselves have increasing problems hiring extra selection capacity. Furthermore, human inspection is prone to false negatives in which sick/diseased potato plants can be missed. This is especially the case when inspecting potato varieties with mild disease symptoms.

Visual crop inspections are done one to three times annually by staff of national inspection agencies. The reliability of their visual observations compared with a laboratory assay (PCR/ELISA) was found to be high (93%) for symptoms caused by viral diseases (K. Boons, NAK, unpublished results). Precision agriculture together with computer vision technologies can be an alternative for human inspection (Bechar and Vigneault, 2016). High tech vision solutions can mitigate the concerns from the high labour cost and increasing potato devaluation costs. If an autonomous machine can replace a human inspector and meanwhile improve the selection quality, this might provide a new business model that is based on these high-tech solutions.

In 2011 WUR Field Crops carried out a project concerning Disease Detection Seed Potatoes in collaboration with WUR Biometris. It concerned a feasibility study with the following question: "it is possible to detect the symptoms of (latent) Erwinia attacks with modern vision techniques under field conditions at a reliable level?" The development of this technique also included the recognition of virus diseases. The main conclusions of the feasibility study were:

1. virus-sick plants are easily recognizable with the hyperspectral technique. However, improvements are necessary because too many healthy plants are still considered to be sick.
2. The same applies to Erwinia detection: plants with clear symptoms are recognized with the hyperspectral technique. It is striking that diseased plants are found that are not recognized by the expert as sick (no symptoms): where the expert found symptoms in 18 of the 61 diseased plants, the spectral camera finds 33 of the diseased plants. Apparently there are effects that are invisible to the human eye, which are picked up by the spectral camera. Many Erwinia plants also

become diseased with the Chlorophyll Fluorescence technique. However, the current set-up gives a false positive classification in 38% of the cases (incorrectly measured as healthy).

Based on these measurement results and discussions, it can be concluded that the techniques are not yet ready to be put into practice. Further research is needed to reach the following objectives:

- measuring in a young and therefore still open crop, measuring not only from above, but also from the side (stems, lower leaf layers). This fits in well with the desire to detect diseases as early as possible.
- the classification protocol (how to distinguish sick and healthy) can be further optimized by focusing more on relevant parts of the plant.
- the use of both the spectral and the chlorophyll fluorescence camera for Erwinia detection.
- For virus diseases also investigate a combination of spectral measurements and 3-D measurements.

The above experiences have served as a basis for the implementation of the present project. This report describes the test results of the detection of virus and bacterial seed potatoes using various techniques including spectral and chlorophyll fluorescence cameras.

2 Objectives of the project

The objective of the project is test sensor technologies in combination with algorithms that enable early detection of virus and bacterial seed potatoes.

In order to achieve successful use in practice, a high detection level of diseased plants is essential to meet the demands of the NAK who do the field inspection of seed potatoes.

The approach of the project was to:

1. test various techniques under lab conditions (2015).
2. continue with the most promising applications (2016)
3. after 1-2 years in the lab to make the step to the field and to test the technology under practical conditions (2017).
4. reach the stage of developing a prototype that can serve as a basis for the development of a commercial application by (or in close cooperation with) the business community.

This approach can be recognized reading this report. The results of 2015 are published (Kamp et al, 2016). However, the technical development of a prototype with a sufficient high quality level well requires a longer lead time as discussed in chapter 6 – Conclusions, discussion and recommendations.

In the report a description is given of the lab testing using specially prepared pot plants of seed potatoes in the first 2 years and the layout of the field tests performed at the NAK farm in the Noordoostpolder (chapter 3). Next the experiences with the 5 tested sensor technologies were described and what the considerations were to choose for the hyperspectral and 3D technology as the most promising ones (chapter 4). Chapter 5 describes the final results of these technologies in combination with Deep Learning algorithms.

3 Experimental setup plant material

3.1 Aim of the experiment

This experiment aims to detect virus – and bacterial diseased seed potatoes in an early phase of plant growth using digital vision techniques (sensors). This means that these diseased plants will be periodically measured using various vision techniques. All techniques are able to monitor plants in a non-destructive way.

3.2 Experimental set-up

In 2011 it was required to grow plants in a glasshouse in pots. The environmental conditions in the glasshouse compartment strongly influenced plant growth resulting in a phenotype that deviated from plants grown in the field. Therefore, in 2015 and 2016 it was de decided to grow plants in pots outdoors. In 2017 and 2018 field experiments were conducted under conditions more realistic in practice.

2015

Experimental setup "Erwinia's"

Tubers were planted in a light clay soil at two dates with a two-weeks period in between. Pots were frequently watered. Due to a cold spring plant development was slow. Therefore measurements started late. Measurements were mainly done on plants from the 1st planting period. In July a limited number of plants with symptoms were selected from the second series.

44 mini tubers of cultivar Kondor were inoculated with Dickeya solani (strain IPO nr. 2222), using two densities of cells (for detailed information, see annex 1). Plants were grown on root fabric in 7.5 l pots in a light clay in a small field plot at Unifarm (Grebbedijk, Wageningen, the Netherlands). Plants were visually examined for disease symptoms at the days plants were also analyzed with the vision techniques. Plants were sampled, plant material extracted and analyzed by dilution planting on double layer CVP (Helias et al., 2012), to confirm the presence of target bacteria. Results are presented in annex 1, including the expert judgement on visible virus symptoms at different dates (the day before lab measurements with the camera systems.

Experimental set up "Viruses"

Virus (secundary-) infected plants were also grown in pots outdoors. The tubers (cv. Bintje) used, were harvested from virus-infected plants grown in the glasshouse. Part of the tubers were lost as a result of damage by crows. In week 25-27, virus-diseased plants were analyzed with vision techniques. Plants were weekly observed for symptom development. In annex 1 the detailed information is presented, also on the type of leaf symptoms connected to 6 potato viruses that were used (like necrotic lesions, yellow spots, leaf roll, crinkle and mosaic).

2016

Experimental set up "Erwinia's"

In 2015 seed potatoes of the susceptible cultivar Kondor were vacuum inoculated with Dickeya solani and grown in pots with clay soil. This only resulted in a limited number of symptomatic plants.
To increase the disease incidence the following was done:

a. we used a light soil instead of a heavy clay (advantage increased warming of soil)
b. supply of less water (advantage less cooling and more drought stress)
c. use of more aggressive bacteria (*P. atrosepticum* and *P. c.* subsp. *brasiliense)*.

Bacteria were grown as described for the experimental set up in 2015. Plants were inoculated with Dickeya solani IPO2222, Pectobacterium atrosepticum IPO1007 or with P. carotovorum subsp. brasiliense IPO3649 or treated with water. For each treatment 40 tubers were planted but only 8 tubers for the water control. Inoculations were evaluated by dilution plating of periderm extracts on double layer CVP (Helias et al., 2012). Typical colonies were found in extracts of the inoculated tubers but not in the extracts of the water control. More details are described in annex 1.

The percentages emerged plants varied largely per treatment and day of planting. At the tubers planted on the 10th of May, between 65% for P. c. subsp. brasiliense and 95% for the other Treatments (Table 3). For tubers planted on the 24th of May the percentage emerged plants were between 20% for P. c. subsp. brasiliense and 100% for the water control.
After emergence the plants inoculated with D. solani and P. c. subsp. brasiliense developed poorly and a strong and rapid symptom development became visible (Fig.1). Frequently, plants hardly grew above the edge of the pots. Plants in inoculated with P. atrosepticum developed much better; the disease incidence was lower and symptom development was more delayed. The water controls remained free of symptoms.
A selection of plants were used for the measurements (Table 4). Plants were measured 1 to 9 times. Plants showed different symptoms, including leaf chlorosis, folding of top leaves, wilting, stem rot and blackleg.

Table 1 *Percentage emerging plants inoculated with Dickeya solani (Dsol), Pectobacterium Atrosepticum (Patro) or P. carotovorum subsp. Brasiliense (Pcb) or treated with water and planted on two different moments (N=20)*

Planting date	Water	Dsol 2222	Patro 1007	Pcb 3649
10-5-2016	95	95	95	65
24-5-2016	100	35	85	20

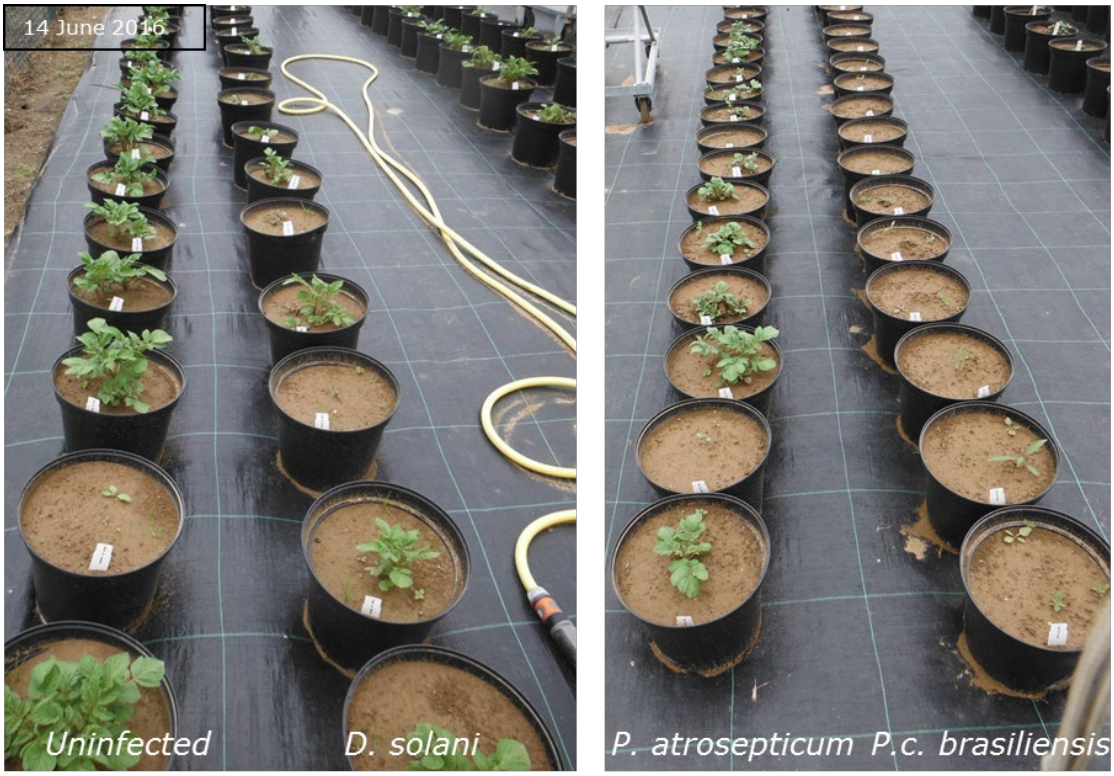


Figure 1 *Situation of the pot experiment on 14 June 2016. Tubers are inoculated with various blackleg causing bacteria or treated with water.*

Experimental set up "Viruses"
In 2015 tubers infected with different viruses were used. During a meeting of the steering committee it was decided that for the follow up research cultivars will be used which differ in susceptibility to PVYNTN. In addition also plants will be used with primary virus infections.

For the availability of secondary infected tubers virus-infected tubers were planted on the 10th of May as indicated for the tubers inoculated with bacteria. The following seed tubers were used: 30 tubers of cultivar Bintje infected with different strains (Y753, Y755, Y772 of Y773) of PVYNTN produced at Wageningen UR, and 9 tubers of cultivar Gineke with a field infection with a PVY complex provided by one of the project partners. In addition, respectively 25 and 9 virus-free tubers were used of cultivars Bintje and Gineke (Table 6). Plants with primary infections were planted in pots on the 10th of May, respectively 25 and 50 virus-free tubers of cultivars Bintje and Kondor were planted in pots as described for tubers inoculated with bacteria. On the 28th of June 25 plants of cultivar Bintje and 25 plants of cultivar Kondor were inoculated with PVYNTN (Bos, 1965).

Plant growth was done as described for the plants inoculated with bacteria. Plants were observed four times for symptom development using the following categories: symptomless (not inoculated), symptomless (inoculated), mosaic, necrosis, crinkle.

Annex 1 gives an overview of the number of plant scans and the symptoms that were shown by the plants. Also data is presented of primary infected plants (the number of plants infected per type of PVY, the emergence and the number of scans performed). All plants were tested for the presence of the PVY viruses using ELISA.

2017
In 2017 and 2018, observations and measurements were done under 'real world' conditions on experimental fields located in the Noordoostpolder (light clay soil, NAK, Tollebeek, the Netherlands). On 11 May, tubers were planted at a distance of 33 cm between tubers in the row. Table 2 shows information on lay out of the experimental field with the cultivars used, the number of tubers and the inoculum per treatment. PVY and seed lots naturally infected with Erwinia were selected by the NAK on the basis of a relatively high infection level according to a laboratory assay. As a reference and control, also minitubers of cultivar Kondor were planted (100 tubers per treatment), inoculated with suspensions (107 cells/ml) of Dickeya solani IPO2222, Pectobacterium atrosepticum IPO1007, or P. carotovorum subsp. brasiliense IPO3649 using a vacuum inoculation method developed at HZPC. Per treatment, plants were distributed over 4 rows of 25 plants per row. Between the treatments tow plant positions were left open to avoid cross-contaminations. After inoculation four tubers per treatment were analyzed for the presence of target bacteria by dilution plating (undiluted and 10-times diluted on double layer CVP, a semi-selective medium. No or hardly any typical colonies grew on the medium indicating that the inoculations had failed.

Table 2 *Description of the testing field in 2017 with naturally infected plant material.*

Row	Cultivar	Row length (m)	Number of tubers	Inoculum
1	Kondor	110	333	Erwinia
2	Kondor	110	333	Erwinia
3	Kondor	110	333	Erwinia
4	Rosa Gold	66	200	PVY
5	Lady Claire	66	200	PVY
6	Vermont	66	200	PVY
7	PCR/11	66	200	PVY

Plants were grown as common in practice, including supply of nutrients and crop protection. Mangane nitrate (0.2 kg Mangene) and magnesium nitrate (total 1.1 kg Magnesium) were extra supplied. For control of Phytophthora infestans 13 full field sprayings were applied. In March (before planting) it was

relatively cold. April was normal for the time of the year, whereas May and June were warmer than average. July was wet and cold. From 30 may onward plants were observed weekly (in total six times) from symptomatic plants.

The health status of each potato plant was determined by a crop expert who visually inspected the plants in the experimental field. Plants that showed Y-virus disease symptoms were geometrically stored with a RTK GNSS rover (Hiper Pro, Topcon, Tokyo, Japan). A VRS signal (06-GPS, Sliedrecht, Netherlands) was used to guarantee a 0.02 m accuracy on the position estimate. The crop expert obtained the position of a diseased plant by placing the rover at the center point of the plant. From the center point, we constructed a geometric plant polygon using the intra- (0.33 m) and inter-row (0.75 m) distance of the potato crop. The constructed plant polygons were stored for offline processing.

2018

Similarly in 2018 a field experiment was conducted. The distribution of the plants/treatments in the field is shown in Figure 2.

Plants were grown as common in practice, including nutrition and crop protection. Mangane nitrate and magnesium nitrate were extra supplied (see details 2017). Between 9 May and 8 August, in total 28 field sprays were applied for weed control, control of *Phytophthora infestans*, fungi and insects. April was relatively wet and warm but thereafter the growing season was very warm and dry. Nevertheless symptom development was as expected both for virus-infected plants as for plants inoculated with bacteria. Plants deteriorated late in the growing season due to the high irradiation and the high soil temperature as a result of a relatively open crop (plant distance 50 cm). From 7 June onward, plants were visually observed weekly (in total seven times) for symptom expression.

