# Development of a crop management method to control the spread of *Potato Virus Y* (PVY)

## Theoretical analysis and practical developments

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Thèse présentée en vue de l'obtention
du grade de docteur en sciences agronomiques
et ingénierie biologique

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*Potato virus Y* (PVY) is a pathogenic plant virus transmitted by aphids. Several PVY control strategies are available but their efficacy is highly variable.
The objective of this thesis is to provide a better understanding of the epidemiology of PVY and propose adapted control strategies to seed potato growers.
The main contributions of the thesis are the following; A PVY survey managed in Switzerland revealed a change over time in the balance of PVY strains. This can be explained by differences in cultivars resistance to the strains and by differences in translocation efficiency of the strains to the progeny tubers. This thesis shows that the decay of the mother tuber physically prevents the virus from translocating from one stem to another, stressing the importance of protecting the crop at early stages. Lastly, this thesis confirmed mineral oil spraying as the most efficient mean to control PVY spread in the field compared to other methods such as elicitors spraying, straw mulching and intercropping. However, the efficacy of mineral oil can be further increased if combined with other methods such as straw mulching.

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Décembre 2017

À Aurèle, à Mélise, à ma famille…

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# ABSTRACT

*Potato virus Y* (PVY) is a pathogenic plant virus transmitted in a non-persistent manner by aphids, and PVY is currently the most important virus challenge faced by seed potato (*Solanum tuberosum* L.) production systems worldwide. Several PVY control strategies are available, the most popular of which are the spraying of insecticides and use of mineral oils. However, insecticides have little impact on PVY spread, while the efficacy of mineral oil is highly variable from one year to the next. Therefore, there is a need to find more efficient control methods.

The objective of this thesis is to provide a better understanding of the epidemiology of PVY and propose more adapted control strategies to seed potato growers. The main findings are as follows:

First, two surveys of PVY isolates genetic diversity in Switzerland revealed an increase in the prevalence of $PVY^{N\text{-}Wi}$ strain, while the prevalence of $PVY^{NTN}$ strain has decreased over the same period. Two hypotheses behind this change in population dynamics are discussed: (i) important differences in the resistance of cultivars to these two PVY strains and (ii) a faster translocation of $PVY^{N\text{-}Wi}$ in comparison to $PVY^{NTN}$.

Second, this thesis sheds new light on mature plant resistance to PVY. An important part of this resistance mechanism is associated with the physiology of the potato plant, as the mother tuber degenerates and physically prevents the virus from translocating from one stem to another. This result further emphasises the need to protect younger plants when the vascular bridge through the mother tuber is still functional.

Third, the thesis suggests intercropping with oat (*Avena sativa* L.) and hairy vetch (*Vicia villosa* Roth) as new efficient PVY control methods. The non-host plants act as virus traps/aphid's stylets cleansing; whereby aphids progressively lose their PVY inoculum as they repeatedly probe the plant(s). However, the neighbouring plants compete for nutrients with the potato plant, leading to significant yield losses. Further research is necessary before these control methods can be widely implemented by seed potato growers.

Finally, this thesis confirmed that mineral oil is the most efficient means of controlling PVY spread in the field compared to insecticides and plant elicitors. However, because the efficacy of mineral oil treatment on interfering with virus transmission is less

significant in the early stages of plant development, this approach needs to be complemented by other control methods such as straw mulching. The results suggest that the synergies between these two methods are significant, paving the way for other combinations of treatments with complementary modes of action.

Taken together, these findings contribute to a better efficacy of PVY control methods and a reduction of their negative impacts on the environment. This in turn helps achieve a more sustainable seed potato production.

**Keywords:** potato, virus, PVY, epidemiology, control, aphid, IPM, oil, insecticide, mulching, intercropping, elicitors, organic.

# RÉSUMÉ

Le *virus Y de la pomme de terre* (PVY) est un phytovirus pathogène transmis de manière non persistante par les pucerons. C'est le virus posant le plus de problèmes pour la production des plants de pomme de terre (*Solanum tuberosum* L.). Plusieurs méthodes de lutte existent pour limiter la dissémination du PVY, dont les plus répandues sont l'application d'insecticides et d'huiles minérales. Cependant, les insecticides ont peu d'effets contre la dissémination de ce pathogène et les huiles minérales présentent une efficacité très variable d'une année à l'autre. Par conséquent, il est important de trouver des méthodes de lutte plus performantes.

Le principal objectif de cette thèse est d'améliorer la compréhension de l'épidémiologie du PVY afin de pouvoir proposer aux producteurs de plants de pommes de terre des méthodes de lutte efficaces.

Les principaux résultats de la thèse sont les suivants :

Premièrement, deux inventaires de souches de PVY réalisés en Suisse ont mis en évidence l'accroissement de la prévalence de la souche $PVY^{N-Wi}$ et une diminution de la prévalence de la souche $PVY^{NTN}$. Deux hypothèses expliquant cette dynamique des populations de souches de PVY sont discutées : (i) des différences importantes de résistance des variétés vis-à-vis de ces souches et (ii) une translocation plus efficace de $PVY^{N-Wi}$ en comparaison de $PVY^{NTN}$.

Deuxièmement, cette thèse propose de nouveaux éléments permettant une meilleure description du phénomène par lequel les plantes de pomme de terre matures sont plus résistantes aux infections par le PVY. Une part importante de cette résistance de l'âge est associée au développement végétatif de la plante et plus particulièrement à la dégénérescence du tubercule mère qui empêche la migration du PVY d'une tige de la plante à une autre. Ce résultat met l'accent sur l'importance de la protection des jeunes plantes contre le PVY, lorsque le pont vasculaire matérialisé par le tubercule mère est encore effectif.

Troisièmement, la thèse propose la culture intercalaire d'avoine (*Avena sativa* L.) et de vesce velue (*Vicia villosa* Roth) comme nouvelles méthodes de lutte efficace contre le PVY. Ces plantes non-hôtes agissent comme des pièges à virus grâce auxquels les pucerons vont progressivement perdre leur charge virale après plusieurs piqûres de

sondage sur ces plantes associées. Cependant, la culture intercalaire entre en compétition avec la pomme de terre pour les éléments nutritifs et l'eau, ce qui a pour conséquences des pertes de rendement significatives. Des recherches supplémentaires sont nécessaires avant que cette méthode ne soit utilisée par les producteurs de plants de pomme de terre.

Enfin, cette thèse confirme que l'application d'huiles minérales est la méthode de lutte la plus efficace contre la dissémination du PVY en comparaison des insecticides et des stimulants de défenses naturelles des plantes. Cependant, l'efficacité des huiles minérales est moindre pour les stades précoces de développement de la plante. Par conséquent, leur application doit être complétée par une autre méthode de lutte comme le paillage par exemple. Les résultats de la thèse ont permis de mettre en évidence des synergies entre ces deux méthodes, ce qui ouvre la voie à d'autres associations de méthodes de lutte complémentaires présentant des modes d'action distincts.

Pour résumer, ces découvertes permettent d'améliorer l'efficacité de la lutte contre le PVY et de réduire l'effet néfaste sur l'environnement des méthodes de lutte actuelles. Par conséquent, cette thèse contribue à l'amélioration de la durabilité de la production de la pomme de terre.

**Mots clés :** pomme de terre, virus, PVY, épidémiologie, lutte, puceron, lutte intégrée, huile, insecticide, paillage, culture intercalaire, stimulants de défenses naturelles, agriculture biologique.

# ZUSAMMENFASSUNG

Das *Y Virus der Kartoffel* (PVY) ist ein pathogenes Phytovirus welches durch die Blattläuse auf nicht persistente Weise übertragen wird. Es handelt sich dabei um das Virus, welches die meisten Probleme in der Pflanzgutproduktion von Kartoffeln (*Solanum tuberosum* L.) verursacht. Es existieren mehrere Bekämpfungsmethoden, um die Ausbreitung des PVY zu begrenzen, wobei das Einsetzen von Insektiziden und Mineralölen die am meist verbreiteten sind. Insektizide haben jedoch wenig Einfluss auf die Ausbreitung dieses Pathogens, und die Wirksamkeit der Mineralöle ist von Jahr zu Jahr sehr variabel. Infolgedessen ist es wichtig, wirksamere Bekämpfungsmethoden zu finden.

Das wesentliche Ziel dieser Dissertation ist es das Verständnis der Epidemiologie des PVY zu verbessern, um den Pflanzgutproduzenten von Kartoffeln wirksame Bekämpfungsmethoden vorschlagen zu können.

Die hauptsächlichen Resultate der Dissertation sind die folgenden:

Als Erstes haben sich bei zwei in der Schweiz durchgeführten Inventaraufnahmen von PVY Stämmen eine Zunahme der Ausbreitung des Stammes $PVY^{N\text{-}Wi}$, sowie eine Verminderung der Ausbreitung des Stammes $PVY^{NTN}$ herausgestellt. Um diese Populationsdynamik zu erklären, werden zwei Hypothesen angeboten: (i) erhebliche Unterschiede in der Sortenresistenz gegenüber diesen Stämmen und (ii) eine effizientere Translokation von $PVY^{N\text{-}Wi}$ gegenüber $PVY^{NTN}$.

Als Zweites zeigt diese Dissertation neue Elemente auf, welche eine bessere Beschreibung des Phänomens der Altersresistenz von reifen Kartoffelpflanzen auf Infektionen von PVY erlauben. Ein wichtiger Teil dieser Altersresistenz ist mit der vegetativen Entwicklung der Pflanze verbunden, und ganz besonders mit der Degeneration des Mutterknollens, welche die Migration des PVY eines Stängels der Pflanze auf einen anderen verhindert. Dieses Resultat hebt die Wichtigkeit des Schutzes von jungen Pflanzen gegenüber dem PVY hervor während dem die die von der Mutterknolle materialisierte vaskuläre Brücke noch wirksam ist.

Als Drittes wird von der Dissertation die Mischkultur mit Hafer (*Avena sativa* L.) und Zottelwicke (*Vicia villosa* Roth) als neue wirksame Bekämpfungsmethode gegen PVY vorgeschlagen. Diese Nichtwirtspflanzen agieren als Virusfallen, da die Blattläuse

nach mehreren Probebohrungen auf diesen Mischpflanzen ihre Virusbelastung schrittweise verlieren (stylet cleansing). Allerdings konkurriert die Mischkultur mit der Kartoffel betreffend Nährstoffen und Bodenfeuchtigkeit, was zu signifikanten Ernteeinbussen führt. Es benötigt zusätzliche Forschung bevor diese Methode von Pflanzgutproduzenten angewendet werden kann.

Letztendlich bestätigt diese Dissertation, dass die Mineralölbehandlung, im Vergleich zu Insektiziden und Stimulatoren der natürlichen Abwehr der Pflanzen, die wirksamste Bekämpfungsmethode gegen die Ausbreitung des PVY ist. Allerding ist die Wirksamkeit der Mineralöle geringer für frühe Entwicklungstadien der Pflanze. Infolgedessen muss deren Anwendung mit einer anderen Methode ergänzt werden, wie zum Beispiel das Ausbringen einer Strohdecke. Die Resultate der Dissertation erlaubten die Evidenz der Synergien dieser zwei Methoden aufzuzeigen, was neue Pisten für andere Assoziationen mit komplementären Bekämpfungsmethoden mit unterschiedlichen Wirkungsweisen erschliesst.

Zusammenfassend erlauben diese Erkenntnisse die Wirksamkeit der Bekämpfung von PVY zu verbessern und den schädlichen Einfluss der aktuellen Bekämpfungsmethoden auf die Umwelt zu reduzieren. Infolgedessen leistet diese Dissertation einen Beitrag zur Verbesserung der Nachhaltigkeit der Kartoffelproduktion

**Stichwörter:** Kartoffel, Virus, PVY, Epidemiologie, Bekämpfung, Blattlaus, integrierte Bekämpfung, Oel, Insektizid, Strohdecke, Mischkultur, Stimulatoren der natürlichen Abwehr, biologische Landwirtschaft.

# SAMENVATTING

Het *aardappel Y virus* (PVY) is een pathogeen plantenvirus dat op een niet-persistente manier door bladluizen wordt overgedragen. PVY is tegenwoordig de belangrijkste virus uitdaging voor de wereldwijde productiesystemen van de aardappel als pootgoed (*Solanum tuberosum* L.). Er zijn een aantal PVY beheersingsstrategieën beschikbaar. Daarvan zijn het spuiten van insecticiden en het gebruik van minerale oliën de meest populaire. Insecticiden hebben echter weinig impact op het verspreiden van PVY, en dat terwijl de doeltreffendheid van minerale oliën van jaar tot jaar zeer variabel kan zijn. Daarom is er nood aan meer efficiënte controlemethodes.

Doel van dit thesis is een beter begrip van de epidemiologie van PVY en het voorstellen van meer toegepaste controlestrategieën naar aardappelpootgoedtelers. De voornaamste bevindingen zijn als volgt:

Ten eerste hebben twee gerichte onderzoeksenquêtes over de genetische diversiteit van PVY-isolaten in Zwitserland een stijging van het overwicht van het $PVY^{N-Wi}$-isolaat onthuld, terwijl het overwicht van het $PVY^{NTN}$-isolaat in dezelfde periode een daling heeft gekend. Er kunnen twee hypothesen achter deze verandering in populatiedynamica voorgesteld worden: (i) belangrijke verschillen in de resistentie van variëteiten tegen deze twee PVY-isolaten en (ii) een snellere translocatie van $PVY^{N-Wi}$ in vergelijking met $PVY^{NTN}$.

Ten tweede heeft dit doctoraatsonderzoek op een nieuwe wijze de resistentie van mature plantenresistentie tegen PVY toegelicht. Een belangrijk deel van deze resistentie is met de ontwikkeling van de aardappelplant geassocieerd, wanneer de moederknol degenereert en de migratie van het virus van stengel tot stengel fysiek vermijdt. Dit resultaat benadrukt verder de nood aan bescherming van jonge planten wanneer de vasculaire hechting met de moederknol altijd effectief blijft.

Ten derde suggereert dit thesis de tussenteelt met haver (*Avena sativa* L.) en bonte wikke (*Vicia villosa* Roth) als nieuwe efficiënte PVY-controlemethode. De niet-gastheerplanten werken als vallen voor bladluizen en ook als styletreinigers. Hierbij verliezen de bladluizen op progressieve manier hun PVY-inoculum terwijl ze herhaaldelijks deze planten toetsen. De naburige planten vechten echter om nutriënten met de aardappelplanten, wat voor een significant oogstverlies zorgt. Meer onderzoek

is nodig vooraleer deze controlemethodes grootschalig door de pootgoedtelers geïmplementeerd kunnen worden.

Tenslotte heeft dit onderzoek minerale oliën als meest efficiënte middel bevestigd ter controle van de PVY-verspreiding in het veld in vergelijking met insecticiden en plantelicitoren. Omdat de doeltreffendheid van de minerale oliebehandeling in het belemmeren met de virustransmissie echter minder significant is tijdens de eerste stadia van de plantontwikkeling, vereist deze benadering om aangevuld te worden met andere controlemethodes zoals mulching. De resultaten duiden aan dat de synergieën tussen beide methodes significant zijn, en de weg plaveien voor andere behandelingscombinaties met complementaire werkingsmodus.

Alles in acht genomen dragen deze bevindingen bij tot een betere effectiviteit van PVY-controlemethodes en een vermindering van hun negatieve impact op het milieu. Dit helpt op zijn beurt om een meer duurzame aardappelpootgoedproductie te bekomen.

**Sleutelwoorden**: Aardappel, virus, PVY, epidemiologie, controle, bladluizen, IPM, Olie, insecticiden, mulching, tussenteelt, elicitoren, biologisch.

# AUTHOR'S CONTRIBUTIONS

The points 1 to 7 of the Introduction were written by myself under the supervision of [1]Christophe Lacomme and [2]Claude Bragard and not published elsewhere. The point 8 of the introduction was co-authored with [2]Claude Bragard, [2]Stuart Carnegie, [2]John Kerr, [3,4]Laurent Glais, [5]Mathuresh Singh, [6]Phillip Nolte, [7]Jean-Louis Rolot, [8]Kürt Demeulemeester, and [1]Christophe Lacomme; and is published in the book "Potato virus Y: biodiversity, pathogenicity, epidemiology and management" published by the PVY-Wide network (Springer Edition; ISBN: 978-3-319-58858-2 [Print] 978-3-319-58860-5 [Online]). The draft version of the manuscript was written by myself and the other authors reviewed the manuscript and provided ad hoc contributions. All authors read and approved the final version of the work. The version presented in this thesis has been modified from the original. The original is presented in Annex 1, with permission of Springer Nature.

Chapter 1 is a research article submitted to "European Journal of Plant Pathology" the 17th of November 2017 with the title: "Resistance of potato cultivars as a determinant factor of Potato Virus Y (PVY) epidemiology" and co-authored with [2]Claude Bragard and [9]Olivier Schumpp. The study was conceived by myself. The sampling in the field was done by [9]Aurelie Buchwalder, [9]Emile Schaer, [9]Henri Gilliand and [9]Werner Wild. The molecular work was done by [9]Aurelie Buchwalder, [9]Emile Schaer, [9]Nathalie Dubuis and [9]Justine Brodard. The greenhouse trials were undertaken by [9]Maud Tallant, [9]Werner Wild, and [9]Peter Frei. The data were analyzed by myself. The draft version of the manuscript was written by myself and the co-authors reviewed the manuscript providing substantial contributions. All authors read and approved the final version of the work.

Chapter 2 is a research article published in "European Journal of Plant Pathology" and is referenced as follows: "Dupuis B, 2017. *The movement of potato virus Y (PVY) in the vascular system of potato plants.* European Journal of Plant Pathology 147, 365-73". The study was conceived by myself. The greenhouse trials were undertaken by [9]Laure Sainthuile, [9]Nicolas Pochon, [9]Carole Morel, [9]Ludovic Piccot, and myself. The serological analysis were done by [9]Werner Wild. The data analysis and the writing of the manuscript was done by myself. The manuscript was reviewed by [9]Fabio Mascher,

[2]Claude Bragard and [9]Jennifer Cadby. The version presented in this thesis has been modified from the original. The original is presented in Annex 2.

Chapter 3 is a research article published in "Journal of Phytopathology" in 2014 (162, 14-18) with the title "Efficacy of Three Strategies Based on Insecticide, Oil and Elicitor Treatments in Controlling Aphid Populations and Potato virus Y Epidemics in Potato Fields", and. co-authored with [9]Ruedi Schwaerzel and [9]Jacques Derron. The field trials have been done before my PhD. The trials were conceived by [9]Jacques Derron and [9]Ruedi Schwaerzel. The field trials were undertaken by [9]Ruedi Schwaerzel and [9]Maud Tallant. Aphid identification was performed by [9]Gabriel Goy and the serological analysis by [9]Charles Fivaz and [9]Werner Wild. The data analysis and the writing of the draft version of the manuscript was done by myself. [10]Mélise Jaud and the co-authors reviewed the manuscript and all authors read and approved the final version of the work. The version presented in this thesis has been modified from the original. The original is presented in Annex 3, with permission of the "Journal of Phytopathology".

Chapter 4 is a research article published in the journal "Plant Pathology" in 2017 (66, 960-969) with the title "Control of potato virus Y (PVY) in seed potatoes by oil spraying, straw mulching and intercropping", and co-authored with [9]Jennifer Cadby, [9]Gabriel Goy, [9]Maud Tallant, [9]Jacques Derron, [9]Ruedi Schwaerzel and [9]Thomas Steinger. The trials were conceived together with [9]Thomas Steinger. The field trials were undertaken by [9]Maud Tallant, [9]Jennifer Cadby, [9]Laure Sainthuile, [9]Solène Pierucci and myself. Aphids identification was done by [9]Gabriel Goy, [9]Floriane Bussereau, and [9]Jennifer Cadby. The serological analysis were performed by [9]Werner Wild, [9]Laure Sainthuile and [9]Solène Pierucci. The data analysis was done by myself under the guidance of [9]Thomas Steinger. The draft version of manuscript was written by myself and [9]Thomas Steinger reviewed the manuscript providing substantial contributions. All authors read and approved the final version of the work. The version presented in this thesis has been modified from the original. The original is presented in Annex 4.

The General Discussion, Conclusions and Perspectives were written by myself under the supervision of [1]Christophe Lacomme and [2]Claude Bragard and not published elsewhere.

Christophe Lacomme[1] and Claude Bragard[2] provided guidance and corrections for the whole manuscript.

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# INTRODUCTION

## 1. Potato Production and Potato Diseases

The potato (*Solanum tuberosum* L.) is an herbaceous of the *Solanaceae* family that produces tubers (Rousselle et al., 1996). Furthermore, the potato is one of the most valuable food crops and the world's number one non-grain food commodity (FAO, 2008). With 368'096'000 tonnes, potato is the fourth world food crop after maize (*Zea mays* L.), rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) (FAO, 2015). Potato is also a recommended food security crop that contributes to protecting low-income countries from the risks posed by rising international food prices (FAO, 2008).

However, potato production is threatened by both biotic and abiotic factors (Bowen, 2003, Harris, 1992), and the potato is prone to more than a hundred diseases caused either by bacteria, fungi, phytoplasma or viruses (Harris, 1992, Franc, 2001).

About 40 viruses can infect potato plants (Valkonen, 2007), and the main viruses infecting potato crops in Europe and North America are presented in Table 1. Most of these viruses are transmitted by aphids such as *Alfalfa mosaic virus* (AMV), *Cucumber mosaic virus* (CMV), *Potato aucuba mosaic virus* (PAMV), *Potato latent virus* (PotLV), *Potato leafroll virus* (PLRV), *Potato virus A* (PVA), *Potato virus M* (PVM), *Potato virus* S (PVS), *Potato virus V* (PVV) and *Potato virus Y* (PVY), by contact such as PAMV, *Potato virus X* (PVX), *Tobacco mosaic virus* (TMV), *Tomato mosaic virus* (ToMV), and by nematodes such as *Tobacco rattle virus* (TRV) and *Tomato black ring virus* (TBRV). Some potato viruses are transmitted by other arthropods such as *Beet curly top virus* (BCTV) and *Potato yellow dwarf virus* (PYDV), which are transmitted by leafhoppers, and *Tomato spotted wilt virus* (TSWV), which is transmitted by thrips. Finally, *Potato mop-top virus* (PMTV) and *Tobacco necrosis virus* (TNV) are transmitted by phytopathogenic fungi, *Spongospora subterranea* and *Olpidium brassicae*, respectively. Most of the viruses presented in Table 1 have a worldwide distribution, except PotLV and PYDV that have been only reported in Northern-America, while PVV, TBRV and ToMV are only reported in Europe. The majority of the viruses are categorised in lists of viruses recommended for regulation as quarantine pests (list A1, A2 or quarantine list) by one or several countries or by plant-protection organisations (Table 1). A survey was undertaken in 2017 to evaluate the prevalence of the potato viruses in Europe and North America and assess the risk posed to seed potato

production by viruses (see questions of the survey in Annex 5). The certification authorities and National Plant Protection Organisations of fourteen countries responded to this survey, namely Belgium, Canada, Czech Republic, Finland, France, Germany, Italy, The Netherlands, Norway, Poland, Scotland, Slovenia, Switzerland and the United States (the contact persons are listed in Annex 6). The results of this survey show that potato viruses are considered as an important threat for seed potato production in most of the European and North-American countries (last line of Table 2). In Belgium, Canada, Czech Republic, Finland, the Netherlands, Poland, Slovenia, Switzerland and the United States, the risk posed to seed potato production by viruses is estimated as “very serious” by growers (Table 2). In these countries, control measures are systematically undertaken by growers to reduce the spread of viruses, irrespective of the potato varieties or location. In France, Northern-Germany and Scotland, the perception of the risk by growers is lower (medium risk). This difference means that the risk due to viruses is well known to growers in these countries and that control measures are undertaken but adjusted to the perception of the risk. For example, the control measures are not systematically applied for more resistant varieties or for fields isolated from external infection sources (e.g. ware potato fields). Finally, in Italy and Norway, the perception of the risk is the lowest (low risk), suggesting that growers are aware that under specific circumstances there is a risk of virus spread in their fields, but it is perceived that no particular measures are needed to control viruses. In this survey, the perception of the risk associated with the European and North-American potato viruses was also assessed (Table 2). Risks were ranked as NP-not present, NI-not important, I-important and VI-very important. When the data is available, the percentage of seed lots infected by each virus is also presented in Table 2. Amongst all viruses infecting potato, the results of the survey show that PVY is the most prevalent in seed potato production, with a minimum of prevalence of 5 to 10% in The Netherlands, Canada, Finland Poland and Scotland and a maximum of above 90% in Italy and Slovenia (Table 2). These percentages refer to the average proportion of potato seed lots in which PVY is detected during yearly surveys. Other viruses are infecting potato seed lots and represent a significant threat for specific countries. For example, PLRV has a high prevalence in Italy (10%), Slovenia (10%), and the Czech Republic (5–10%, see Table 2), particularly in some susceptible cultivars (Peter Dolničar, personal communication). Nevertheless, the prevalence of PLRV has been drastically reduced after the introduction of insecticide

treatments in seed potato production (Jean-Louis Rolot, personal communication). PMTV is sporadically detected in the potato seed lots in the Netherlands, Poland and Switzerland (Table 2), but PMTV causes severe quality problems in potato production in the Nordic countries (Santala et al., 2011). It is yearly detected in the seed potato production of Finland (5% of prevalence) and Norway (2% of prevalence), but it is also regularly detected in Sweden, Denmark, Russia, Estonia, Latvia and Lithuania (Kvarnheden et al., 2011). The prevalence of PVA is high in Norway (18%) and it is regularly detected in Czech Republic (<1% of prevalence, see Table 2). Symptoms of PVA are rarely observed in the seed potato fields in Scotland (<1% of prevalence, see Table 2) but still significant in seed potato production in the United Kingdom (Adrian Fox, personal communication). The prevalence of PVM is low in Europe and North America (Table 2), while the highest prevalence of this virus is in Slovenia (10%), Czech Republic (less than 5%) and Norway (2%). Furthermore, a sampling performed in Estonia in 2011 revealed 5% of prevalence of PVM in plant samples randomly collected in ware potato fields (Liiv et al., 2011). PVS is often detected ($\geq$10% of prevalence) in potato seed lots in Norway, Slovenia, the Czech Republic and the United States (Table 2). It is also reported in the Balkans, with about 10% of prevalence in Serbia (Ristic et al., 2017). The prevalence of TRV is low in most of the European and North-American countries (Table 2). Nevertheless, it can be responsible for important damage in ware potato production dedicated to the processing of fried products. These outbreaks in ware potato production are sporadic but were noticed in Germany (Kerstin Linder, personal communication) and Switzerland (Olivier Schumpp, personal communication). In the plains of Turkey, potato viruses including PVA, PVS, PLRV, PVX, and PVY are widespread (Sertkaya & Sertkaya, 2014). In Western Russia, almost all the seed potato crops of susceptible varieties are infected by PVY, while PVM and PLRV are often detected (Ruedi Schwaerzel, personal communication).