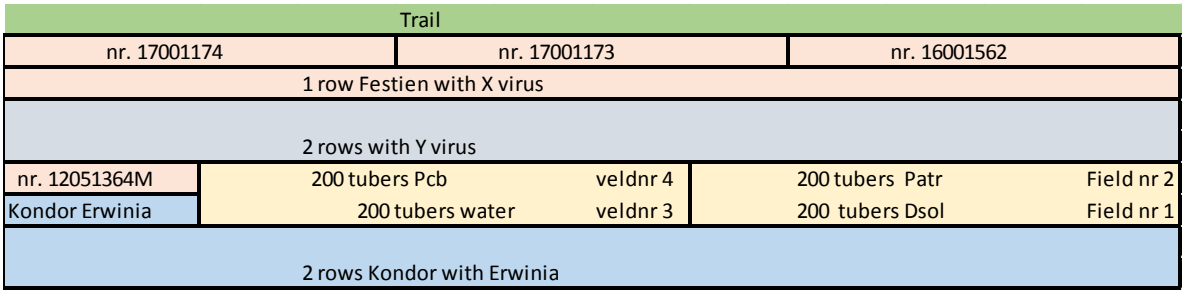


Figure 2 *Experimental set up field experiment in 2018. Total length is field is 263 meter (maximal). Per row maximal 526 planten were planted at a distance of 50 cm between plants. Row 3 is material of cultivar Kondor inoculated with Pectobacterium carotovorum subsp. brasiliense (Pcb), P. atrosepticum (Patr) or Dickeya solani (Dsol). In rows 7 and 8 the virus diseased (symptomatic) plants in the row were labeled before measurements.*

In addition, minitubers of cultivar Kondor (200 per isolate) were inoculated with suspensions (107 cells/ml) of Dickeya solani IPO2222, Pectobacterium atrosepticum IPO1007, or P. carotovorum subsp. brasiliense IPO3649 using the vacuum inoculation method as developed at HZPC. Inoculation was evaluated by analyzing extracted periderms of tubers by dilution plating (undiluted and 10-times diluted) on platen double layer CVP. Typical colonies for Pectobacterium and Dickeya were found from inoculated tubers but not or hardly from the water treated control tubers. During crop growth plants were visually observed for the development of symptoms typical for blackleg or virus diseased plants.

The health status of each potato plant was again determined by a crop expert who visually inspected the plants in the experimental field as was done in 2017.

4 Technologies used

This chapter describes the different techniques that were used in the project for disease detection and data analysis. At the start 5 potentially promising techniques were selected. After year 1 a selection was made for the two most promising one.

4.1 Spectral measurements

During the first two years (2015-2016) plants were cultivated in pots and scanned in a lab environment using a hyperspectral camera (Specim V10e). Wavelength range 400-1000 nm, with a spectral resolution of 3 nm (FWHM). Image and spectral analysis was done in Matlab using the image processing toolbox and the pattern recognition toolbox provided by perClass. Virus detection was based on spectra from the whole plant. For Erwinia detection the plants were classified into leaf and stem parts. This could easily be done using the spectral data due to high water absorption in the stems.
Classifiers were trained on random selection of pixels using leave one out cross-validation.
Linear and non-linear classifiers were tested. The Gaussian classifier in the end appeared to give the best performance on this dataset.

During the third and fourth year of the project (2017-2018) plants were grown under field conditions. An image acquisition device was built for measuring the plants in the field.
Hyperspectral data acquisition was done in a larger scope where several sensor techniques were explored for disease detection in the field. An imaging box was designed for measuring the potato plants. The box consists of two equally sized compartments (150 cm × 75 cm × 150 cm). The first compartment was equipped with an RGB-Depth camera while the other was equipped with a Specim FX10 hyperspectral line scan camera (wavelength range 400–1000 nm). For this paper we only focused on the hyperspectral data. An embedded PC (Nexcom NISE3500) was installed to acquire the hyperspectral images. Ambient light was blocked by use of light curtains placed around the measurement box (Figure 3).

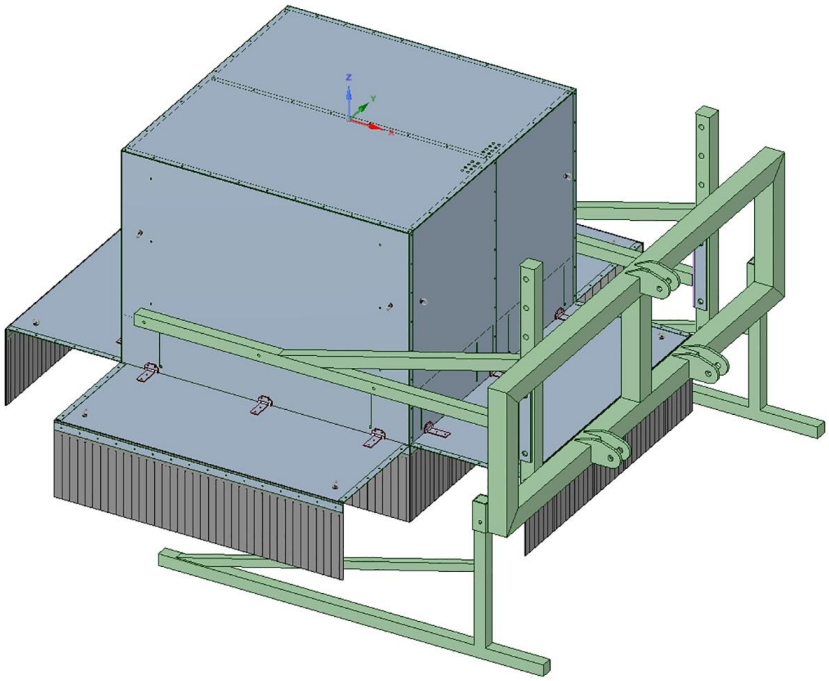


Figure 3 *Drawing of the measurement box, consisting of two compartments, the first one for the hyperspectral camera and the second one for an RGB-D imaging system. Ambient light is blocked by two rows of rubber flaps. The box can be mounted on the front of a tractor.*

The measurement box was placed 3.1 m in front of a tractor that drove at a constant speed of 300 m per hour (0.08 m/s) during the measurements. Figure 4.shows the system operating in the test field.

Figure 4 *Picture of the system while doing field measurements.*

The FX10 is a pushbroom hyperspectral line scan sensor. The frame rate was 60 f/s, resulting in an interval of 5 mm in the driving direction of the tractor. The full sensor resolution of the FX10 is 1024 pixels in the spatial by 224 bands spectral. In order to improve light sensitivity and speed, the images were binned by a factor 2 in the spatial direction and a factor 4 in the spectral direction, resulting in line images of 512 pixels × 56 pixels (Figure 5.

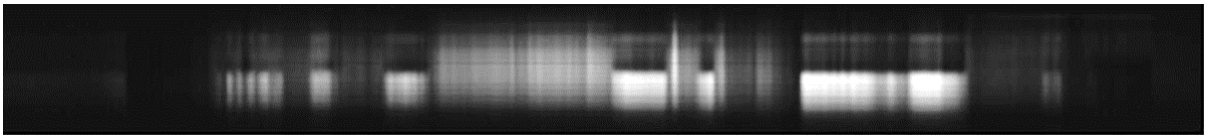


Figure 5 *One single hyperspectral line image, horizontally showing the spatial information of one line, vertically showing the spectral reflection between 400 and 1000 nm***.**

As the bands at the start and the end of the spectrum were noisy, only the central 35 bands were kept for further processing.

Plants were illuminated by:

- 2017: 13 Tungsten Halogen lamps (Osram Decostar 51 PRO, 14 Watt, 10°, Dichroic) placed in a row.
- 2018: 25 Tungsten Halogen lamps (Osram 35 Watt, 36°, aluminium reflector)

This way the plants were evenly illuminated. White and black references were taken at the start of each measurement cycle. The white reference object was a gray (RAL 7005) PVC plate. The black reference was taken with the camera shutter closed. Reflection images were calculated by: $R = \frac{I-B}{W-B}$ where I is the raw hyperspectral Image, B is the black reference and W is the White reference.

On each measurement day, we obtained the real-world position of the hyperspectral line images using the RTK-GNSS receiver of the tractor (Viper 4, Raven Europe, Middenmeer, Netherlands). The GGA message of the GNSS receiver (NMEA-0183), containing the WGS-84 coordinates and the GNSS precision status, were passed to the embedded PC at a frequency of 10 Hz and stored for offline processing.

We processed the data in such a way that only the hyperspectral line images were assessed where a RTK-fix signal was guaranteed. In this way the exact position of all hyperspectral images could be determined with a precision of 0.02 m.

In 2017 and 2018 deep learning was used as classification method. A novel FCN is used to detect plant diseases based on hyperspectral image data. The network used was a fully convolutional neural network (FCN), but had a non-standard decoder (final) portion. Usually with FCNs the output is a two-dimensional segmentation. Here, we outputted a one-dimensional segmentation (it was also a lower resolution 1D strip).

The model was trained and tested on a system containing an Intel Xeon E5-1650 CPU with a 3.5 GHz clock speed and 16 Gb of RAM. The system also housed an Nvidia GTX 1080 Ti GPU with 11 Gb of video memory. All data preprocessing, Deep Learning training and validation was done using the PyTorch deep learning framework (Paszke *et al.*, 2017). At test time, a forward pass through the neural network took a total of 8.4 ms, of which 4.0 and 1.1 ms were the times taken to move data to and from the GPU, respectively. This time is negligible compared to the time taken to acquire the images.

The 2017 experiment was published in Polder *et al*. (2018).

4.2 Chlorophyll fluorescence measurements

An interaction of the virus with photosynthesis can cause irreversible damage. The reduction in photosynthesis efficiency is be measured with fluorescence images. The fluorescence measurements were made with the Crop Reporter (see figure 6) by measuring the plants from above in the virus test and laterally in the Erwinia test. The measurement protocol consisted of adapting the plants for at least ½ hour in the dark and then carrying out a measurement. With this protocol the maximum value of the photosynthesis efficiency, Fv / Fm, is determined. This method provides the greatest contrast in the image between affected and healthy parts of the plant.

The fluorescence images are analyzed with in-house written image analysis software (CRopReporter Analysis V330) specially for fluorescence images. With this software, the average efficiency of photosynthesis is calculated per image. Another parameter can also be calculated: the relative surface of the plant with a value of the photosynthesis efficiency lower than a threshold value. This means that the area of the plant is calculated by counting the number of pixels that have an Fv / Fm value lower than, for example, 70%, the set threshold value. The relative area is then calculated by dividing this number by the area of the plant.

Figure 6 *The lab setup of the Crop Reporter used for recording the fluorescence images.*

The damage due to viruses can best be calculated by determining the relative area with these reduced Fv / Fm values. The images are calculated with image analysis software on the relative surface with Fv / Fm less than 70%. This is shown in Figure 13 for the three measurement days. The results of measurements gave the best results on the first measurement day (Figure 7). For the next two measurement days, there was less distinctive character between the healthy and viral diseases.

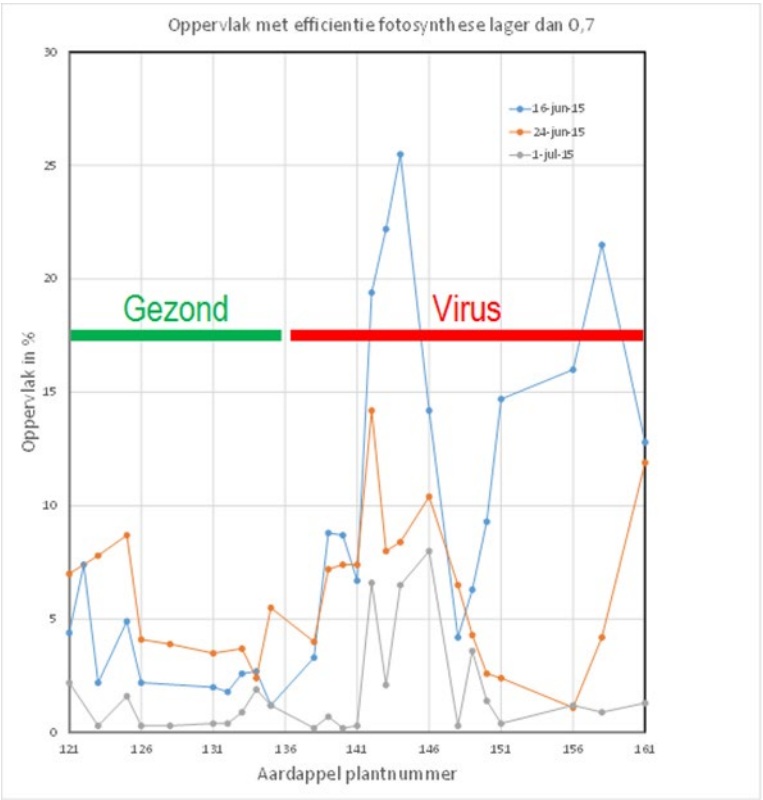


Figure 7 *Overzicht van de resultaten bereikt op drie meetdagen voor de mate van aantasting van de planten uitgedrukt in het relatieve oppervlak van de plant met een Fv/Fm lager dan 0,7.*

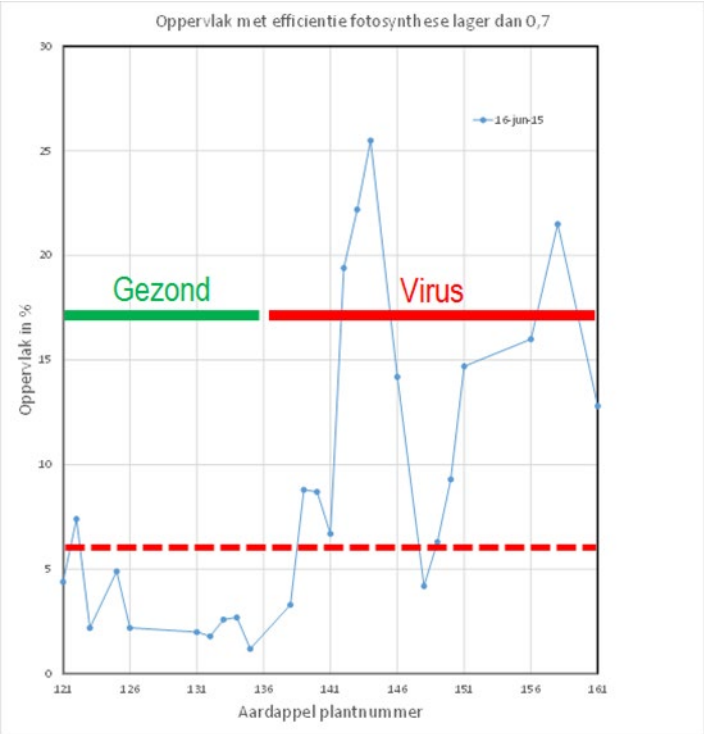


Figure 8 *Overview of the results of the first measurement day for the degree of infection of the plants expressed in the relative surface of the plant with an Fv / Fm lower than 0.7. The dotted line indicates the sorting limit where above this limit the plants are affected by the virus and healthy below.*

For better assessment a sorting limit has been set at 6% damage percentage of the plant (Figure 8). These results show that diseased plants are recognized with this method in 13 out of 15 cases (2x not). On the other hand, only 1 plant is wrongly assessed as being sick against 9 times good.

Table 3 compares the classification of healthy / viral plants with the scores of the crop expert. This gives the impression that 88% of the plants are classified as comparable by the expert. In 2 of the 25 cases, a virus-diseased plant was classified as healthy (false negative) and in 1 case, a healthy plant was classified as sick (false positive).

Table 3 *Classification results for healthy and virus sick plants compared to expert judgement of health status of the plants.*

Real health status of the plant	Disease status plant by classifier		Total
	Healthy	**Sick**	
Healthy	9	1	10
Sick	2	13	15
Total	11	14	25

By playing with the threshold, e.g. from 6% to 8% damage percentage, the number of false positive (healthy plants classified as sick) plants decreases to zero, but the number of false negative plants (sick plants classified as healthy) increases to 4. This means that it's easy to find on optimum setting to minimize false negatives or false positives.

Evaluation of Chlorophyll Fluorescence technology

The results of the chlorophyll fluorescence method for detecting viruses are quite good. At a limit of 6%, 13 of the 15 diseased plants were assessed as being sick and 2 were wrongly considered to be healthy. Of the 10 healthy plants, 9 plants were assessed as healthy and 1 incorrectly as sick. The best results were obtained at a young planting stage.

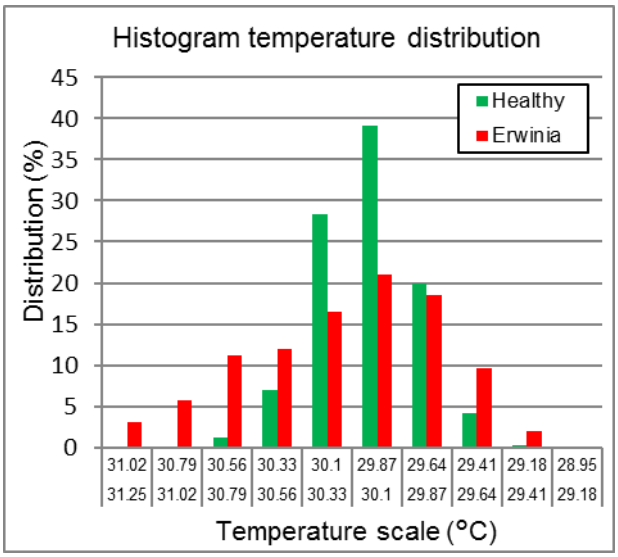
In the Erwinia trial all healthy plants are also classified as healthy, while 4 of the 9 plants of the diseased plants are classified as sick. The high percentage of 33% incorrectly classified as healthy classified plants by this technique can be explained by the fact that Erwinia diseased plants may be internally affected by the disease but do not or hardly show this at the surface and therefore a "healthy" photosynthesis in give measurements. This chlorophyll fluorescence method is very successful when plants have clearly visible symptoms on the outside of the plants.

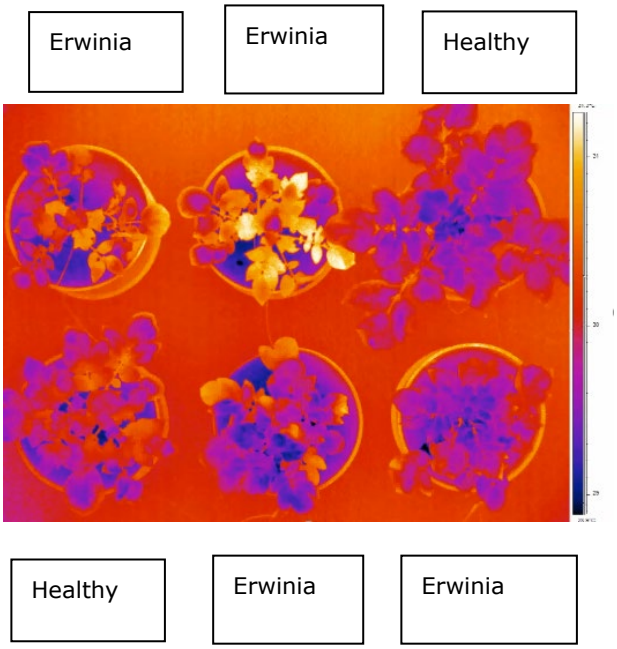
The fact that plants have to be adapted to the dark is a problem for measuring in the practical situation. For the time being, only measuring at night with a search engine is not a realistic option. The light adaptation of the plants is also desirable for Erwinia detection. This is the reason why this technique, which does not “perform” significantly better than (hyper) spectral recordings, has not been included in the study for the time being.

4.3 Feasibility research with thermal imaging

In 2015, we started with a feasibility study on the applicability of two state-of-the-art sensor technologies, which were thermal imaging and three-dimensional (3D) imaging (chapter 3.4). These technologies were believed to detect plant wilting and growth stagnation. With thermal imaging we measured the heat radiation of the leaves of the potato plant. The hypothesis was that when a potato plant starts to wilt, there will be a rise in leaf temperature due to water stress. These (local) temperature differences can be detected by a thermal camera, as we proved by our experiments in a laboratory setup.

However, we also experienced a need for frequent camera calibration when there is a change in the ambient temperature. This feature limits the practical application of a thermal camera, so we decided not to continue with this sensor for the other growing seasons (2016-2018).





4.4 3D technology

In 2015 the testing was done using a 3D camera, a Microsoft Kinect v1. We hypothesized that this camera can provide us information on plant growth. Then, infected plants can be detected when the growth stagnates. The results showed that these effects can be detected with a 3D camera. Because a 3D camera does not need frequent calibration, we concluded that this sensor was more appropriate for the extended laboratory test in 2016.

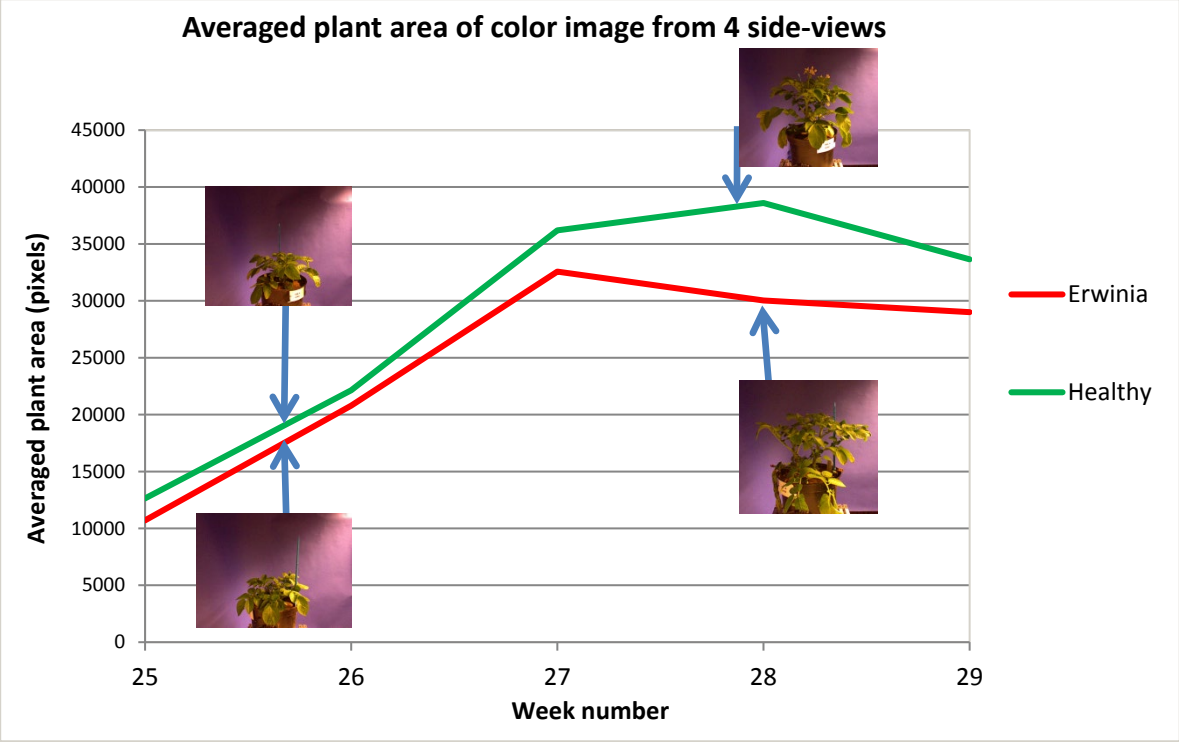


Figure 9 *Monitoring of the plant growth development with a 3D camera.*

In 2016 we extended our laboratory research by conducting plant volumetric estimations using the same 3D camera. We did two experiments; first we determined the complete 360 degrees plant volume in 3D and then we determined the extrapolated plant volume when only using the top-view image of the 3D camera (2.5D). Our research showed a similar trend between the 2.5D top-view image analysis and the complete 3D volume. This was desired, as the complete 3D volumetric estimations are time-consuming and practically impossible to execute due to the leaf occlusion. From the 2016 research, we concluded that the 2.5D top-view image analysis was a candidate technique to robustly detect Erwinia in field conditions.

In 2017, we validated the 2.5D image analysis in the field using an industrial 3D camera (IDS Ensenso N35). We used various machine learning techniques for the detection of Erwinia in the 2.5D images.

Figure 10 *Image acquisition with a 3D camera (right) under controlled light conditions (left).*

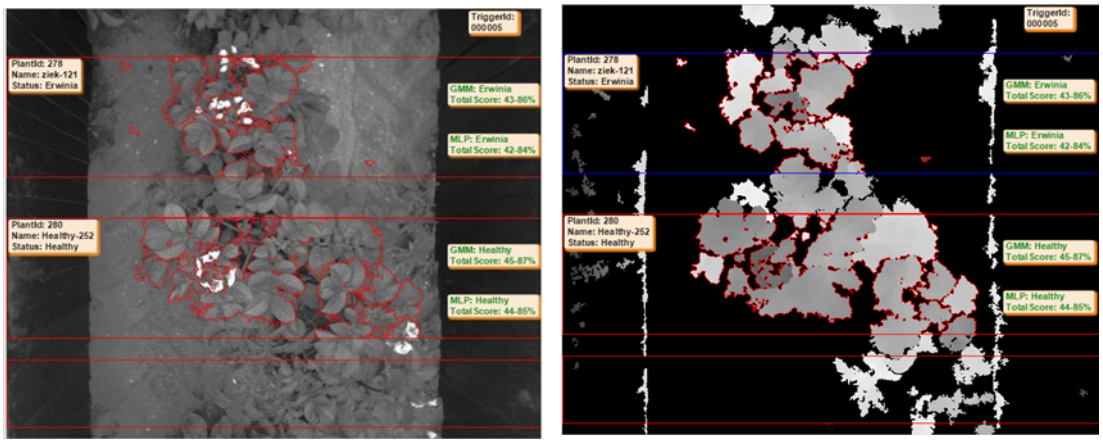


Figure 11 *Grey-scaled image of the potato crop (left) and 2.5D image analysis using machine learning (right).*

The focus of the research in 2018 is related to combining 3D measurement data with Deep Learning. Deep Learning has emerged as the state-of-the-art technique for image analysis and computer vision problems such as semantic segmentation, object detection, and classification. These methods are being used increasingly in computer vision in agriculture for applications such as automatic harvesting, yield estimation, phenotyping, disease and pest detection (Kamilaris and Prenafeta-Boldú, 2018). The advantage of training convolutional neural networks (CNN) over traditional machine vision methods, like our 2017 classifier, is their ability to discern discriminative features that would be difficult to hand-craft with data with a lot of variability. CNN-based classifiers have been recently applied for classifying images of individual plant leaves into healthy or diseased (Mohanty et al., 2016). In our conclusive research on Erwinia detection, we applied a CNN to differentiate between healthy and Erwinia-disease potato plants in the field.

Dataset

In 2018 we used the same hardware setup to acquire images from the experimental potato fields. From the image dataset, we selected 532 images across six different dates. The following criteria were used to process the images:

1. The potato plant must be isolated from other neighboring plants without overlap or contact. Therefore, only plants from row numbers 3 and 4 in the experimental field were selected.
2. The images were resized to 224 x 224 pixels using aspect ratio retention to prevent image distortion. The resolution of 224 x 224 pixels corresponds to the preprocessing transformation in the deep learning software.
3. It was ensured that the selected images were realistic representations of each class (healthy or Erwinia). As such, we avoided trivial classification, for instance healthy detections with large sized plants and Erwinia detections with smaller sized plants.

The image dataset was randomly split into a training and testing set, with a train-test ratio of 80:20, leading to a training set with 426 images (218 healthy, 208 Erwinia) and a test set with 106 images (60 healthy, 46 Erwinia). The test set was used for independent benchmarking of the CNN classifier. We only used the RGB-color images to make transfer-learning possible in the CNN training process.

Software and Setup

The PyTorch framework was used to code the deep learning classifier in Python, on a workstation with one NVIDIA GeForce GTX 1080 Ti 11GB GPU, 12 core Intel Xenon E5-1650 processor and 64GB DDR4 RAM running Linux Mint 18.2 and CUDA 9.0.

Algorithm

The Residual Network (ResNet) architecture (He et al., 2016), either with 18 and 50 layers, was used to extract the features that were used to perform the classification. We chose for a residual architecture, because this network alleviates the vanishing gradient problem. The fully connected (FC) layer was redefined by a classifier that linearly combined the output of the FC layer into a size 512

vector, over which a rectified linear unit (RELU) activation was applied. The last network layer consisted of a 2 class linear classifier using logarithmic soft max activation to enable our binary classification (healthy versus Erwinia).

For network weight initialization, we applied transfer-learning using a model that was pretrained on the ImageNet dataset. For weight optimization, we used the Adam optimizer with a learning rate of 1e-4 for ResNet18 and 5e-5 for ResNet50. Both networks were trained for 100 epochs, using a mini-batch size of 12. To prevent model overfitting, we applied two forms of explicit regularization. The first was random neuron disconnection (drop-out) and the second was network weight decaying (L2-regularization). For ResNet18, we used a drop-out with 1% probability (0.01) and for ResNet50 a drop-out with 20% probability (0.2). The L2-regularization was 0.05 for both networks. Each network was trained for 5 times on random splits of the training set.

Evaluation procedure
The evaluation was performed on the test set. For each test image, the decision healthy or Erwinia was decided through majority voting over the 5 trained models of each network.

4.5 Force-A

Force-A is a French company specialized in sensing solutions with experience in disease detection in wine growing (Figure 10). Two different Force-A sensors were included in the trials to test the successful disease detection in seed potatoes. The sensors used were the MX-330 and MX-375 handhelds, which can detect changes in plant piglets stilbenes and flavanols, respectively. Change in stilbenes is related to fungal infections and plant diseases. The amount of Flavanols are influenced by plant stress and/or nitrogen deficiency.

Figure 12 *handheld Force-A sensor as used in disease detection in vineyards.*

In 2015 all plants were measured using the 2 Force-A sensors (24 measurements per plant in total: each technique - per plant: 3 top leaves, 3 middle leaves, 3 bottom leaves, 3 scans of the bottom side of the stem).

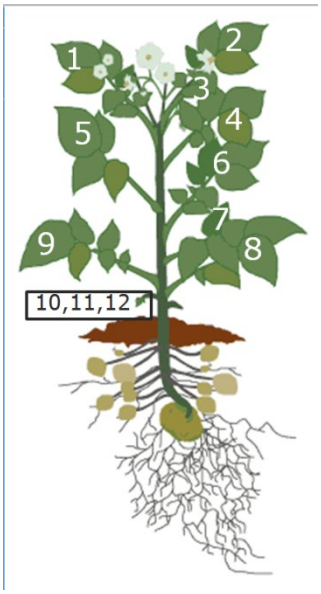


Figure 13 *Locations of measurements (both MX-330 and MX-375 handheld sensors).*

Results

The first analysis of Force-A focused on the chlorophyll index. Figure 14 shows small differences between healthy ("False") and sick plants ("True"). The differences are statistically non significant.