*Table 1: Viruses infecting cultivated potatoes in Europe and Northern-America. The table presents the name of the virus, the Genus and family (ICTV, 2017), the main vector (Valkonen, 2007), the occurrence (EPPO, 2017, Valkonen, 2007) and the categorisation on the A1, A2 and quarantine lists of Europe and North America (EPPO, 2017). The A1 list presents the quarantine organisms not present in that area, the A2 list shows the quarantine organisms present in that area but not widely distributed there and being officially controlled, and the quarantine list shows the organisms of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled. In the categorisation lists, the countries are presented with their ISO ALPHA-3 code and the organisations with their initials (EPPO = European and Mediterranean Plant Protection Organisation; EU = European Union; TUR = Turkey; CAN = Canada; GBR = United Kingdom; NOR = Norway; USA = United States), NA stands for "information not available" and "-" means that the virus is not listed in the corresponding list.*

| Species (acronym) | Genus, subfamily* & family | Vector | Occurrence | Categorization | | |
|---|---|---|---|---|---|---|
| | | | | A1 list | A2 list | Quarantine list |
| *Alfalfa mosaic virus* (AMV) | Alfamovirus, Bromoviridae | Aphids | Worldwide (uncommon) | - | - | - |
| *Beet curly top virus* (BCTV) | Curtovirus, Geminiviridae | Leafhoppers | Arid areas worldwide | EPPO, EU, TUR | - | CAN |
| *Cucumber mosaic virus* (CMV) | Cucumovirus, Bromoviridae | Aphids | Worldwide (uncommon) | - | - | - |
| *Potato aucuba mosaic virus* (PAMV) | Potexvirus, Alphaflexiviridae | Contact, aphids | Worldwide (uncommon) | NA | NA | NA |
| *Potato latent virus* (PotLV) | Carlavirus, Quinvirinae, Betaflexiviridae | Aphids | N-America | - | - | - |
| *Potato leafroll virus* (PLRV) | Polerovirus, Luteoviridae | Aphids | Worldwide | - | TUR | NOR |
| *Potato mop-top virus* (PMTV) | Pomovirus, Virgaviridae | *Spongospora subterranea* | Worldwide | CAN, TUR | - | USA |
| *Potato virus A* (PVA) | Potyvirus, Potyviridae | Aphids | Worldwide | - | TUR | NOR |
| *Potato virus M* (PVM) | Carlavirus, Quinvirinae, Betaflexiviridae | Aphids | Worldwide | - | TUR | NOR |
| *Potato virus S* (PVS) | Carlavirus, Quinvirinae, Betaflexiviridae | Aphids | Worldwide | - | - | NOR |
| *Potato virus V* (PVV) | Potyvirus, Potyviridae | Aphids | N-Europe | CAN, TUR | - | NOR, USA |
| *Potato virus X* (PVX) | Potexvirus, Alphaflexiviridae | Contact | Worldwide | - | TUR | NOR |
| *Potato virus Y* (PVY) | Potyvirus, Potyviridae | Aphids | Worldwide | - | TUR | |
| *Potato yellow dwarf virus* (PYDV) | Nucleorhabdovirus, Rhabdoviridae | Leafhoppers | N-America | EPPO, EU, TUR, GBR | - | - |
| *Sowbane mosaic virus* (SoMV) | Sobemovirus, – | ? | Worldwide (uncommon) | NA | NA | NA |
| *Tobacco mosaic virus* (TMV) | Tobamovirus, Virgaviridae | Contact | Worldwide (uncommon) | NA | NA | NA |
| *Tobacco necrosis virus* (TNV) | Alphanecrovirus, Tombusviridae | *Olpidium brassicae* | Europe, N-America, | - | - | - |
| *Tobacco rattle virus* (TRV) | Tobravirus, – | Nematodes | Worldwide | TUR | CAN, EU | US |
| *Tomato black ring virus* (TBRV) | Nepovirus, Comovirinae, Secoviridae | Nematodes, TPS** | Europe | - | CAN, EU | NOR, USA |
| *Tomato mosaic virus* (ToMV) | Tobamovirus, Virgaviridae | Contact | Europe | - | - | - |
| *Tomato spotted wilt virus* (TSWV) | Orthotospovirus, Tospoviridae | Thrips | Worldwide | EU | EPPO, EU, TUR | NOR |

* when available; ** true potato seed

*Table 2: Results of an international survey giving two pieces of information: (1) the qualitative evaluation of the occurrence of the main potato viruses (NP-not present, NI-not important, I-important, VI-very important). When the prevalence of the virus is quantitatively known (percentage of seed lots infected by the virus), it is given in brackets (≈0% means that the prevalence of the virus is close to 0% and the asterisk means that the virus is sporadically detected in seed lots). In the last line, (2) the perception of the threat caused by potato viruses in general to local seed potato production (high, medium, low, absence).*

| Virus | BEL | CAN | CZE | FIN | FRA | DEU-N | DEU-S | ITA | NLD | NOR | POL | GBR | SLV | CHE | USA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AMV | NI | NI | NP | NP | NI | - | - | I (10%) | NP | NP | NP | NP | NI | NP | NI (<1%) |
| BCTV | NP | NI | NP | NP | NP | - | - | NP | NP | NP | NP | NP | NP | NP | NI |
| CMV | NP | NI | NP | NP | NP | - | - | NI (<1%) | NP | NP | NP | NP | NP | NP | NI |
| PAMV | NP | NI | NP | NP | NP | NI | - | NP | NP | NI (<1%) | NP | NI (≈0%) | NI | NP | NP |
| PotLV | NP | NI | NP | NP | NP | NP | - | NP | NP | NP | NP | NP | NP | NP | NP |
| PLRV | I (1-2%) | NI (≈0%) | VI (5-10%) | NI (≈0%) | I (1.5%) | I | I (3%) | I (10%) | NI (≈0%) | NP | NI | I (<1%) | I (10%) | I (5%) | NI |
| PMTV | NP | I | NP | I (5%) | NI (0.01%) | NP | - | NP | NI (≈0%)* | I (2%) | NP (<1%)* | I (<1%) | NP | NI (≈0%)* | NI (<1%) |
| PVA | NI (≈0%) | NI | I (<5%) | NI (1%) | NI (0.1%) | NI | NI (0.1%) | NI (<1%) | NI (≈0%) | I (18%) | NI | I (<1%) | NP | NI | NI (<1%) |
| PVM | NP | NI | I (<5%) | NI (1%) | NI (0.1%) | I | NI (1%) | NI (<1%) | NI (≈0%) | NI (2%) | I | NI (≈0%) | I (10%) | NI | NI (<1%) |
| PVS | I (1-2%) | NI | VI (>70%) | I (3%) | NI (0.1%) | I | NI (0.1%) | NI (<1%) | NI (≈0%) | I (19%) | I (5%) | NI (≈0%) | I (30%) | NI | I (>20%) |
| PVV | NP | NP | NP | NP | NI (≈0%)* | NI | - |  | NP | NI | NI | (<1%,∃! cv. | NP | NP | NI |
| PVX | I (1-2%) | NI | I (<5%) | NP | I (2.4%) | NI | NI (≈0%) | NI (<1%) | NI (≈0%) | NI (≈0%) | NI | I (<1%) | NP | NI | NI (<1%) |
| PVY | VI (30-60%) | I (10%) | VI (10-70%) | VI (10%) | VI (25%) | VI | VI (20%) | VI (90%) | VI (5-10%) | I (20%) | VI (10%) | I (≈5%) | VI (90%) | VI (50%) | VI (20-30%) |
| PYDV | NP | NP | NP | NP | NP | NP | - | - | NP | NP | NP | NP | NP | NP | NP |
| SoMV | NP | NP | NP | NP | NP | - | - | - | NP | NP | NP | NP | NP | NP | NP |
| TMV | NP | NP | NP | NP | NP | NI | - | NI (<1%) | NP | NP | NP | NP | NP | NP | NI |
| TNV | NP | NP | NP | NP | NP | NI | - | - | NI (≈0%)* | NP | NP | NP | NP | NP | NI |
| TRV | NI | NP | NP | NI | NI (0.05%) | NI | NI (0.1%) | NI (<1%) | NI (≈0%) | NI (0.2%) | NI | I (<1%) | NP | NI (≈0%) | NI (<1%) |
| TBRV | NP | NP | NP | NP | NP | - | - | NP | NP | NI | NP | NI (≈0%) | NP | NP | NP |
| ToMV | NP | NP | NP | NP | NP | - | - | NI (<1%) | NP | NP | NP | NP | NP | NP | NP |
| TSWV | NP | NP | NP | NP | NP | NP | - | - | NP | NP | NP | NP | NP | NP | NI |
| **Threat** | **High** | **High** | **High** | **High** | **Medium** | **Medium** | **Medium** | **Low** | **High** | **Low** | **High** | **Medium** | **High** | **High** | **High** |

*Note: Belgium (BEL), Canada (CAN), Czech Republic (CZE), Finlan (FIN), Northern-Germany (DEU-N), Southern-Germany (DEU-S), Italy (ITA), The Netherlands (NLD), Norway (NOR), Poland (POL), Scotland (GBR), Slovenia (SLV), Switzerland (CHE), United States (USA)*

## 2. PVY Epidemiology and Pathosystem

Robert (2001) defined the epidemiology of plant virus diseases as *a cyclical development of virus diseases within plant populations in time and space.* Epidemiology deals with how and why a virus is spread by vectors in an ecosystem and is fundamental to the choice and improvement of control methods in agriculture (Robert, 2001). It includes a dimensional aspect of the factors determining the spread of the virus into a given situation, as it is influenced by several factors such as: the environment, rate of virus reproduction, mode of dispersion, efficiency of virus survival, aggressiveness of the virus and level of host plant resistance/susceptibility (Sastry et al., 2014, Hull, 2013). Understanding PVY epidemiology is vital to formulating sustainable disease management practices in a given agro-ecosystem. Factors that influence the development and progress of plant disease epidemics do vary between different geographical areas; hence, researchers working in different regions have to understand disease epidemics before attempting to control virus and virus-like diseases (Sastry et al., 2014, Robert, 2001).

A pathosystem is a subsystem of an ecosystem and is defined by the phenomenon of parasitism (Robinson, 1977). A pathosystem has three main components: the host, the parasite and the environment. A virus pathosystem includes a fourth component which is the vector of the virus, if there is one. Finally, a crop pathosystem has an additional main component, which is human intervention, who has a profound influence on the other components of the pathosystem (Robinson, 1977, Döring, 2011). The PVY pathosystem is highly complex, as it involves the interactions between the plant, the pathogen, the vector and the environment (Döring, 2011). Figure 1 shows a tentative representation of the PVY pathosystem as a pyramid, a variation in three dimensions of the classical disease triangle (Jones, 2014), showing the interactions between the four components of the pathosystem: the plant (potato cultivars), the virus (PVY strains), the vector (aphid species) and the environment (field environment). Figure 1 is further used in this document to characterise the epidemiology of PVY in potato fields and the effect of the PVY control strategies that can be used to reduce the spread of the virus.

The following sections discusses the whole PVY pathosystem with a description of its various components: the virus, the host, the interaction between the plant and the virus, the vectors and the environment.

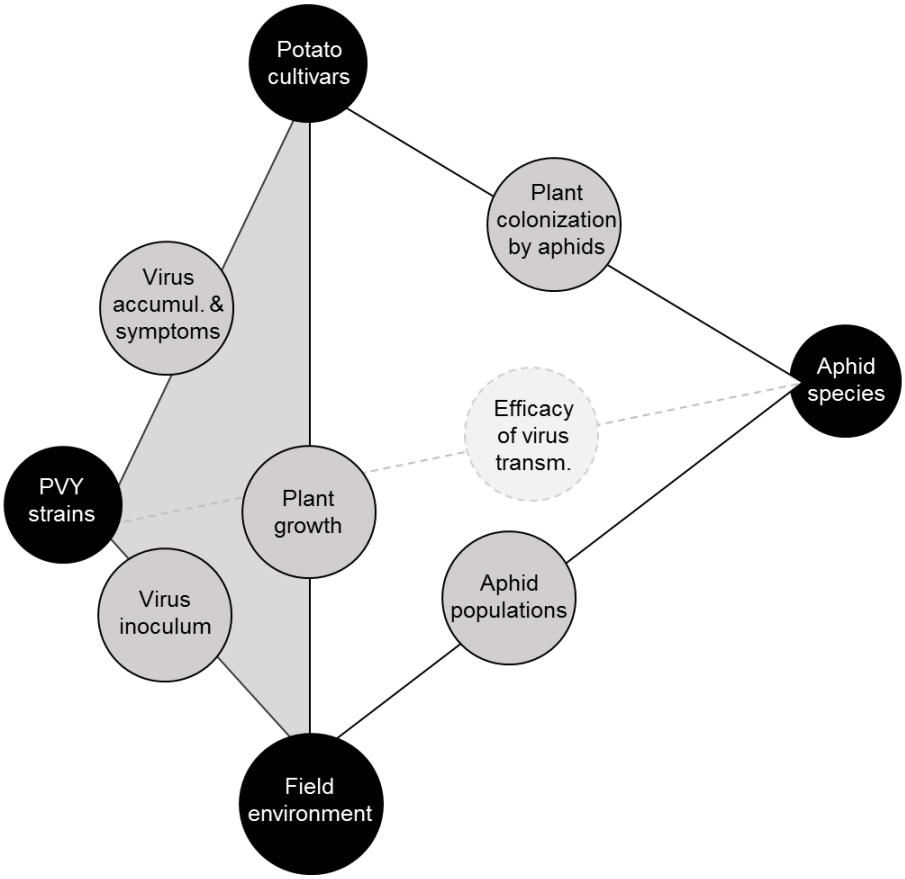


*Figure 1: Diagram presenting the PVY pathosystem with the four components of the system in black and the interactions between one another in grey.*

# 3. The Virus

## 3.1. PVY Genome and Viral Proteins

PVY virions have a length of 730–740 nm and a diameter of 12 nm (Figure *2*). The genome consists of a single strand genomic ribonucleic acid (RNA) positive sense of approximately 9.7 kb covalently linked to a single molecule of the viral-encoded protein genome-linked (VPg) at its 5´-end and a polyadenosine (poly A) tail at its 3´-end (Figure *3*a) (Quenouille, 2013).

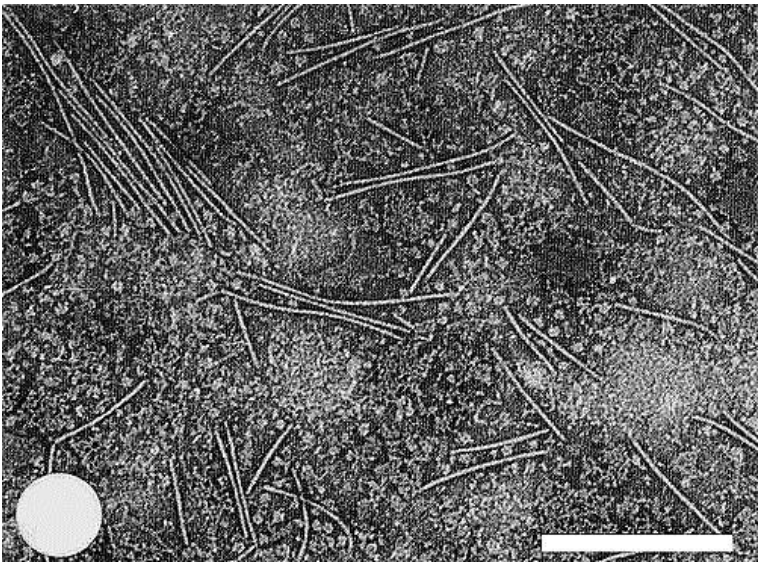

*Figure 2: Partially purified virus particles negatively stained with 2% potassium phosphotungstate, pH 6.5, in water. Bar represents 500 nm [from de Bokx and Huttinga (1981)].*

Most of the proteins of PVY are multifunctional. P1 is the first protein and is encoded by the 5´ region of the PVY genome. It is the most variable protein among potyviruses (Verchot et al., 1991). Furthermore, P1 is involved in virus genome amplification and in the suppression of plant defences through RNA silencing mechanisms (Verchot & Carrington, 1995, Pruss et al., 1997). In addition, the HC-Pro protein is involved in various aspects of the virus infectious cycle: in aphid transmission, virus multiplication, cell-to-cell and systemic movements, in the suppression of RNA silencing and

symptom intensity (Saenz et al., 2002, Quenouille, 2013). P3, together with the 6K2 protein, are the two potyviral membrane proteins. P3 is involved in virus replication, systemic infection, pathogenicity and movement (Eiamtanasate et al., 2007, Restrepo-Hartwig & Carrington, 1994, Chu et al., 1997, Merits et al., 1999), while the function of the 6K1 protein is unknown (Quenouille, 2013). The CI protein is involved in the cell-to-cell virus movement and virus replication (Carrington et al., 1998, de Cedron et al., 2006, Roberts et al., 1998, Wei et al., 2010b, Fernández et al., 1997, Merits et al., 1999). The 6K2 protein plays an essential role in virus replication by anchoring the viral replication complex to the endoplasmic reticulum (Schaad et al., 1997, Wei et al., 2010a). The first nuclear inclusion protein, the NIa, possesses two domains: the VPg and a protease domain which cleaves all proteins at the C-terminal half of the precursor polyprotein (Quenouille, 2013, Carrington & Dougherty, 1987). The VPg has the capacity to interact physically with viral and plant proteins binding PVY with the host (Deom et al., 1997, Robaglia & Caranta, 2006). The second nuclear inclusion protein, the NIb, is the RNA-dependent RNA polymerase (RdRp) involved in the replication of the viral RNA (Quenouille, 2013). The last protein, the CP, is required for virion assembly, cell-to-cell and systemic movements and aphid transmission (Rojas et al., 1997, Andersen & Johansen, 1998, Atreya et al., 1990, Blanc et al., 1997).

## 3.2. Classification of PVY Strains

Initially identified as Potato virus C (Salaman & Le Pelley, 1930), the virus was renamed PVY. PVY isolates have traditionally been classified into strains according to the symptoms they elicit on various indicator plants (Table 3), namely $PVY^{C}$, $PVY^{O}$, $PVY^{Z}$, $PVY^{N}$ and $PVY^{E}$ (Karasev & Gray, 2013b).

$PVY^{C}$ induces hypersensitive reaction (HR) in potato cultivars expressing the *Nc* gene (Table 3), $PVY^{O}$ induces HR in potato cultivars expressing the *Ny* gene, and $PVY^{Z}$ induces HR in potato cultivars expressing the *Nz* gene (Table 3). $PVY^{N}$ can be distinguished from $PVY^{C}$ and $PVY^{O}$ by its ability to induce vein necrosis when infecting tobacco (*Nicotiana tabacum*) and $PVY^{E}$ by its ability to induce symptoms of mosaic on tobacco (Table 3). Moreover, $PVY^{N}$ and $PVY^{E}$ can overcome *Nc, Ny* and *Nz* and do not elicit any HR in potato (Rolland et al., 2008, Kerlan et al., 2011, Karasev & Gray, 2013b). The complete genome sequences are known for isolates from $PVY^{O}$, $PVY^{C}$,

and $PVY^{N}$ strains. However, little information is available for $PVY^{Z}$ and $PVY^{E}$ (Karasev & Gray, 2013b), because these strains are rare, with few isolates sequenced. Therefore, we do not refer to $PVY^{Z}$ and $PVY^{E}$ in the remainder of this thesis.

*Table 3: Genetic classification of PVY strains, with serological and molecular properties of the main genetic strains of PVY. Data compiled according to Karasev and Gray (2013b), Kehoe and Jones (2016), Kerlan et al. (2011), and Glais et al. (2017).*

| **Plant** | **Genetic background** | **$PVY^{O}$** | **$PVY^{C}$** | **$PVY^{Z}$** | **$PVY^{N}$** | **$PVY^{E}$** | **$PVY^{NTN}$** | **$PVY^{N-Wi}$** |
|---|---|---|---|---|---|---|---|---|
| Desiree, Pentland Crown | *Ny:nc:nz* | HR | s | s | s | s | s | s |
| King Edward | *ny:Nc:nz* | s | HR | s | s | s | s | s |
| Maris Bard, Pentland Ivory | *Ny:Nc:Nz* | HR | HR | HR | s | s | s | s |
| Russet Burbank | *ny:nz* | s | HR | - | s | - | - | - |
| Yucon Gold | *Ny:Nz* | HR | HR | - | HR | - | s | s |
| Tobacco | - | mos | mos | mos | VN | mos | VN | VN |
| Serotype | | O | O | N | N | N | N | O |
| Genome type | | NR | NR | R | NR | R | R | R |

*HR : hypersensitive response ; s : susceptible; mos : mosaic ; VN : vein necrosis ; O : serotype $PVY^{O}$; N : serotype $PVY^{N}$ ; NR : non-recombinant; R: recombinant, and "-" : no symptoms.*

Multiple recombinant of $PVY^{O}$ and $PVY^{N}$ genomes have recently been identified, and the most common ones are $PVY^{NTN}$, $PVY^{N:O}$, and $PVY^{N-Wi}$ (Karasev & Gray, 2013b). Most of PVY recombinant induces vein necrosis on tobacco (Table 3), and $PVY^{NTN}$ is associated with a potato disease, inducing tuber necrosis in some susceptible cultivars (Table 4), which is called potato tuber necrotic ringspot disease (PTNRD). Interestingly, $PVY^{N:O}$ and $PVY^{N-Wi}$ strains present a $PVY^{O}$ serotype due to their recombinant nature (Karasev & Gray, 2013b). The naming of these last two recombinant strains is still under discussion. Mello et al. (2011) suggest considering $PVY^{N:O}$ and $PVY^{N-Wi}$ as equivalent strains, because only some rare isolates of $PVY^{N-Wi}$ present different recombinant junctions than $PVY^{N:O}$ (Singh et al., 2008). Further in this document, the name $PVY^{N-Wi}$ includes $PVY^{N:O}$ and $PVY^{N-Wi}$, as presented in Table 4.

*Table 4: The commonly described isolates, strain groups, synonyms and definitions of PVY, adapted from Singh et al. (2008)*

| Strain name | Strain group | Synonyms | Definition | Refs. |
|---|---|---|---|---|
| $PVY^{O}$ | $PVY^{O}$ | $PVY^{O5}$ | Common or ordinary strain group, isolates elicit HR mediated by the gene *Ny* | Singh *et al.*, 2008 |
| $PVY^{N}$ | $PVY^{N}$ | $PVY^{EU-N}$, $PVY^{NA-N}$, NA-$PVY^{N}$, $PVY^{R}$, $PVY^{TVN}$ | Tobacco veinal necrosis strain group, isolates not known to cause PTNRD | Singh *et al.*, 2008 |
| $PVY^{NTN}$ | $PVY^{N}$ | EU-$PVY^{NTN}$, Eu-$PVY^{NTN}$, $PVY^{EU-NTN}$, $PVY^{NN}$, $PVY^{NA-NTN}$, NA-$PVY^{NTN}$ | Isolates able to cause PTNRD Isolates do not elicit HR mediated *by Ny, Nc* or *Nz* and cause necrosis in tobacco. Isolates do not elicit HR mediated by *Ny, Nc* or *Nz* and cause necrosis in tobacco. | Singh *et al.*, 2008 |
| $PVY^{N-Wi}$ | $PVY^{N}$ | $PVY^{N-Wilga}$, $PVY^{N-W}$, $PVY^{N-Wi-P}$, $PVY^{N:O}$ | Recombinant isolates, phenotypically $PVY^{N}$ but serologically $PVY^{O}$. Isolates do not elicit HR mediated by *Ny*, *Nc* or *Nz* nor necrosis in tobacco. | Singh *et al.*, 2008 |
| $PVY^{NTN-NW}$ | $PVY^{N}$ | $PVY^{SYR}$ | Recombinant isolates, phenotypically $PVY^{N}$ but serologically $PVY^{O}$, and able to cause PTNRD | Singh *et al.*, 2008; Kehoe et al., 2015; Chikh Ali et al., 2010 |
| $PVY^{C}$ | $PVY^{C}$ | $PVY^{C1}$, $PVY^{C2}$ | Strain group C, isolates elicit HR mediated by the gene *Nc* | Singh *et al.*, 2008 |
| $PVY^{D}$ | $PVY^{C}$ | | Strain group C, isolates do not elicit HR mediated by the gene *Nc* | Kehoe *et al.*, 2015 |
| $PVY^{Z}$ | $PVY^{Z}$ | | Strain group Z, isolates elicit HR mediated by the proposed gene *Nz* | Singh *et al.*, 2008 |
| $PVY^{E}$ | $PVY^{E}$ | $PVY^{ZE}$ | Strain group E, isolates do not elicit HR mediated by *Ny*, *Nc* or *Nz* and no necrosis in tobacco | Singh *et al.*, 2008 |

## 3.3. Molecular Variability of PVY

$PVY^{NTN}$ and $PVY^{N-Wi}$ genomes can be considered as recombinant, made of segments of $PVY^{O}$ and $PVY^{N}$ genomes (Figure 3b), presenting one to four recombinant junctions (RJs) (Karasev & Gray, 2013b). The first 500 nucleotides (nt) of the recombined genome of $PVY^{N-Wi}$ have a typical $PVY^{O}$ sequence, and after which, the next 1890 nt (nt 501–2390) have a typical $PVY^{N}$ sequence. These nucleotides are then followed by a typical $PVY^{O}$ sequence up to the end of the genome (nt 2390–9259) (Revers et al., 1996, Visser & Bellstedt, 2009, Schubert et al., 2007). The first 2390 nt of the recombined genome of $PVY^{NTN}$ consist of a typical $PVY^{N}$ sequence, and after these,

the next 1890 nt (nt 2390–5715) have a typical PVY$^{O}$ sequence. These nucleotides are then followed by a typical PVY$^{N}$ sequence up to 9170 nt (nt 5175–9170). Lastly, these nucleotides are then followed with a PVY$^{O}$ sequence up to the end of the genome (nt 9170–9259) (Schubert et al., 2007, Visser & Bellstedt, 2009). Most of these RJs are conserved in their position in the PVY genome (Hu et al., 2009).

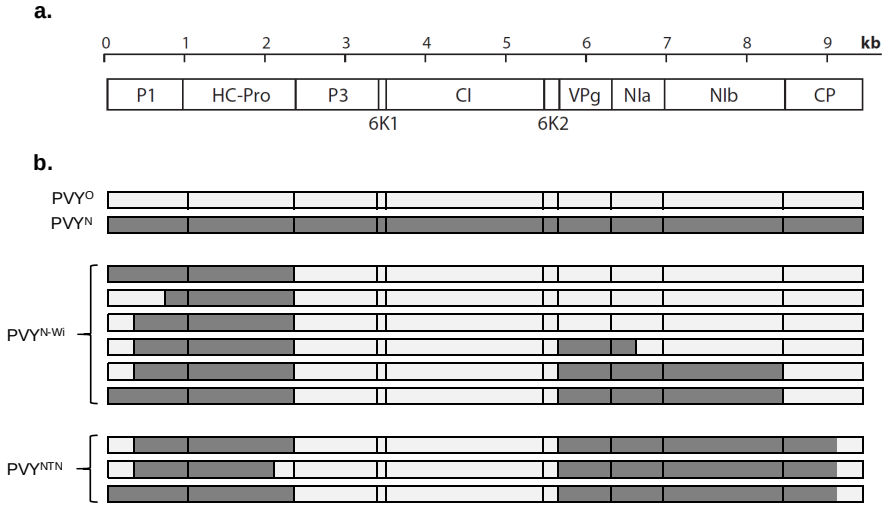


*Figure 3: (a) Schematic representation of the ten genes of PVY, adapted from Karasev and Gray (2013a) and (b) a schematic diagram of the main types of PVY recombinant found in Europe and North America — PVY$^{NTN}$, and PVY$^{N-Wi}$, adapted from Hu et al. (2009). Different shading marks the origin of genome fragments either from PVY$^{N}$ (dark grey) or PVY$^{O}$ (light white) parental genome segments.*

Some variations can be observed for the location of the RJs, mainly in the P1, HC-Pro, NIa and CP parts of the genome of PVY (see Figure 3b). This genetic variability can be explored in further detail, using genome sequencing on the entire genome of the virus [e.g. by Karasev et al. (2011) and Kehoe and Jones (2016)], or on a part of the PVY genome [e.g. the sequencing of the CP part of the genome by Visser and Bellstedt (2009)]. After sequencing the genome, phylogenic studies can be undertaken to identify clades of PVY strains/subgroups with genetic similarities (see the example in Figure 4). These clades are then compared to the classical biological characterisation of the strains to identify the corresponding PVY strain. Indeed, the strain concept is based on the biological response and not the genome of the virus. This methodology

improves the characterisation of the strains and highlights the genetic diversity of the PVY strains and subgroups (Kerlan et al., 2011, Kehoe & Jones, 2016).

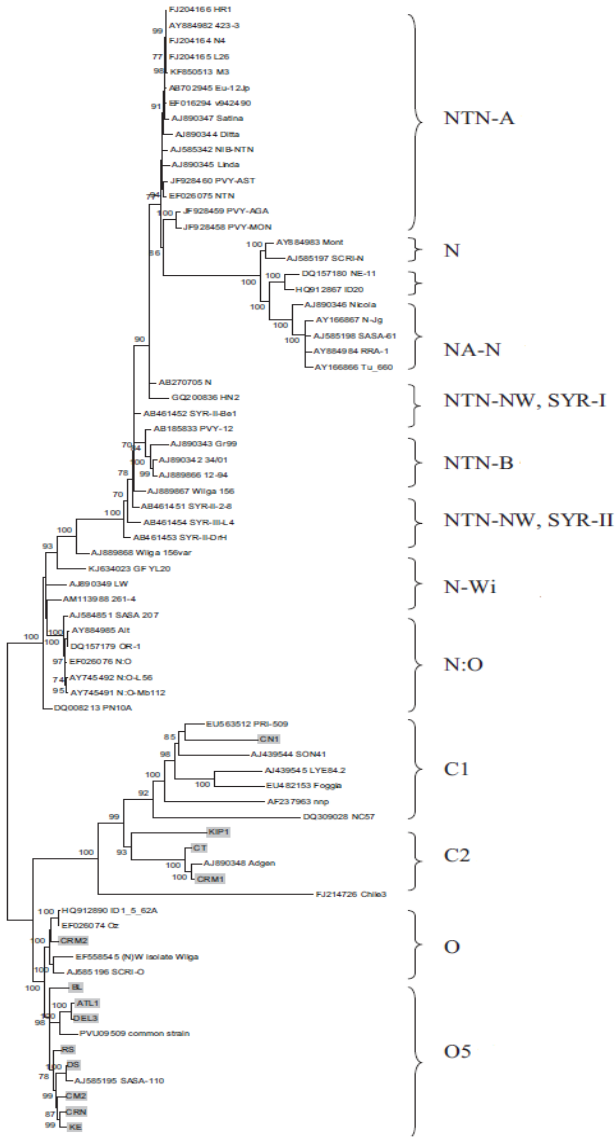


*Figure 4: Maximum likelihood phylogram adapted from Kehoe and Jones (2016) obtained from the alignment of 73 complete Potato virus Y genomes from Australia and the United Kingdom . Branch lengths indicate the number of substitutions per site.*

## 3.4. Evolution of PVY Populations

Three evolutionary forces drive the genetic evolution of plant virus populations generated by mutation and recombination: natural selection, genetic drift, and gene flow (Moya et al., 2004, Roossinck, 2003). Natural selection is a process by which strains that are more adapted to a certain environment increase their frequency in the population whereas other strains less adapted decrease their frequency (Rubio et al., 2013). The genetic drift consists of changes in the frequency of alleles in a finite population due to the random sampling of genes at reproduction (Moya et al., 2004). Gene flow from one geographical area to another, from plant to plant or among different parts of the same plant is an important factor determining the balance of strains of viral populations. Important migration of strains favours genetic uniformity of virus populations and decreases the genetic diversity of these populations (Moya et al., 2004, Garcia-Arenal et al., 2003). Mutations and recombination are intrinsic of the virus genome and generated during the replication process. On the other hand, natural selection, genetic drift and gene flow are affected by the biology of the virus (e.g., host type, host range and vectors), environmental conditions, and population parameters (e.g., population size) (Rubio et al., 2013).

An evolution of the PVY populations has been observed since the beginning of the strains surveys. In the end of the 1970s and beginning of the 1980s, the $PVY^{O}$ strain was prevalent in Europe (Sigvald, 1985, Gugerli, 1978, De Bokx et al., 1975). By the end of the 1980s and the beginning of the 1990s, the prevalence of the $PVY^{N}$ strains had increased in Europe, Canada and the United States (Nie & Singh, 2002, Weidemann, 1988, McDonald & Kristjansson, 1993, Crosslin et al., 2006). Afterwards, the “ancestral” $PVY^{O}$ and $PVY^{N}$ non-recombinant strains were gradually replaced by the recombinant strains $PVY^{NTN}$ and $PVY^{N-Wi}$. This shift occurred differently in Europe and North America. In the early 2000s in the United States and Canada, $PVY^{O}$ was still the predominant strain (69%) and followed by $PVY^{N-Wi}$ (25%) and $PVY^{NTN}$ (3%), with the remainder being mixed infections and unidentified strains (Gray et al., 2010). In Switzerland, at the same period, $PVY^{NTN}$ strains were predominant (78%) and followed by $PVY^{O}$ (10%), $PVY^{N-Wi}$ (6%) and $PVY^{N}$ (2%), with the remainder consisting of mixed infections (Rigotti et al., 2011). The results of this Swiss survey of PVY isolates provide an overview of PVY strains diversity throughout Western Europe, as

Switzerland imports most of its seed potatoes from the main potato producing countries (mainly from France, Germany and the Netherlands).