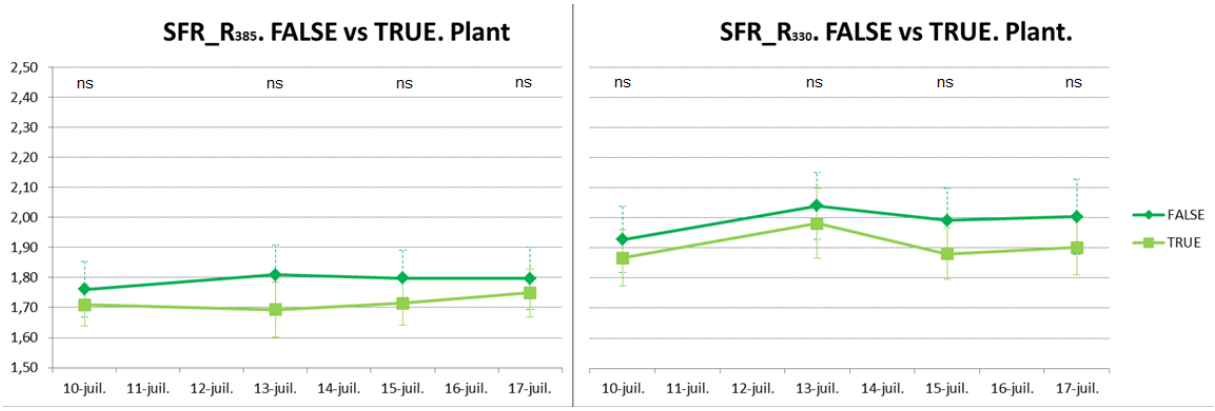


Figure 14 *On the left the chlorophyll index output of the MX-375 and on the right that of the MX-330. The light green "True" lines relate to the Erwinia diseased plants; the dark green line "False" the healthy plants. NS indicates a non-significant difference.*

The second analysis has been done on the flavanols, see figure 15. A slight increase in the flavanols for both objects over time can be seen, but no significant difference can be demonstrated.

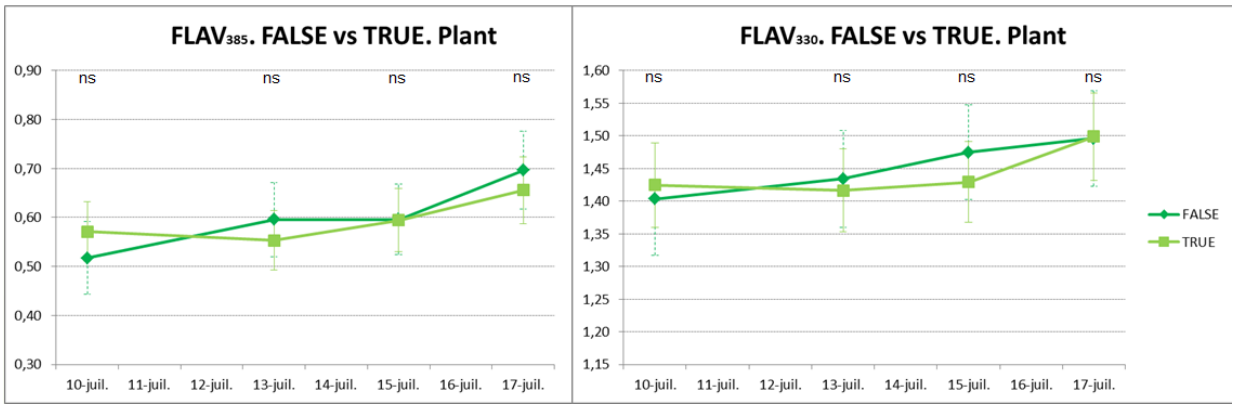


Figure 15 *Flavanols output van de MX-375 (left) and of the MX-330 (right hand side)*.

The ratio between blue and "far" red fluorescence at 330 nm (BRR) shows a significant difference on 1 measurement day (July 13) between the stems of Erwinia diseased and healthy plants (Figure 16). The other measurement days are not significant.

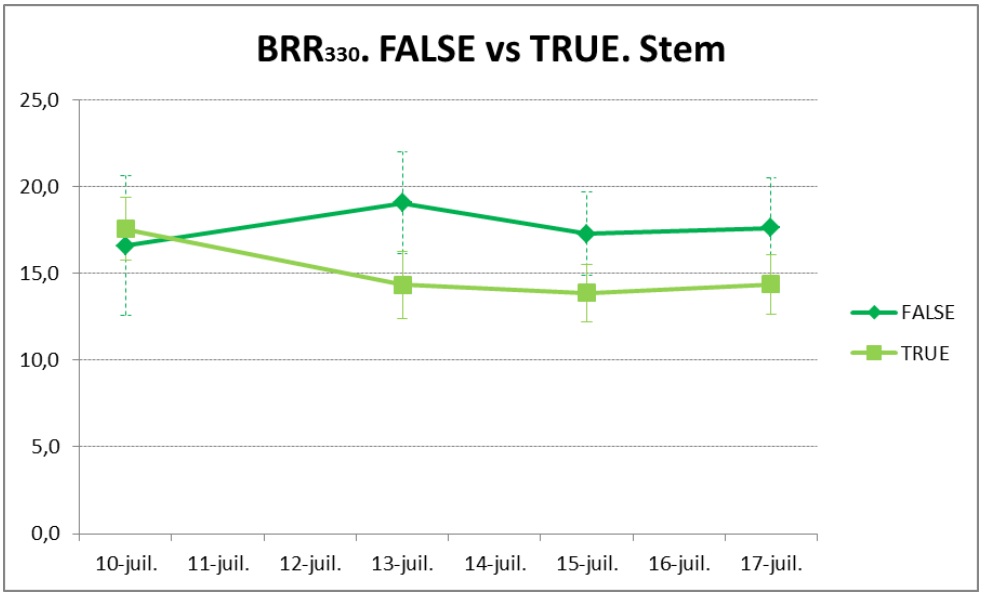


Figure 16 *The ratio between the blue and "far" red fluorescence is lower for Erwinia diseased plants, but only statistically significant on 13th of July.*

Evaluation of the experiments

Both Force-A sensors show no distinctive character between Erwinia diseased and healthy plants when it comes to the chlorophyll index and the flavanols. The sensors perform spot measurements and because they don't perform full plant imaging, potential sick spots are missed. The current MX-330 and MX-375 are contact sensors and therefore less suitable for field measurements.

At this stage of development the Force-A sensors are not distinctive enough to detect an Erwinia diseased plant and are therefore not included in the next year's trial.

4.6 Evaluation of technologies

In this chapter five technologies are described that were tested in 2015. Both the spectral and the 3D technology were evaluated positively based on 1) distinctive character regarding virus and/or Erwinia diseased plants, 2) the potential working speed of a device using this technology including the processing speed of data involved, and 3) the robustness when dealing with changes in the direct environment of the device (e.g. sunny – clouds related to thermal sensing).

As a summary: after 2015 we continued with only spectral imaging and 3D sensing, as also described in this chapter. In the period 2016 – 2018 we have been improving the data analysis by enlarging the data sets. And even more important, from 2017 on we started to implement the deep learning techniques for the analyses of data sets for both technologies. In chapter 4 the main focus is on the results of 2018, but also some intermediate results are presented.

5 Results

5.1 Spectral imaging – virus detection

In this paragraph a summary of the results is given for each year. After intensively analysing the data sets the results are presented in the form of confusion matrices, comparing the "real disease situation" with the classification of the machine (or deep) learning systems.

5.1.1 Results 2015

The experiment of 2015 was a lab experiment with artificial infected plants. Results of hyperspectral classification against real infection is compared to scores of the crop experts.

Table 4 *Classification results of individual plants first week, based on stem pixels (secundairy infection).*

Real spectra	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	9	1	10
Diseased	1	13	14
Total	10	14	24

Table 5 *Classification results of individual plants first week, based on leaf pixels.*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	10	0	10
Diseased	1	14	15
Total	11	14	25

Table 6 *Score on individual plants first week based on symptoms of the crop expert.*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	10	0	10
Diseased	1	14	15
Total	11	14	25

Table 7 *Total score virus detection for all measure weeks.*

Week	*Date*	*Leaves*	*Stem*	*Expert*
1	16/6/2015	0.96	0.92	0.96
2	24/6/2015	0.52	0.52	0.96
3	1/7/2015	0.68	0.68	1

5.1.2 Results 2016

During the lab experiment of 2016 both primary and secondary infections were analyzed.

Table 8 *Score individual plants in the first week (28) based on symptoms by the crop expert (secondary infected plants)*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	4	0	4
Diseased	5	0	5
Total	9	0	9

Table 9 *Score on individual plants in the first week based on machine vision analysis (secondary infected plants)*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	4	0	4
Diseased	0	5	5
Total	4	5	9

In this week the expert considered all plants to be healthy, while the algorithm already found 5 sick plants.

In week 34 measurements on the occurrence of primary virus infections were analyzed. The results from the crop expert (table 10) and the machine algorithm (Table 11) were compared with the real plant status (lab analysis). We only had a very limited number of plants available.

Table 10 *Score individual plants in last week (34) based on symptoms by the crop expert (primary infections)*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	6	0	6
Diseased	1	9	10
Total	7	9	16

Table 11 *Score on individual plants in the last week (34) based on machine vision analysis (primary infections)*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	4	2	6
Diseased	2	8	10
Total	6	10	16

In week 34 the expert only missed one sick plant, while the algorithm missed two sick plants and decided two healthy plants to be sick. The algorithm performed poorly compared to the expert at a later stage of the crop. When the crop starts to deteriorate it's more difficult to extract the right symptoms.

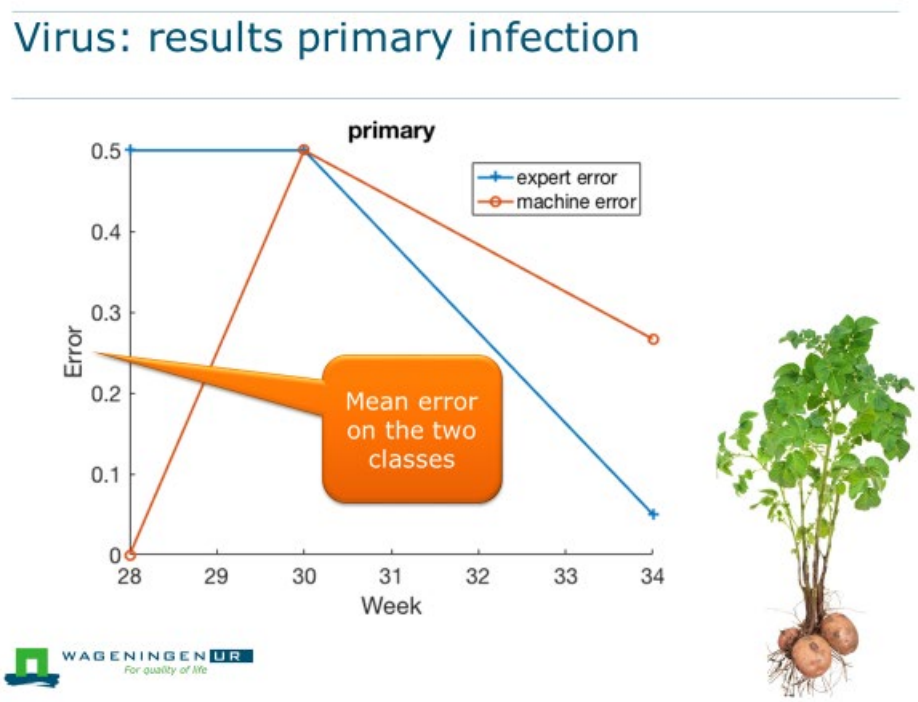


Figure 17 *Mean error machine vision and expert for primary infected potatoes*

In this experiment we only had limited numbers of plants, which makes it difficult to draw conclusions. From the analysis of the primary virus infection we can conclude that in the first week (28) the machine analysis performed much better than the expert. Later on both the machine learning algorithm as the expert didn't perform optimally, but the algorithm performed worse.

Confusion matrices were also created based on measurements of the secondary infections. In week 25 measurements on the occurrence of secondary virus infections were analyzed. The results from the crop expert (table 12) and the machine algorithm (Table 13) were compared with the real plant status (lab analysis). This experiment was repeated in week 29 (Table 14 and 15).

Table 12 *Score individual plants in first week (25) based on symptoms of secondary virus infection by the crop expert*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	3	1	4
Diseased	1	14	15
Total	4	15	19

Table 13 *Score on individual plants in the first week (25) based on symptoms of secondary virus infection by machine vision analysis*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	2	2	4
Diseased	0	15	15
Total	2	17	19

In this week the expert took a wrong decision on two plant, comparable to the algorithm (missed one sick plant and decided one healthy plant to be sick)

Table 14. *Score individual plants in last week (29) based on symptoms of secondary virus infection by the crop expert*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	2	2	4
Diseased	0	12	12
Total	2	14	16

Table 15. *Score on individual plants in the last week (29) based on symptoms of secondary virus infection by machine vision analysis*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	0	4	4
Diseased	0	12	12
Total	0	16	16

In week 29 the expert only decide wrong on two healthy plant, while the algorithm decided four healthy plants to be sick. Percentage wise the algorithm performed 12,5% less well than the expert.

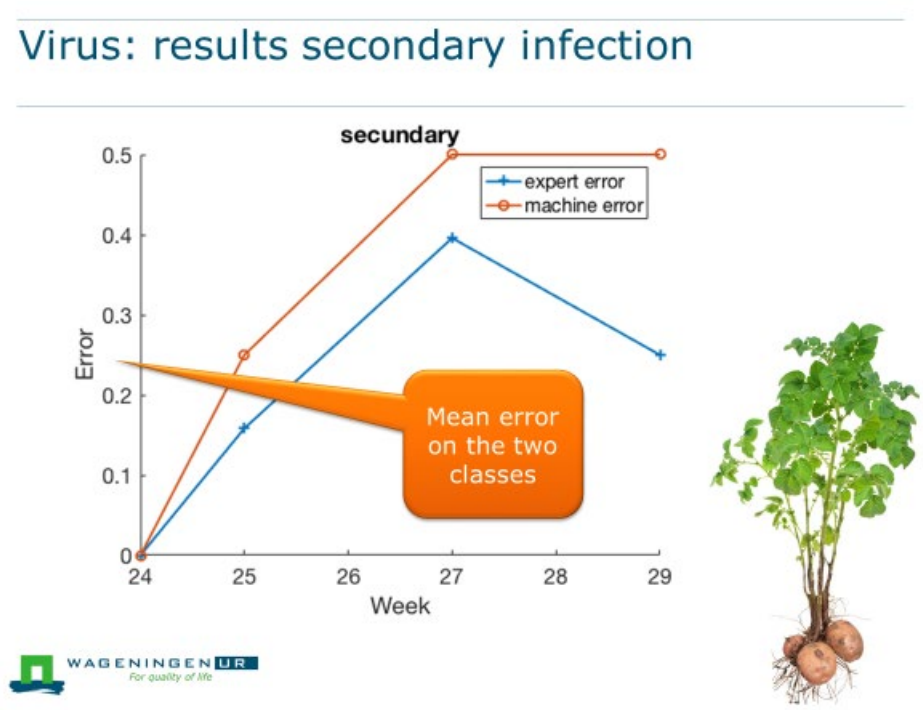


Figure 18 *mean error machine vision and expert for secondary infected potatoes*

Also for the secondary virus infections we can conclude that in the first week (28) the machine analyse performed as good as the expert. Later on both the machine learning algorithm as the expert didn't perform optimally, but the algorithm performed worse.

5.1.3 Results 2017

In 2017 the first field experiment took place. Compared to the previous years a large number of plants were tested. Contrary to the lab experiments no Elisa tests were done. Ground truth for machine learning in this case were the crop expert scores.
Due to a small mismatch in the localization of the plants caused by the GPS system some corrections were made for neighboring plants of the virus infected ones.

In 2017 deep learning was used as classification method for the first time. The network used was a fully convolutional neural network (FCN), but had a non-standard decoder (final) portion (see also chapter 3.1.

The confusion matrices of row 6 **(A,B)** and 7 **(C,D)**, measured on 2017/06/27 **(A,C)** and 2017/07/03 **(B,D)** are shown below.