The explanation of the switch from non-recombinant strains to recombinant strains is not well known (Mello et al., 2011). A first hypothesis is that aphids can more efficiently transmit recombinant strains than their non-recombinant counterpart. Several experiments were performed to test this hypothesis and revealed an interaction between the PVY strain and the aphid species regarding the efficiency of transmission of the virus. For example, the experiments of Srinivasan et al. (2012) showed that although $PVY^{O}$, $PVY^{N\text{-}Wi}$, and $PVY^{NTN}$ were transmitted with similar efficiency by *Myzus persicae* (Sulzer), $PVY^{NTN}$ was transmitted more efficiently than $PVY^{O}$ or $PVY^{N\text{-}Wi}$ when it was present in mixed infections with one of those two strains. Mondal et al. (2016) also observed a better efficacy of $PVY^{NTN}$ transmission by *Myzus persicae* than $PVY^{O}$ and $PVY^{N\text{-}Wi}$, even from plants infected with $PVY^{NTN}$ alone. The same experiment performed with *Rhopalosiphum padi* (L.) gave similar results. *Rhopalosiphum padi* and five other aphid species, namely *Acyrthosiphon pisum* (Harris)*, Aphis fabae* (Scopoli)*, Aphis nasturtii* (Kaltenbach)*, Aphis* spp. and *Phorodon humuli* (Schrank)*,* also transmit $PVY^{N\text{-}Wi}$ more efficiently than $PVY^{N}$ (Verbeek et al., 2010). The only report of a lower aphid transmission of a recombinant strain has been for $PVY^{N\text{-}Wi}$ by *Aphis frangulae* (Kaltenbach) (Verbeek et al., 2010). Another hypothesis to explain the increase of the prevalence of the recombinant strains in Europe and North America may be that these strains accumulate better or translocate faster to progeny tubers than the non-recombinant ones. This hypothesis is supported by experiments performed with non-recombinant strains showing that $PVY^{N}$ migrates faster than $PVY^{O}$ strains from the leaves to the progeny tubers (Beemster, 1976, Basky & Almasi, 2005). Nevertheless, this work has never been performed with recombinant strains, and additional results are required to confirm this second hypothesis.

The emergence of new recombinant PVY isolates is a challenge for breeders, as there is a constant need to replace old varieties that become unprofitable due to their susceptibility to new PVY recombinant strains. For example, the production of cv. Erntestolz in Switzerland ended in the 1990s following the emergence of the $PVY^{NTN}$ strains due to the susceptibility of this variety to PTNRD (Werner Reust, personal communication).

# 4. The Hosts

## 4.1. Range of Hosts

Hosts of PVY can be found in crops and weeds. Mainly solanaceous crops can be infected by PVY; this includes potato, tobacco (*Nicotiana tabacum*), pepper (*Capsicum* spp.), tomato (*Solanum esculentum*), eggplant and ground cherries (*Physalis* spp.) (Ali et al., 2008, Kerlan, 2006, Quenouille et al., 2013). Solanaceous weeds can be infected by PVY, such as hairy nightshade (*Solanum sarrachoides*) in North America (Cervantes & Alvarez, 2008, Cervantes & Alvarez, 2011), black nightshade (*Solanum nigrum*) in Europe, North Africa and Australia (Ali et al., 2008, Coutts & Jones, 2015) and bittersweet nightshade (*Solanum dulcamara*) throughout the northern hemisphere (Beemster & de Bokx, 1987).

Quenouille et al. (2013) reviewed the infectivity of PVY isolates from five non-recombinant PVY strains ($PVY^{C1}$; $PVY^{C2}$; $PVY^{O}$; $PVY^{N}$ and $PVY^{Chile}$) in crop plants of the *Solanaceae* family and showed that the susceptibility of the solanaceous plants varies depending on the PVY strain (Table 5). $PVY^{C1}$ is rarely found in potato but is frequent on pepper (*Capsicum* spp.), tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*). $PVY^{C2}$ is also rarely found on potatoes, and natural infections on pepper, tobacco, tomato and eggplant (*Solanum melongena*) have never been reported. $PVY^{O}$ is frequent on potato and tobacco and has been reported on pepper, tomato and eggplant. Furthermore, $PVY^{N}$ has the same distribution as $PVY^{O}$, but it has yet to be reported on eggplant. Finally, $PVY^{Chile}$ has only been reported on pepper and tobacco.

Edwardson and Christie (1997) found 36 weed species belonging to 13 different botanical families identified as potential host plants for PVY (in natural and/or artificial conditions). (Kaliciak & Syller, 2009) undertook a survey of potential non-solanaceous weed hosts of PVY by mechanical inoculation of plants. This survey revealed that two plants of the Geraniaceae family are symptomless hosts of PVY, namely redstem firalee (*Erodium cicutarium*) and small-flowered crane's bill (*Geranium pusillum*). One plant of the Asteraceae family, namely prickly lettuce (*Lactuca serriola*), and one plant of the Lamiaceae family, purple deadnettle (*Lamium purpureum*), were also revealed to be symptomless hosts of PVY. This study also indicates that the whole range of PVY hosts in weed plants is probably not yet determined, given the large diversity of weed

plants worldwide. Additional surveys are required to have a better perspective of the potential PVY reservoir of the main weed species in the potato production areas across the world, and the reservoir of PVY in the environment can be assumed to be higher in the areas where potatoes are regularly grown.

*Table 5: Simplified Potato virus Y (PVY) phylogeny (excluding the recombinant isolates) and infectivity of PVY isolates from the five major clades in crop plants of the family Solanaceae. This table is adapted from Quenouille et al. (2013).*

| **PVY strain** | **Potato** *(Solanum tuberosum)* | **Pepper** *(Capsicum* spp.*)* | **Tobacco** *(Nicotinan tabacum)* | **Tomato** *(Solanum lycopersicum)* | **Eggplant** *(Solanum melongena)* |
|---|---|---|---|---|---|
| $PVY^{C1}$ | Rare | Frequent worldwide | Frequent worldwide | Frequent worldwide | Reported |
| $PVY^{C2}$ | Rare | Not reported and not infectious in the laboratory | Not reported but infectious in the laboratory | Not reported but infectious in the laboratory | Not reported |
| $PVY^{O}$ | Frequent worldwide | Reported but poorly infectious in the laboratory | Frequent worldwide | Reported | Reported |
| $PVY^{N}$ | Frequent worldwide | Reported but poorly infectious in the laboratory | Frequent worldwide | Reported | Not reported |
| $PVY^{Chile}$ | Not reported and not infectious in the laboratory | Reported on *Capsicum baccatum* in Chile | Reported in Chile | Not reported but infectious in the laboratory | Not reported |

## 4.2. Resistance Mechanisms to PVY

Potato cultivars have various levels of resistance (or susceptibility) to PVY (Schwaerzel et al., 2016) due to the different types of resistance mechanisms developed by plants. The main mechanisms of resistance to plant viruses are described as follows, namely gene-for-gene resistance, tolerance, immunity, mature plant resistance and systemic acquired resistance.

### *4.2.1. Gene-for-gene Resistance*

There are two types of gene-for-gene resistance of plants to viruses: extreme resistance (ER), and hypersensitive response (HR).

ER is associated with a very low accumulation of the virus in plant tissues (Valkonen, 1994). In the case of ER, the titre of the virus is either too low to be detected by current diagnostic techniques or detected but shown to have a very low concentration in the plant tissues. In plants expressing ER, no symptoms are visible on mechanically inoculated plants, or possibly very small systemic necrosis called *pin-point* lesions (Valkonen et al., 1996).

HR includes cell death, which occurs in cells at the site of infection, and discrete necrotic lesions appear in phenotypically normal tissue (Soosaar et al., 2005, Valkonen et al., 1996). The virus is confined to the lesion and to the cells immediately surrounding it; thus, the virus cannot spread from lesions into adjacent healthy tissues, which prevents the systemic infection of the whole plant (Soosaar et al., 2005, Tian & Valkonen, 2015).

In potatoes, HR and ER are mediated by distinct resistance genes that are expressed against specific viruses, such as PVY (Valkonen, 1994). Resistance genes can also be expressed against specific PVY strains of a virus species (Table 3). ER is conferred by single dominant genes designated *Ry* that were introgressed from wild potato species such as *Solanum stoloniferum* (gene $Ry_{sto}$), *S. tuberosum* ssp. *andigena* (gene $Ry_{adg}$), and *Solanum chacoense* ($Ry_{chc}$) (Valkonen et al., 2008). In contrast, HR is mediated by the *N* gene that is present in some potato cultivars. The most common *N* genes are $Nc_{tbr}$, $Ny_{tbr}$ and $Nz_{tbr}$. Each cultivar that harbour one of these *N* genes develops a HR when it is infected by a specific PVY strains on a gene-for-gene basis (Table 3) (Tian & Valkonen, 2015).

### *4.2.2. Tolerance*

Tolerance is defined as a lack of symptoms and yield loss. However, tolerance cannot be considered as a type of resistance *stricto sensu*, as symptomless plants can support high levels of virus accumulation. Disease tolerance in a crop species may also be defined as the ability of a plant to produce a greater yield than would normally be expected from the observed disease (Newton & Thomas, 1994). For example, the

potato cultivars *Red LaSoda* is susceptible to PVY but do not show symptoms when the plant is infected (Draper et al., 2002). Thus, infected tolerant plants can act as a source of viruses without being identified during crop inspection and removal from the field (roguing) (Valkonen, 1994). The consequences of producing these cultivars on PVY spread is discussed in section 8 of the introduction.

### *4.2.3. Immunity*

The immunity of a plant to viruses is characterised by a lack of infection at the cellular level. However, this type of virus resistance has never been characterised for potatoes (Cooper & Jones, 1983, Valkonen, 1994).

### *4.2.4. Mature Plant Resistance*

The relationship between plant age and resistance to several diseases has been described and investigated in many plant-pathogen systems (Kus et al., 2002). Potato plants can also acquire some resistance to PVY during aging, which this phenomenon is called *mature plant resistance* (MPR). Bagnall (1992) observed that the in-field natural infections barely stopped on average 50 days after emergence (Figure 5). In this experiment, batches of 20 plants of the PVY-susceptible cultivars Russet Burbank protected by a screen house were successively exposed to aphid flights for 7 to 15 days and then covered again. It showed that few infected plants were found in the plants exposed to aphids 50 days after emergence or later. This trial was repeated for 13 years, and MPR was observed for years with both low and high PVY pressure (Figure 5).

MPR was firstly described for PVY on potatoes by Debokx (1964) and is associated with changes in the metabolisms of older plants, inducing a reduction of the speed of the translocation of the virus (Beemster, 1976, Debokx, 1964). A significant decrease of ribosomes, glycoproteins and RNA content was observed in the old leaves infected by PVY compared with the youngest fully expanded leaves (Venekamp & Beemster, 1980, Venekamp et al., 1980). This observation suggests that the development of MPR is associated with a decrease of the metabolic activity of the plant. Other MPR indicators were tested such as dry matter, organic nitrogen, chlorophyll and soluble protein contents, and peroxidase activity, but none of them proved to be reliable

markers in establishing or predicting the time of occurrence of MPR (Braber et al., 1982).

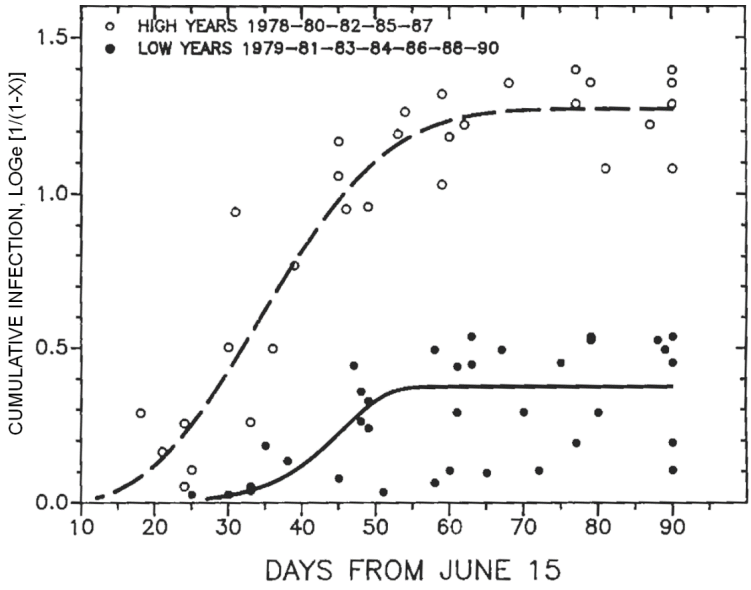


*Figure 5: Cumulative infection curves of PVY (Fredericton, New Brunswick, Canada, 1978–1990), calculated from indexing tubers harvested each year from trial plots that were exposed in sequence for 7- to 14-day periods but covered with screened cages at other times. Cumulated percentages were concerted by means of the equation* $y=\log_e[1/(1-x)]$*. The dashed line and solid line represent the means calculated from combined points of each group for high years and low years, respectively [adapted from Bagnall (1992)].*

Other MPR indicators were tested such as dry matter, organic nitrogen, chlorophyll and soluble protein contents, and peroxidase activity, but none of them proved to be reliable markers in establishing or predicting the time of occurrence of MPR (Braber et al., 1982). Studies performed with the tobacco-*Phytophthora infestans* pathosystem have shown that the susceptibility of young plants is due to a lack of induction of salicylic acid signalling (Shibata et al., 2010). This study suggests that salicylic acid could play a role in signalling the status of MPR in the whole plant. This type of resistance is also strain-dependent, as it is more pronounced for $PVY^O$ strains than for $PVY^N$ strains (Debokx, 1964). It appears about five weeks after emergence and

increases to reach complete protection of the progeny tubers eight to ten weeks after emergence (Sigvald, 1985, Gibson, 1991), but MPR varies among varieties (Gibson, 1991). For example, MPR status might be observed earlier for cv. Désirée compared to cvs. King Edward, Record and Maris Piper (Figure 6). Furthermore, the phenomenon of MPR seems to be low or absent in some varieties (e.g. cv. Russet Norkotah) (Zhang, 2014).

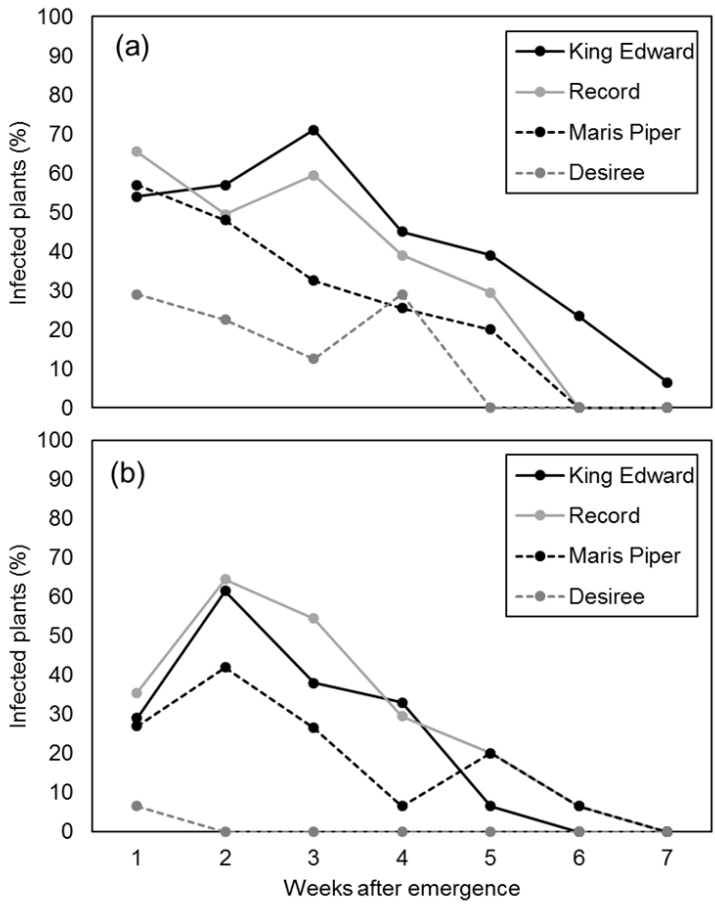


*Figure 6: Evolution of mature plant resistance for 4 cultivars and 2 PVY strains: $PVY^O$ (a) and $PVY^N$ (b). The graphs have been created from published data (Gibson, 1991).*

### *4.2.5. Systemic Acquired Resistance*

Systemic Acquired Resistance (SAR) is defined as a process where a plant successfully resists to a pathogen following a previous exposure. New exposure to the pathogen leads to resistance through localized tissue necrosis. In this process, the whole plant becomes highly resistant to subsequent infection (Navarre et al., 2003, Conrath, 2006). SAR is effective against the original pathogen, but also against a wide range of other pathogens likely to infect the plants thereafter (secondary pathogens) which include viruses, bacteria, oomycetes, and fungi (Dempsey et al., 1999, Conrath, 2006). SAR is characterised by the development of an enhanced and long-term resistance (ranging from weeks to months) in the distal, uninoculated plant organs (Conrath, 2006, Dempsey et al., 1999). This effect in distal organs suggests a long-distance signal for SAR, but this mechanism is still unknown (Conrath, 2006). Salicylic Acid (SA) is required to induce resistance where a secondary infection by another pathogen occurs (Nakayama et al., 1996, Vasyukova & Ozeretskovskaya, 2007, Conrath, 2006). In addition, in model plants such as *Arabidopsis*, it has been demonstrated that SA signalling also interacts with jasmonate and ethylene signalling pathways, activating both identical and different sets of genes (Schenk et al., 2000). Nevertheless, SA levels are clearly different in potato from those in *Arabidopsis* (Navarre et al., 2003). SAR against fungus, oomycetes, bacteria and viruses have been described for various solanaceous crops (Alexandersson et al., 2016). For potatoes, SAR mechanisms have been described in response to challenge by *Phytophthora infestans* (oomycete), *Fusarium solani* (fungus) and *Dickeya solani* (bacteria) but no SAR is known against viruses (Alexandersson et al., 2016). The effect of SAR should be assessed against PVY, as efforts have been made to use SAR for crop protection, and several chemical companies are currently marketing putative elicitors of SAR (Navarre et al., 2003). Unfortunately, the preliminary results obtained on tobacco suggest that SAR does not provide strong protection against PVY (Pennazio & Roggero, 1988). Despite those negative results obtained with tobacco, elicitors of SAR have yet to be tested on potatoes.

### *4.2.6. Barriers to the Use of Resistance Mechanisms to Control PVY*

Resistant cultivars can be used to reduce the prevalence of PVY in seed potato stocks. In practice, the cropping choices made by growers are not primarily based on disease

resistance to viruses, but they are mainly on quality features requested by the market (e.g. tuber shape and colour, starch content, and thickness of the skin). As a result, 60% of the cultivars produced in Switzerland in 2016 are highly susceptible to PVY (Schwaerzel et al., 2016). Consequently, breeders should make efforts to propose new resistant cultivars corresponding to the needs of the market to increase the percentage of resistant cultivars produced and therefore reduce losses associated to the rapid spread of PVY in susceptible crops.

# 5. Virus Infectious Cycle

## 5.1. PVY Transmission and Plant Infection

Potato plants can become infected by PVY either through mechanical wounding or through aphid feeding, this type of infection is termed *primary infection* (Fereres & Moreno, 2009). Human or animal activity can provoke mechanical transmission of viruses as well as plant-to-plant contact, although to a much lower efficacy for PVY than for contact-transmissible viruses such as PVX. This occurs when sap from a diseased plant spreads to healthy plants. Hane and Hamm (1999) showed that a healthy plant of a very susceptible cultivar can be infected at the end of the growing season by an adjacent infected plant. Nevertheless, mechanical transmission is considered a slow and inefficient mean of transmission of PVY compared to aphid-transmission. PVY is transmitted by aphids in a non-persistent fashion (Bradley, 1954, Kanavaki et al., 2006). Consequently, the acquisition and inoculation access periods of the virus are very short (seconds to minutes), and aphids remain viruliferous for a very short period (minutes to a few hours) (Fereres & Collar, 2001, Pirone & Perry, 2002). Aphids can acquire PVY during brief probing events (5 s) into epidermal cells, but longer acquisition period with longer probing events (10 s to 1 min) increase transmission efficiency. Nevertheless, prolonged feeding (more than 5 to 10 min) results in poor transmission or no transmission at all (Bradley, 1954, Watson & Roberts, 1939, Singh et al., 1983). Starved aphids are more efficient PVY vectors than non-starved ones, aphid fasting prior to acquisition access is therefore another factor affecting PVY transmission (Bradley, 1954, Watson & Roberts, 1939, Upreti & Nagaich, 1971, Singh et al., 1983).

## 5.2. Virus Replication

The PVY infectious cycle is presented schematically in Figure 7 and is divided into several overlapping stages. The first stage is the infection *per se* and the movement of the virus in the cytoplasm of the leaves cells or trichome cells of the potato plant. During the second stage, which is the uncoating stage, the positive-sense RNA of the virus is released from the capsid that coats the viral RNA. In the third stage, the virus recruits the translation machinery from the host to produce virus-encoded proteins (i.e. P1, HC-Pro, P3, CI, VPg, NIa, NIb, and CP). The fourth stage of the infectious cycle is the replication of the virus. During the replication process, the RNA of the virus acts as a template for negative-strand RNA transcription, which in turn serves as a template for the synthesis of progeny positive-strand RNAs (Mandahar & Mandahar, 2006, Visser, 2012). Then, this progeny positive-sense RNA is encapsidated by the capsid (coat) protein subunits (Jiang & Laliberte, 2011, Visser, 2012).

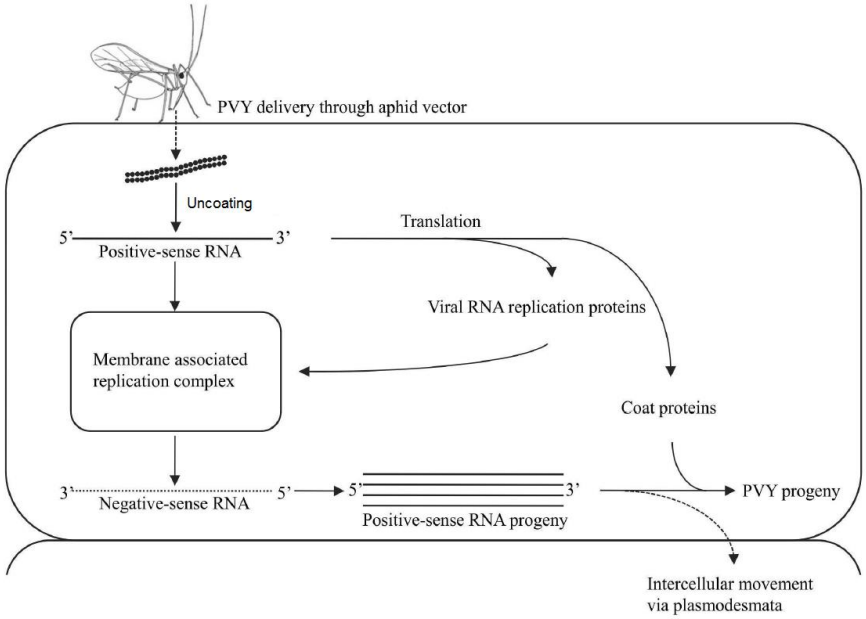


*Figure 7: A graphical representation of replication of a virus with a positive-sense RNA genome in a plant cell [adapted from Noueiry and Ahlquist (2003)].*

## 5.3. PVY Movement into the Plant

Concomitantly to the replication event, the PVY virions progeny spreads from cell-to-cell through plasmodesmata which links neighbouring cells (Lucas et al., 2009). This intercellular movement continues until the virus reaches the phloem vessels associated cells from where it systemically spreads throughout the plant in a source-to-sink manner (Maule, 1991). The movement of the virus is directly related to the developmental stage of the plant tissue, and to its physiological status as either a source of photosynthetic assimilates or sink for those photoassimilates (Figure 8). Thus, in the potato, PVY is actively transported with other plant products from the mother-tuber or mature leaves to young developing leaves (Basky & Almasi, 2005, Turgeon, 2006). Later on, PVY is transported from mature leaves to the uppermost leaves and to the developing progeny tubers, acting as physiological sinks (Basky & Almasi, 2005). The movement of the virus from the above ground plant parts to the progeny tubers is called translocation (Beemster, 1972, Beemster, 1976), and the spread of PVY in the vascular system of the plant requires the involvement of virus-encoded movement proteins to facilitate the transport of virions and viral RNA complexes (Wei et al., 2010b). Infected seed potatoes planted in the field will lead to infected plants. This type of infection is termed *secondary infection* (Malnoe et al., 1994)

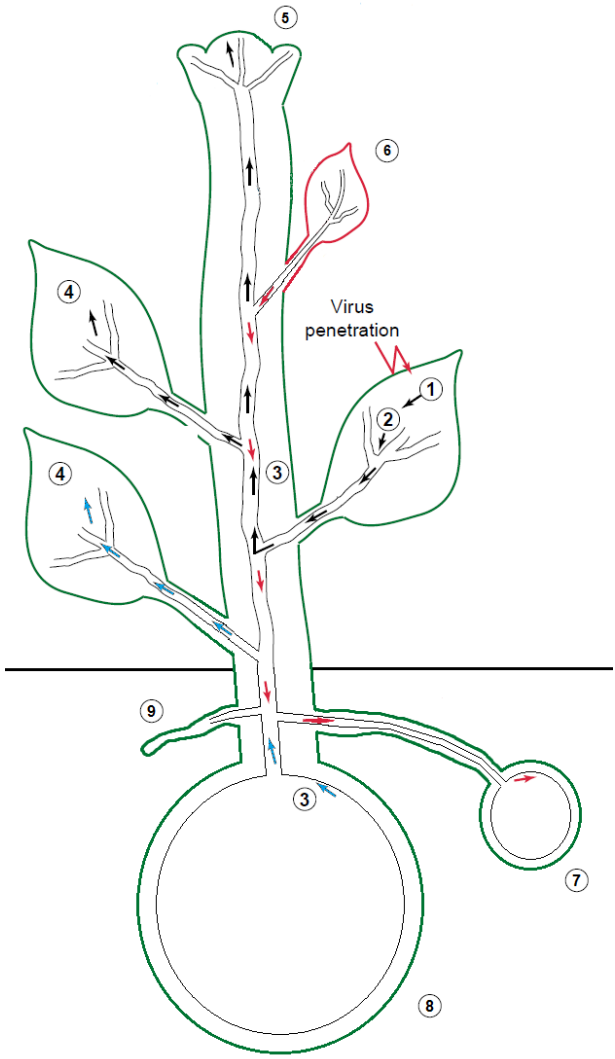


*Figure 8: Systemic RNA and virions transport in an infected potato plant, adapted from Citovsky and Zambryski (2000). (1) cell-to-cell transport of viral RNA, (2) entry of viral RNA into phloem, (3) systemic transport of viral RNA – black=primary infection – blue=secondary infection – red=virus translocation, (4) exit of viral RNA from phloem, (5) apical region, (6) infected leave, (7) progeny tuber, (8) mother tuber, (9) root.*

# 6. The Vectors

## 6.1. Diversity of Vectors

Many aphid species (more than 70) can acquire and transmit PVY to host plants (Pirone & Harris, 1977, Sigvald, 1984, Al-Mrabeh et al., 2010, Radcliffe & Ragsdale, 2002, Debokx & Piron, 1990, Boukhris-Bouhachem et al., 2011). Among these species, the green peach aphid, *Myzus persicae* (Sulzer), is considered to be the most efficient vector of PVY in potatoes (Fernandez-Calvino et al., 2006, Sigvald, 1984). The efficacy of PVY transmission from plant-to-plant by other aphid species was evaluated initially using relative efficiency factors (REFs). REFs assessment relies on the following experimental procedure: after a starvation period, individual aphids are set to probe on a PVY-infected plant and subsequently transfer to a healthy plant (bait plant). After the inoculation access period, the aphids are killed. Then, after a given time, allowing for the accumulation of PVY in the bait plants, the plants are tested for PVY infection (Debokx & Piron, 1990). REFs values are calculated for each aphid species by dividing the transmission rate value (infected plants divided by the total number of bait plants) of each individual species by the transmission rate value of *M. persicae* (Debokx & Piron, 1990, Mondal et al., 2016, Sigvald, 1984, Verbeek et al., 2010, Basky, 2003). Important differences in the REFs assessment methodologies used in the different laboratories lead to significant variability in the results, even for the same aphid species (*Table 6*).

The efficiency of transmission also varies between PVY strains (Figure 9 and section 6 of the introduction). Additionally, differences in REFs between individuals of a specific aphid species (*Myzus persicae*) are observed (Figure 9), suggesting that the genetic variability within an aphid species significantly affects the efficiency of transmission of PVY (Verbeek et al., 2010). Pelletier et al. (2012) have used a different method to identify the potential vectors of PVY: they analysed by polymerase chain reaction (PCR) wild aphids trapped in seed potato fields by yellow water traps to detect the presence of the virus in their mouthparts. Using this method, they identified 45 potential new vector species of PVY. Furthermore, Pelletier et al. (2012) suggest that, under certain conditions, most (if not all) aphid species can act as vectors for PVY. This method assesses the presence of a virus in an aphid and not its capacity to transmit it. Therefore, this method must be completed by performing transmission experiments to assess the REFs of the aphid species in which the virus was detected.