Table 16. *Score individual plants in row 6 (A) based on symptoms of primary virus infection (crop expert defined the real plant status) – 27-6-2017*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	12 (92.3%)	1 (1.7%)	13
Diseased	11 (6.4%)	160 (93.6%)	171
Total	23	161	184

Percentage of all well classified plants (sick and healthy): 93%

Table 17. *Score on individual plants in row 6 (B) based on symptoms of primary virus infection (crop expert defined the real plant status) –3-7-2017*

Real status plant	**Classified plant status**		**Total**
	Healthy	**Diseased**	
Healthy	12 (92.3%)	1 (7.7%)	13
Diseased	18 (10.5%)	153 (89.5%)	171
Total	30	154	184

Percentage of all well classified plants (sick and healthy): 90%

Table 18. *Score individual plants in row 7 (C) based on symptoms of primary virus infection (crop expert defined the real plant status) – 27-6-2017*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	7 (87.5%)	1 (12.5%)	8
Diseased	6 (3.4%)	173 (96.6%)	179
Total	13	174	187

Percentage of all well classified plants (sick and healthy): 96%

Table 19*. Score on individual plants in row 7 (D) based on symptoms of primary virus infection (crop expert defined the real plant status) –3-7-2017*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	6 (75%)	2 (25%)	8
Diseased	20 (11.1%)	159 (88.9%)	179
Total	26	161	187

Percentage of all well classified plants (sick and healthy): 89%

The results in this test is very good. The scores of well predicted disease status is between 89% and 96%. The perspective for an application based on this technology is more or less at the level that is appreciated by a farmer.

5.1.4 Results 2018

In 2018 a comparable field experiment took place as in 2017. Again a large number of plants were tested. Ground truth for machine learning in this case were the crop expert scores.

Based on the successful experiences in 2017 again in 2018 deep learning was used as classification method (fully convolutional neural network -FCN).

In 2018 two rows with virus infected plants were tested row 5 and 6. Measurements were done during a period of 4 weeks. Like in the 2017 experiment some corrections were needed for localization uncertainty.

Results of the first week:

Table 20*. Score individual plants in row 5 based on symptoms of primary virus infection (crop expert defined the real plant status) – first week*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	130 (97.0%)	4 (3.0%)	134
Diseased	1 (6.7%)	14 (93.3%)	15
Total	131	18	149

Percentage of all well classified plants (sick and healthy): 92%

Table 21*. Score individual plants in row 6 based on symptoms of primary virus infection (crop expert defined the real plant status) – first week*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	123 (92.5%)	10 (7.5%)	133
Diseased	4 (28.6%)	10 (71.4%)	14
Total	127	20	147

Percentage of all well classified plants (sick and healthy): 90%

Results per row, all weeks combined

Table 22*. Score individual plants in row 5 based on symptoms of primary virus infection (crop expert defined the real plant status) – all weeks (combined)*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	498 (92.9%)	38 (7.1%)	536
Diseased	10 (16.7%)	50 (83.3%)	60
Total	508	88	596

Percentage of all well classified plants (sick and healthy): 92%

Table 23. *Score individual plants in row 6 based on symptoms of primary virus infection (crop expert defined the real plant status) – all weeks (combined)*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	479 (90.0%)	53 (10.0%)	532
Diseased	13 (23.2%)	43 (76.8%)	56
Total	492	96	588

Percentage of all well classified plants (sick and healthy): 89%

Results per week (both rows combined)

Table 24. *Score individual plants in week 1 based on symptoms of primary virus infection (crop expert defined the real plant status) – (rows combined)*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	253 (94.8%)	14 (5.2%)	267
Diseased	5 (17.2%)	24 (82.8%)	29
Total	258	38	296

Percentage of all well classified plants (sick and healthy): 93.6%

Table 25. *Score individual plants in week 2 based on symptoms of primary virus infection (crop expert defined the real plant status) – (rows combined)*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	254 (95.1%)	13 (4.9%)	267
Diseased	7 (24.1%)	22 (75.9%)	29
Total	261	35	296

Percentage of all well classified plants (sick and healthy): 93.2%

Table 26*. Score individual plants in week 3 based on symptoms of primary virus infection (crop expert defined the real plant status) – (rows combined**)***

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	247 (92.5%)	20 (7.5%)	267
Diseased	5 (17.2%)	24 (82.8%)	29
Total	252	44	296

total: 91.6%

Table 27. *Score individual plants in week 4 based on symptoms of primary virus infection (crop expert defined the real plant status) – (rows combined**)***

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	223 (83.5%)	44 (16.5%)	267
Diseased	6 (20.7%)	23 (79.3%)	29
Total	229	67	296

Percentage of all well classified plants (sick and healthy): 83.1%

Summary by row and week
Finally, the results were ordered by

Table 28. *Percentage of Healthy plants correctly classified (true negatives)*

	Week 1		Week 2	Week 3	Week 4
Row 5	97,0%		99,2%	91,0%	84,3%
Row 6	92,5%		91,0%	94,0%	82,7%

Table 29. *Percentage of sick plants correctly classified (true positives**)***

	Week 1	Week 2	Week 3	Week 4
Row 5	93,3%	73,3%	80,0%	86,7%
Row 6	71,4%	78,6%	85,7%	71,4%

Summary by row and week:

Again in 2018 the results of detecting viruses by deep learning are better in the first two weeks of the analysis. The score to 93,6% well scored plants in these weeks is considered to be good. When plants are getting older, there seems to be more "noise" in the images caused by deteriorating or slightly damaged leaves.

5.2 Detection of Erwinia based on 3D technology

In chapter 4 the technology was described that was used in the different years. After good results in 2015, the 3D technology was continued in the years 2016, 2017 and 2018. The main improvements over the years relate to using 2,5D images as a basis (showing a high correlation with 3D imaging) and changing from machine learning algorithms to Deep Learning in 2018.

5.2.1 Result 2016

Based on measurements on diseased and healthy potato plants growing in pots, plant volumetric estimations were conducted, using images from both the same 3D camera as in 2015 and a top-view 3D camera (2.5D). A similar trend between the 2.5D top-view image analysis and the complete 3D volume was found (Figure 4). From the 2016 research, we concluded that the 2.5D top-view image analysis was a candidate technique to robustly detect Erwinia in field conditions.

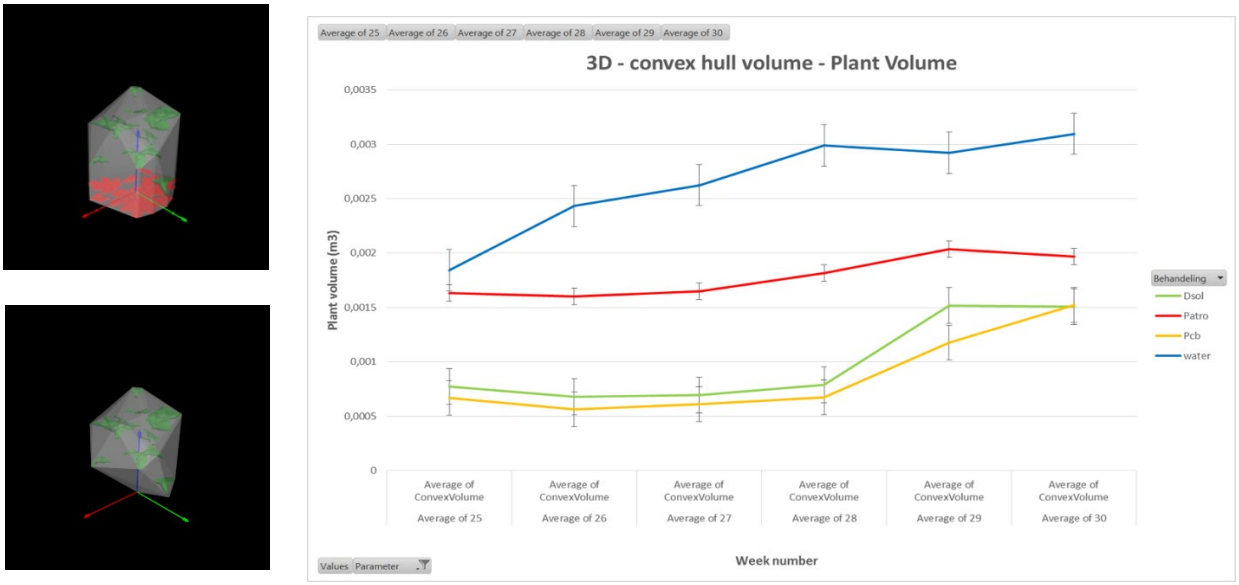


Figure 19 *Monitoring of the plant growth development using the top-view image of the 3D camera (2.5D).*

5.2.2 Result 2017

In 2017, we validated the 2.5D image analysis in the field using an industrial 3D camera (IDS Ensenso N35) We used various machine learning techniques for the detection of Erwinia in the 2.5D images . We found that a shallow neural-network (multi-layered perceptron) had a detection accuracy of 87% on 2.5D images acquired in the field. However, as Table 30 indicates, there was a suboptimal recall on the Erwinia infections. With the shallow neural network, only 41% of the Erwinia infections in the field were detected. The high overall accuracy (87%) originated from the high recall (97%) and precision (89%) on the detection of the healthy potato plants. As such, we needed to improve the detection on the Erwinia infected plants. A candidate technique is deep-learning that was researched in 2018.

Table 30. *Confusion matrix of the Erwinia detection results in 2017 using a shallow neural network*

Real status plant	Classified plant status			Total
	Erwinia Healthy	Erwinia Diseased	Y-virus	
Erwinia Healthy	424 (97.5%)	8 (1.8%)	3 (0.7%)	435
Erwinia Diseased	43 (52.4%)	34 (41.5%)	5 (6.1%)	82
Y-Virus	8 (88.9%)	0 (0%)	1 (11.1%)	9
Total	475	42	9	526

Percentage of all well classified plants (sick and healthy): 87.3%

5.2.3 Results 2018

In 2018 the same hardware setup was used to acquire images from the experimental potato fields. From the image dataset, we selected 532 images across six different dates. After training the Deep Learning algorithm as described in chapter 4, two Residual Network (ResNet) architecture (He et al., 2016), either with 18 and 50 layers, was used to extract the features that were used to perform the classification. The evaluation was performed on the test set. For each test image, the decision healthy or Erwinia was decided through majority voting over the 5 trained models of each network.

The binary classification results for each Resnet-architecture are presented in the confusion matrices (table 31 and 32)). A confusion matrix shows the breakdown of true healthy and true Erwinia images, and how many of them were correctly classified. The evaluation metrics are also included in the matrices. The recall values for each class is presented in parentheses in the right most column and the precisions are shown in the parentheses in the bottom column. The recall is number of true positives divided by the sum of true positives and false negatives. The precision is number of true positives divided by the sum of true and false positives. The overall accuracy was calculated as the number of correct classifications divided by the sum of correct and incorrect classifications and is indicated in parenthesis in the right-bottom corner of the matrices.

From the confusion matrix of ResNet18 (Table 31), we see that 94% of the images were classified correctly. For the class healthy, the precision was 97% and the recall was 93%. For Erwinia, these metrics were 92% and 96%. Thus, the CNN classification proved to be better than the shallow neural network that was researched in 2017. It can also be seen from the examples of inference using ResNet18 in Figure 5 and Figure 6 that the few misclassified instances were very hard examples, which visually offer no clue to their true class.

Table 31*. Confusion matrix for classification using ResNet18*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	56 (93.3%)	4 (6.7%)	60
Diseased	2 (4.3%)	44 (95.7%)	46
Total	58	48	106

Percentage of all well classified plants (sick and healthy): 94.3%

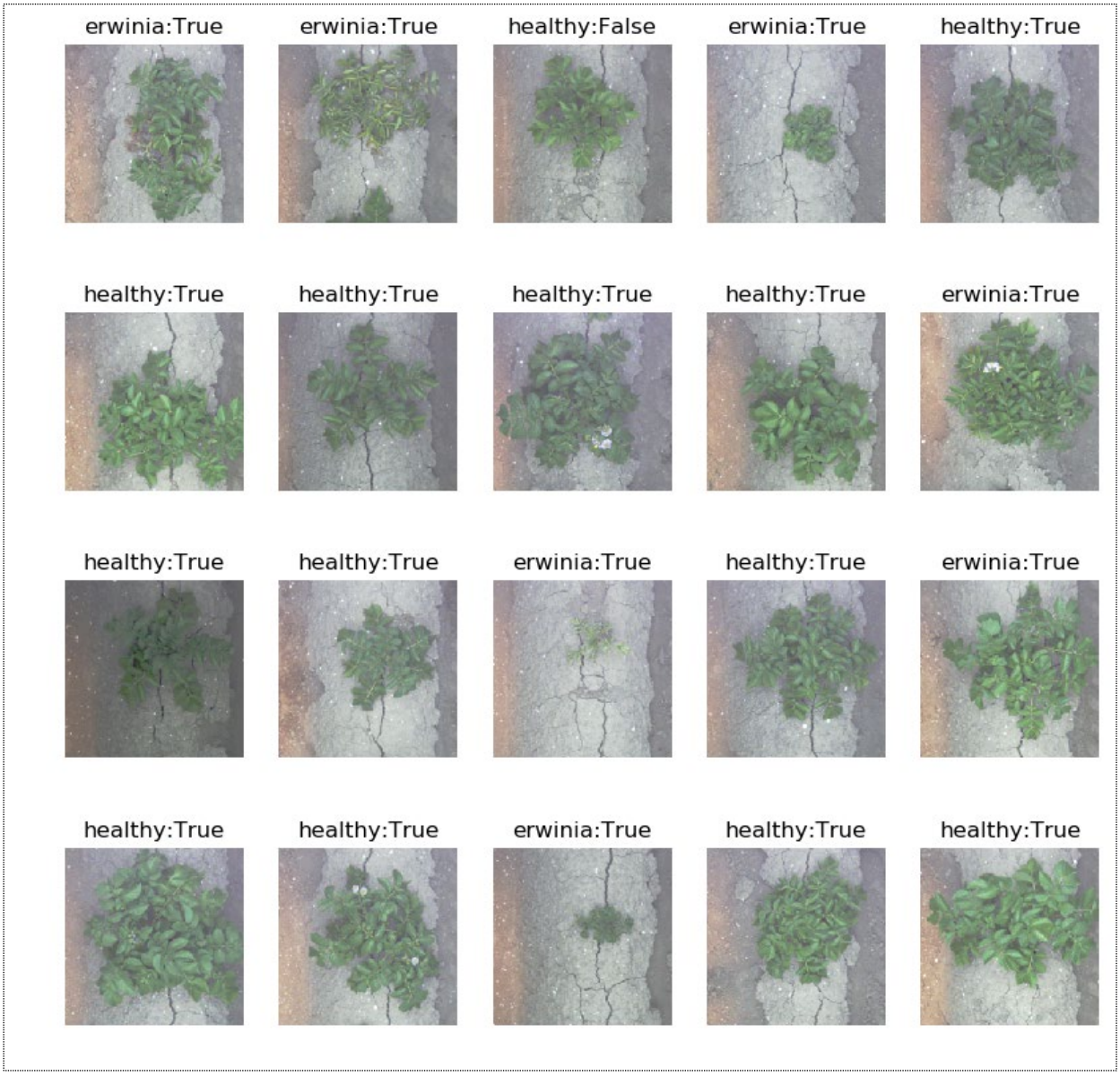


Figure 20 *Examples of correct and incorrect inferences with ResNet18. The first class name indicates the detection decision, followed by the correctness to the ground truth.*

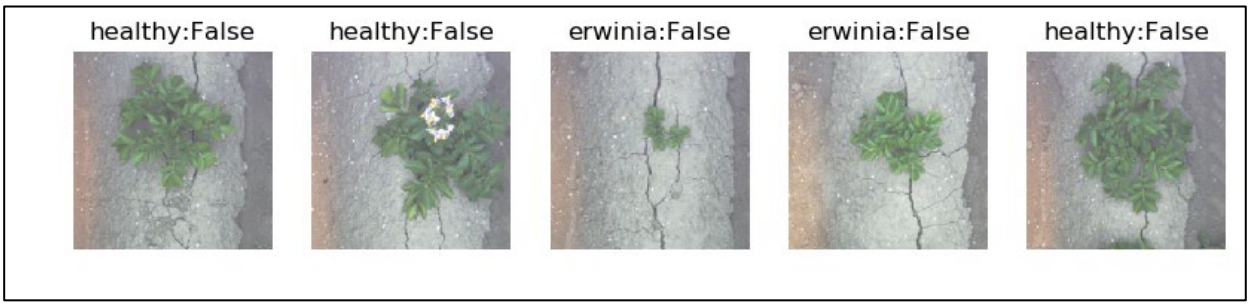


Figure 21. *Examples of incorrect inferences using ResNet18. The first class name indicates the detection decision, followed by the correctness to the ground truth.*

For ResNet50, it can be seen from the confusion matrix in Table 32 that the number of misclassifications is higher and the values of the accuracy, precision and recall per class are lower than ResNet18. This can be explained by the fact that ResNet50 has many more layers and weights than ResNet18 and therefore is more prone to overfitting leading to an inferior classification. It can be seen from table 23 that the average image analysis time is fast enough for practical applications. The detection system can process 133 frames per second when using the ResNet50 classifier, while 217 frames per second can be analyzed with ResNet18. These results show that ResNet18 is the preferred classifier for Erwinia detection in the field.

Table 32. *Confusion matrix for classification using ResNet50*

Real status plant	Classified plant status		Total
	Healthy	Diseased	
Healthy	**51 (85,0%)**	**9 (15.0%)**	60
Diseased	**10 (21.7%)**	36 (78.3%)	46
Total	61	45	106

Percentage of all well classified plants (sick and healthy): 82.1%

Table 33. *Computation times for training and testing*

	ResNet18	ResNet50
Training (100 epochs, 426 images)	3 minutes	9 minutes
Testing (per image)	4.6 milliseconds	7.5 milliseconds

5.3 Conclusions and recommendations

The results from this conclusive research show that deep learning can work as a robust detector for Erwinia. In future research, we can improve the detection performance:

- By using additional image channels other than RGB, such as the hyperspectral channels and depth registered to RGB
- By increasing dataset size and using data augmentation

The next step would be to deal with overlapping plants by applying an object detector such as Mask R-CNN (He et al., 2017). This would be a useful disease classifier for improved application in the field.

6 Conclusions, discussion and recommendations

In the production of seed potatoes both viruses and Erwinia are very much of importance. It was therefore important to focus on both diseases. From the start of the project it was clear that the detection of viruses based on hyperspectral information was giving fair to good detection levels. This was different with Erwinia. After choosing for 3D as the technique with the best perspective, the results were still quite poor. Based on machine learning technology only 41% of the Erwinia infected plants were detected. When comparing this to the low tolerance of the inspection results of the Dutch Arable Inspection Board (NAK), only 1% of the plants being Erwinia sick will result in lower quality certificate or rejection, the results must be higher.

The step by using deep learning technology in combination with RGB imaging or Hyperspectral imaging is a big step forward. By changing from lab measurements to field measurements we were able to enlarge the number of plants in the experiment drastically, which is very much needed in order to be successful with Deep Learning. For learning, testing and implementing the system, the dataset has to be split up. The bigger the data set and the better covering the different ways the disease can express itself, the better the results will be. Based on only one variety for Erwinia detection that has been tested, the test result of 94% success rate is quite good and comparable with the success rate of Y-virus detection.

Nevertheless, there are still a number of challenges to come to a robust, ready-to-use system for the detection of virus and bacterial diseases.

1. Translation into a real-time application
   It is necessary to be able to recognize sick from healthy on the go. This requires a high speed solution. It requires optimization between speed and quality.
   Which technology will be able to do this? By changing from hyperspectral to multispectral or RGB imaging as a basis will make the solution quite a lot cheaper, but will it be as good?
   And do we need different systems for virus and Erwinia detection, or is it possible to create one integrated system without losing performance.

2. Required level of performance of a technical solution: what quality level is necessary, before a farmer is interested in implementing it. This requires field test combined with inspections by farmers and inspectors of the NAK.

3. Camera design from hyper to multispectral: determination of spectral bands;
   It was expected that a hyperspectral camera has a big advantage compared to RGB, but that has to be investigated. The current hyperspectral camera is a line scan based device that operates slowly and under special light conditions. Being able to switch over to multispectral or RGB cameras without losing to much quality is highly promising.

4. Varietal effects: varieties vary strongly in the way the virus infection presents itself. Being able to tackle many different ways and level of presentation, requires a high investment in collected big datasets under a lot of different circumstances and using a lot of different varieties. Also the annotation of these large datasets is labor intensive.

5. Different Erwinia subspecies: Erwinia has 3 common types (subspecies): Dickeya solani, Pectobacterium atrosepticum, P. carotovorum ssp. brasiliense. These too have different syndromes. It is necessary to recognize either of these subspecies.

6. Effects of varying light conditions, influence of dew / relative humidity. All measurements till now have been done in a more or less controlled environment: covered camera setup with artificial lighting and always dry conditions (dry crop).

7. Translation of the above into a cost / benefit analysis of the application (as a sharpening of a previous market analysis). At the start of the project an estimation was made of the cost of on farm seed potato selection and losses due to lowering the quality level of the potatoes or full rejection as seed potato. This resulted in an estimate of a cost level of €30-35k for a 40ha seed potato farm. When knowing the investment and the productivity (working speed) of practical solution, then a cost-benefit analysis can be made. In connection to this, detection using drones might be a feasible option. Further research is needed in this field.

8. Consequences of the new selection technology for the inspection system (eg cost reduction through logging and sharing measurement data). The cost of inspection is paid by the farmer and when a trustworthy solution is available to reduce these cost, it will be highly appreciated by the farmers. This option requires first to collect experiences with integral field inspected by an inspection implement. This discussion is therefore useful as soon as a commercial device is in sight.

In terms of TRL (Technology Readiness Level) we are now at the stage of TRL5 (technology component of systems validation in a relevant environment). Several steps are needed to develop to TRL level 7-8 (prototype demonstration in an operational environment – actual system completed). We aim to do so in an initiative called Synergia, a public – private partnership.

7 Communication

During the project the progress and intermediate results were communicated with the Dutch arable sector.

2016

- presentation on the Delphy Pootaardappeldag (Emmeloord)
- article in the newsletter of BO-Akkerbouw
- Several messages on Twitter
- Press release
- News message regarding progress of Smart disease detection.

2017

- Open day 6th July 2017 at NAK research farm.
- Several news articles in agricultural magazines.
- presentation on AgroFood Tech fair (Den Bosch 13-14 december 2017).

Nieuwe Oogst (15-7-17)

Detectie ziek pootgoed met Smart-zoeker getest in het veld

ACHTERGROND

DOOR JANET BEEKMAN

Dit jaar rijdt er voor het eerst een meetbak met speciale camera's op een proefveld om ziekte in poters tijdig op te sporen. Zo komt Smart-technologie voor telers van pootgoed weer dichterbij.

Vroege detectie en verwijdering van met virus- en bacterieziekten besmet pootgoed kan veel schade voorkomen. Praktijkonderzoek AGV, Glastuinbouw en PRI van Wageningen UR onderzoeken sinds 2011 inzet van smart-technieken voor detectie van erwinia en virusziek. Het is onderdeel van het project Op naar Precisielandbouw 2.0. "In het laboratorium zijn de hyperspectraalcamera-techniek en 3D-plantanalyse het veelbelovendst", zegt Jan Kamp, projectleider Smart Ziekzoeken Pootaardappelen. "De hyperspectraalcamera kan virusziekten goed herkennen. We hebben aanwijzingen dat erwinia zo ook te detecteren is, maar dan in combinatie met 3D-beelden."

Er is een Smart Ziekzoeker ontwikkeld met een meetbak waarin camera's hangen. De bak is afgesloten van buitenlicht en hangt in de fronthef van een trekker. De led- en halogeenverlichting in de meetbak geeft een specifiek lichtspectrum. De hyperspectraalcamera meet de lichtweerkaatsing van een plant. Een snelle 3D-camera meet volume en vorm van het bladpakket en de structuur van een oppervlakte. Door ziekte verandert de bladstructuur, de camera kan dit identificeren.

FOTO: PRAKTIJKONDERZOEK AGV

De Smart Ziekzoeker bestaat uit een meetbak met camera's.

Sinds juni 2017 doet de Smart Ziekzoeker metingen op een proefveld in Tollebeek. "Parallel aan die resultaten, analyseren we ook gewasdata vastgelegd met een drone. Zo zien we of met een drone ziek pootgoed is op te sporen. En we laten een NAK-expert in het veld zieke planten opsporen met koppeling van gevonden locaties aan gps-coördinaten."

Kamp schat ziekteschade in pootgoed voor een teler met 40 hectare op gemiddeld €30.000 tot €40.000 per jaar. Dit zijn kosten voor selectie, klasseverlaging en afkeuringen. Op termijn investeren in een Smart-zoeker is voor grotere pootgoedbedrijven rendabel te maken. Kamp: "Zeker als het lukt met Smart-technologie de ziekzoeker zover te ontwikkelen dat deze ziektes eerder ontdekt dan het menselijk oog. Dan kunnen telers nog beter selecteren en de kwaliteit van het Nederlandse pootgoed verder verbeteren."

Het ideale scenario is een ziekzoekrobot die volautomatisch zieke planten detecteert en met een grijper verwijdert. "Dat is nog toekomstmuziek, eerst moet blijken of de ziekzoeker in staat is om zieke planten betrouwbaar te herkennen. We zijn nog volop bezig dit te onderzoeken. In 2018 willen we het detectiesysteem verder vervolmaken en daarna zoeken naar een goede techniek voor toepassing in het veld."

Website Boerderij – 12 juli 2017

http://www.boerderij.nl/Akkerbouw/Achtergrond/2017/7/Detectie-ziek-pootgoed-getest-in-het-veld-157192E/?cmpid=NLC|boerderij_vandaag|2017-07-12|Detectie_ziek_pootgoed_getest_in_het_veld

Vakblad Boerderij – 11-7-17

'Smart Ziekzoeker moet ziekten eerder zien dan teler'

IVAN REINDERS

De Smart Ziekzoeker kan met speciale camera's virus- en Erwinia-zieke planten in pootaardappelen herkennen. Projectleider Jan Kamp van Wageningen University & Research ziet kansen.

INTERVIEW

Welk doel heeft de Smart Ziekzoeker?

'Een techniek ontwikkelen waar de pootgoedteler op kan vertrouwen. De Smart Ziekzoeker moet bij voorkeur de ziekten eerder zien dan de teler of selecteur.'

Wat is er tot nu toe gebeurd?

'In 2015 en 2016 hebben we in een laboratorium met opgepotte aardappelplanten gekeken naar virus en Erwinia. De focus lag op vroegtijdige detectie. De resultaten van 2015 moettechnisch zijn vergeleken met die van een selecteur. De hyperspectraal- en 3D-camera bieden het meeste perspectief.'

Is het veld is een stuk lastiger?

Ja. In het lab kun je aardappelplanten van opzij met camera's bekijken en dat lukt in het veld niet. Je moet van boven werken of schuin van opzij. Daarvoor hebben we een meetbak ontworpen met speciale camera's en aangepaste belichting.

'De machine doet het niet gek, maar statistisch gezien hebben we meer data nodig. De detectie in het veld is belangrijk voor de robuustheid van het systeem.'

Nog tegen problemen aangelopen?

'De Specim FX10-hyperspectraalcamera was nog maar kort op de markt. Er zaten kinderziekten in en de camera moest terug naar Finland. Met de proef zijn we daardoor iets later gestart.

'De Specim FX10 maakt een lijnscan van de planten en dat zorgt qua snelheid voor een beperkende factor. We rijden met een bedrevend lage snelheid van 400 meter per uur. Het veld in gaan met de machine is wel een belangrijke stap in het proces.'

Na vier jaar is er een machine?

'De techniek is nog niet praktijkrijp. We hopen in dit project een techniek te kunnen afleveren op basis waarvan Evenaland, een van de partners van het project, een machine kan maken. Bijvoorbeeld een autonoom werkend voertuig met camera's voor de aardappelselectie.'

'De detectie in het veld levert veel data op en dat is belangrijk voor de robuustheid van het systeem.'

Jan Kamp, Wageningen University & Research — Foto: Ivan Reinders

Wat is het economische belang?

'De schade door klasseverlaging en afkeuring in pootgoed is in Nederland jaarlijks 20 à 25 miljoen euro. De kosten van selectie bedragen 8 tot 10 miljoen euro. Voor een bedrijf met 40 hectare pootgoed gaat het om 30.000 tot 35.000 euro aan kosten en kwaliteitsderving.

'BO Akkerbouw financieert het project uit restmiddelen van het voormalig Productschap Akkerbouw. Agrico, HZPC, NAK en de Pootaardappelacademie doen ook mee.'

Meuwissen tv

Artikel Nieuwe Oogst 8-7-17

Smart-ziekzoeker pootgoed nu in volgende fase

Lelystad – Praktijkonderzoek AGV test het automatisch opsporen van ziekte pootaardappelen voor het eerst op het veld. Hyperspectraal- en 3D-camera's zijn hiervoor het meest geschikt.

"Na twee jaar testen van detectietechnieken van ziek pootgoed in een laboratorium, testen we dit seizoen geschikte technieken uit in het veld", zegt Jan Kamp, projectleider van het project Smart Ziekzoeken Pootaardappelen.

Vanaf 2015 voeren Praktijkonderzoek AGV, Glastuinbouw en PRI het project Smart Ziekzoeken Pootaardappelen uit. Het vierjarige project loopt tot eind 2018 en is onderdeel van 'Op naar Precisielandbouw 2.0'.

Vanaf vorige maand rijdt een nieuwe meetunit Smart Ziekzoeker voor het eerst op een proefveld in Tollebeek. Uit resultaten in 2015 en 2016 in een laboratorium blijkt 3D-plantanalyse en de hyperspectraalcameratechniek het meest veelbelovend zijn. "We willen de Smart-technologie zover ontwikkelen dat de ziekzoeker ziektes eerder ontdekt dan het menselijk oog. Telers kunnen dan de kwaliteit van het Nederlandse pootgoed nog verder verbeteren", zegt Kamp.

De Smart-ziekzoeker bestaat uit een meetbak waarin camera's en led- en halogeenverlichting hangen. De bak is afgesloten van buitenlicht en hangt in de fronthef van een trekker. De verlichting geeft een specifiek lichtspectrum. De hyperspectraalcamera meet de lichtweerkaatsing van een plant. Een snelle 3D-camera meet het volume en de veranderde bladstructuur van een zieke aardappelplant. De Smart-ziekzoeker verricht metingen op een NAK-proefveld met pootgoed met verschillen in besmettingsgraad van Erwinia en virus. "Ook analyseren we gewasdata vastgelegd met een drone om te zien of we hiermee ziek pootgoed kunnen detecteren. En we laten een NAK-expert in het veld zieke planten opsporen met koppeling van gevonden locaties aan GPS-coördinaten."

FOTO: PRAKTIJKONDERZOEK AGV

De Smart-ziekzoeker bestaat uit een meetbak met camera's.

Poster (pitch) Agrofood Tech – Den Bosch (13-14 december 2014)

2018:

- Presentation on the Delphy pootaardappeldag (Tollebeek, 27-6-18)

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Annex 1 Overview of inoculation procedures of seed potatoes in pots 2015 - 2016

In 2011 it was required to grow plants in a glasshouse in pots. The environmental conditions in the glasshouse compartment strongly influenced plant growth resulting in a phenotype that deviated from plants grown in the field. Therefore, in 2015 and 2016 it was de decided to grow plants in pots outdoors. In 2017 and 2018 field experiments were conducted under conditions more realistic in practice.