*Table 6: Relative efficiency factors (REFs) of the main aphid species reported in the literature: [1] van Hoof (1980); [2] Van Harten (1983); [3] Piron (1986); [4] Gibson et al. (1988); [5] Harrington and Gibson (1989); [6] Debokx and Piron (1990); [7] Derron and Goy (1990); [8] Sigvald (1992); [9] Basky and Almasi (2005); [10] Verbeek et al. (2010); [11] Boquel et al. (2011); [12] Mello et al. (2011); [13] Mondal et al. (2016); [14] Fox et al. (2017). "-" means that no data is available.*

| PVY strain | *M. persicae* | *A. pisum* | *A. fabae* | *A. nasturtii* | *B. helichrysi* | *C. aegopodii* | *H. pruni* | *M. euphorbiae* | *M. dirhodum* | *Ph. humuli* | *R. insertum* | *R. padi* | *S. avenae* | References |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $PVY^{N}$ | 1 | 0.28 | 0.48 | - | 0 | 0 | - | 0.58 | 0.06 | 0.7 | 1 | 0.04 | 0 | [1] |
| NA | 1 | 0.05 | 0.1 | - | 0.01 | - | - | 0.1 | 0.02 | 0.15 | 0.05 | 0.02 | - | [2] |
| $PVY^{N}$ | 1 | 0.17 | 0.14 | 0.39 | 0.17 | 0.05 | 0.19 | 0.2 | 0.14 | 0.25 | 0.19 | 0.16 | 0.02 | [3] |
| $PVY^{O}$ | 1 | - | - | - | - | - | - | 0.24 | | | | 0.32 | - | [4] |
| $PVY^{N}$ | 1 | - | - | - | - | - | - | 0.15 | | | | 0.32 | - | [4] |
| NA | 1 | 0.45 | 0.9 | - | 0.7 | 0.02 | - | 0.91 | 0.06 | 0.58 | 0.09 | 0.28 | 0.01 | [5] |
| $PVY^{N}$ | 1 | 0.11 | 0.07 | 0.42 | 0.21 | 0 | 0.07 | 0.07 | 0.1 | 0.13 | 0.13 | 0.14 | 0.02 | [6] |
| $PVY^{N}$ | 1 | 0.08 | 0.25 | 0.25 | 0.25 | 0.01 | 0.01 | 0.25 | 0.01 | 0.25 | - | 0.08 | 0.01 | [7] |
| $PVY^{O}$ | 1 | 0.7 | 0.1 | 0.4 | - | - | - | - | 0.3 | - | - | 0.4 | 0.01 | [8] |
| $PVY^{O}$ | 1 | - | 0.00 | - | - | - | - | - | - | - | - | 0.00 | - | [9] |
| $PVY^{N}$ | 1 | - | 1.10 | - | - | - | - | - | - | - | - | 0.11 | - | [9] |
| $PVY^{N}$ | 1 | 0.08 | 0.03 | 0.46 | - | 0 | - | 0 | 0.02 | 0.22 | - | 0 | 0 | [10] |
| $PVY^{NTN}$ | 1 | 0.07 | 0.04 | 0.5 | - | 0 | - | 0 | 0 | 0.23 | - | 0.01 | 0 | [10] |
| $PVY^{N\text{-}Wi}$ | 1 | 0.11 | 0.13 | 0.69 | - | 0 | - | 0.01 | 0.01 | 0.3 | - | 0.04 | 0 | [10] |
| $PVY^{NTN}$ | 1 | 0.08 | 0 | - | - | - | - | 0.32 | - | - | - | 0 | 0.2 | [11] |
| $PVY^{O}$ | 1 | - | - | 0.46 | - | - | - | - | - | - | - | 0.26 | - | [12] |
| $PVY^{N\text{-}Wi}$ | 1 | - | - | 0.49 | - | - | - | - | - | - | - | 0.02 | - | [12] |
| $PVY^{O}$ | 1 | - | - | - | - | - | - | 0.09 | - | - | - | 0.44 | - | [13] |
| $PVY^{N\text{-}Wi}$ | 1 | - | - | - | - | - | - | 0.05 | - | - | - | 0.33 | - | [13] |
| $PVY^{NTN}$ | 1 | - | - | - | - | - | - | 0.07 | - | - | - | 0.38 | - | [13] |
| $PVY^{O}$ | 1 | 1.08 | 0.06 | - | - | 0.77 | - | 0.3 | 0 | - | - | 0.77 | 0.33 | [14] |
| $PVY^{N}$ | 1 | 0.29 | 0 | - | - | 0.49 | - | 0.66 | 0.87 | - | - | 0.57 | 1.1 | [14] |
| $PVY^{NTN}$ | 1 | 0.49 | 0.13 | - | - | 0.96 | - | 0.38 | 0.07 | - | - | 0.95 | 0.49 | [14] |
| Nb. | 23 | 14 | 16 | 9 | 6 | 11 | 3 | 18 | 13 | 9 | 5 | 23 | 13 | |
| Mean | 1 | 0.29 | 0.22 | 0.45 | 0.22 | 0.21 | 0.09 | 0.24 | 0.13 | 0.31 | 0.29 | 0.25 | 0.17 | |
| St. dev. | 0 | 0.30 | 0.33 | 0.12 | 0.26 | 0.36 | 0.09 | 0.25 | 0.24 | 0.20 | 0.40 | 0.26 | 0.32 | |

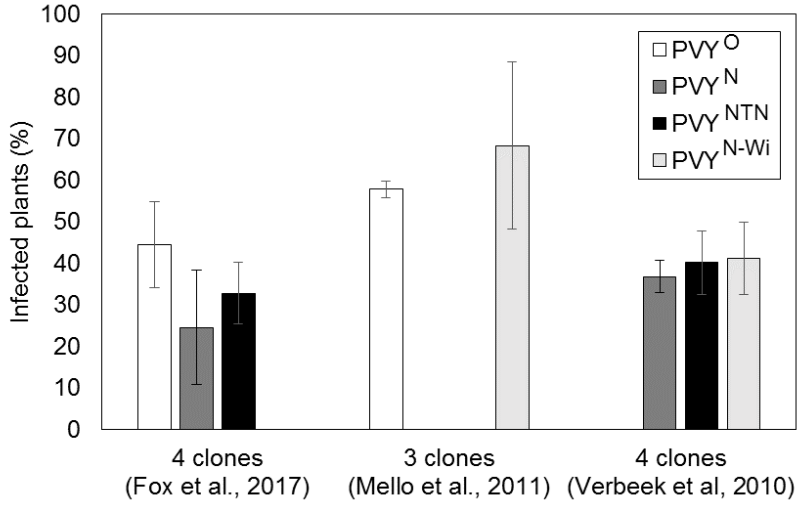


*Figure 9: Percentage of infected tobacco in three experiments (Fox et al., 2017, Mello et al., 2011, Verbeek et al., 2010) done with different clones of Myzus persicae. Error bars show the standard deviation of the mean.*

## 6.2. Life Cycle of Aphid

Understanding the behaviour of aphid species in potato crops is of the uttermost importance to understand the risk of PVY spread, since aphid phenology will be associated with a higher risk of PVY transmission. Apterous aphids contribute little to PVY spread, because their dispersal and thus virus transmission is limited to neighbouring plants (Nemecek, 1993, Boiteau, 1997). The behaviour of winged aphids, which are more likely to transmit PVY, depends on the species and its life cycle. Aphid species can be divided into three groups based on their cycles on different types of host plants: (i) the monoecious species performing their entire cycle on herbaceous hosts, (ii) the monoecious species performing their cycle on woody hosts (trees or bushes) and (iii) the dioecious species performing their cycle alternatively on woody and herbaceous hosts. Usually, the winged dioecious aphid species leave woody plants (the primary host) in spring to colonise specific herbaceous plants (the secondary host). This flight is called the *migration flight*. Then, a series of short-distance flights, called *dissemination flights*, occur in early summer. During the fall, a

new generation of winged aphid then migrate back to the primary host. It is called the *remigration flight* (Turpeau et al., 2011, Bell et al., 2012, Döring, 2014).

## 6.3. Host Selection Process

The host selection process by winged aphids during migration and dissemination flights implies a complex set of behaviours mainly governed by climatic conditions, visual and olfactory stimuli (Döring, 2014, Kring, 1972). Döring (2014) compared the behavioural sequences of the flights of aphids and presented observations of several publications. These can be summarised as follows: before take-off, the aphid is in resting mode on the winter host. When climatic conditions are favourable, the aphid starts a long-distance migration flight and is oriented by light (positive phototactic). Then, the aphid changes from migratory to targeted flight and lands on plants for probing. After contact with a non-host plant, the aphid takes off from the plant and continues with the host-finding process. After host contact, two sequences are possible: the aphid can settle on the plant and deposit the larvae underneath the leaves, or the aphid can resume flight and fly further to find another host plant. This second sequence can also be observed in the field and be explained by the host preference of aphid species or by the fact that the host plant is already colonised by aphids (Döring, 2014). Visual contrast seems to play an important role in the host selection process and, in particular the contrast between plants and the background of bare soil (Döring & Chittka, 2007). Thus, crop density might influence the landing rate of aphids. For example, the contrast between the bare soil and the plant is at the maximum nearly after emergence due to the gaps between plants. These gaps increase the risk of infection by PVY, as more aphids land at crop margins (Davis et al., 2009). The colour of the plant is also important in the host-selection process. Several studies have shown that aphids colour selection is directed towards yellow over green and red (Döring & Chittka, 2007). This has strong implications for virus spread in general and for PVY spread in particular. Virus infection of plants generally induces the yellowing of the leaves, such as mosaic on PVY-infected plants, making them more attractive to aphids (Hodge et al., 2011). Nevertheless, experiments performed using *M. persicae* could not demonstrate that winged aphids were more attracted to PVY-infected plants (Castle et al., 1998, Boquel et al., 2012). After landing, the aphid feeding behaviour is modified by PVY-infected plants, resulting in an

increase of the duration of the ingestion of sap by the aphids (Boquel et al., 2012). Finally, olfactory stimuli also play a role in the host-selection process. Aphids respond to volatile compounds emitted by plants and to pheromones emitted by aphids (Döring, 2014). Infected plants produce volatiles that can attract aphids (Jimenez-Martinez et al., 2004), but this has never been tested for potatoes infected by PVY. This suggests a multimodal interaction of visual, olfactory and probably other stimuli influencing aphid host-selection behaviour. This paragraph highlights the fact that the behaviour of potato aphids in the field is likely to play an important role in explaining PVY spread as opposed to the observations in laboratory conditions regarding their relative efficacy in transmitting the virus (Sigvald, 1987).

## 6.4. Plant Contact and Probing

After alighting (sequence 1), Powell et al. (2006) defined five additional stages of aphid feeding as follows: (2) initial plant contact and assessment of surface cues before stylet insertion, (3) probing the epidermis, (4) stylet pathway activity, (5) sap element puncture and salivation and (6) phloem acceptance and sustained ingestion.

The efficiency of the transmission of PVY relies on the early stages of 1, 2 and 3 (Powell & Hardie, 2000) and on the brief probes (5 to 10 seconds) made in the epidermis during stage 3, which provide the aphid with information regarding the gustatory quality of the plant sap (Powell, 1991). These probes in the epidermis imply the injection of saliva into the epidermal and mesophyll cells and, thereafter, the ingestion of a saliva/cytoplasm mixture, which occurs a few seconds later. During these probes, PVY is acquired and inoculated during salivation (Powell, 2005, Fereres & Moreno, 2009). Thus, any aphid that alights on a potato plant and that probes the plant may be a potential vector of PVY (Boquel et al., 2012).

## 6.5. Aphids Flights and PVY Spread

To evaluate the risk of PVY spread in the field, aphid flights have been studied for different aspects: composition, timing and intensity (Steinger et al., 2015, Thomas et al., 1997, Van Harten, 1983, DiFonzo et al., 1997, Sigvald, 1987, Sigvald, 1989). All these studies demonstrated the relationship between the intensity of the aphids' flights

and the spread of PVY in the field. With this in mind, decision support systems have been created to forecast the risk of PVY spread using aphid-flight data (Steinger et al., 2015, Sigvald, 1987, Gabriel, 1981, Bertschinger et al., 1995, Nemecek, 1993, Madden et al., 2000). Nevertheless, decision support systems allowing an efficient evaluation of the risk of PVY spread in the field are difficult to implement in practice (Sigvald, 1987, Van Harten, 1983). This is mainly due to the difficulty of calculating a cumulative aphid pressure based on aphids captures effectively correlated with a risk of PVY spread (Steinger et al., 2015). Indeed, when REFs are used to calculate the cumulative aphid pressure, bias can be introduced when estimating the risk. The first bias is related to the omission of potential PVY vectors (Pelletier et al., 2012) and the second bias is related to the omission of the behaviour of the aphids in the crops (Sigvald, 1987). While some aphid species have relatively low REF values, their importance as PVY vectors is directly dependent on their behaviour in potato fields. For example, the number of *Brachycaudus helichrysi* (Kaltenbach) captured during the potato growing period by suction traps in Switzerland is a good predictor of the incidence of PVY in Swiss seed potato production, while counts of *M. persicae* were not correlated with the virus incidence in potato-seed lots (Steinger et al., 2015). This statement is consistent with the data obtained from a field experiment performed for 14 years in Switzerland in two distinct locations (Changins-409m a.s.l and Reckenholz-443m a.s.l) in the immediate vicinity of an aphids suction trap (Rothamsted trap) (Dupuis et al. (2016); see proceedings in Annex 7). In this field experiment, it was observed that the strongest correlation between the number of aphids and PVY incidence in the harvested tubers was obtained with *B. helichrysi* and the aphids of the Genus Aphis (Table 7).

Figure 10 shows the average catches at the suction trap of the eight main aphid species in the two locations of the field experiment mentioned, and the data of the first 7 weeks of catches are presented. We observe that, for *Brachycaudus helichrysi* and *Aphis* spp., a higher incidence occurs during the first 3 weeks after emergence, when the potato plants are still susceptible to PVY infections due to the lack of mature plant resistance. The incidence of *M. persicae* were abundant, but they were seen mainly after the fifth week of potato growth, when mature plant resistance is already present (Sigvald, 1985, Gibson, 1991). This argument is supported by a survey undertaken by DiFonzo et al. (1997) in the Red River Valley of Minnesota and North Dakota (United States). This study indicates that eight main aphid species (representing 91% of the

captures) were identified at the time of PVY infection in the field (monitored by trap plants). The species were *Acyrthosiphon pisum* (Harris), *Aphis helianthi* (Monell), *Capitophorus elaeagni* (Del Gercio), *Lipaphis erysimi* (Kaltenbach), *Rhopalosiphum maidis* (Fitch), *Rhopalosiphum padi* (L.), *Schizaphis graminum* (Rondani), and *Sitohion avenae* (F.); while *M. persicae* accounted for only 1.7% of the aphid species.

*Table 7 Results of correlation analysis (significance and coefficient of determination) between the aphids captures at the Rothamsted '12-m' suction trap and PVY infection of the harvested tubers (cv. Bintje) (Dupuis et al., 2016).*

| Variables | p-values | $R^2$ |
|---|---|---|
| *Acyrthosiphon pisum* | 0.98 | 0.00 |
| *Aphis fabae* | 0.15 | 0.08 |
| *Aphis* spp. | **0.01** | **0.28** |
| *Brachycaudus helichrysi* | **0.01** | **0.26** |
| *Macrosiphum euphorbiae* | 0.11 | 0.11 |
| *Myzus persicae* | 0.25 | 0.05 |
| *Phorodon humuli* | 0.11 | 0.10 |
| *Rhopalosiphum padi* | 0.46 | 0.02 |
| Total aphids | 0.18 | 0.07 |

*Values in bold are different from 0 with a significance level alpha<0.05*

These results challenge the importance of *M. persicae* as the major vector of PVY in the field, or they at least illustrate the complexity of evaluating the risk of PVY spread in the field. Similarly, the number of catches of *M. persicae* from suction trap (Rothamsted trap) is no longer used in Switzerland to evaluate the risk of spread of PVY in the field (Thomas Steinger, personal communication). The new model created in 2015 is based on *B. helichrysi* phenology to assess the risk of PVY spread in the Swiss potato fields (Steinger et al., 2015).

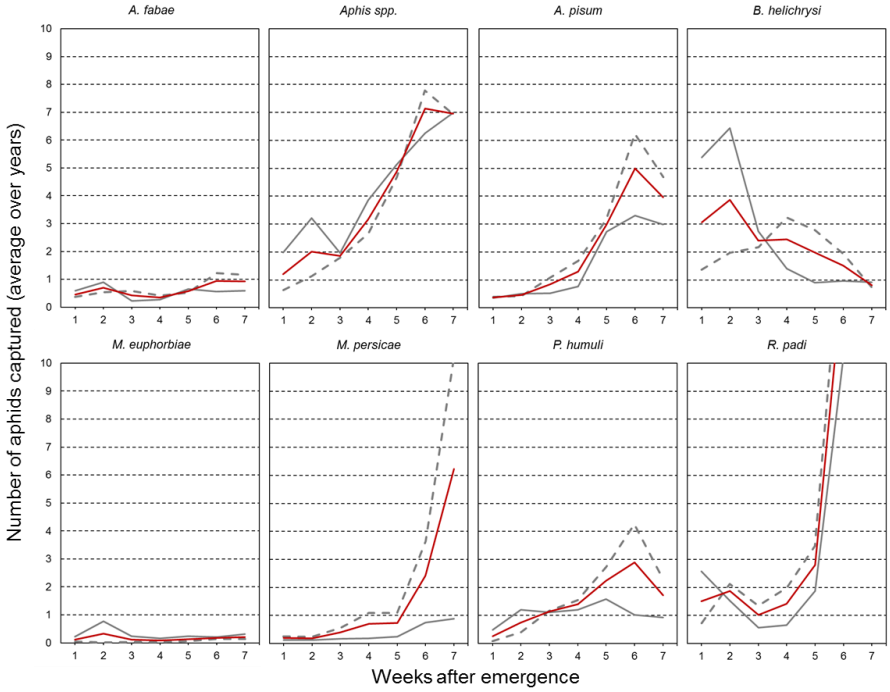


*Figure 10: Number of aphid captures in Changins-Switzerland (dotted line) and in Reckenholz-Switzerland (continuous line), as well as the average (red line). The values are averaged over 14 years of captures in Changins and 10 years in Reckenholz [adapted from Dupuis et al. (2016)].*

# 7. The Environment

## 7.1. Reservoir of PVY Inoculum

The presence of the inoculum in the field depends on the percentage of infected tubers in the seed lot, on the presence of potato volunteers and on the presence of weeds infected by PVY (Steinger et al., 2014, Takacs et al., 2002, Rakotonindraina et al., 2011).

A high percentage of PVY inoculum in the field is known to be the main risk factor for the subsequent spread of PVY to the healthy plants of the field (Steinger et al., 2014, Kerlan et al., 1987). An infected seed lot will generate secondary infected plants which will be directly correlated to the initial percentage of infection of the parent seed lot.

The role of secondary infected plants in promoting PVY spread is widely documented. For example, Hansen and Nielsen (2012) observed in a field experiment performed with cv. Désirée that a 4% of secondary infection in a potato lot can reach 97% of infection of the progeny tubers in absence of control methods.

The role of weeds and volunteers in PVY spread is less documented, but the role of volunteers as PVY inoculum sources can be hypothesised to depend on the susceptibility of the cultivar of these volunteers. Indeed, volunteers of susceptible cultivars have a high probability of being infected by PVY, as no control method is used to prevent their infection. Most of the weed species in temperate regions are annual plants (Simić et al., 2016) growing in the spring after germination of the seed. PVY is known not to be transmitted through seeds of annual plants, but the annual plants are only infected to low levels (Sastry, 2013), suggesting that most of the annual weeds are PVY-free in spring and become infected later in the season. This suggests that weeds become inoculum sources when potatoes are less susceptible to PVY infection due to the mature plant resistance mechanism (Sigvald, 1985, Beemster, 1972, Gibson, 1991). Although it has not been tested experimentally, it is likely that weeds present a lower risk for PVY spread than volunteers that are infectious from the early stages of the crop. For the same reasons, neighbouring potato fields infected by PVY represent a higher risk as reservoirs of PVY inoculum than wild plants susceptible to PVY that are in most annual plants, because the transmission of PVY through the seed from one year to the next is low (Sastry, 2013, Kaliciak & Syller, 2009). The risk of infection of a potato field by an inoculum located outside of the field depends on the capacity of the aphids to bring the virus into the plot from the outside. For an individual viruliferous aphid, the risk seems to be very low, as (i) he has little control over his flight (Haine, 1955, Kring, 1972) and (ii) he generally transmits the virus over short distances (Rolot, 2005). While the risk of infection is low at the level of a single aphid, it probably increases significantly with the large number of aphids landing on a field during the season, because many of them may carry the virus (Sigvald, 1989, Sigvald, 1987, Basky, 2003, Boiteau et al., 1988, Milocevic & Petrovic, 1997, Van Harten, 1983). Nevertheless, the importance of the arrival of PVY inoculum in a field through aphids has not been tested yet, and the new technologies of PVY detection in single flying aphids will certainly contribute to answering this question (Kim et al., 2016, Zhang et al., 2013).

## 7.2. Aphid Populations

Migration and dissemination flights involving PVY vectors are potential sources of PVY spread. Consequently, the biodiversity of plants (woody and herbaceous) in a potato production area influences the population of aphids, and hence PVY spread. For example, the primary host of *M. persicae* are plants of the genus *Prunus*, such as peach trees (*Prunus persica*) (Turpeau et al., 2011). Therefore, an area with a high number of peach trees sustains high levels of *M. persicae,* which is an efficient vector of PVY (see *the vector* paragraph). In addition, the biodiversity of herbaceous wild plants and crops in potato field areas also has an influence on aphid populations. For example, cereal aphids such as *Rhopalosiphum padi*, vector of PVY (Verbeek et al., 2010, Mondal et al., 2016), migrate from barley (*Hordeum vulgare*) fields in July when the plants mature, as the quality of the phloem sap that the aphids have been feeding on decreases (Döring, 2014). The cereal aphids then explore neighbouring fields such as potato fields before establishing again on *Poaceae* (Turpeau et al., 2011). These flights due to host change can be responsible for the late PVY spread for cultivars with low (or no) mature plant resistance (Zhang, 2014).

## 7.3. Natural Predators of Aphids

Natural predators of aphids are known to be efficient in controlling aphid populations in potatoes (Nakata, 1995, Shands et al., 1972). With complex landscapes, interactions between natural predators are largely complementary and lead to a strongly positive effect on the control of aphids. Increasing the availability of seminatural habitats in agricultural landscapes may thus benefit natural predators and the effectiveness of aphid natural pest control (Martin et al., 2015). Nevertheless, these biocontrol agents control the development of the colonies but do not prevent the arrival of alate aphids coming from outside of the field, having a higher impact on PVY spread than aphids of established colonies on potato plants (Boiteau, 1997, Fisken, 1959).

## 7.4. Fertilisation and Soil Factors

It is commonly assumed that PVY symptoms on plants can be masked by a high nitrogen fertilisation regime (Whitworth et al., 2006). However, this assumption is

rebutted by the higher titre of PVY measured in plants supplied with nitrogen compared to unfertilised ones (Mierzwa et al., 1986) and by the fact that increased nitrogen does not significantly mitigate the yield reduction due to PVY infection (Whitworth et al., 2006). Thus, it is more likely that nitrogen fertilisation promotes the accumulation of PVY in the plant, but it is unknown whether this effect has an influence on virus acquisition by aphids. Furthermore, increase in nitrogen supply has the effect of darkening the potato foliage through increasing the chlorophyll content (Güler, 2009). We discussed previously (section 6.3) that aphids are more attracted to yellow than green colours (Döring & Chittka, 2007). As a result, we can assume that potato plants presenting a dark green colour due to a high nitrogen fertilisation could be less attractive to aphids than other less fertilised plants. Nevertheless, this has not been tested experimentally. The effect of other soil-associated factors on PVY spread, such as soil types and the presence of other nutrients than nitrogen, is also unknown and has yet to be assessed.

## 7.5. Low Temperature

Climate conditions (mainly temperature, wind and rain) have an effect on potato crops, the vectors populations and their predators. Temperature is the most important of these factors, as it affects the development rate of aphid populations and their natural enemies (Jones, 1979, Nakata, 1995). Winter temperature affects aphid phenology, a 1 °C increase in average of winter temperature advances aphid migration by 4–19 days depending on aphid species (Zhou et al., 1995). Spring temperatures also influence the initiation of the migration of aphids. When temperatures are above a certain threshold, winged aphids leave their primary hosts and migrate to their secondary hosts, which this threshold ranges from 10 to 20°C depending on the aphid species (Cockbain, 1961, Walters & Dixon, 1984, Wiktelius, 1981). For example, 70% of the winged aphids of *Sitobion avenae* take-off when the temperature remains at 15°C over a period of 16 hours (Walters & Dixon, 1984). Thus, for low temperatures in winter and/or spring, a delay of the initiation of the migration flights in spring is observed (Nakata, 1995, Turpeau et al., 2011). For example, the highest incidence of flights of *Myzus persicae* occurs in May in southern France (Mediterranean climate), whereas it occurs from June to July for the rest of the country (Turpeau et al., 2011). In addition, potato growth and development widely depend on photoperiod and temperature.

Experiments performed in the laboratory with five cultivars have shown that the optimal combination of temperature and day length for the highest yield is on average 20°C for a day length of 12 hours and 16°C for a day length of 24 hours, with some variations between cultivars (Wheeler & Tibbitts, 1986). For lower temperature, the rate of the growth of haulm and stolons is reduced, and the tuber initiation is hastened (Burt, 1964). Experiments in controlled conditions shown that plants of cv. Katahdin (day length of 14 hours) grow at a temperature starting at 10°C with an optimum at 25°C (Benoit et al., 1983). For higher temperatures, haulm growth is enhanced at the expense of tuber growth and can reach to the absence of yield for extreme temperatures (Yean-Uk & Woo, 2016). This information indicates that potatoes can grow below the temperature threshold required for the initiation of the migration flight of several aphid species vectors of PVY. This in turn means that in cold spring weather conditions, the main aphid flights occur when the vegetative development of the crop is advanced and has already developed some resistance to infection through the mature plant resistance mechanism (Sigvald, 1985, Beemster, 1972, Gibson, 1991).

## 7.6. High Temperature and Drought

Water shortage slows potato growth and reduces yield when it comes after tuber initiation (van Loon, 1981). Water shortage also reduces cell turgor, which limits food uptake by aphids due to a lower sap pressure, and therefore slows the development of aphids (Kennedy et al., 1958, Wearing & Van Emden, 1967, Simpson et al., 2012). Water shortage has a positive effect by enriching the phloem sap, which is of better quality for aphid nutrition, and these positive effects recorded with various aphid species may be associated with less severe or intermittent water stress in the plant when the reduced quantity of sap obtainable due to reduced cell turgor is more than compensated by its improved quality (Kennedy et al., 1958).

Water stress is generally associated with high average temperatures, and temperatures above 30°C are lethal for the larvae of several aphid species (Asin & Pons, 2001). For example, among three aphid species, *Rhopalosiphum padi* (L.), *Sitobion avenae* (Fabricius) and *Metopolophium dirhodum* (Walker), *R. padi* is the only species whose larvae survive at such high temperatures. Temperatures above 40°C are considered as the thermal death-point for the majority of the aphid species (Asin &

Pons, 2001, Broadbent & Hollings, 1951). Hot and dry conditions are unfavourable to PVY spread, but these conditions rarely occur in spring, which is when potato plants are the most susceptible to PVY infections (Gibson, 1991, Robert et al., 2000, Sigvald, 1985).

### 7.7. Wind and Rain

Rain is favourable to plant growth but is detrimental to aphid development, especially in the case of heavy rains (Jones, 1979). Additionally, rain is negatively correlated with the flight of aphids (Xu et al., 2016, Du et al., 2014), which lowers the risk of PVY spread during rainy days. A recent laboratory experiment demonstrated that potato plants can become infected by PVY through water uptake by the roots (Mehle et al., 2014). However, the impact of water transmission of PVY in field conditions remains to be assessed, and whether PVY can be present and remain infective in irrigation water in field conditions is uncertain. Although there is currently no answer to this question, water should be considered in future research on PVY epidemiology. Finally, strong wind has an adverse effect on aphid take-off and aphid development (Jones, 1979, Nakata, 1995, Walters & Dixon, 1984, Haine, 1955) and reduces the importance of the migration flights (Du et al., 2014). Consequently, windy areas are suitable for seed potato production, as aphids are less efficient in transmitting PVY.

## 8. Control, Management and Seed Certification Programs

### 8.1. Preamble

This part of the introduction is co-authored with Claude Bragard, Stuart Carnegie, John Kerr, Laurent Glais, Mathuresh Singh, Phillip Nolte, Jean-Louis Rolot, Kürt Demeulemeester, and Christophe Lacomme; and is published in the book “Potato virus Y: biodiversity, pathogenicity, epidemiology and management” written by the PVY-Wide network (Springer Edition; ISBN: 978-3-319-58858-2 [Print] 978-3-319-58860-5 [Online]). The version presented in this thesis has been modified from the original. The original is presented in Annex 1, with permission of Springer Nature.

## 8.2. Overview of the PVY Control Strategies

Several strategies to control potato viruses in general and PVY in particular have been published and reviewed by different authors (Zitter & Simons, 1980, Weidemann, 1988, Radcliffe & Ragsdale, 2002, Kerlan et al., 1987). The main PVY control strategies reported in the literature are presented in Figure 11: the use of mulching, border crops, intercropping, aphid pheromones of alarm, oil and insecticide spraying, early haulm destruction, removal of diseased plants (roguing), planting of healthy seeds, isolation of the field, pre-sprouting, early planting, use of plant elicitors and the use of resistant cultivars. In Figure 11, the blue arrows indicate the location of the main effect of the control strategies on the PVY pathosystem, the references behind this assignment are given in the body of the text of this section of the introduction. Isolation of the field, the planting of healthy seed and roguing have an effect in reducing the PVY inoculum in the environment of the crop. The use of resistant cultivars, the spraying of plant elicitors or the development of early mature plant resistance (with pre-sprouting and early planting) have an effect in reducing the accumulation of the virus in the plants and, hence, in the crop. Mulching, the planting of border crops, intercropping and the use of insect pheromones are reducing the colonization of the crop by the aphids. Oil and insecticide spraying are reducing the transmission of the virus by the vectors, and insecticides have an additional effect by reducing the aphid population. Finally, early haulm killing is acting on the potato plants by stopping the growth and subsequently avoiding the transmission of the virus to the plant. The existing PVY control strategies, their efficacy and their mode of action are further detailed in the remainder of the introduction.

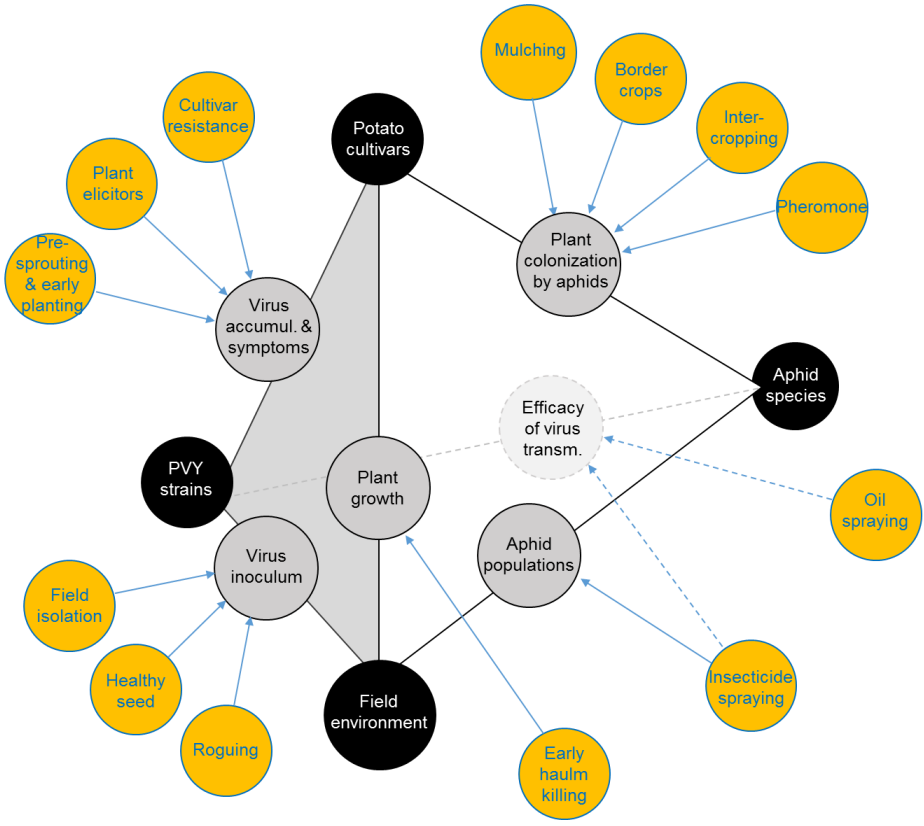


*Figure 11: Diagram presenting the PVY pathosystem with the four components of the system in black, the interactions between each other in grey and with the main control strategies in orange. The blue arrow indicates the location of the main effect of each control strategy.*

## 8.3. Cultural Methods

### *8.3.1. Prophylactic Measures*

*8.3.1.1. Use of virus-free seed potatoes*

The risk of PVY spread can be reduced by planting seed potatoes which are virus-free or have a very low incidence of tuber infection by PVY, thus minimising the number of inoculum sources within a crop (Kerlan et al., 1987, Rolot, 2005, Steinger et al., 2014). This will be discussed in the paragraph relating to seed potato certification programs.

*8.3.1.2. Use of Virus-Resistant Cultivars*

The most effective means of controlling virus in potato crops is to grow resistant potato cultivars. This strategy allows potatoes to be produced in areas where aphid pressure is high and hence unsuitable for potato production because of disease. There are several breeding programs worldwide whose goal is to breed new potato cultivars that are resistant to PVY (see section 4.2 of the introduction, *The host*).

In the USA and Canada, breeders have developed a number of widely grown cultivars which are tolerant to PVY, such as cv. Russet Norkotah which displays mild symptoms of PVY (Whitworth et al., 2010) and cv. Red LaSoda which is fully susceptible to infection by PVY but does not express symptoms (Draper et al., 2002). This could be one of the reasons for the re-emergence of PVY in these potato production areas (Gray et al., 2010, Schramm et al., 2011). In Germany and France, the selection programs for new cultivars is different. Breeders mainly aim to develop potato cultivars resistant to the multiplication of PVY and avoid the selection of cultivars susceptible to Potato Tuber Necrotic Ringspot Disease (PTNRD) (Le Romancer & Kerlan, 1991). However, it is widely believed that breeding for cultivars displaying high level of PVY resistance might often not being seen as a major goal due to poor economic return on investments (Ruedi Schwaerzel, personal communication).

*8.3.1.3. Choice of the location*

Location of crops is important in determining the risk of PVY transmission by aphids (Kerlan et al., 1987). Low risk areas are usually those with low aphid populations, often as a consequence of low mean temperatures (Robert et al., 2000, Gabriel, 1965).

Klueken et al. (2009) studied the flight behaviour of alate cereal aphid *Sitobion avenae* (Fabricius) and demonstrated that, for a 16h period, no aphids flight occurred at 10°C; while the proportion of aphids flying increased to 70% at 15°C to reach 100% at 20°C. They also showed that the temperature threshold was 3°C higher for *Metopolophium dirhodum* (Walker) (bird cherry oat aphid), another cereal aphid. This implies that, for cold temperature, these aphid species are likely to stay on their *winter host* instead of colonising crops (*summer hosts*). Under these conditions, vector pressure and aphid-transmission of PVY are minimised. In Switzerland, it was shown that an increase in altitude from 400 m to 800 m resulted in a decrease in infection of potatoes by 57%, mainly due to an average colder temperature at the higher altitude (Steinger et al., 2014). In addition, strong wind speeds (>0.8 km/h) are not favourable for alate aphid displacement but also for aphid feeding and development, so windy areas tend to be more suited to seed potato production because there is less opportunity for aphids to transmit virus (CIP, 1979, Walters & Dixon, 1984). The presence of plants infected by PVY in neighbouring crops can also pose a risk with regard to inoculum sources of PVY, especially if the seed potatoes planted are not certified and the crop is being grown for consumption without management measures to control aphids. Virus is likely to be more prevalent in farm-saved seed potatoes than in certified seed potatoes (Kerlan et al., 1987).

#### *8.3.1.4. Time of Planting and Haulm Killing*

The physiological state of a plant may affect the transmission of PVY by aphids because older plants appear to be less susceptible to PVY infection than younger plants (Gibson, 1991, Robert et al., 2000, Sigvald, 1985, Dupuis, 2017), a phenomenon termed mature plant resistance (see section 4.2.4). Sigvald (1985) reported that potato plants are at their most susceptible state up to 25 days post emergence, becoming more resistant thereafter at the rate of 10% every week. Managing the physiology of plants could, therefore, be integrated into a program to control PVY in potato crops. Advancing the time of planting and haulm destruction could be important in reducing the risk of PVY spread. Pre-sprouting seed potatoes can enable plants to emerge earlier and daughter tubers to bulk earlier, thus allowing earlier haulm destruction than with unsprouted tubers. However, the effectiveness of pre-sprouting in controlling PVY spread will depend to a great extent on the timing of

aphid flights. The efficacy of pre-sprouting in controlling PVY spread has been assessed by Saucke and Doring (2004) in a three years experiment (in 2000, 2001 and 2002) with the cv. Christa. In 2000, no effect was observed and in 2001 and 2002, pre-sprouting allowed a reduction of PVY spread of respectively 50 and 68%. Pres-sprouting was not efficient in 2000 as the vector activity was concentrated early in the year. If aphid flights are late in the growing season, pre-sprouting could be an effective measure. However, if spring aphid flights are earlier than normal this could pose a greater risk to crops which have been planted with sprouted seed tubers because the plants will have emerged when aphids are flying whereas those from unsprouted seed potatoes will not (Saucke & Doring, 2004). Early haulm destruction will reduce the time of exposure of the crop to aphid flights, thereby reducing the risk of infection (Basky, 2003, Kerlan et al., 1987, Steinger et al., 2014). In some conditions, it has been reported that delaying haulm destruction can increase the incidence of PVY infection by 3.5% per day (Steinger et al., 2014). For mature crops, the choice of herbicide or the use of mechanical haulm destruction has a limited impact in reducing virus transmission [Dupuis and Schwaerzel (2011); see proceedings in Annex 8]. Nevertheless, rapid and total haulm destruction is required to reduce the risk of late PVY transmission. If new foliage develops on desiccated stems, transmission of PVY by aphids could be possible as the relatively immature leaves are potentially susceptible to PVY transmission (Sigvald, 1985). New growth that develops after haulm destruction can be susceptible to aphid-borne infection especially when significant aphid pressure is still observed late in the growing season. However, quantitative data are still lacking to assess accurately the impact of late of infection of new growth and PVY incidence at post-harvest.

### *8.3.2. Roguing and Weed Control*

For effective control of PVY in crops, it is essential to eliminate any sources of inoculum. These can be plants from infected seed tubers, from weeds or from volunteer plants i.e. potato plants derived from tubers or parts of a tuber left in the soil after harvesting a previous crop (Jones et al., 1996). The use of certified seed potatoes according to official tolerances can provide assurance regarding the maximum amount of virus disease which could develop in a crop, but cannot rule out the absence of viruses in a crop. When a crop is being grown for marketing as seed potatoes, roguing

is a key component in maintaining the health of a crop. Roguing is the removal of potato plants which are atypical of the cultivar in appearance or diseased including virus (Kerlan et al., 1987). For virus diseases, roguing is efficient in reducing PVY spread but the effect can vary from none to a 20% reduction (Broadbent et al., 1950). To be effective, roguing should be conducted as soon as possible after plant emergence to minimise the opportunity for aphids to acquire virus from infected plants within a crop. The plants should be removed from the field, as aphids are able to probe and acquire the virus even on wilted plants abandoned on the soil between the rows (Boquel et al., 2017). However, roguing will be ineffective if a cultivar is tolerant to a virus because infected plants will be symptomless and, therefore, not recognised and removed. There is a risk that excessive amounts of virus can build up in crops of such cultivars cultivated over several generations and pose a serious threat to the health of crops of susceptible cultivars from aphid transmission (Ragsdale et al., 2011). It is also possible that roguing could enhance the transmission of PVY by aphids if too many plants are removed in a small area creating bare patches. Aphids land preferentially in areas where there is a contrast between bare soil and potato plants [reviewed in Döring (2014)]. Excessive gaps might promote the landing of aphids and, consequently, increase the risk of infection of plants surrounding a gap. The risk of infection increases with the size of the gap (Davis et al., 2009). The incidence of PVY infection was 13% around gaps of ≤0.6 $m^2$, and 29% around gaps of ≥0.6 $m^2$.

Volunteer plants are a major concern in potato-growing areas. These volunteers plants grow from tubers remaining in the field following harvest because a significant proportion of tubers are too small to be collected by harvesters and will remain in the soil over the winter (Yves Le Hingrat, personal communication). Some of these tubers will survive the winter if soil temperatures are not sufficiently cold enough. The extent of tuber overwintering will vary with their depth in soil the severity and length of cold periods (Lutman, 1977, Boydston et al., 2006, Cooke et al., 2011). The growth of plants from surviving tubers (volunteer potatoes) even after several years of crop rotation could be impaired by the foliar growth of the newly planted crop, depending on its capacity to cover and shade volunteer potato plants. In France between 2007 and 2011, the density of volunteer plants in fields in the Brittany region was estimated to range from none to 6 stems per $m^2$ depending on cultivars, cropping practices and succeeding crop (Rakotonindraina et al., 2011). In addition to volunteer potatoes being

a source of varietal mixtures in potato crops and posing weed control issues in other field crops, they can also act as a reservoir for potato diseases and could impact on the phytosanitary status of seed potatoes. A survey was conducted in the UK in 1996 in which volunteers plants were collected from three different sites and the percentage of volunteers plants infected by PVY ranged from 2 to 54% (Jones et al., 1996). Inoculum from within or near a crop can also originate from weeds. In the late 90s, 36 weed species belonging to 13 different botanical families were identified as potential host plants for PVY (in natural and/or artificial conditions) (Edwardson & Christie, 1997). More recently, seven additional weed species have been identified as potential hosts (Kaliciak & Syller, 2009, Kazinczi et al., 2004). It is almost certain that other weed hosts for PVY will be reported in the future. However, the epidemiological role and impact of those weeds in the field is not fully understood, and it is unclear whether the presence of weed species will contribute significantly to the spread and prevalence of PVY in potato crops (see section 7.1).

### *8.3.3. Crop Borders*

Crop borders can be planted in order to limit the amount of virus introduced into a crop (Boiteau et al., 2009, Difonzo et al., 1996). Crop borders display two distinct modes of action, serving as a “virus barrier” and also as a “virus sink” (Boiteau et al., 2009). Viruliferous aphids landing on a border crop will probe the plants and shed any virus particles that they might be carrying into the barrier plant. In this case, the border crop serves the role of “virus sink” by retaining the virus. To be effective, border crops must be planted with a plant species which is not susceptible to PVY. Border plants can also act as a physical barrier interrupting aphids flight (Simons, 1957). To provide this type of protection, the border plants have to be taller than the potato plants at all stages of growth. A border must also be wide enough to maximize the probability of aphids landing on it (Boiteau et al., 2009, Difonzo et al., 1996). With a soybean border of 24m, Difonzo et al. (1996) obtained an efficacy of PVY control of 27 and 60% over the 2 years of an experiment while Boiteau et al. (2009) obtained 32% efficacy for an experiment with a narrower border of 4 m sown with grass. This technique has the advantage of being effective for the entire cropping season, whatever the environmental conditions. Nevertheless, the use of crop borders requires relatively large areas of a field to be removed from potato production with no commercial return

so the method may not be practical, especially if fields are relatively small. To solve this issue, a potato cultivar resistant to PVY can be used as a border. A border of 4m of cv. Kennebec (known to be relatively resistant to PVY), was used in a three-year field trial in Canada to protect a central plot of cv. Russet Burbank, susceptible to PVY and its effectiveness was compared with applying mineral oil (Boiteau et al., 2009). The border crop alone reduced PVY transmission by about 20% in the first two years of the trial and 60% in the third year, while mineral oil applications alone reduced PVY transmission by 20% in the first two years and by 70% in the third year.

### *8.3.4. Mulching and Intercropping*

Straw mulching (Figure 12) is effective in controlling PVY spread (Heimbach et al., 2004, Saucke & Doring, 2004, Kirchner et al., 2014, Dupuis et al., 2010). The mode of action of straw mulching is not well understood but the main hypothesis is that straw impacts on the visual perception of a crop by an aphid (Döring, 2014). The contrast between potato foliage and the yellow straw is considerably lower than potato foliage and bare soil, so that potato plants in mulched plots are less easily seen by aphids (Döring, 2014, Döring & Schmidt, 2007). This was confirmed when fewer aphids were captured in mulched plots compared with those in bare soil plots (Heimbach et al., 2004, Saucke & Doring, 2004). In the study reported by Saucke and Doring (2004), aphids were counted on the foliage of potato plants in straw-mulched and bare soil plots. An average reduction of 54% in aphid numbers was recorded in the mulched plots. This reduction of aphid populations on foliage was 18% in an independent study (Heimbach et al., 2004), while, in the same experiment, 85% fewer winged aphids were captured by sticky net traps within the mulched plots. Thus, the lowest number of winged aphids landing on potato plants in mulched plots might be the main factor in controlling PVY spread by mulching. PVY control by mulching is more effective in the early stages of crop development, declining as the crop canopy develops over the mulch (Heimbach et al., 2004). This was shown clearly in a field experiment undertaken over a three year period (Saucke & Doring, 2004) in which the efficacy of PVY control recorded in the mulched plots was about 48% in the first year, 33% in the second year and 27% in the third year. In the first year of the trial, aphids were most prevalent a few days after potato emergence in mid-May, whereas in the third year of the trial, aphid were most active two weeks later. In the second year of the trial, aphid activity

remained low during the entire growing period. Intercropping was also tested for its effectiveness in reducing PVY spread in potato crops (Dupuis et al. (2010); see proceedings in Annex 9).

*Figure 12: Wheat straw mulch in potatoes, 2'500 kg/ha of straw in cv. Charlotte (Photo: Maud Tallant)*

*Figure 13: Oat intercropping (Avena sativa, 60 kg/ha) in potatoes (cv. Charlotte), 7 days after spraying oats with tepraloxydim (200 g/l) to stop their development (Photo: Maud Tallant).*

Oats sown between rows of potatoes but killed before becoming large enough to provide unwanted competition for the potatoes (Figure 13), resulted in a significant reduction in PVY spread (of 37%, see Figure 14). The mode of action of intercropping is comparable to the “sink effect” of a border crop in which inoculum being carried by aphids is lost during probing on the associated crop (Boiteau et al., 2009). Intercropping offers an additional mode of action called the “dilution effect” by significantly decreasing the probability of an aphid landing on potato plants. Given the same plot surface, plots containing an associated crop presents many more plants to potential vectors, thereby reducing the risk of potatoes being infected.

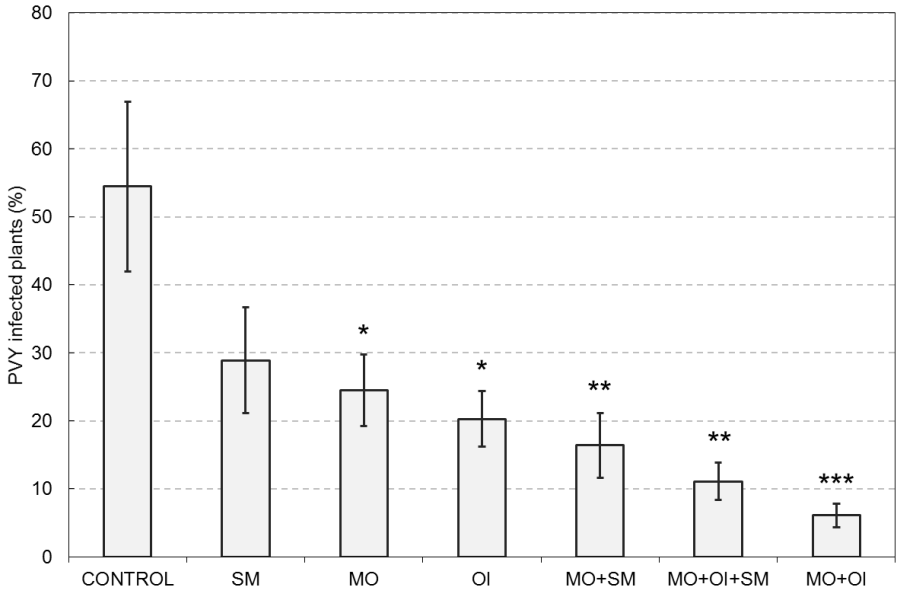


*Figure 14: Percentage of PVY infected plants assessed by testing daughter tubers for 6 treatments and untreated (one year; 4 replications of 100 plants per plot). The acronyms for the treatments are: SM=straw mulching; MO=mineral oil; OI=oat intercropping. Error bars show the standard error and stars shows the treatments with a percentage of infection significantly lower than untreated (Dunnett test; * for p<0.05, ** for p<0.01, and *** for p<0.001) (Dupuis et al., 2010).*

## 8.4. Chemical Methods

### *8.4.1. Oil Treatments*

Spraying oil on to potato plants has been reported to provide a means to control the spread of PVY (Boiteau et al., 2009, Bell, 1980, Bell, 1989, Boiteau & Wood, 1982, Bradley et al., 1966, Bradley et al., 1962, Dewijs, 1980, Dupuis et al., 2014b, Hansen & Nielsen, 2012, Kirchner et al., 2014, Martin-Lopez et al., 2006, Milosevic, 1996, Steinger et al., 2014, Rolot, 2005). Mineral oil was found to be more effective than vegetable oil (Martin-Lopez et al., 2006, Rolot, 2005, Wrobel, 2012), although among the latter, refined oils were more effective than raw oils (Martin-Lopez et al., 2006). The mechanism of action of the oils is not fully understood. It has been suggested that oil on aphid mouthparts reduces acquisition, retention of PVY by aphids. Oil may impede binding of virus particles on to the stylet (Bradley, 1963, Boquel et al., 2013, Loebenstein et al., 1964, Powell, 1992) and/or shorten the duration of virus retention in the stylets (Wróbel, 2009). Mineral oil has also been shown to reduce virus replication and accumulation in inoculated plants, possibly due to a general activation of defense mechanisms in a plant (Loebenstein et al., 1964, Peters & Lebbink, 1973, Martoub, 2010, Al-Daoud et al., 2014). This hypothesis is supported by recent research showing that oil treatment induces the expression of plant-virus interaction related genes in both local and systemic potato tissues (Khelifa, 2017). Oil also has the ability to kill aphids by interfering physically with their respiration (Martin-Lopez et al., 2006, Hesler & Plapp, 1986). Studies have investigated the effects of mineral oil treatment of potato plants on selection of host plant, growth, and reproduction of the colonizing potato aphid, *Macrosiphum euphorbiae* (Thomas) (Ameline et al., 2009). Olfactometry experiments showed that mineral oil treatment induced a transient repellent effect shortly after spraying that lasted a day. While probing behaviour was not drastically affected in oil-treated plants, the treatment resulted in antagonistic effects with a significant reduction in nymph survival shortly after treatment and concomitantly a higher fitness and fecundity rate in adult aphids (Ameline et al., 2009). Other studies have reported antagonistic effects of mineral oil on *M. euphorbiae* that were dose-dependent; topical contact at the highest concentration of oil resulted in complete mortality, while lower concentrations and exposure to oil volatiles enhanced aphid fecundity but had no effect on aphid survival (Martoub, 2010). Tan et al. (2005) suggested that, after spraying, oil is “absorbed” in various plant tissues and cells and

transported throughout the plant , resulting in a decrease in local concentrations and the induction of physiological changes (Tan et al., 2005), altering photosynthesis (Helson & Minshall, 1951, Wedding et al., 1952), and triggering the expression of pathogenesis related proteins (Kachroo et al., 2001, Lin et al., 1996).

In practice, the protection achieved with mineral oil spraying varies greatly depending on the time of application (Figure 14, Figure 16 and Figure 18). Mineral oils are more effective on older plants in reducing the speed with which PVY moves in the vascular system (Al-Daoud et al., 2014). The reduction in the transmission of PVY by oil treatments is likely to vary among different potato cultivars and with different aphid and virus pressures. Steinger et al. (2014) reported that oil treatments reduced the average incidence of PVY by 39% ($p$<0.001) over a 4-year period. The protective effect of mineral oil was slightly greater in the year in which the incidence of PVY was greatest (50% decrease in infection $N_{treated}$ =432, $N_{non\text{-}treated}$ =86) than in other years, and with the susceptible cvs Bintje and Charlotte (54% decrease in infection, $N_{treated}$ =819, $N_{non\text{-}treated}$ =79) compared with more resistant cultivars. In a separate study over three years of field trials in UK, mineral oil reduced PVY infection 2- to 3-fold in cvs King Edward and Maris Piper (N=160 plants per cultivar per treatment per year) during a year of relatively high virus pressure (overall 30% incidence of plants infected by PVY) (Dawson et al., 2015). However, there was no significant effect of mineral oil treatments on the incidence of PVY in years of low virus pressure (3% - 13% PVY incidence). In this study, significant variation in the effectiveness of the oil treatment in controlling PVY suggests that local differences in aphid phenology and aphid vector pressure strongly influenced the effectiveness of the treatment. These results are consistent with the expected reduction in the incidence of PVY for oil-based treatments compared with untreated plants, usually between 30% – 60% [reviewed by Al-Mrabeh et al. (2010)].

Mineral oils are usually applied weekly to a seed potato crop. The protection provided by oil is generally limited to the leaf surfaces exposed to the spray being applied (Simons et al., 1977). Effective coverage of foliage is easier to achieve on older plants than on younger plants on which new leaves are constantly developing. The rate of foliar development should be considered when determining the optimum interval between sprays for the effective application of oil. An increase in the frequency of foliar applications on young plants should be considered for maximum effectiveness in order to protect recently opened leaves (Fageria et al., 2014a, Fageria et al., 2014b,

Demeulemeester, 2013). The usual practice in France to protect high grade seed is to spray mineral oil three times a week starting at 30% plant emergence and continuing until complete emergence followed by weekly applications thereafter. In Belgium, the practice is very similar except that only two applications are recommended during the early period of crop growth followed by one each week until haulm destruction is complete (Yves Le Hingrat and Pierre Lebrun, personal communication).

Mineral and vegetable oils must be used with caution to avoid plant phytotoxicity (Kirchner et al., 2014). It is essential to use a labeled product (Dewijs, 1980). Paraffinic oils are preferably used for the treatment of plants instead of naphtenic oils (phytotoxic) and aromatic oils (phytotoxic and unstable) (Rolot, 2005). In the rest of the document, “mineral oils” refers to paraffinic oils approved for the treatment of plants to prevent the spread of viruses. The linear structure of saturated paraffinic oils is relatively stable and not phytotoxic. Dewijs et al. (1979) demonstrated that the viscosity of the oil, which is related to the number of carbon atoms in the molecular chain, is an important character determining the efficacy of the oil. Nevertheless, when the chains have more than 25 carbon atoms, phytotoxicity can be observed (Walsh, 2000). The phytotoxicity of the paraffinic oil also depends on its degree of refining. Less than 8% of sulfonated residues (residues reacting with sulfuric acid) are required for plant treatments (Walsh, 2000). For treatments with paraffinic oils, it is also required to not exceed the maximum rate and to avoid application during hot weather because an oil can become so hot that it can burn potato foliage on application after being heated in the sprayer pipes during prolonged sun exposure. Mineral oil treatment can alter plant physiology and, in some cases, be phytotoxic. This can have an adverse effect on the appearance of plants and potentially affecting ability to identify cultivars and virus symptoms in a growing crop during inspection. A recent study in the UK of the effect of oil treatments on a range of virus-infected and healthy plants of various potato cultivars concluded that the ability of inspectors to identify both cultivars and virus symptoms was not diminished by applications of mineral oil to foliage (Dawson et al., 2015), even although phytotoxic symptoms (localised necrotic spots) were occasionally observed (Figure 15). However, some loss of tuber yield was reported for some cultivars after mineral oil treatment, emphasizing the necessity for a cautious use of mineral oil to control PVY (Kirchner et al., 2014). Delaying haulm destruction by several days could compensate for this reduction in yield (Dawson et al., 2015).


*Figure 15: Phytotoxic symptoms (localised necrotic spots and leaf midrib necrosis) developing on leaves of potato cv Desiree following mineral oil spraying (Dawson et al., 2015). Note the beading of rain water on the foliage of sprayed plants (Photo: Courtesy of SASA Crown copyright).*

### *8.4.2. Insecticide Treatments*

The acquisition and inoculation periods of non-persistent viruses like PVY by their aphid vectors are extremely short (seconds to minutes) (Bragard et al., 2013). To be effective, an insecticide has to kill or incapacitate an aphid very quickly to limit infection of a plant. However, the effect of an insecticide may impair the capacity of a viruliferous aphid to fly from a treated plant to another plant. Various insecticides and their formulations have been tested for their effectiveness in controlling PVY transmission in the field (Table 8). Pyrethroids have a near- instantaneous "knock-down" effect; and can provide a reduction in PVY transmission in controlled conditions (Perring et al., 1999, Gibson et al., 1982, Collar et al., 1997). Unfortunately, results obtained in field grown potato crops with the same products have proven to be variable (Table 8). Another group of insecticides with different modes of action can potentially interfere with the transmission of viruses either by repelling aphids [deltamethrin, Rice et al.

(1983)], altering feeding behaviour [flonicamid, imidacloprid, pymetrozine and thiametoxam, Morita et al. (2007), Cho et al. (2011), Boquel et al. (2014)] and reducing aphid's movement [aldicarb, Boiteau et al. (1985)]. However, no significant reduction of PVY transmission was reported in field trials for imidacloprid, pymetrozine (Table 8) and flonicamid (Figure 16). Pyrethroids such as deltamethrin (Gibson et al., 1982) and cypermethrin (Collar et al., 1997) can provide a significant degree of PVY protection in controlled conditions but it is not known whether virus acquisition or transmission is affected. Lambda-cyhalothrin, dimethoate and pymetrozine reduce PVY acquisition by aphids (Boquel et al., 2014, Margaritopoulos et al., 2010) but the effect of these insecticides is too slow to prevent PVY spread in the field (Table 8). In summary, for most of the field trial studies reported, the efficacy of insecticide in controlling PVY spread was either not significant, of a limited impact or highly variable between independent experiments. One of the reasons that insecticides have been demonstrated to be either ineffective or only of limited effectiveness is because many aphids which transmit PVY do not colonize plants in a crop and thus only come in contact with the insecticides for a very limited period [reviewed in Perring et al. (1999)].