2015

Experimental setup "Erwinia's"

Tubers were planted in a light clay soil at two dates with a two-weeks period in between. Pots were frequently watered. Due to a cold spring plant development was slow. Therefore measurements started late. Measurements were mainly done on plants from the 1st planting period. In July a limited number of plants with symptoms were selected from the second series.

Bacteria growth and inoculation of tubers. Dickeya solani (strain IPO nr. 2222) was grown for 48 h on Trypticase Soy Agar (Oxoid). Bacteria were suspended from colonies on plates in water and diluted to a densitiy of approximately 10^7 cells/ml. Tubers were inoculated with respectively 10^6 and 10^7 cells/ml. Minitubers of cultivar Kondor were submerged in a mesh sac (48 tubers per bag) in a 2L container in the bacterial suspension or in water (control) and subsequently placed into a exicator. A vacuum was created using a vacuum pump of -600 to -800 mbar. Tubers were left in the vacuum for 10 min to release air from the lenticels. The vacuum was released at which cells migrated in the open spaces (lenticels) in the tubers. Tubers were left for 10 min at atmospheric pressure after which they were dried in an air flow during which the mesh sac was turned several times.

Growth of plants. Plants were grown on root fabric in 7.5 l pots in a light clay in a small field plot at Unifarm (Grebbedijk, Wageningen, the Netherlands). Plants were visually examined for disease symptoms at the days plants were also analyzed with the vision techniques. Plants were sampled, plant material extracted and analyzed by dilution planting on double layer CVP (Helias et al., 2012), to confirm the presence of target bacteria.

Results. Results are summarized in Table 1. A total of 21 from 44 plants were not infected, 9 were symptomless infected. The other 14 plants were symptomatic. We have no clear explanation for the relatively low number of infected plants. Water-inoculated plants were symptom-free.

Table 1. *Dickeya solani inoculated plants*

Symptoms	N	Plant nr.
Not infected	21	22, 23,25,28,33,34,38, 39,42,43,45,47,50,53,54,55,57,59,94,113
Symptomless infected	9	24,27,40,44,48,49,51,52,58
Internal vascular discoloration	5	21,26,36,37,41
Wilting	7	29,30.32.46.56.87.89
Blackleg	1	31
Stem rot	1	103

Experimental set up "Viruses"

Growth virus-infected plants. Virus (secundary-) infected plants were also grown in pots outdoors. The tubers (cv. Bintje) used, were harvested from virus-infected plants grown in the glasshouse. Part of

the tubers were lost as a result of damage by crows. In week 25-27, virus-diseased plants were analyzed with vision techniques. Plants were weekly observed for symptom development.

Results. Symptom development is summarized in Table 2. In all virus-infected plants symptoms were visually observed. In leaves that developed later in the growing season only weak (but still visible) symptoms were observed. Resistance develops due to ageing as the plants responds on the presence of the virus by RNAi silencing. Symptoms of virus S and M were relatively weak compared with plants infected with virus Y. In plants infected with PLRV strong leaf rolling was observed with yellowing of veins and pointed leaf tips. Vision techniques should also be evaluated with primary infected plants in which symptoms are more difficult to observe visually.

Table 2. *Virus-diseased plants*

Virus	Leaf symptoms	N	Plant nr.
PVS	Necrotic lesions	1	138
PAMV	Yellow spots	3	139,140,141
PLRV	Leaf roll	3	142,143,144
PVA	Crinkle and mosaic	1	146
PVV	Crinkle and mosaic	3	148,149,150
PVY	Crinkle and mosaic	4	151,156,158,161

Virus inoculation plants – disease symptoms (expert judgement)

Virusplanten - waarnemingen per datum (op dag voorafgaand aan de metingen)														
datum	15-6-2015				datum	23-6-2015				datum	30-6-2015			
plant	inoculum	planth. (cm)	symptomen	ras	plant	inoculum	planth. (cm)	symptomen	ras	plant	inoculum	planth. (cm)	symptomen	ras
121	geen	15	geen	Bintje	121	geen	30	geen	Bintje	121	geen	44	geen	Bintje
122	geen	18	geen	Bintje	123	geen	28	geen	Bintje	123	geen	42	geen	Bintje
123	geen	14	geen	Bintje	125	geen	28	geen	Bintje	125	geen	39	geen	Bintje
125	geen	15	geen	Bintje	126	geen	27	geen	Bintje	126	geen	37	geen	Bintje
126	geen	11	geen	Bintje	128	geen	28	geen	Bintje	128	geen	35	geen	Bintje
131	geen	12	geen	Bintje	131	geen	27	geen	Bintje	131	geen	41	geen	Bintje
132	geen	10	geen	Bintje	132	geen	25	geen	Bintje	132	geen	42	geen	Bintje
133	geen	11	geen	Bintje	133	geen	25	geen	Bintje	133	geen	41	geen	Bintje
134	geen	13	geen	Bintje	134	geen	30	geen	Bintje	134	geen	41	geen	Bintje
135	geen	12	geen	Bintje	135	geen	29	geen	Bintje	135	geen	43	geen	Bintje
138	PVS 009	13	vroeg diepnervigheid	IJsselster	138	PVS 009	28	vroeg diepnervigheid	IJsselster	138	PVS 009	37	vroeg diepnervigheid, e.a. *)	IJsselster
139	PAMV 202	7	geen	Friso	139	PAMV 202	17	diepnervigh.+licht bontig	Friso	139	PAMV 202	31	diepnervigheid + licht bontig	Friso
140	PAMV 202	10	diepnervigheid	Friso	140	PAMV 202	22	diepnervigh.+licht bontig	Friso	140	PAMV 202	33	diepnervigheid + licht bontig	Friso
141	PAMV 202	11	diepnervigh.+licht bontig	Friso	141	PAMV 202	21	diepnervigh.+licht bontig	Friso	141	PAMV 202	33	diepnervigheid + licht bontig	Friso
142	PLRV 303	5	groeivertraging	Bintje	142	PLRV 303	13	groeivertr, lichte bladrol	Bintje	142	PLRV 303	19	groeivertraging, lichte bladrol	Bintje
143	PLRV 303	7	groeivertraging	Bintje	143	PLRV 303	12	groeivertr, lichte bladrol	Bintje	143	PLRV 303	21	groeivertraging, lichte bladrol	Bintje
144	PLRV 303	8	groeivertraging	Bintje	144	PLRV 303	12	groeivertr, lichte bladrol	Bintje	144	PLRV 303	21	groeivertraging, lichte bladrol	Bintje
146	PVA 410	16	krinkel en bont	Sirtema	146	PVA 410	29	krinkel en lichte bont	Sirtema	146	PVA 410	31	krinkel en lichte bont	Sirtema
148	PVV 502	15	krinkel	Gladblaadje	148	PVV 502	25	krinkel	Gladblaadje	148	PVV 502	31	krinkel	Gladblaadje
149	PVV 502	16	krinkel	Gladblaadje	149	PVV 502	26	krinkel	Gladblaadje	149	PVV 502	34	krinkel	Gladblaadje
150	PVV 502	10	krinkel en bont	Gladblaadje	150	PVV 502	25	krinkel en bont	Gladblaadje	150	PVV 502	32	krinkel en bont	Gladblaadje
151	PVY 753	13	bont	Bintje	151	PVY 753	23	licht bont	Bintje	151	PVY 753	25	licht bont	Bintje
156	PVY 755	11	krinkel en bont	Bintje	156	PVY 755	20	krinkel	Bintje	156	PVY 755	31	krinkel	Bintje
158	PVY 772	13	krinkel en bont	Bintje	158	PVY 772	19	krinkel en bont	Bintje	158	PVY 772	26	krinkel en bont	Bintje
161	PVY 773	18	krinkel en bont	Bintje	161	PVY 773	25	krinkel en bont	Bintje	161	PVY 773	24	krinkel en bont	Bintje
					symptomen weer iets meer uitgesproken t.o.v. vorige week					symptomen weer iets meer uitgesproken t.o.v. vorige week				
25					25					25				
Toelichting:														
- groeivertr = groevertraging														
*) vroeg diepnervigheid, beginnende bronsvlekken, bruine bladranden atypisch voor virus														

Erwinia inoculation plants – disease symptoms (expert judgement)

Beoordeling Erwinia besmette planten

plant	serie	inocu-lum	datum	planth. (cm)	symp-tomen		meting 19-6	meting 23-6
001	A	water	18-6-2015	14	geen		x	
002	A	water	18-6-2015	9	geen		x	
003	A	water	18-6-2015	12	geen		x	
004	A	water	18-6-2015	10	geen		x	
005	A	water	18-6-2015	10	geen		x	
006	A	water	18-6-2015	7	geen		x	
007	A	water	18-6-2015	10	geen		x	
008	A	water	18-6-2015	7	geen		x	
009	A	water	18-6-2015	10	geen		x	
010	A	water	18-6-2015	10	geen	9,4	x	
011	A	water	18-6-2015	8	geen			x
012	A	water	18-6-2015	7	geen			x
013	A	water	18-6-2015	8	geen			x
014	A	water	18-6-2015	9	geen			x
015	A	water	18-6-2015	5	geen			x
016	A	water	18-6-2015	8	geen			x
017	A	water	18-6-2015	8	geen			x
018	A	water	18-6-2015	10	geen			x
019	A	water	18-6-2015	11	geen			x
020	A	water	18-6-2015	15	geen			x
021	A	Ds E6	18-6-2015	9	geen		x	
022	A	Ds E6	18-6-2015	9	geen		x	
023	A	Ds E6	18-6-2015	10	geen		x	
024	A	Ds E6	18-6-2015	9	geen		x	
025	A	Ds E6	18-6-2015	10	geen		x	
026	A	Ds E6	18-6-2015	10	geen		x	
027	A	Ds E6	18-6-2015	9	geen		x	
028	A	Ds E6	18-6-2015	8	geen		x	
029	A	Ds E6	18-6-2015	10	geen		x	
030	A	Ds E6	18-6-2015	11	geen	9,7	x	
031	A	Ds E6	18-6-2015	11	geen			x
032	A	Ds E6	18-6-2015	6	geen			x
033	A	Ds E6	18-6-2015	10	geen			x
034	A	Ds E6	18-6-2015	11	geen			x
035	A	Ds E6	18-6-2015	9	geen			x
036	A	Ds E6	18-6-2015	10	geen			x
037	A	Ds E6	18-6-2015	12	geen			x
038	A	Ds E6	18-6-2015	11	geen			x
039	A	Ds E6	18-6-2015	10	geen			x
040	A	Ds E6	18-6-2015	9	geen			x
041	A	Ds E7	18-6-2015	8	geen		x	
042	A	Ds E7	18-6-2015	8	geen		x	
043	A	Ds E7	18-6-2015	10	geen		x	
044	A	Ds E7	18-6-2015	11	geen		x	
045	A	Ds E7	18-6-2015	8	geen		x	
046	A	Ds E7	18-6-2015	4	geen		x	
047	A	Ds E7	18-6-2015	8	geen		x	
048	A	Ds E7	18-6-2015	9	geen		x	
049	A	Ds E7	18-6-2015	8	geen		x	
050	A	Ds E7	18-6-2015	7	geen	8,21	x	
051	A	Ds E7	18-6-2015	10	geen			x
052	A	Ds E7	18-6-2015	8	geen			x
053	A	Ds E7	18-6-2015	8	geen			x
054	A	Ds E7	18-6-2015	8	geen			x
055	A	Ds E7	18-6-2015	9	geen			x
056	A	Ds E7	18-6-2015	7	geen			x
057	A	Ds E7	18-6-2015	10	geen			x
058	A	Ds E7	18-6-2015	8	geen			x
059	A	Ds E7	18-6-2015	7	geen			x
							30	29

plant	serie	inocu-lum	datum	planth. (cm)	symp-tomen	planth. (cm)	meting 26-6	meting 30-6
001	A	water	25-6-2015	19	geen		x	
002	A	water	25-6-2015	19	geen		x	
003	A	water	25-6-2015	20	geen		x	
004	A	water	25-6-2015	18	geen		x	
005	A	water	25-6-2015	18	geen		x	
006	A	water	25-6-2015	13	geen		x	
007	A	water	25-6-2015	17	geen		x	
008	A	water	25-6-2015	16	geen		x	
009	A	water	25-6-2015	18	geen		x	
010	A	water	25-6-2015	15	geen	17,25	x	
011	A	water	25-6-2015	16	geen			x
012	A	water	25-6-2015	16	geen			x
013	A	water	25-6-2015	17	geen			x
014	A	water	25-6-2015	15	geen			x
015	A	water	25-6-2015	11	geen			x
016	A	water	25-6-2015	18	geen			x
017	A	water	25-6-2015	18	geen			x
018	A	water	25-6-2015	19	geen			x
019	A	water	25-6-2015	19	geen			x
020	A	water	25-6-2015	23	geen			x
021	A	Ds E6	25-6-2015	16	geen		x	
022	A	Ds E6	25-6-2015	16	geen		x	
023	A	Ds E6	25-6-2015	17	geen		x	
024	A	Ds E6	25-6-2015	14	geen		x	
025	A	Ds E6	25-6-2015	14	geen		x	
026	A	Ds E6	25-6-2015	16	geen		x	
027	A	Ds E6	25-6-2015	17	geen		x	
028	A	Ds E6	25-6-2015	16	geen		x	
029	A	Ds E6	25-6-2015	16	geen		x	
030	A	Ds E6	25-6-2015	17	geen	16,55	x	
031	A	Ds E6	29-6-2015	18	2			x
032	A	Ds E6	25-6-2015	17	1			x
033	A	Ds E6	25-6-2015	20	geen			x
034	A	Ds E6	25-6-2015	18	geen			x
035	A	Ds E6	25-6-2015	15	geen			x
036	A	Ds E6	25-6-2015	17	geen			x
037	A	Ds E6	25-6-2015	16	geen			x
038	A	Ds E6	25-6-2015	17	geen			x
039	A	Ds E6	25-6-2015	18	geen			x
040	A	Ds E6	25-6-2015	16	geen			x
041	A	Ds E7	25-6-2015	12	geen		x	
042	A	Ds E7	25-6-2015	14	geen		x	
043	A	Ds E7	25-6-2015	13	geen		x	
044	A	Ds E7	25-6-2015	15	geen		x	
045	A	Ds E7	25-6-2015	13	geen		x	
046	A	Ds E7	25-6-2015	6	geen		x	
047	A	Ds E7	25-6-2015	12	geen		x	
048	A	Ds E7	25-6-2015	14	geen		x	
049	A	Ds E7	25-6-2015	12	geen		x	
050	A	Ds E7	25-6-2015	15	geen	13,42	x	
051	A	Ds E7	25-6-2015	15	geen			x
052	A	Ds E7	25-6-2015	12	geen			x
053	A	Ds E7	25-6-2015	14	geen			x
054	A	Ds E7	25-6-2015	16	geen			x
055	A	Ds E7	25-6-2015	17	geen			x
056	A	Ds E7	25-6-2015	14	geen			x
057	A	Ds E7	25-6-2015	15	geen			x
058	A	Ds E7	25-6-2015	14	geen			x
059	A	Ds E7	25-6-2015	12	geen			x
							30	29