*Table 8: Insecticides tested in field trials to control the spread of Potato Virus Y (PVY). The table shows the name of the active compound and its chemical group, the type of treatment applied in the trial, the authors and the corresponding protective efficiency expressed as a percentage of reduction in the incidence of tuber infection in comparison with that for untreated plants (NS=test not significant or no protective effect).*

| ***Active compound*** | ***Chemical group*** | ***Application*** | ***Protection*** | ***Authors*** |
|---|---|---|---|---|
| Cypermethrin | Pyrethroid | Foliage | 7%<br>29%<br>NS | Bell (1989)<br>Gibson and Cayley (1984)<br>Martin-Lopez et al. (2006) |
| Demeton-S-methyl | Organophosphate | Foliage | NS | Milosevic (1996) |
| Esfenvalerate | Pyrethroid | Foliage | 29%; NS; NS (Year 1, 2 & 3) | Kirchner et al. (2014) |
| Imidacloprid | Neonicotinoid | In furrow at planting | NS | Boiteau and Singh (1999)<br>Alyokhin et al. (2002) |
| | | Seed tubers | NS | van Toor et al. (2009) |
| | | Foliage | 53%<br>NS | Alyokhin et al. (2002)<br>Boiteau and Singh (1999) |
| Lambda-cyhalothrin | Pyrethroid | Foliage | 10%<br>NS<br>NS | Dupuis et al. (2014b)<br>Hansen and Nielsen (2012)<br>van Toor et al. (2009) |
| Permethrin | Pyrethroid | Foliage | 22% | Bell (1989) |
| Pymetrozine | Pyridine | Foliage | NS | van Toor et al. (2009)<br>Rolot et al. (2006) |
| Thiomethon and Tau-fluvalinate | Organophosphoru-s and Pyrethroid | Foliage | 8% | Rolot (2005) |
| Tau-fluvalinate and thiacloprid | Pyrethroid and neonicotinoid | Foliage | NS | Kirchner et al. (2014) |
| Thiamethoxam | Neonicotinoid | Foliage | NS | Kirchner et al. (2014) |

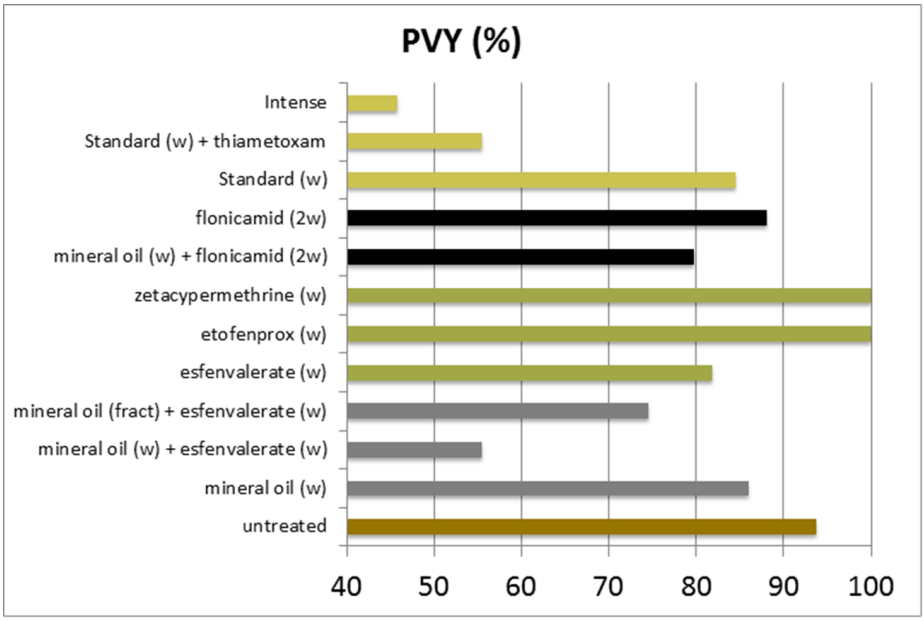


*Figure 16: Percentage of PVY-infected daughter tubers produced by plants treated with a range of insecticides and mineral oil. The insecticides used belong to different chemical groups such as: pyrethroids (esfenvalerate, etofenprox, zeta-cypermethrin), neonicotinoids (thiacloprid, thiamethoxam) and pyridine (flonicamid, pymetrozine). The "Standard" program refers to a weekly application of mineral oil, together with a pyrethroïd in the first week after emergence (esfenvalerate) and a systemic insecticide every two weeks (one treatment with flonicamid followed by pymetrozin and thiacloprid). The "Intense" program is similar to the "standard" program with, in addition, two applications of mineral oil in the first week after emergence. The letter (w) means weekly application, (2w) means 1 applications every two weeks and (fract) means two applications per week with ½ doses for the first three weeks after emergence and thereafter one application per week (Demeulemeester, 2013).*

### *8.4.3. Limitation of Chemical Control: Insecticide Resistance in Aphids.*

The (intensive) use of insecticides has led to the development of different mechanisms of resistance in some aphid populations. The main aphid vectors displaying various levels of resistances to insecticides worldwide are *Myzus persicae* (Sulzer), *Aphis nasturtii* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas) and *Sitobion avenae* (Fabricius) (Foster et al., 2002, Foster et al., 2014). Different types of biochemical and molecular mechanisms of insecticide resistance can be found with *Myzus persicae*

(Bass et al., 2014), these includes: (i) the overproduction of carboxylesterases [confering broad spectrum resistance to members of the organophosphate (OP), (mono-methyl) carbamate and, to a much lesser extent, pyrethroid classes], that hydrolyse the active compound before it affects the nervous system of the insect, (ii) mutation of the acetylcholinesterase enzyme (MACE- Modified AcetylCholinEsterase) resulting in the insensitivity of target site of the insecticides, the acetylcholinesterase enzyme, (iii) mutation of the voltage-gated sodium channel, also named `knockdown resistance' (kdr or super-kdr for the enhanced allelic form), which is conferred by a mutation of a transmembrane ion channel that plays an essential role in the initiation and propagation of action potentials in neurons, and (iv) the mutation of the nicotinic acetylcholine receptor (nAChR), a neurotransmitter-gated ion channel that plays an important role in nerve signalling (Bass et al., 2014). In addition, there are new types of resistance that are currently being identified (such as resistance to Pyridine compounds) [Table 9; Bass et al. (2014)].

Table 9 presents the chemical groups of insecticides frequently used in potato production and the corresponding resistance mechanism displayed by *Myzus persicae*. The incidence of *Myzus persicae* susceptible to the insecticides listed in Table 9 is highly variable from one year to another. For example, in aphid samples collected in the UK in 2004, about 60% of the sampled aphids were genotypically associated with carboxylesterases-mediated resistance, 70% with MACE and 80% with super-kdr; while 5 years before, this proportion was about 20%-5%-80% respectively (IRAG, 2008). *Myzus persicae* individuals resistant to neonicotinoids (with alteration of the nAChR) have not yet been found in the UK, however, a survey performed in Southern France and Northern Spain in 2010 revealed that resistance to neonicotinoids is relatively widespread in these regions, with an average of 76% of resistance to thiamethoxam (Slater et al., 2012). Combinations of the three main resistance mechanisms (MACE, carboxylesterase and kdr) can be found in individuals of *Myzus persicae*, but the most common association of resistance found in the field in the United Kingdom are MACE with super-kdr (IRAG, 2008). Consequently, growers are recommended to minimise whenever possible the use of insecticides mixtures in order to prevent the outbreak of *Myzus persicae* populations displaying cross-resistances. Populations of *Aphis nasturtii* (Buckthorn-potato aphid) sampled in Belgium and northern France in the 1990's were found to be resistant to insecticide,

however, resistance in *Aphis nasturtii* seems to be a relatively rare event (Duvauchelle et al., 1997). *Macrosiphum euphorbiae* has developed a resistance mechanism based on overproduction of an esterase after treatment with pirimicarb and λ-cyhalothrin. This mechanism of resistance is analogous to the one found in *M. persicae*. However, it is not known if this mode of resistance is effective when treatment are applied at the recommended rate (Foster et al., 2002). Kdr resistance to pyrethroïds (λ-cyhalothrin) was found in *Sitobion avenae* in the United Kingdom (Foster et al., 2014), and the prevalence of resistant individuals in 2015 varied from 45 to 75% depending on the location of sampling (Malloch et al., 2016).

*Table 9 Examples of chemical groups and active compounds available for aphid control in potatoes and types of resistance developed by Myzus persicae (IRAG, 2014, Bass et al., 2014).*

| **Chemical group** | **Active compound(s)** | **Resistance mechanism (period of first reported resistance)** |
|---|---|---|
| Dimethyl carbamate | Pirimicarb | MACE (1940s) and/or Carboxylesterase (1950s) |
| Neonicotinoid | Acetamprid, Thiacloprid, Thiamethoxam | Alteration of nAChR (1990s) |
| Pyrethroid | Esfenvalerate, λ-cyhalothrin, Etofenprox, Cypermethrin | Super-kdr (1970s) |
| Pyridine azomethine | Pymethrozine | Action on the Chordotonal organ TRPV channel modulators (1990s) |
| Pyridine carboxamide | Flonicamid | Action on the Chordotonal organ TRPV channel modulators (2010) |

### *8.4.4. Synthetic Pheromones*

Numerous aphid species secrete pheromones as a behavioural mechanism when attacked by natural enemies (Kislow & Edwards, 1972, Nault et al., 1973). (E)-β-farnesene (EβF) is the main alarm pheromone (Pickett et al., 1992, Zhang et al., 1997). EβF has a repellent effect on aphid colonies, and affects the development and fecundity of the aphid populations (Su et al., 2006, Gibson et al., 1984). Additionally, it

was shown in the laboratory that EβF inhibits the acquisition and subsequent inoculation of PVY by *Myzus persicae* (Sulzer) (Gibson et al., 1984). Another laboratory experiments performed with tobacco plantlets using apterous aphids [*Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas)], revealed a higher spread of PVY when the aphids are in contact with EβF (Lin et al., 2016). This result suggests an increase of the movement of apterous aphids from plant to plant in the presence of EβF. Apterous aphids are known to be able to move from plant to plant even without overlapping of crop canopy, by walking on the soil surface (Alyokhin & Sewell, 2003, Narayandas & Alyokhin, 2006). Nevertheless, winged aphids are more motile in the crop by flying from plant to plant and, hence, have a higher impact on PVY spread in the field (Boiteau, 1997). A one-year experiment undertaken in field conditions showed that PVY spread was identical with or without diffusion of EβF (Crutzen et al., 2014). While pheromone treatment might have a limited impact on aphid transmission of PVY, the efficacy of insecticide in controlling aphid populations can be improved by using EβF treatments, as reported in Chinese cabbage fields treated simultaneously with imidacloprid and EβF (Cui et al., 2012). The strategy of combining EβF diffusion with alternative treatments or control strategies to control PVY spread has yet to be tested and might represent an interesting approach to control aphid populations and consequently virus transmission.

### *8.4.5. Elicitors*

Several chemicals are able to induce systemic resistance of plants to pathogens (Kessmann et al., 1994). Among those chemicals, salicylic acid (SA) is known to induce resistance to a wide range of pathogens including viruses (Nakayama et al., 1996, Vasyukova & Ozeretskovskaya, 2007). It was shown that *Potato Virus X* (PVX) accumulation in tobacco leaves is reduced after SA treatment (Naylor et al., 1998). This effect was also observed in tomato leaves inoculated with PVY and treated with acibenzolar-S-methyl (Bion®), a functional analogue of SA (Petrov & Andonova, 2012). The analogy of the structure of the molecules of acibenzolar-S-methyl and SA is clearly visible in Figure 17.

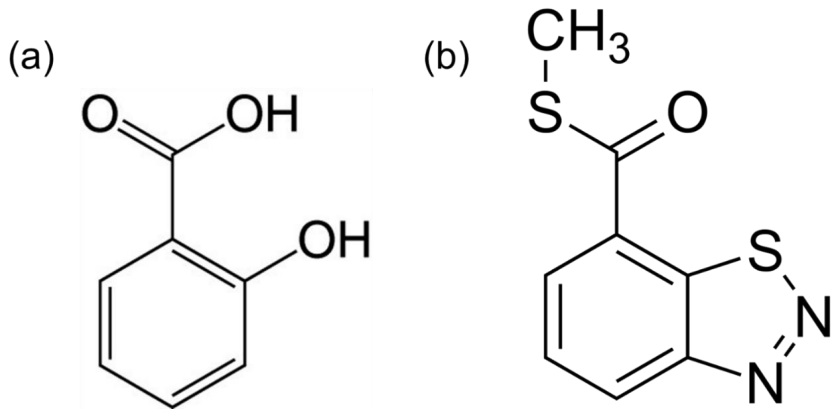


*Figure 17: Molecular structure of (a) salicylic acid and (b) acibenzolar-S-methyl.*

## 8.5. Combined Methods

Many of the techniques cited above have often been tested in combination. Crop borders and mineral oil treatments have been tested together and the combination of those two techniques was more effective than when they were applied individually. In the field trials of Boiteau et al. (2009), the combination of applying oil sprays to potato crops and a border crop improved the efficacy of PVY control compared with oil treatment and borders used alone: 47–59% reduction in incidence of PVY in the first year, 57–63% in the second year and 79–97% in the third year of field trials. Insecticide treatment of a border crop did not produce a reduction in the incidence of tuber infection by PVY (Difonzo et al., 1996). A synergistic effect of applying straw mulching and mineral oil applications together was recorded in a one-year trial in Switzerland (Figure 14) but no synergy was found in a similar trial in Finland (Kirchner et al., 2014). In the Swiss trial, the efficacy of PVY control with straw mulching was about 47% while spraying mineral oil produced a similar efficacy of a 55% reduction in PVY. However, the efficacy of 70% was achieved when both techniques were used together. In the Finnish trial, straw-mulching produced a reduction of 26% in PVY infection but a combined mineral oil spraying and straw mulching treatment gave only a 19% reduction. It is likely that the extent of any synergistic effect of mineral oil with straw mulch may depend upon the experimental conditions of a trial. As explained above, the efficacy of mineral oil treatment increases the older the plants are when they are

sprayed while the efficacy of straw mulching is greatest early in the season before the soil surface becomes covered by the crop canopy. Thus, the timing and location of the peak of aphid activity (data not shown) could explain the differences between those two trials. Oat intercropping was also tested with mineral oil applications and some synergy was recorded (Figure 14). Oat intercropping resulted in 63% reduction in PVY infection in the potato crop and the efficacy of PVY reduction reached 89% when oat intercropping was used together with mineral oil spraying. The combination of insecticides and mineral oil treatments can also provide some synergy, increasing the protection of treated crops against PVY. A Canadian survey of 56 crops of seed potatoes being multiplied using distinct cropping techniques revealed that mineral oil was more effective when combined with insecticide applications, particularly when used early in the season (MacKenzie et al., 2014, MacKenzie et al., 2016). This synergy was not found in all circumstances. A weekly treatment with esfenvalerate together with mineral oil gave better protection (42%) of a crop against PVY spread than a weekly spray of mineral oil alone (9%; $p<0.05$) (Figure 16). Contrastingly, the combination of a weekly treatment of thiomethon + fluvalinate and a twice weekly treatment with mineral oil did not improve the protection of the crop against PVY infection ($p>0.05$ for all years of trial) compared to when treatments were applied individually (Figure 18).

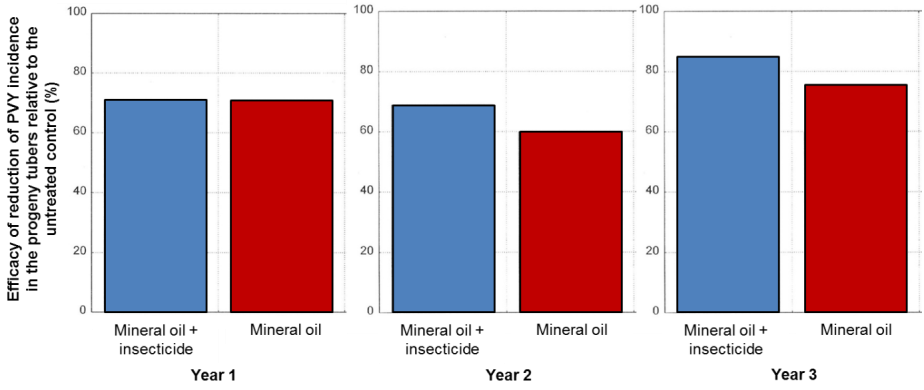


*Figure 18: Efficacy in reduction of the incidence of PVY in progeny tubers in response to foliar treatments with either mineral oil (3 l twice a week) sprayed alone or in combination with a pyrethroid insecticide (0.6 l of thiomethon (200g/l) + fluvalinate (72g/l) once a week) over a 3-year period. Each year, the trials were conducted in three different locations with three replications of each treatment (Rolot, 2005).*

Additional studies conducted in a greenhouse have shown a significant reduction in transmission of PVY in plants treated with mineral oil and insecticides (Martin-Lopez et al., 2006, Gibson & Rice, 1986). This synergistic effect of insecticides and oil may be influenced by the particular chemistry of insecticide e.g. synthetic pyrethroids (Figure 16), and by the time of the application because mineral oil alone is less efficient when applied early in the season (MacKenzie et al., 2014, Gibson & Cayley, 1984, Demeulemeester, 2013).

## 8.6. Seed Certification Schemes

### *8.6.1. History and Evolution.*

Potato is a vegetatively propagated crop and consequently tubers can potentially be contaminated with a large range of organisms (Franc, 2001). These pathogens can then be transmitted from the seed tuber to the new plant in the next generation of multiplication and also to healthy plants in the crop. The pathogens can range from fungi to viroïds and can be carried within and/or on the surface of a tuber. The diseases caused by the pathogens can have a serious effect on the yield and quality of a crop. Seed potatoes are potatoes intended for planting, unlike those intended for end uses such as consumption and processing. As commercial trade in seed potatoes developed, a perceived need arose for some form of independent verification of the quality of seed potatoes being marketed. This resulted in the establishment of seed potato certification schemes under official control with tolerances for quality aspects of seed potatoes being applied at each year of propagation (Rousselle et al., 1996, EPPO, 1999). The first potato seed certification schemes were established in Europe (Germany) in the first years of the 20$^{th}$ century and about ten years later in the United States (Shepard & Claflin, 1975). Similar schemes were introduced in New-Zealand in the late 1920's and in Australia in the 1930's (Maunder, 2005, Crump, 2008). Until early 1970s, production of the initial planting material was largely by *clonal selection* (or *positive selection*) which involved selecting disease-free tubers from apparently healthy mother plants (Gildemacher et al., 2011). As far as possible, testing was conducted on mother plants and tubers to ensure freedom from viruses known to be present in a country. However, appropriate testing was not available to confirm freedom from bacterial and fungal tuber pathogens. Subsequently, tissue culture

techniques were developed for producing initial planting material in which greater security of plant health could be achieved through an extensive testing program (Espinoza et al., 1984, Dodds, 1988). In Scotland, the first technique used was 'stem cuttings' starting in late 1960s with aim of eliminating latent tuber-borne pathogens, especially those causing blackleg, from the initial planting material (Jeffries, 1986, Hall, 1993) but this was superseded by micropropagation to produce 'nuclear stock'. Nowadays, this method is used as the starting point in almost all certification schemes worldwide because it allows material to be tested comprehensively for quality and notifiable pathogens and pests (EPPO, 1999, Donnelly et al., 2003, Frost et al., 2013). One exception is the Netherlands where clonal selection is also used, together with micropropagation, to produce the initial planting material (NIVAP, 2016). Tubers produced from the initial planting material are then multiplied for a number of generations in the field as seed potatoes under certification control until finally marketed for end use (Frost et al., 2013). In Asia, Africa and South America 'informal' seed potato production systems account for 94% of the market (Thomas-Sharma et al., 2016). In informal seed potato production systems, seed tubers are sourced on-farm, neighbouring farms, local markets, and unofficial specialist producers (Hirpa et al., 2010). The health status of this type of seed potatoes can be very variable and consequently crop yields fall far short of their potential. This is largely due to the inherent high amounts of virus in the potatoes and greater aphid pressure in more favourable environments for spread. Gildemacher et al. (2011) assessed the economic return of three different seed potato schemes: (i) the use of the farmer seed stock, (ii) the use of the 'positive selection' technique and (iii) the use of certified seed from the market. This showed that buying high-quality seed is generally more economically effective than other schemes because the yield obtained with certified seed is usually greater. This does assume that farmers can afford buying high-quality seed potatoes, and that the required cultivar is available for planting.

### *8.6.2. Objectives of Seed Potato Certification Schemes.*

The main goal of a potato certification scheme is to assure the quality of seed potatoes being marketed through an independent process of verification and testing conducted by a designated certifying authority. The aspects covered by certification are identity of cultivars, purity of crop, diseases and pests affecting quality or yield, external quality,

physiology, size and labelling (UNECE, 2015). Seed potatoes are propagated over a number of years so genealogy and traceability are key elements of a well-developed certification scheme. In order to maintain the health of seed potatoes during propagation, tolerances for disease and quality characteristics are set for each generation, being strictest for first generation and becoming slightly more relaxed for subsequent generations in the field (EPPO, 1999). Verification of tolerances is largely conducted by visual inspection of plants or tubers supported by testing as appropriate (Franc, 2001, Frost et al., 2013).

### *8.6.3. Principles of Certification.*

Although varying degrees of complexity exist, the basic principles of seed certification remain the same (EPPO, 1999). Currently, the first step is the multiplication of an *in vitro* pathogen-free plant (nuclear stock) which can then be multiplied in an approved facility to produce large numbers of *in vitro* plantlets or microtubers (i.e. tubers produced in vitro by a micropropagated plantlet). Nuclear stock is normally subject to an extensive testing program to ensure its freedom from a range of specified pathogens before being used to produce minitubers in insect free greenhouses or screenhouses (Frost et al., 2013, EPPO, 1999). These tubers are then planted in the field the following year to produce the first generation of seed potatoes (Franc, 2001). The import of healthy plant material or clonal selection can be used as an alternative to tissue culture (Franc, 2001, Schulte-Geldermann et al., 2012). Several years of field multiplication will ensue before being used by ware potato producers. In European countries, most certification schemes classify seed potatoes in three categories: Pre-basic, Basic and Certified in accordance with United Nations Economic Commission for Europe (UNECE) Standard for Seed Potatoes (UNECE, 2015). At least two classes within each category are normally set nationally although the possibility of agreed common classes is being explored by EU (EU, 2002). The number of years that seed potatoes can be multiplied is also limited in a scheme, normally less than nine (Frost et al., 2013), and with a maximum of nine generations allowed in the EU since 2016 (directives 2014/20/CE and 2014/21/CE). Some schemes, especially in North America, are based solely on generation number although the number does not always correspond to the number of generations in the field from initiation (Willem Schrage, personal communication). Each year, crops are visually examined by seed potato

inspectors for varietal purity and a range of faults including virus diseases around the time when plants are flowering. Inspected crops have to comply with specific tolerances for faults for the class for which they have been entered, otherwise the crop is downgraded to the appropriate class or, in extreme circumstances, not accepted as being suitable for marketing as seed potatoes (Frost et al., 2013). The UNECE has developed an international standard for certification and marketing of seed potato that sets out a common terminology and minimum commercial quality requirements for the certification of high-quality seed intended for marketing internationally (UNECE, 2015). This standard is a useful blueprint to facilitate seed potato trade between countries that already have their own certification schemes in accordance with the UNECE standard. It is also a good model for countries aiming to develop their own seed potato certification scheme.

### *8.6.4. Virus Diagnostics Methods used in Certification Schemes.*

Historically, the incidence of viruses, including PVY, in seed potato crops was assessed visually at two or more inspections according to disease symptoms reported as severe mosaic or mild mosaic (see Chapter 5). Knowledge of the virus causing symptoms was not essential for certification purposes but was nevertheless collected for future analysis. If any tolerance is exceeded at inspection, a crop is downgraded as appropriate or rejected (Shepard & Claflin, 1975). This method is still used in seed potato production areas where PVY pressure is low (e.g. Scotland). Diseased plants seen at inspection are generally the result of secondary transmission from infected tubers although symptoms of primary infection can sometimes be observed but this can underestimate the true extent of such infection. The incidence of symptomatic plants derived from primary infection varies with environment and time of infection. Late season infection of a susceptible potato plant will probably result in no development of foliar symptoms although virus may be translocated to daughter tubers, highlighting the necessity for post-harvest testing (Franc, 2001, Frost et al., 2013). Therefore, in some environments, relying on visual inspection may be insufficient to provide assurance that seed potatoes will meet the expectations of customers (Lindner et al., 2015). Additional measures such as a targeted post-harvest assessment were introduced in some certification schemes to check for late season virus infection or, in some cases, symptomless infection of tolerant cultivars.

There are numerous post-harvest diagnostic methods for viruses. The first method to be developed was termed ‘post-harvest grow-out assay’ or ‘growing-on assay’. A sample of a prescribed number of tubers is collected at random from a crop before harvest or shortly after the harvest. Seed pieces consisting of a single eye are removed from each tuber and planted (with or without a treatment to break dormancy) in a greenhouse or outdoors if the climate of a country is suitable. When these tests were first conducted the plants grown from each seed piece were then examined visually for symptoms of virus infection (Franc, 2001). Microscopy (Igel-Lange test) or serological (radial-immunodiffusion) techniques were used to support these assessments (Shepard & Claflin, 1975, Gugerli & Fries, 1983). Visual observation was later replaced by an enzyme-linked immunosorbent (ELISA) test for detection of the virus. France and Belgium still use ELISA for this type of testing.

A variation of this method is the so-called *winter test*. After breaking tuber dormancy, a standard sample from a crop is shipped to a *warmer* location for planting. After emergence, each plant is visually inspected and/or a leaf sample is taken for further testing by serological or molecular methods. The northern states of the USA use this approach and ship seed samples to southern states for a ‘winter test’. These programs are expensive and are gradually being replaced by laboratory-based tuber tests (Shepard & Claflin, 1975, Franc, 2001). Laboratory-based tests of tubers were first developed in Switzerland in the early 80’s. After dormancy of the tubers is broken by fumigation with Rindite (a mixture of 2-chloroethanol, 1,2-dichloroethane and carbon tetrachloride 7:3:1), sap from the basis of the sprouts of each tested tuber is tested by ELISA (Gugerli & Gehriger, 1980). In Europe, this product is banned due to its toxicity and is replaced by a solution of gibberellic acid. In this case, ELISA test is performed on recently emerged potato leaflets. ELISA is gradually being replaced by molecular techniques such as real-time RT-PCR, which has been employed in seed potato certification schemes in The Netherlands and Scotland since 2013 and in Switzerland and France since 2015 (this list of countries is not exhaustive).

### *8.6.5. The Role of Seed Certification Schemes for PVY Control and Future Challenges*

Seed certification schemes are essential in managing and controlling non-persistent viruses such as PVY in seed potato production through inspection of seed potato crops and statutory virus testing. They provide a purchaser with assurance that seed potatoes have been produced according to scheme requirements and standards, particularly with regards to health and cultivar purity. A post-harvest test should provide a reasonable estimate of the incidence of PVY in the daughter crop, provided the sample collected is representative of the crop (Frost et al., 2013). Certification, therefore, offers the opportunity to discard or *flush through* crops which pose a risk to health of subsequent crops. The population dynamics of PVY are changing. Ordinary and veinal necrotic PVY strains (i.e. $PVY^{O}$ and $PVY^{N}$) are gradually becoming less prevalent in Europe, and are being replaced by recombinant strains $PVY^{NTN}$ and $PVY^{N-Wi}$. $PVY^{O}$ and $PVY^{N}$ are generally believed to be more aggressive strains than the now prevalent above-mentioned recombinant strains, and elicit severe foliar symptoms on potato plants, although this does vary between cultivars. On certain cultivars, the recombinant $PVY^{NTN}$ and $PVY^{N-Wi}$ strains tend to produce milder symptoms, and in some occasions, higher copy numbers of viral RNA was found in leaves displaying milder symptoms (Lindner et al., 2015, Karasev & Gray, 2013a). This suggest an apparent lack of correlation between symptoms severity, virus concentration and virus strains, therefore adopting different tolerances with respect to the severity of virus symptoms, might no longer be appropriate in certification schemes when it comes to control PVY (Lindner et al., 2015). Consequently, the Specialised Section on Standardisation of Seed Potatoes at UNECE has revised the UNECE Standard (S-1) Seed Potatoes and adopted new virus tolerances irrespective of the severity of viral symptoms observed in growing crops (UNECE, 2014). As mild mosaic/viral symptoms may be missed during crop inspection, particularly if conditions are less favourable for visual inspection of plants (*e.g.* bright sunlight or water on foliage) or, as stated above, when plants are infected by less aggressive virus strains or if a cultivar is tolerant to some virus species, visual inspections should be complemented by post-harvest virus testing, as undertaken by most developed certification schemes worldwide.

# OBJECTIVES OF THE THESIS

The **main objective** of this thesis is to analyse new key elements for a better understanding of the epidemiology of PVY and take advantage of these elements to propose new control strategies to seed potato growers. The critical aspects of PVY epidemiology that have to be considered for the development of new control strategies are twofold: first, the susceptibility of potato cultivars to the main PVY strains, and second, the evolution of this susceptibility from emergence until the senescence/haulm destruction of the crop. A better knowledge of these two key elements allows us to propose new PVY control strategies adapted to the environment of PVY strains and implemented when the vulnerability of the crop is the highest. To efficiently perform the main objective of the thesis, four major emerging **research questions** must be addressed.

**(1) What is the role of the resistance status of the potato cultivars against PVY strains in the selection and the biodiversity of PVY strains in the environment?**

It is well-known that cultivars have different levels of susceptibility to PVY strains. This characteristic of cultivars is commonly used to characterise PVY strains. We hypothesise that the level of PVY resistance-susceptibility of the cultivars influences the diversity of PVY strains in potato crops.

**(2) Does the vascular movement of PVY strains in the plant influence the epidemiology of PVY in the field?**

The vascular movement of PVY in the potato plant at different ages is not well-known. We hypothesise that the mother tuber plays an important role as a vascular bridge between the different stems of the plant and that this role changes over time and depends on the PVY strains.

**(3) Which is the most effective strategy to control PVY spread in the field?**

All the PVY control strategies that have been tested to control PVY spread in the field have proven to be partially efficient and/or have significant variability in efficacy. We hypothesise that new control methods and/or the adaptation of existing control methods should allow increased protection of potato fields from PVY spread.

**(4) Can we reduce PVY spread through the combined use of different control strategies?**

The existing PVY control strategies have distinct modes of action. We hypothesise that the combination of control strategies should increase the control of PVY through an additive effect of the efficacy of the combined strategies.

To address these research questions, the experimental approaches are developed in four chapters. The **first chapter** addresses the first research question by focussing on the interactions between PVY recombinant strains ($PVY^{NTN}$ and $PVY^{N\text{-}Wi}$) and the resistance of potato cultivars. This interaction is explored through two PVY strains surveys managed in Switzerland in 2012 and 2014 and through inoculation trials managed in the greenhouse with different cultivars. This chapter also presents the consequences of these interactions on the recent evolution of PVY strains in the potato crops and on the management of PVY by planting resistant cultivars. The **second chapter** responds to the second research question by presenting the dynamic of movement of the PVY recombinant strains in the vascular system of the plant and its implications for the development of the *mature plant resistance* mechanism. The movement of PVY in the plant is studied through inoculation trials performed in the greenhouse with plants of different ages. This chapter stresses the importance of the physiological age of the crop in the implementation of an efficient PVY control strategy. The **third chapter** contributes to answering the third research question by comparing the efficacy of three strategies of PVY control in the field using oils, insecticides and plant elicitors. The three control strategies have been tested in a two-year field experiment. This chapter shows that the three strategies are effective at several stages of the PVY pathosystem and therefore behave differently from one another. Finally, the **fourth chapter** helps answer the third research question by proposing intercropping as a PVY control strategy. This chapter also contributes to the fourth research question by testing the combination of mineral oil with straw mulching to control PVY spread in the field. These PVY control strategies have been tested for at least two years in field trials. This chapter also provides a tentative description of the mode of action of these strategies and discusses possibilities of implementation in the field.

# CHAPTER 1: RESISTANCE OF POTATO CULTIVARS AS A DETERMINANT FACTOR OF PVY EPIDEMIOLOGY

## 1. Preamble

The objective of this chapter is to characterize the main strains of PVY found in cultivated potatoes in Switzerland and their potential association with specific potato cultivars.

To achieve this objective, two new surveys were set up to describe the current distribution of PVY strains in Switzerland. The results were compared to previous surveys with the view to assess the relative prevalence of certain PVY strains at the expense of others. We also study how the differential susceptibility of potato cultivars to different PVY strains may affect the selection of certain strains. Finally, a greenhouse experiment was undertaken to better characterize the interactions between potato cultivars and PVY strains. The differential susceptibility of the potato cultivars Agria and Charlotte to PVY recombinant strains ($PVY^{NTN}$ and $PVY^{N\text{-}Wi}$) was characterized following mechanical inoculation. The consequences of these interactions on PVY epidemiology are discussed.

## 2. Introduction

The PVY recombinant strains, $PVY^{NTN}$ and $PVY^{N\text{-}Wi}$, spread rapidly during the last 30 years into European seed-potato production and almost replaced the historical strains (Kerlan et al., 2011). The spread of $PVY^{N\text{-}Wi}$ was more recent than the spread of $PVY^{NTN}$ as this strain was first reported in Poland in the early 1990s (Chrzanowska, 1991) and has since been reported in several countries, including France (Rolland et al., 2008, Glais et al., 2005), the USA, Canada (Gray et al., 2010), Spain (Blanco-Urgoiti et al., 1998), Germany, the UK (Schubert et al., 2007), the Netherlands (van der Vlugt et al., 2007), Belgium (Kamangar et al., 2014) and Switzerland (Rigotti et al., 2011). In Switzerland, the prevalence of $PVY^{N\text{-}Wi}$ was of about 17% in 2008 (Rigotti et al., 2011). It was comparable/slightly higher in the USA and Canada, with 18% and 33%, respectively, in 2006, but it was much higher in Germany, with 63% in 2011 (Lindner et al., 2015). The prevalence of $PVY^{N\text{-}Wi}$ seems to have increased in Switzerland by +12% from 2003 to 2008 (Rigotti et al., 2011) and in Canada by +9%

from 2004 to 2006 (Gray et al., 2010). $PVY^{N-Wi}$ seems to have been relatively stable in Germany, with an increase of +4% from 2009 to 2011 (Lindner et al., 2015) and to have decreased in the USA by -9% from 2004 to 2006 (Gray et al., 2010). Since then, this percentage has significantly increased to reach 47% in 2012 (+23% since 2009), $PVY^{N-Wi}$ becoming the most prevalent strain in the USA (Stewart Gray, personal communication). The prevalence of $PVY^{N-Wi}$ seems to increase in the countries where periodic PVY strain monitoring has been performed. The following hypothesis have been proposed to explain the increase of the prevalence of $PVY^{N-Wi}$ strains: climate change and changes in cultural practices (Rolland et al., 2008), a higher transmission rate of $PVY^{N-Wi}$ strains by aphids (Verbeek et al., 2010), a faster translocation of $PVY^{N-Wi}$ than $PVY^{NTN}$ (Dupuis, 2017), a faster spread in the field [Dupuis et al. (2014a), see proceedings in Annex 10], a lower expression of symptoms by $PVY^{N-Wi}$-infected plants, which are then less susceptible to being eliminated from fields by roguing (Chrzanowska, 1991, Glais et al., 2005), acquisition of an enhanced pathogenicity (Bellstedt & Visser, 2016) and finally, a shift in the varieties that are produced, with the progressive introduction of varieties susceptible to $PVY^{N-Wi}$ and resistant to other PVY strains (Lindner & Billenkamp, 2005, Lindner et al., 2015, Funke et al., 2017). Indeed, it was shown through surveys in Germany and the USA that the susceptibility of the varieties varies among PVY strains. For example, in surveys managed in Germany between 2009 and 2011, all PVY-infected plants of cv. Agria (n=240) were found to be infected by $PVY^{N-Wi}$, while the prevalence rate of $PVY^{NTN}$ in the PVY-infected plants was 34% in this country over the same period (Lindner et al., 2015). Surveys managed from 2011 to 2015 in the Columbia Basin in the Pacific Northwest of the USA also proved strain-specific resistance exhibited by potato varieties grown in the area (Funke et al., 2017). This resistance resulted in a significant drop of the $PVY^{O}$ strain in favour of the recombinant strains $PVY^{NTN}$ and $PVY^{N-Wi}$.

This Chapter has two objectives. Firstly, to characterize the incidence of the PVY strains in Switzerland between 2008 and a new PVY strain survey performed in 2012. Secondly, to assess the strain-specific susceptibility of the two main cultivars grown in Switzerland in 2012, namely cv. Agria (22% of the surface) and cv. Charlotte (14% of the surface) (Swisspatat, 2012). The results of the survey of 2012 were combined with the results of another survey performed two years later to assess the incidence of the strains $PVY^{NTN}$ and to $PVY^{N-Wi}$ in these cultivars. In addition to the surveys, a

greenhouse trial was performed in which plants of cv. Agria and Charlotte were mechanically inoculated with $PVY^{NTN}$ and $PVY^{N-Wi}$ in order to assess their susceptibility to both strains under controlled conditions and the corresponding development of symptoms.

# 3. Materials and Methods

## 3.1. Plant Material

In June 2012, 338 potato leaf samples with severe or mild symptoms were collected from the following post-harvest fields in Switzerland: Grange-Verney (canton of Vaud), Rütti (canton of Bern), Uttewil (canton of Fribourg), Wallierhof (canton of Solothurn), Liebegg (canton of Aarau) and Strickhof (canton of Zürich). The samples were collected on 25 different potato cultivars (Agata, Agria, Amandine, Annabelle, Antina, Bintje, Celtiane, Charlotte, Désirée, Ditta, Fontane, Gourmandine, Hermes, Innovator, Jelly, Lady Rosetta, Lady Claire, Lady Jo, Malou, Markies, Nicola, Panda, Pirol, Stella and Victoria). For each sample, three leaflets were selected and stored in plastic bags at −20°C until use for serological and molecular analysis (detailed below). In June 2014, 91 additional potato leaf samples of Agria and Charlotte with severe or mild symptoms were collected from the same fields and analysed in the same way.

Agria and Charlotte were also challenged with PVY strains in greenhouse trials. The trials were repeated three times, with cv. Charlotte tested in trials 1, 2 and 5 and cv. Agria tested in trials 3, 4 and 5. Virus-free tubers were planted in plastic pots (19x19x18cm) filled with compost. The pots were randomized and maintained in an insect-proof greenhouse (20°C, 70% relative humidity and 12-hour photoperiod). Three leaves of each plant were inoculated seven days after plant emergence using carborundum (Perez 2011). The inoculum is prepared from fresh tobacco leaves showing PVY symptoms crushed in an inoculation buffer (0.02 M phosphate + 0.01 M DIECA). At least 20 plants were inoculated with two PVY isolates (Agroscope collection): $PVY^{N-Wi}$ 1315 isolate (28 in trial 1, 22 in trial 2, 21 in trial 3, 25 in trial 4 and 22 in trial 5) and with $PVY^{NTN}$ 1317 isolate (105 in trial 1, 35 in trial 2, 20 in trial 3, 25 in trial 4, and 22 in trial 5). A leaf sample (3 leaves per plant) was taken six weeks after inoculation for serological analysis. The symptoms were observed at the same time in trials 3, 4 and 5. As the control for symptomless plants, 6, 15 and 6 plants, respectively,

were kept non-inoculated. The symptoms were classified following a four-grade scale: 0 = no visible symptoms; 1 = small necrotic spots; 2 = large necrotic spots and presence of dry leaflets and 3 = completely dry leaves. For trial 4, the tuber yield/plant was measured (g/plant).

## 3.2. Samples Analysis

The serological and molecular methods used for the analysis of the leaf and tuber samples were the same as the methods described in Rigotti et al. (2011). In summary, both leaf and tuber samples were analysed by a double antibody sandwich (DAS)-ELISA using PVY monoclonal antibodies (mAb). The plates were coated with anti-PVY-NOC mAb (210369, Bioreba, Basel, Switzerland) and the $PVY^N$ strains were detected using the anti-PVY-N mAb (191073, Bioreba). A sample was considered infected by a $PVY^O$ strain if it reacted negatively to anti-PVY-N mAb and positively to anti-PVY-NOC mAb (210369, Bioreba), another mAb detecting both $PVY^N$ and $PVY^O$ strains. All the collected isolates were ELISA tested. A sample was considered positive if the optical density value was three times higher than the optical density of sap extracted from a healthy potato tuber (control on each ELISA plate).

Part of the leaf samples of the survey was analysed by RT-PCR (numbers in Table 12), as proposed by Rigotti et al. (2011). The one-step RT-PCR was done in a 50µl reaction containing 2µl of RNA extract (about 200 ng of total RNA), 2% (w/v) of sucrose, 0.1mM of cresol red, 3.5mM $MgCl_2$, 0.8mM of each dNTP, 0.4 µM of each primer, 12 units (U) of RNasin ribonuclease inhibitor (Promega), 3U AMV RT (Promega) and 2.5U of *Tth* polymerase in 10× reaction buffer (Promega). The amplifications were carried out in a thermocycler (Biometra TGradient) programmed as follow: 1 cycle at 42 °C for 1 h, 1 cycle at 94 °C for 2 min, 35 cycles at 94 °C for 30 s, 57 °C for 1 min and 72 °C for 1 min. RT-PCR products were separated on a 1.5% agarose gel in TBE buffer (89mM Tris pH8, 89mM boric acid, 2mM EDTA) and observed by UV illumination after staining with ethidium bromide. The three primer pairs PVYc3/f, PVY3+/3− and CP2+/1− were used (Rigotti and Gugerli 2007). The sequences of the primers are presented in Table 10.

*Table 10: Sequences and genomic location of the oligonucleotide primers [adapted from Rigotti and Gugerli (2007)]*

| Primers | Sequence 5'–3' | Genomic location* |
|---|---|---|
| PVYc3 | CAACGCAAAAACACTCA(CT)AAA(AC)GC | 34–57 |
| PVYf | TAAGTG(AG)ACAGACCCTCT(CT)TTCTC | 667–690 |
| PVY3+ | TGTAACGAAAGGGACTAGTGCAAAG | 4530–4554 |
| PVY3− | CCGCTATGAGTAAGTCCTGCACA | 5622–5644 |
| CP2+ | CCAGTCAAACCCGAACAAAGG | 8637–8657 |
| CP1− | GGCATAGCGTGCTAAACCCA | 9149–9168 |

** Numbered in PVY$^{NTN}$-H [Thole et al. (1993); accession number M95491].*

The triplex RT-PCR allowed classification of the PVY isolates into five groups (i) PVY$^{O}$, (ii) PVY$^{C}$, (iii) PVY$^{N}$, (iv) PVY$^{NTN}$, and (v) PVY$^{N-Wi}$ with distinction between French (Glais et al., 2000, unpublished data; accession number AF248500) and Polish isolates (Chachulska et al., 1996, unpublished data; accession number Z70238). The sizes of the amplified RT-PCR fragments are listed in Table 11, and their location on the PVY genome is presented in Figure 19. To simplify presentation of the data, PVY$^{N-Wi}$ Polish and French isolate were grouped as PVY$^{N-Wi}$.

*Table 11: Amplified bands by one-step triplex RT-PCR using the primers PVYc3/f, PVY3+/3−, CP2+/1− for the detection of different PVY strains [adapted from Rigotti and Gugerli (2007)]*

| | PVYc3/f [kb] | PVY3+/3− [kb] | CP2+/1− [kb] |
|---|---|---|---|
| PVY$^{N}$ | 0.44 | 1.11 | – |
| PVY$^{NTN}$ | 0.44 | – | – |
| PVY$^{O}$ | 0.66 | – | 0.53 |
| PVY$^{N-Wi}$ (PVY$^{N}$Wi-P) | – | – | 0.53 |
| PVY$^{N-Wi}$ (PVY$^{N}$N242) | 0.44 | – | 0.53 |
| PVY$^{C}$ | 0.66 | – | – |

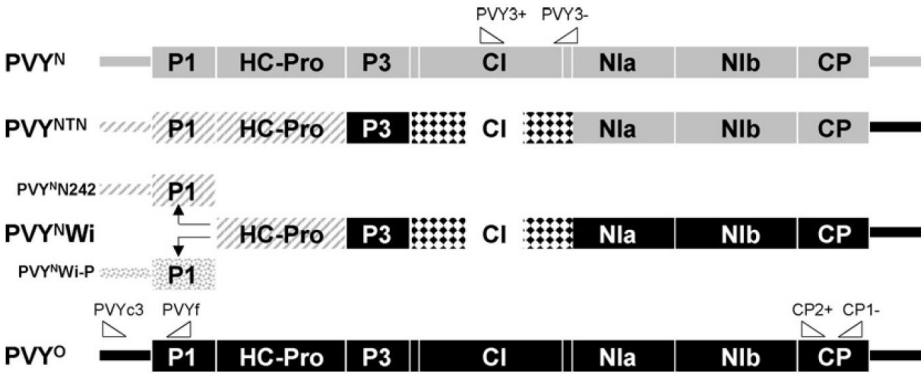


*Figure 19: Schematic presentation of the PVY genome adapted from Glais et al. (2002) with genomic location of the oligonucleotide primers. Restriction patterns of PVY$^N$-type in solid grey ; similar to PVY$^N$ type in grey stripes; PVY$^O$-/PVY$^N$-/PVY$^{NTN}$-/other-type in dotted grey ; PVY$^O$-type in black ; similar to PVY$^O$-type: in B&W chessboard [adapted from Rigotti and Gugerli (2007)].*

## 3.3. Data Analysis

The 2012 distribution for each PVY strain was compared to the corresponding distribution of 2003 and 2008 surveys presented by Rigotti et al. (2011). Mixed infections were counted as positive samples for each of the identified virus strains. The distribution of the strains between years was compared with a Chi-squared test (significance level of 5%). The distribution of the strains infecting cv. Agria was compared to the distribution of the stains infecting cv. Charlotte also with another Chi-squared test (significance level of 5%).

For the greenhouse trial, we used the general linear model to evaluate the effects of PVY strains on the symptom expressions on the foliage, on the percentage of infected plants and on the tuber yield. The trials were included in the model as the replication factor for the analysis of the symptoms on the foliage and percentage of infected plants. For the analysis of the tuber yield data, the plants were used as the replication factor. The data of each cultivar were analysed separately as they were tested in different trials (except trial 5).

The software Statistica® (StatSoft Inc., Tulsa, USA) was used to perform all analyses.

# 4. Results

## 4.1. Survey 2012

In 2012, 176 of the collected samples tested positive to PVY by ELISA, among which 130 were $PVY^{N}$ positive (74%) and 46 $PVY^{O}$ positive (26%). For the majority of the varieties (19 out of 25), the $PVY^{N}$ serotype was most frequently detected in the samples (Figure 20). Three cultivars, namely Markies, Panda and Antina had the same proportion of $PVY^{N}$ and $PVY^{O}$ serotype in their samples. While three other cultivars, namely Lady Rosetta, Agria and Jelly had a majority of sample in which $PVY^{O}$ serotype was detected. We note that the average number of samples per variety is about 7, meaning that the percentage of occurrence of PVY serotypes in the varieties tested has to be considered with caution.

We further characterized 103 isolates from the 2012 survey by molecular testing, with 57 isolates serologically positive for $PVY^{N}$ and 46 isolates serologically positive for $PVY^{O}$ (example of RT-PCR gel in Figure 21). In 2012, only one mixed-infection was found between $PVY^{N}$ and $PVY^{N\text{-}Wi}$.

The data and distribution of each PVY group are presented in Table 12 and compared with data from the 2003 and 2008 surveys published by Rigotti et al. (2011). The Chi-squared test shows that the distribution of the PVY strains significantly changed from 2003 to 2008 ($p<0.001$), from 2008 to 2012 ($p<0.05$) and from 2003 to 2012 ($p<0.01$). Table 12 presents these changes in details, as well as their significance. A change in prevalence of a virus strain from one survey to the other can be considered as significant when there is no overlap between the confidence intervals of both surveys (see Table 12). The $PVY^{NTN}$ group remains the most prevalent in Switzerland but has decreased from 78.9% of prevalence in 2003, to 70.4% in 2008, and 71.3% in 2012. This important decrease (-7.6% between 2003 and 2012) is marginally significant as there is only 0.4% of overlap between the higher bound of the confidence interval of 2003 and the lower bound of the confidence interval of 2012. This decrease of the prevalence of the $PVY^{NTN}$ strain is compensated by an important increase in the prevalence of the $PVY^{N\text{-}Wi}$ strain. The prevalence of this strain has been increasing from 5.5% in 2003 to 17.3% in 2008 and 18.8% in 2012. This increase of 13.3% from 2003 to 2012 also compensates the decrease of the $PVY^{O}$ strain observed during the same period (-5.9%). The prevalence of this strain has importantly decreased between

the first two surveys (-8.4%) with 13.3% in 2003 and 4.9% in 2008. Then, the prevalence of $PVY^O$ remained relatively stable with 7.4% of prevalence in 2012. Finally, $PVY^N$ populations has been increasing from 2003 to 2008 with 2.3% of prevalence in 2003 and 7.4% in 2008. This increase of 5.1% was almost compensated by a decrease of 4.8% between 2008 and 2012, with 2.6% of occurrence in the last survey.

*Table 12: Frequency of PVY types as assessed by triplex RT-PCR in selected leaf samples collected in Swiss seed-potato control fields in 2003, 2008 and 2012. The data for the years 2003 and 2008 were retrieved from the article of Rigotti et al. (2011). The binomial confidence interval of sample proportion is presented between squared brackets. The significance of the evolution of the occurrence is presented between brackets with a "+" for a significant increase, a "-" for a significant decrease and a "NS" for a non-significant effect.*

| Strain | Year | Percentage of occurrence | Confidence interval | Evolution of the occurrence |
|---|---|---|---|---|
| $PVY^{NTN}$ | 2003* | 78.9 | [75.3 ; 82.5] | |
| | 2008 | 70.4 | [65.3 ; 75.5] | (NS) |
| | 2012** | 71.3 | [66.8 ; 75.7] | (NS) |
| $PVY^N$ | 2003* | 2.3 | [1.0 ; 3.7] | |
| | 2008 | 7.4 | [4.5 ; 10.3] | (+) |
| | 2012** | 2.6 | [1.0 ; 4.2] | (-) |
| $PVY^{N-Wi}$ | 2003* | 5.5 | [3.5 ; 7.5] | |
| | 2008 | 17.3 | [13.1 ; 21.5] | (+) |
| | 2012** | 18.8 | [14.9 ; 22.6] | (NS) |
| $PVY^O$ | 2003* | 13.3 | [10.3 ; 16.3] | |
| | 2008 | 4.9 | [2.5 ; 7.3] | (-) |
| | 2012** | 7.4 | [4.8 ; 10.0] | (NS) |

** Mixed infections in Rigotti et al. (2011) were counted as positive samples for each of the identified virus strains.*

*** The percentages of occurrence were weighted by the balance of $PVY^O$ and $PVY^N$ strains obtained after serological analysis.*

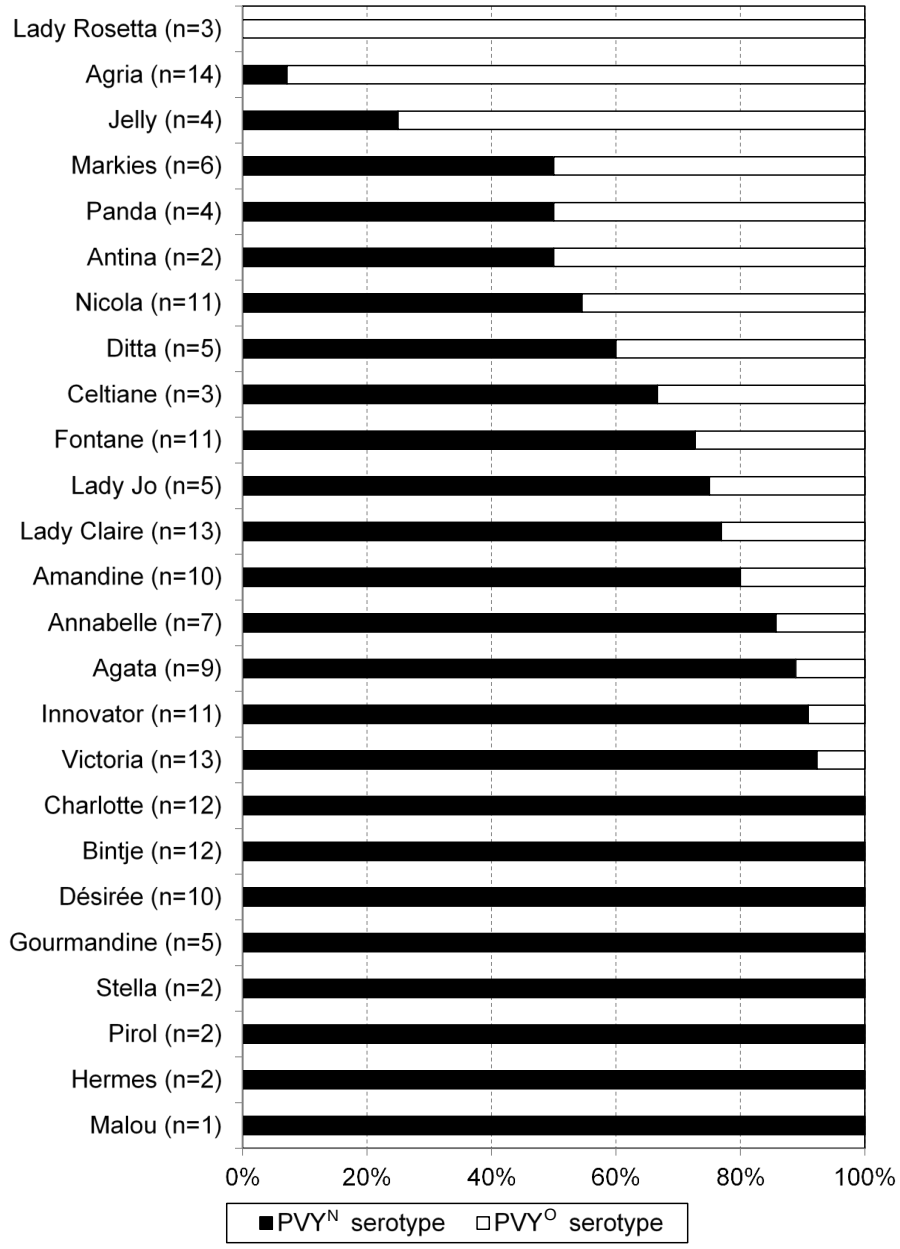


*Figure 20: For each variety collected during the PVY survey of 2012, proportion of samples infected by $PVY^O$ and $PVY^N$ after detection by ELISA, "n" is the amount of samples collected for each variety.*

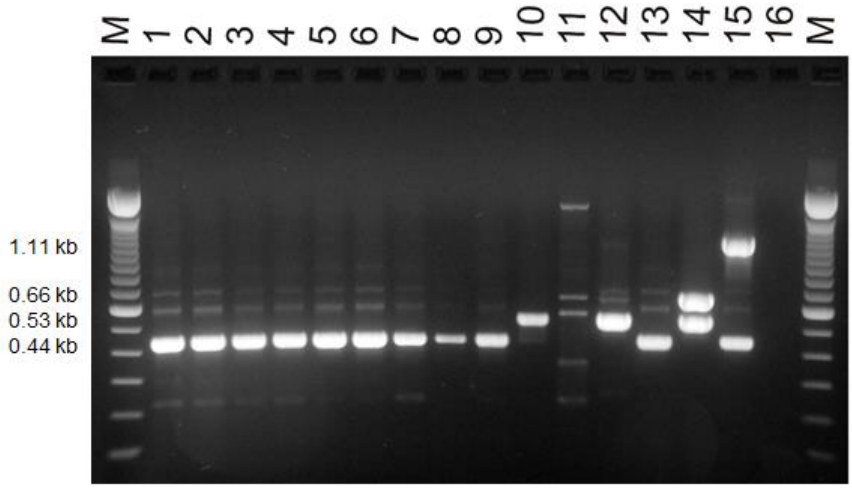


*Figure 21: Triplex RT-PCR amplifications by using the primers PVYc3/f, PVY3+/3− and CP2+/1− on total RNA of cv. Desiree infected with PVY$^{NTN}$ (lane 1), cv. Innovator infected with PVY$^{NTN}$ (lane 2), cv. Lady Claire infected with PVY$^{NTN}$ (lane 3), cv. Victoria infected with PVY$^{NTN}$ (lane 4), cv. Panda infected with PVY$^{NTN}$ (lane 5), cv. Nicola infected with PVY$^{NTN}$ (lane 6), cv. Markies infected with PVY$^{NTN}$ (lane 7), cv. Stella infected with PVY$^{NTN}$ (lane 8), cv. Fontane infected with PVY$^{NTN}$ (lane 9), cv. Agria infected with PVY$^{N-Wi}$ (lane 10), cv. Agata not infected (lane 11), Nicotiana tabacum cv. Xanthii infected by PVY$^{N-Wi}$ (accession number 1315 – lane 12), N. tabacum cv. Xanthii infected by PVY$^{NTN}$ (accession number 1317 – lane 13), ), N. tabacum cv. Xanthii infected by PVY$^{O}$ (accession number 803 – lane 14) and N. tabacum cv. Xanthii infected by PVY$^{N}$ (accession number 605 – lane 16). Lane 16: negative control (no RNA). Lanes M: molecular mass marker (100 nt DNA ladder).*

## 4.2. Agria and Charlotte Surveys

The data from the 2014 survey were combined with the data from the 2012 survey in Table 13 to assess the prevalence of the main PVY strains in the cultivars Agria and Charlotte. Results of both years are presented together in Table 13, as there was not enough positive samples for each single year (n=26 in 2012 and n=85 in 2014) to be able to determine tendencies by year. In both surveys, 58 cv. Agria plants and 53 cv. Charlotte plants were found to be infected by PVY. The Chi-squared test showed that the distribution of the PVY strains was significantly different in cv. Agria compared to cv. Charlotte ($p$<0,001). Cultivar Agria was mainly infected by PVY$^{N-Wi}$ (81%) and PVY$^{O}$ (16%) strains, while only eight plants were found to be infected by PVY$^{NTN}$ strains (3%)

and no plants by $PVY^{N}$. In contrast, cv. Charlotte was mostly infected by $PVY^{NTN}$ strains (90%), whereas only 6% of the plants were infected by $PVY^{N-Wi}$. One and two plants, respectively, of cv. Charlotte were found to be infected by $PVY^{N}$ and $PVY^{O}$ strains.

*Table 13: Frequency of PVY types in cultivars Agria and Charlotte as assessed by triplex RT-PCR in selected leaf samples collected in Swiss seed-potato control fields in 2012 and 2014. The 90% binomial confidence interval of sample proportion is presented between squared brackets.*

| Cultivar | Strain | Percentage of occurrence* | Confidence interval |
|---|---|---|---|
| Agria | $PVY^{NTN}$ | 3.4 | [1.2 ; 5.7] |
| | $PVY^{N}$ | 0.0 | [0.0 ; 0.0] |
| | $PVY^{N-Wi}$ | 81.0 | [76.1 ; 85.9] |
| | $PVY^{O}$ | 15.5 | [11.0 ; 20.0] |
| Charlotte | $PVY^{NTN}$ | 90.1 | [85.9 ; 94.4] |
| | $PVY^{N}$ | 2.3 | [0.2 ; 4.4] |
| | $PVY^{N-Wi}$ | 6.0 | [2.7 ; 9.4] |
| | $PVY^{O}$ | 1.5 | [-0.2 ; 3.2] |

** The percentages of occurrence were weighted by the balance of $PVY^{O}$ and $PVY^{N}$ strains obtained after serological analysis.*

## 4.3. Inoculation Trials

In the greenhouse assays, the inoculation rate of cv. Charlotte was similar ($p$>0.05) to $PVY^{N-Wi}$ (57%) and $PVY^{NTN}$ (69%) strains, while the inoculation rate of cv. Agria was significantly higher for $PVY^{N-Wi}$ ($p$=0.029), with 65% compared to $PVY^{NTN}$ with 29% (Figure 22).