plant	serie	inocu-lum	datum	planth. (cm)	symptomen	planth. (cm)	meting 3-7	meting 7-7
001	A	water	2-7-2015	30	geen		x	
002	A	water	2-7-2015	30	geen		x	
003	A	water	2-7-2015	29	geen		x	
004	A	water	2-7-2015	28	geen		x	
005	A	water	2-7-2015	29	geen		x	
006	A	water	2-7-2015	27	geen		x	
007	A	water	2-7-2015	30	geen		x	
008	A	water	2-7-2015	32	geen		x	
009	A	water	2-7-2015	31	geen		x	
010	A	water	2-7-2015	29	geen	28,8	x	
011	A	water	2-7-2015	27	geen			x
012	A	water	2-7-2015	25	geen			x
013	A	water	2-7-2015	28	geen			x
014	A	water	2-7-2015	29	geen			x
015	A	water	2-7-2015	24	geen			x
016	A	water	2-7-2015	29	geen			x
017	A	water	2-7-2015	30	geen			x
018	A	water	2-7-2015	31	geen			x
019	A	water	2-7-2015	29	geen			x
020	A	water	2-7-2015	29	geen			x
021	A	Ds E6	2-7-2015	23	geen		x	
022	A	Ds E6	2-7-2015	23	geen		x	
023	A	Ds E6	2-7-2015	28	geen		x	
024	A	Ds E6	2-7-2015	27	geen		x	
025	A	Ds E6	2-7-2015	25	geen		x	
026	A	Ds E6	2-7-2015	26	geen		x	
027	A	Ds E6	2-7-2015	27	geen		x	
028	A	Ds E6	2-7-2015	28	geen		x	
029	A	Ds E6	2-7-2015	27	4		x	x
030	A	Ds E6	2-7-2015	29	4	26,4	x	x
031	A	Ds E6	2-7-2015	22	2, beginnend 6		x	x
032	A	Ds E6	2-7-2015	18	1		x	x
033	A	Ds E6	2-7-2015	33	geen			x
034	A	Ds E6	2-7-2015	27	geen			x
035	A	Ds E6	2-7-2015	29	geen			x
036	A	Ds E6	2-7-2015	26	geen			x
037	A	Ds E6	2-7-2015	27	geen			x
038	A	Ds E6	2-7-2015	30	geen			x
039	A	Ds E6	2-7-2015	29	geen			x
040	A	Ds E6	2-7-2015	24	geen			x
041	A	Ds E7	2-7-2015	25	geen		x	
042	A	Ds E7	2-7-2015	28	geen		x	
043	A	Ds E7	2-7-2015	27	geen		x	
044	A	Ds E7	2-7-2015	26	geen		x	
045	A	Ds E7	2-7-2015	27	geen		x	
046	A	Ds E7	2-7-2015	20	4		x	x
047	A	Ds E7	2-7-2015	24	geen		x	
048	A	Ds E7	2-7-2015	25	geen		x	
049	A	Ds E7	2-7-2015	26	geen		x	
050	A	Ds E7	2-7-2015	29	geen	25,9	x	
051	A	Ds E7	2-7-2015	27	geen			x
052	A	Ds E7	2-7-2015	25	geen			x
053	A	Ds E7	2-7-2015	27	geen			x
054	A	Ds E7	2-7-2015	27	geen			x
055	A	Ds E7	2-7-2015	30	geen			x
056	A	Ds E7	2-7-2015	23	beginnend 2		x	x
057	A	Ds E7	2-7-2015	28	geen			x
058	A	Ds E7	2-7-2015	27	geen			x
059	A	Ds E7	2-7-2015	22	geen			x
071	B	water					x	x
087	B	Ds E6					x	x
089	B	Ds E6					x	x
103	B	Ds E7						x
							36	36

symptomen	
0	geen
1	verwelken topbladeren
2	donker groene bovenste bladeren
3	uitdrogen van de bladranden onderste bladeren
4	verwelken en uitdrogen totale blad
5	vergeling van de bladeren
6	zwartpoot
7	stengelnatrot
8	uitdrogen van de gehele stengel
9	plant is dood

plant	serie	inocu-lum	datum	planth. (cm)	sympto	bloei
019	A	water	10-7-2015	30	geen	ja
029	A	Ds E6	10-7-2015	30	4	ja
030	A	Ds E6	10-7-2015	30	4	ja
031	A	Ds E6	10-7-2015	27	2, begir	nee
032	A	Ds E6	10-7-2015	26	1	nee
046	A	Ds E7	10-7-2015	20	4	nee
056	A	Ds E7	10-7-2015	28	geen	nee
071	B	water	10-7-2015	25	geen	nee
087	B	Ds E6	10-7-2015	21	?	nee
089	B	Ds E6	10-7-2015	22	?	nee
094	B	Ds E6	10-7-2015	19	geen	nee
103	B	Ds E7	10-7-2015	16	stengel	nee
113	B	Ds E7	10-7-2015	19	geen ba	nee

plant	serie	inocu-lum	datum	planth. (cm)	symptomen	bloei
019	A	water	16-7-2015	26	geen	uitgebloei
029	A	Ds E6	16-7-2015	30	4	ja
030	A	Ds E6	16-7-2015	28	4	ja
031	A	Ds E6	16-7-2015	24	2, beginnen	nee
032	A	Ds E6	16-7-2015	29	1	nee
046	A	Ds E7	16-7-2015	22	gedrongen (	nee
056	A	Ds E7	16-7-2015	30	geen	nee
071	B	water	16-7-2015	23	geen	nee
087	B	Ds E6	16-7-2015	32	?	ja
089	B	Ds E6	16-7-2015	19	?	nee
094	B	Ds E6	16-7-2015	23	geen	nee
103	B	Ds E7	16-7-2015	22	geïnoculeer	nee
113	B	Ds E7	16-7-2015	19	geen bacter	ja

2016

Experimental set up "Erwinia's"

In 2015 seed potatoes of the susceptible cultivar Kondor were vacuum inoculated with *Dickeya solani* and grown in pots with clay soil. This only resulted in a limited number of symptomatic plants.
To increase the disease incidence the following was done:

a. we used a light soil instead of a heavy clay (advantage increased warming of soil)
b. supply of less water (advantage less cooling and more drought stress)
c. use of more aggressive bacteria (*P. atrosepticum* and *P. c.* subsp. *brasiliense)*.

Bacteria were grown as described for the experimental set up in 2015. Plants were inoculated with *Dickeya solani* IPO2222, *Pectobacterium atrosepticum* IPO1007 or with *P. carotovorum* subsp. *brasiliense* IPO3649 or treated with water. For each treatment 40 tubers were planted but only 8 tubers for the water control. Inoculations were evaluated by dilution plating of periderm extracts on double layer CVP (Helias et al., 2012). Typical colonies were found in extracts of the inoculated tubers but not in the extracts of the water control.

Plants were grown on root fabric in 7.5 l pots in a light clay in a small field plot at Unifarm under nets to prevent bird damage (Grebbedijk, Wageningen, the Netherlands). Tubers were planted on 10 May and 24 May. During plant growth restricted water was provided. Fertilization was done on 28 June and 16 July. On 4 July plants were treated against Phytophthora with Curzate and thereafter treatment against early blight was done as is usual in practice. Plants were visually examined weekly for disease symptoms at the days plants were also analyzed with the vision techniques. On most days 6 water-treated plants and 14 inoculated plants were selected for analysis with vision techniques from the following categories: symptomless, yellowing leaves, upward folding of top leaf, wilting, stem rot or blackleg. Symptomatic stems were marked with a label and plants were measured from the top and front side. After the last measurement, plants were transported back to the experimental field. Plants which were symptomless or showed atypical symptoms were sampled, plant material extracted and analysed by dilution planting on double layer CVP (Helias et al., 2012), to confirm the presence of target bacteria.

Results. The percentages emerged plants varied largely per treatment and day of planting. At the tubers planted on the 10th of May, between 65% for *P. c.* subsp. *brasiliense* and 95% for the other Treatments (Table 3). For tubers planted on the 24th of May the percentage emerged plants were between 20% for *P. c.* subsp. *brasiliense* and 100% for the water control.
After emergence the plants inoculated with *D. solani* and *P. c.* subsp. *brasiliense* developed poorly and a strong and rapid symptom development became visible (Fig.1). Frequently, plants hardly grew above the edge of the pots. Plants in inoculated with *P. atrosepticum* developed much better; the disease incidence was lower and symptom development was more delayed. The water controls remained free of symptoms. Obviously there was a large difference in the virulence between the bacterial strains used for inoculation. Results confirmed observations in previous field experiments in which similar strains were used (Van der Wolf et al., 2016). A later moment of planting seems to result in a higher disease incidence. Possibly the ageing of the seed during cold storage or alternatively, the weather conditions after planting may have influenced symptom expression. In particular plants inoculated with *P. atrosepticum* were useful for the evaluation of the visison techniques.
A selection of plants were used for the measurements (Table 4). Plants were measured 1 to 9 times. Plants showed different symptoms, including leaf chlorosis, folding of top leaves, wilting, stem rot and

blackleg (Table 5). During crop growth also dwarfing was found for a number of plants. From July onward black spots developed on leaves (also on the water treated plants) which may have influenced measurements. The stems of non-symptomatic plants or plants with atypical symptoms were analyzed by dilution plating: 7 water-treated, 4 plants inoculated with *D. solani*, 3 with *P. atrosepticum* and 1 with *P. c.* subsp. *brasiliense*. Independent on the treatement, densities of pectinolytic bacteria were low (10^2 – 10^4 cfu/g; data not shown). Infections of stems of water-treated plants may have occurred during as plants grew at close proximity. Nevertheless, at the low densities not effect on physiology or measurements can be expexted.

Table 3. *Percentage emerging plants inoculated with Dickeya solani (Dsol), Pectobacterium atrosepticum (Patro) or P. carotovorum subsp. brasiliense (Pcb) or treated with water and planted on two different moments (N=20)*

Planting date	Water	Dsol 2222	Patro 1007	Pcb 3649
10-5-2016	95	95	95	65
24-5-2016	100	35	85	20

Table 4. *Summary of the number of scan days, the number of plants measured per treatment and the number of scans per plant for two days in 2016. Plants were inoculated with Dickeya solani (Dsol), Pectobacterium atrosepticum (Patro) or P. carotovorum subsp. brasiliense (Pcb) Scans were made on 16/6, 23/6, 24/6, 27/6, 29/6, 30/6, 5/7, 6/7, 12/7, 14/7, 19/7, 21/7, 26/7 and 28/7.*

Planting date	Water	Dsol 2222	Patro 1007	Pcb 3649
10-5-2016	95	95	95	65
24-5-2016	100	35	85	20

		Plant date	
		10-5-2016	24-5-2016
Number of scan days		9	5
Water	Number of plants	12	8
	Number scans/plant	1 – 9	1 – 5
Dsol 2222	Number of plants	9	4
	Number scans/plant	2 – 8	1 – 5
Patro 1007	Number of plants	16	14
	Number scans/plant	1 – 7	1 – 5
Pcb 3649	Number of plants	6	2
	Number scans/plant	2 – 7	3 – 5

Table 5. *Symptoms of potato plants inoculated with Dickeya solani (Dsol), Pectobacterium atrosepticum (Patro) or P. carotovorum subsp. brasiliense (Pcb) at the latest scan.*

Symptoms	water	Dsol 2222	Patro 1007	Pcb 3649
	N	N	N	N
No	20	4	2	2
Leaf yellowing	0	0	4	0
Folding top leaves	0	2	6	1
Wilting	0	0	0	1
Desiccation of stems	0	1	7	2
Blackleg	0	1	6	0
Dying stems due to blackleg	0	5	4	2

Experimental set up "Viruses"

In 2015 tubers infected with different viruses were used. During a meeting of the steering committee it was decided that for the follow up research cultivars will be used which differ in susceptibility to PVY^{NTN}. In addition also plants will be used with primary virus infections.

Cultivation of plants from secondary infected tubers. On the 10th of May virus-infected tubers were planted as indicated for the tubers inoculated with bacteria. The following seed tubers were used: 30 tubers of cultivar Bintje infected with different strains (Y753, Y755, Y772 of Y773) of PVY^{NTN} produced at Wageningen UR, and 9 tubers of cultivar Gineke with a field infection with a PVY complex provided by one of the project partners. In addition, respectively 25 and 9 virus-free tubers were used of cultivars Bintje and Gineke (Table 6)

Cultivation of plants with primary infections. On the 10th of may respectively 25 and 50 virus-free tubers of cultivars Bintje and Kondor were planted in pots as described for tubers inoculated with bacteria. On the 28th of June 25 plants of cultivar Bintje and 25 plants of cultivar Kondor were inoculated with PVY^{NTN} (Bos, 1965). The inoculum came from *N. tabacum* 'White Burley' infected with PVY^{NTN} (isolate 757). Plants were consecutively sprayed with carborundum powder (500 mesh), inoculated by rubbing leaves with the index fingers with the infectious sap spraying plants with water. After the last scan day, 12 (6 control and 6 inoculated) scanned plants of cultivar Bintje and 6 (3 inoculated and 3 control) plants of cultivar Kondor were analyzed with ELSIA for infections with PVY.

Plant growth was done as described for the plants inoculated with bacteria. Plants were observed four times for symptom development using the following categories: symptomless (not inoculated), symptomless (inoculated), mosaic, necrosis, crinkle.

Results

Secondary infected. The percentage emergence varied per cultivar and virus strain between 75 and 100% (Table 6). The differences cannot be explained by the virus strain but other physiological parameters or pathogens may have influenced emergence. The number of plants per treatment analyzed with vision techniques varied between 2 and 11 (Table 7). The number of scans per plant varied between one and four (Table 7). Infected plants of both cultivars exposed predominantly a light mosaic pattern (Table 8). These symptoms were not visible in the non-inoculated control plants.

Primary infected. None of the inoculated plants showed virus symptoms. Nevertheless, it could not be excluded that plants were latently infected and therefore plants of cultivar Bintje were measured anyway (Table 9). Plants of cultivar Kondor were excluded due to the strong development of black spots on leaves.

After this last day of scanning, plants were tested for the presence of PVY using ELISA. One out of six tested control plants and all six inoculated plants of cultivar Bintje were positive for the virus. ELISA results further showed that inoculated cultivar Kondor plants were positive. When the plants showed a light to moderate mosaic pattern in August, plants were also analyzed with the vision techniques. (Table 9). Due to ageing, the plants of cultivar Bintje were not suitable any more for analysis.

Table 6. *Emergence percentage of tubers secondary infected with various strains of PVY^{NTN}*

Cultivar	virus-isolate	Number of tubers	% emergence
Bintje	No	25	96
Bintje	Y753	7	85
Bintje	Y755	16	100
Bintje	Y772	3	100
Bintje	Y773	4	75
Gineke	No	9	100
Gineke	Y complex	9	100

Table 7. *Summary of the number of scan days, the number of plants per treatment scanned, the number of scans per plant for two potato cultivars with secondary infections with PVY^{NTN}. Plants were scanned on the following days in 2016: 16/6, 22/6, 7/7, 13/7, 20/7 and 27/7.*

		Cultivar	
		Bintje	Gineke
Number of scan days		4	3
Virus free	Number of plants	7	4
	Number of scans per plant	1 - 4	1 – 3
Y 753	Number of plants	4	0
	Number of scans per plant	3 - 4	—
Y 755	Number of plants	11	0
	Number of scans per plant	1 - 4	—
Y 772	Number of plants	3	0
	Number of scans per plant	1 - 4	—
Y 773	Number of plants	2	0
	Number of scans per plant	1 - 4	—
Y complex	Number of plants	0	5
	Number of scans per plant	—	1 - 3

Table 8. *Summary of the number of scanned plants for two cultivars secondary infected with PVY^{NTN} and the number of plants with a light or clear mosaic pattern.*

Cultivar	Virus isolate	Number of	Expression of mosaic pattern	
		Plants scanned	Very light-light	Moderate - severe
Bintje	No	7	0	0
Bintje	Y753	4	4	0
Bintje	Y755	11	10	1
Bintje	Y772	3	1	2
Bintje	Y773	2	2	0
Gineke	No	3	0	0
Gineke	Y complex	5	4	1

Table 9. *Summary of the number of scan days, the number of plants scanned per treatment for two cultivars primary infected with PVY^{NTN}. Plants of Bintje were scanned on the following days in 2016: 13/7 en 27/7. Plants of cultivar Kondor were only scanned on 25/8.*

		Cultivar	
		Bintje	Kondor
Number of scan days		2	1
Virus free	Number of plants	6	6
	Number of scans/plant	1 - 2	1
Y 757	Number of plants	6	10
	Number of scans/plant	1 - 2	1

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