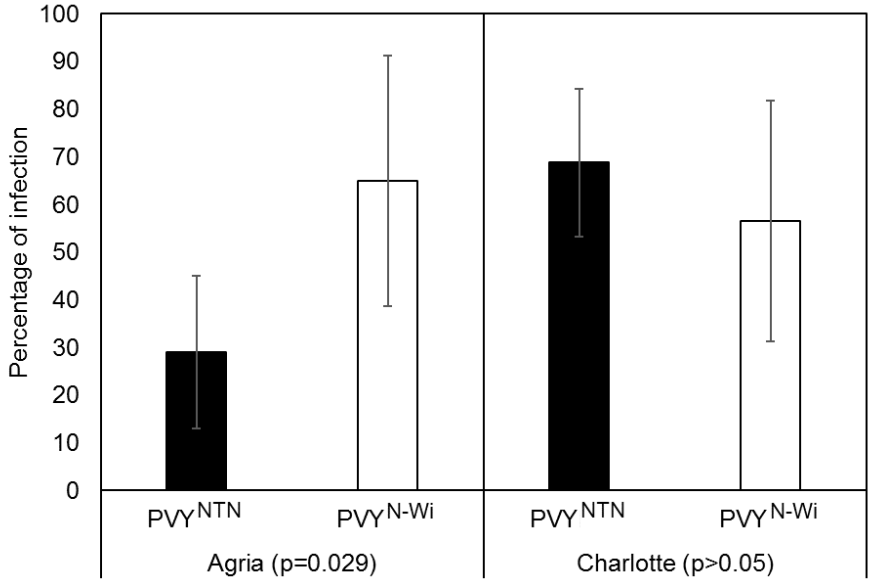


*Figure 22: Percentage of infected plants of cv. Agria and Charlotte after mechanical inoculation with PVY$^{N-Wi}$ 1315 and PVY$^{NTN}$ 1317 strains. The number under brackets in the legend gives the p value of the analysis of variance.*

The plants of cv. Agria infected with PVY$^{NTN}$ showed more severe symptoms ($p$<0.001) compared to plants infected with PVY$^{N-Wi}$, with necrosis (local and systemic necrosis) on nearly all leaflets and a high number of totally dry leaflets and possible systemic reactions (Figure 23 ; Figure 24). The plants inoculated with PVY$^{N-Wi}$ mainly showed small necrotic spots on the foliage (Figure 23, Figure 24). The plants of cv. Charlotte showed severe symptoms regardless of the strain used for the inoculation (Figure 24).


*Figure 23: Symptoms of PVY strains on leaflets, 60 days after mechanical inoculation of plants of cv. Agria. (a) Uninoculated plants, (b) plants inoculated with PVY$^{N-Wi}$ and, (c) plants inoculated with PVY$^{NTN}$.*

No significant difference of tuber yield was observed between the cv. Agria plants inoculated with different PVY strains ($p$=0.607) with an average of 49 g of tubers for the control plants and 59 g and 62 g, respectively, of tubers for the plants inoculated with $PVY^{NTN}$ and $PVY^{N\text{-}Wi}$.

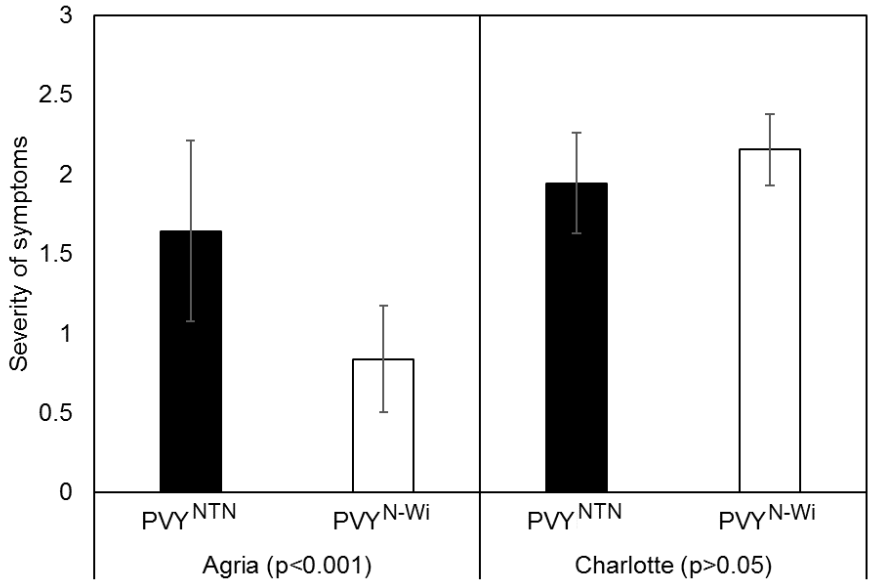


*Figure 24: Severity of symptoms (0 = non-visible symptoms; 1 = small necrotic spots; 2 = big necrotic spots and presence of dry leaflets and 3 = leafs completely dry). The number under brackets in the legend gives the p value of the analysis of variance.*

## 5. Discussion

The surveys for PVY revealed a slow yet progressive increase of the prevalence of $PVY^{N\text{-}Wi}$ strains in Switzerland and, simultaneously, a gradual decrease of the prevalence of $PVY^{NTN}$ and $PVY^{O}$ strains in Swiss seed-potato crops. The $PVY^{N}$ strain is still present albeit at low levels. This result is consistent with the increase of the prevalence of $PVY^{N\text{-}Wi}$ strains observed in Germany, Canada, and the United States (Gray et al., 2010, Lindner et al., 2015). Such increase in the prevalence of $PVY^{N\text{-}Wi}$ might be associated instead to factors specific to each country. In Switzerland, the role of importation of infected seed potatoes is excluded, as they represent only 15% of the

seed potatoes with low PVY infection rates (0.1 to 0.2% - Peter Frei, personal communication).

Consistent with surveys performed in Germany (Lindner et al., 2015, Lindner & Billenkamp, 2005), our surveys revealed that Agria is more frequently infected by $PVY^{N-Wi}$ (81% prevalence). In our greenhouse trial, this cultivar resisted $PVY^{NTN}$ better than it resisted the $PVY^{N-Wi}$ inoculation. This cultivar's specific resistance to $PVY^{NTN}$ leads to a higher detection of $PVY^{N-Wi}$ in the symptomatic plants, as observed in our surveys. We observed in the inoculation trials that Agria display less severe symptoms in response to $PVY^{N-Wi}$ infection. Given that symptom expressions strongly influence the efficacy of elimination of symptomatic plants from fields by roguing (Chrzanowska, 1991, Glais et al., 2005), the resistance of Agria to $PVY^{NTN}$ and the lower expression of symptoms of Agria infected by $PVY^{N-Wi}$ might both contribute to $PVY^{N-Wi}$ prevalence compared to $PVY^{NTN}$. The cultivar-specific property of developing milder symptoms when infected by $PVY^{N-Wi}$ was also reported in cv. Elipsa by Chrzanowska (1991). In our greenhouse trial, Agria plants infected by $PVY^{NTN}$ displayed local necrosis in inoculated leaves or systemic necrosis with total desiccation of the infected stems followed by leaf-drop syndrome. Such symptoms in potato plants can be associated with hypersensitive resistance (HR) response in spite of the fact that the virus can still spread systemically (Funke et al., 2017, Valkonen et al., 1998). This HR response of cv. Agria has been previously described by Petrov et al. (2015), in a trial with mechanically inoculated plants with $PVY^{NTN}$. This trial revealed the blocking of the replication of $PVY^{NTN}$ in the newly grown leaves. The inoculated leaves remained infected and collapsed after desiccation, while the virus was absent from the newly grown parts of potato plants.

By contrast, no difference in symptom expressions or cultivar-specific resistance is observed in other cultivars, such as cv. Charlotte inoculated with the $PVY^{NTN}$ or $PVY^{N-Wi}$ strains. In the field, however, the observed prevalence of $PVY^{NTN}$ in this cultivar is close to 90%, to the detriment of the plants being infected by $PVY^{N-Wi}$. This level exceeds the average prevalence of $PVY^{NTN}$ in the country by 20%. This shows that other factors are probably playing a role in the higher prevalence of $PVY^{NTN}$ in cv. Charlotte.

Thus, a change in the cultivars grown in the fields is likely to be a determinant factor in the distribution of PVY strains. It has been shown by Funke et al. (2017) in the context

of seed-potato production in the Columbia Basin (USA), where the prevalence of $PVY^{O}$ dropped down sharply due to the introduction of potato cultivars resistant to this strain. In Switzerland, the increase in the prevalence of $PVY^{N\text{-}Wi}$ from 17% to 19% between 2008 and 2012 is in line with the increase in surface area planted with cv. Agria from 20% to 22% during the same period.

# CHAPTER 2: THE MOVEMENT OF PVY IN THE VASCULAR SYSTEM OF POTATO PLANTS

## 1. Preamble

The objective of this chapter is to compare the translocation of the two main recombinant strains $PVY^{N\text{-}Wi}$ and $PVY^{NTN}$, and better understand the phenomenon of *mature plant resistance*

The first part of the chapter further examines the reasons behind the increase in prevalence of $PVY^{N\text{-}Wi}$ strains in Switzerland, as described in Chapter 1. A greenhouse experiment is designed to compare the efficacy of translocation to the progeny tubers of a $PVY^{N\text{-}Wi}$ and a $PVY^{NTN}$ strain. The hypothesis is that a strain with a more efficient translocation would increase its prevalence to the detriment of the others. In the second part of the chapter we focus on *mature plant resistance* and conduct a second greenhouse experiment to better understand how PVY colonizes plants with different physiological ages. The end of the chapter discusses the practical implications of our results for the control of PVY during the growing season.

This chapter is published in “European Journal of Plant Pathology” and is referenced as follows: “Dupuis B, 2017. *The movement of potato virus Y (PVY) in the vascular system of potato plants.* European Journal of Plant Pathology 147, 365-73. The original version of the article is presented in Annex 2.

## 2. Introduction

The breaking of the tuber’s dormancy activates transport mechanisms through the phloem of the young tissues of the growing sprouts, which are actively draining sap out of the mother tuber. In PVY-infected tubers, this sap is loaded with virus particles that will concentrate at the rose end of the tuber where most of the sprouts are located (Basky & Almasi, 2005, Gugerli & Gehriger, 1980). Roots will grow horizontally from the base of the sprout while its apex will grow vertically, emerge from the soil, and give rise to a stem with branches and leaves. It is unknown whether all the stems growing

from the same tuber will be infected through an upward systemic movement of PVY particles from the mother tuber.

The descending flow of sap from the stems to the progeny tubers, which allows the translocation of the virus, is better documented. Debokx (1964) was the first to study PVY translocation in potato plants, and to identify differences between varieties with respect to the rate of tuber infection in primary infected plants. Beemster (1976) confirmed those results and demonstrated that the efficacy of the translocation varied according to the PVY strain. He observed that $PVY^N$ primary infected plants presented a higher rate of infected progeny tubers compared to $PVY^O$ strains.

Basky and Almasi (2005) further investigated within-plant translocation and observed that $PVY^N$ migrates faster than $PVY^O$ from infected leaves to other plant organs. After a cell-to-cell movement from the infected leaf cells to the vascular system, the virus was detected either in the upper or the lower segments of the stem, meaning that it can be transported by the flow of sap up and down the stem (Basky & Almasi, 2005, Valkonen & Rokka, 1998, Draper et al., 2002). It was also demonstrated that when a sprout is inoculated, the virus can systemically migrate to the other sprouts and develop symptoms in the stems later on (Basky & Almasi, 2005).

Taken together, these results show that the virus is able to migrate through the phloem of the infected stem, and after reaching the vascular ring of the mother tuber it is able to colonize the vascular system of the other growing stems. This physical continuity of the vascular ring of the mother tuber with the vascular systems of the growing sprouts is clearly visible in Figure 25. As each stem has its own set of progeny tubers, if PVY succeeds in colonizing all the stems of the plant, it may also be capable of infecting all the plant's progeny tubers as well.


*Figure 25: Longitudinal section of a potato tuber with two growing stems. Legend: (a) vascular system of the first stem; (b) junction of the stems and mother tuber vascular systems; (c) vascular ring of the mother tuber; (d) vascular system of the second stem.*

The first objective of this research is to track the spread of two PVY strains ($PVY^{NTN}$ and $PVY^{N\text{-}Wi}$) from an infected mother tuber to the above-ground parts of the plant, and then from the haulms to the progeny tubers. The second objective of this research is to establish whether the earliness/moment of primary infection in one stem may influence the infection of the other stems of the same plant. This result will contribute to a better understanding of the resistance mechanisms implemented in mature plants. To achieve these two objectives, two greenhouse trials were managed in Changins (Switzerland): a “movement and translocation trial”, and a “early and delayed inoculation trial”.

# 3. Materials and Methods

## 3.1. Plant Material

For both greenhouse trials, the PVY-susceptible cultivar Charlotte was used (Schwaerzel et al., 2014). For the movement and translocation trial, 30 tubers infected

by the $PVY^{NTN}$ 1317 strain (Agroscope collection) and 30 tubers infected by the $PVY^{N-Wi}$ 1315 strain (Agroscope collection) were used. Those PVY infected tubers were produced the previous year in the field in separated plots of 100 plants grown from healthy tubers. In each plot four percent of plants were inoculated with aforementioned PVY strains. The virus was then naturally transmitted to the neighboring plants through aphids. The identity of the inoculum was confirmed using a triplex RT-PCR as described by Rigotti and Gugerli (2007) and the identity of the strains in the 60 progeny tubers was confirmed using an enzyme-linked immunosorbent assay (ELISA). For the early and delayed inoculation trial, healthy tubers of cultivar Charlotte were used. They were analyzed using the same ELISA method to verify the presence/absence of the virus. Details of the ELISA method are presented below.

## 3.2. PVY Detection Method

PVY infections were tested in sprouting tubers using an ELISA method as described in Gugerli and Gehriger (1980). After the harvest, a Rindite [ethylene chlorhydrin, dichlorethane and carbon tetrachloride (7:3:1)] treatment was used to break dormancy and the treated tubers were then stored in the dark for 5 weeks at 22°C and 80-90% of relative humidity to promote sprouting. After this storage period, sap is extracted from the vascular tissue underneath the sprouts with a drill (Bioreba AG, Reinach, Switzerland) and analyzed by ELISA. Two sets of monoclonal antibodies were used to verify the identity of the serotype, on the one hand an anti-PVY-N (191073, Bioreba, specific for N strain) and on the other hand an anti-PVY-NOC (210369, Bioreba). The samples reacting positively to the anti-PVY-NOC and negatively to the anti-PVY-N were declared infected by $PVY^{N-Wi}$ 1315 strain whereas the samples reacting positively to both antibodies were declared to be infected by $PVY^{NTN}$ 1317 strain. For PVY detection in the stems, three leaflets are collected from the same stem: one in the upper, one in the medium, and one in the lower part of each stem. The three leaflets are ground together using Homex 6® (Bioreba AG, Reinach, Switzerland) and the sap is analyzed by ELISA. The same sets of antibodies as for tubers analysis are used to verify the identity of the PVY serotype in the leaflets (Bioreba AG, Reinach, Switzerland). A sample is considered positive when the optical density value is three times higher than the optical density of sap extracted from a healthy potato tuber.

## 3.3. Management of Trials

For the movement and translocation trial, sprouts of the infected tubers were removed in order to maintain only three sprouts per tuber: one close to the rose end, one close to the heel end, and one in the middle. Following PVY detection underneath each sprout, the tubers were half-buried in a perlite bed in plastic pots (19x19x18 cm). The pots were then dispatched in four distinct insect-proof greenhouses (20°C, 70% relative humidity, and 12 hours photoperiod) following a randomized block design. After development of the three stems (from the three sprouts), the stems were accordingly numbered and the pots were filled with compost. The plants were then grown until flowering and a leaf sample was taken for PVY detection. At the beginning of natural senescence, a sample of progeny tubers was analyzed by ELISA.

For the early and delayed inoculation trial, the heathy tubers were planted in plastic pots (19x19x18cm) filled with compost. The pots were randomized and maintained in an insect-proof greenhouse (20°C, 70% relative humidity, and 12 hours photoperiod). The plants were distributed into three groups. The first group was inoculated seven days after plant emergence (DAE). In order to do that, three leaves from one stem—one in the upper, one in the medium, and one in the lower part of each stem—were mechanically inoculated with the $PVY^{NTN}$ 1317 strain (Agroscope collection) using carborundum (Perez, 2011), and the inoculated stem was tagged. The other stems were numbered for traceability. The inoculum is based on crude sap from frozen infected material samples 1:2 diluted in sodium phosphate buffer (0.02 M Na-phosphate, 0.01 M sodium diethyldithiocarbamate [DIECA], pH 7.6). The second group of plants was inoculated 21 DAE and the last group 56 DAE.

The identification of the strain used for the experiment was done by RT-PCR, as proposed by Rigotti et al. (2011). The one-step triplex RT-PCR was performed using the three primer pairs PVYc3/f, PVY3+/3− and CP2+/1−. This triplex RT-PCR allows for the discrimination of the PVY isolates belonging to the following groups: $PVY^{O}$, $PVY^{C}$, $PVY^{N\text{-}Wi}$ and $PVY^{NTN}$.

After inoculation, leaf samples were collected on each potato stem one, two, four, and six weeks after inoculation, in order to determine whether the stems were infected by PVY. The plants were grown until the beginning of natural senescence, around 90 days after emergence. This trial was repeated with an eight-month interval between trials.

For the first trial, 11 plants were allocated to each inoculation group, and for the second trial 15 plants were allocated to each group. For the replication of the trial, the tubers of each plant were counted and weighted. A sample of progeny tubers was analyzed by ELISA and it was established whether the tubers were coming from the inoculated stem or from any other stems of the plant.

### 3.4. Statistical Analysis

The data on infection percentages were transformed using the angular data transformation method (Dagnelie, 1975) prior to running an analysis of variance (ANOVA). The software STATISTICA® (StatSoft, Tulsa, USA) was used for all analysis. For the movement and translocation trial, another two-factors ANOVA was run to compare the percentage of infected sprouts, stems, and tubers between the two PVY strains. The effects of the factors “PVY strain” and “sprout location” were tested as well as the interaction between those factors, and the “greenhouse” effect was used as a replication factor. For the early and delayed inoculation trial, a Cochran Q test (Cochran, 1950) was used to test the effect of inoculation dates (7, 21, or 56 DAE) on the probability of infection of stems after inoculation, and the probability of a new stem to be infected through the vascular system of the plant. A simple regression analysis was used to evaluate the effect of the infection date on the yield. Finally, a two-factors ANOVA was run to compare the percentage of infected stems. The effects of the factors “day of inoculation” and “replication of the trial” were tested, as well as the interaction between these factors.

## 4. Results

### 4.1. Movement and Translocation Trial

We did not observe any effect of the insertion site of the sprout on the probability of sprout becoming infected ($p$>0.05). However, all the sprouts growing from infected tubers finally became stems in which the virus was detected (Table 14), proving that even if the infection was not present in the vascular system of the sprout itself, it may have arrived later from other parts of the mother tuber. Part of the sprouts never grew up (Table 14). As presented in Table 14, the rate of PVY-infected sprouts were

significantly higher ($p$<0.001) for $PVY^{N-Wi}$ infected tubers (84%) than for $PVY^{NTN}$ infected tubers (69%). The $PVY^{N-Wi}$ stem infection was efficiently transmitted to the progeny tubers, as 99% of them were infected (Table 14). The transmission of $PVY^{NTN}$ was significantly lower than $PVY^{N-Wi}$, with no more than 43% of the progeny tubers infected ($p$<0.001).

*Table 14: Assessment of the PVY infection rate of potato plants during the different phases of their development from sprouts to stems and progeny tubers. Two groups are presented: the plants infected by PVYN-Wi and by PVYNTN strains. For each group, the location of the sprout on the mother-tuber is specified.*

| PVY strain | Percentage of infected sprouts | | Percentage of infected stems | | Percentage of infected tubers | |
|---|---|---|---|---|---|---|
| ***$PVY^{NTN}$*** | ***69%*** | ***(n=90)*** | ***100%*** | ***(n=51)*** | ***43%*** | ***(n=202)*** |
| Rose end sprout | 63% | (n=30) | 100% | (n=22) | 35% | (n=106) |
| Middle sprout | 73% | (n=30) | 100% | (n=15) | 42% | (n=56) |
| Heel end sprout | 70% | (n=30) | 100% | (n=14) | 59% | (n=40) |
| ***$PVY^{N-Wi}$*** | ***84%*** | ***(n=90)*** | ***100%*** | ***(n=57)*** | ***99%*** | ***(n=225)*** |
| Rose end sprout | 83% | (n=30) | 100% | (n=23) | 100% | (n=95) |
| Middle sprout | 93% | (n=30) | 100% | (n=14) | 98% | (n=72) |
| Heel end sprout | 77% | (n=30) | 100% | (n=20) | 99% | (n=58) |
| ***Total*** | ***77%*** | ***(n=180)*** | ***100%*** | ***(n=108)*** | ***74%*** | ***(n=427)*** |

## 4.2. Early and Delayed Inoculation Trial

The results indicate that not all the inoculated stems developed infections: the later the inoculation was done, the fewer infected stems were observed ($p$<0.01). At early plant senescence times, PVY was detected in 92% and 81% of the stems inoculated at 7 and at 21 days after emergence, respectively. In contrast, PVY was detected in only 50% of the stems inoculated at 56 days after emergence (Figure 26). However, all the progeny tubers (n=36) harvested from the inoculated stems were infected by PVY, whatever the date of inoculation.

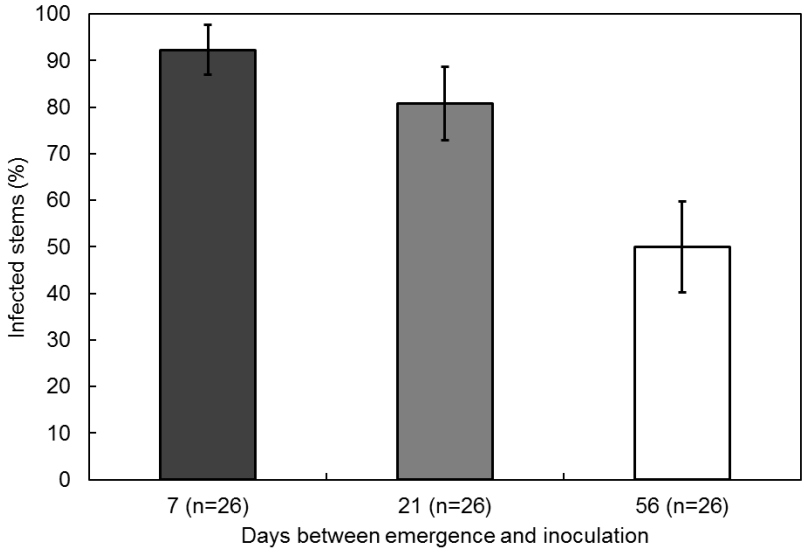


*Figure 26: Percentage of stem infection after inoculation, function of the inoculation schedule. Error bars present the standard error of the mean and "n" values indicate the number of plants considered in the statistical analysis.*

The probability for a non-inoculated stem to get infected through the movement of the virus particle along the vascular system of the mother tuber also varied as a function of the inoculation date ($p$<0.001). The results of the last ELISA analysis done before plant senescence show that 92% of the stems of early-inoculated plants (7 DAE) were infected (Figure 27). This percentage was less than half for the plants inoculated 21 days after emergence (39%), and even lower (13%) for the late-inoculated plants (56 DAE). The percentage of infected progeny tubers harvested from non-inoculated stems of early-inoculated plants (7 DAE) was 89% (n=35), 58% (n=12) from plants inoculated 14 days later, and 10% (n=10) from plants inoculated 56 DAE.

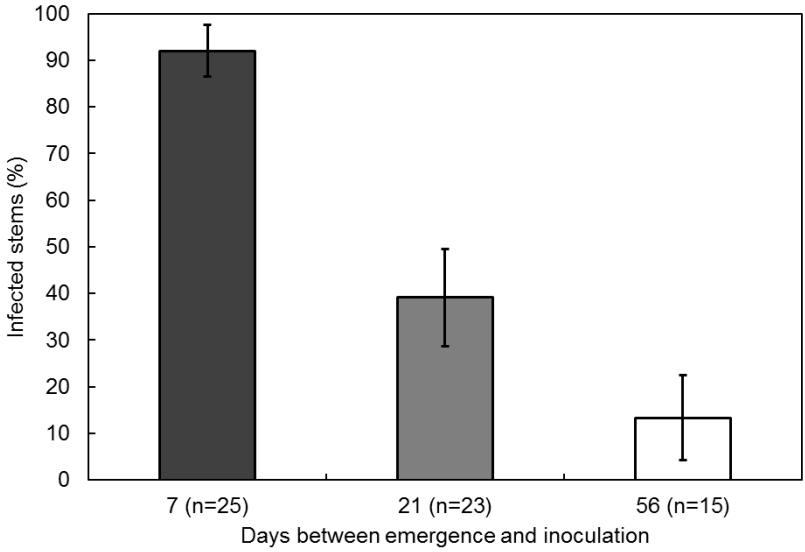


*Figure 27: Percentage for non-inoculated stems getting infected, function of the inoculation schedule. Error bars present the standard error of the mean and "n" values indicate the number of plants considered in the statistical analysis.*

After inoculation of one stem, the virus migrated faster to the other stems of the plant when the inoculation was done shortly after emergence (7 DAE) compared to later inoculations (21 DAE). As shown in Figure 28, the percentage of infected stems in plants inoculated 7 and 21 DAE is low, and not significantly different at one week and two weeks after inoculation ($p$>0.05). Four weeks after inoculation, the percentage of infected stems in early-inoculated plants increased sharply, reaching around 71%, while this percentage remained low (10%) for the plants inoculated 21 DAE ($p$<0.001). The percentage of infected stems continued to increase 6 weeks after inoculation to reach 83% for the early-inoculated plants (7 DAE) and 30% for the late-inoculated plants (21 DAE). Due to the decay of the plants inoculated 56 days after emergence, the evolution of the percentage of infected stems could not be monitored properly and therefore is not presented in Figure 28. However, the evolution was slow, with no stems infected one and two weeks after inoculation and only 10% infected 4 weeks after inoculation.

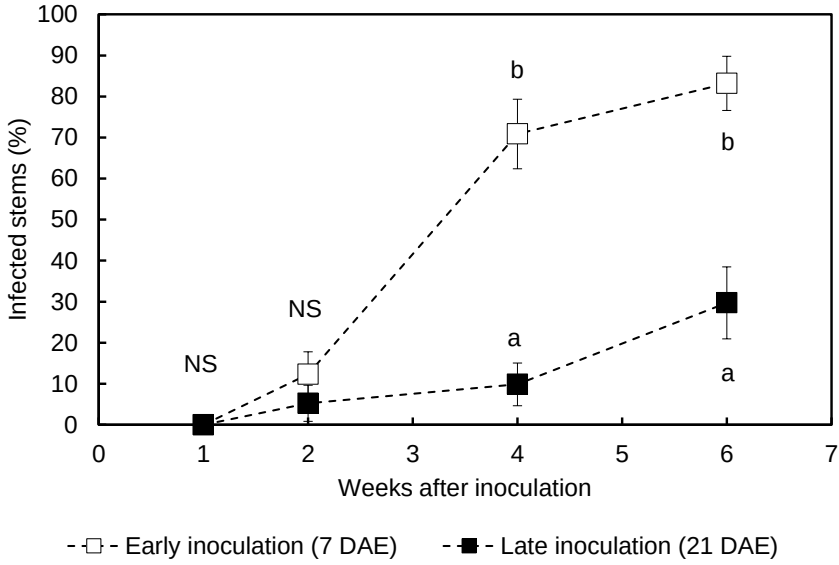


*Figure 28: Evolution of the percentage of infected stems: 1, 2, 4, and 6 weeks after inoculation for plants inoculated 7 and 20 days after emergence (DAE). Error bars present the standard error of the mean. Different letters indicate that the percentage of stems are different among the groups of plants inoculated at different dates ($p<0.05$ after analysis of variance ANOVA). "NS" indicates that the ANOVA results were not significant ($p>0.05$).*

From our results we could observe that the later the inoculation was done, the higher the yield was ($p$=0.001; equation of the regression: Y = 2.9*X+505.4).

Nevertheless, the model's coefficient of determination is low ($R^2$=0.3). Finally, no significant linear correspondence was found between the inoculation schedule and the number of progeny tubers harvested ($p$>0.05; $R^2$=0.042; equation of the regression: Y = 3.2E-02*X+13.9).

## 4.3. Comparison of two Methods of Data Analysis

We have compared the results of two methods of data analysis: general linear model (GLM) on the number of stems and GLM on the percentage on infected stems.

For both methods, no "trial" effect was observed ($p$>0.05 for all dates of stem sampling). A "date of inoculation" effect was observed (Table 15 below) and the results

of both statistical methods lead to the same conclusions. The difference of significance between the methods observed four weeks after inoculation may be due to a high variability in stems numbers in the data, leading to an underestimation of the variability of the data for the analysis of the “number of stems”. Finally, for both methods, no interaction was observed between “trial” effect and “date of inoculation” effect ($p$>0.05 for all dates of stem sampling).

*Table 15: Results of the general linear model (GLM) on the number of stems and GLM on the percentage of infected stems.*

| **Weeks after inoculation** | ***Number of infected stems*** | | | ***Percentage of infected stems*** | | |
|---|---|---|---|---|---|---|
| | df | F | p | df | F | p |
| 2 | 1 | 0.44 | =0.52 | 1 | 0.46 | =0.50 |
| 4 | 1 | 11.25 | <0.01 | 1 | 46.96 | <0.001 |
| 6 | 1 | 7.09 | <0.05 | 1 | 6.46 | <0.05 |

## 5. Discussion

The initial source of inoculum in the field is known to be the main risk factor for the subsequent spread of PVY to the healthy plants of the field (Steinger et al., 2014). The in-field source of inoculum comes from the infected seed tubers planted that offer PVY-infected foliage to the flying aphids, the vectors of the virus. Our results showed that all the stems of these tubers will become infected sooner or later. However, we also observed that not all the sprouts of an infected tuber are infected by PVY. We can hypothesize that the ELISA test was not sensitive enough to detect latent PVY infections in some of the sprouts, or that the virus came from other parts of the tuber and infected all the stems after the ELISA test. This suggests that all stems growing from an infected tuber will, eventually, become a source of inoculum in the field. In order to reduce the in-field sources of inoculum, it is generally recommended to remove the PVY-symptomatic plants as well as their progeny tubers (Davis et al., 2009). Some plants may, however, escape the vigilance of the seed grower and produce progeny tubers, which will present a risk for the following year. We also observed that the infection rate of these progeny tubers varied depending on the PVY strain. This

variation of the infection rate is well-documented for primary infections (Sigvald, 1985, Beemster, 1976, Gibson, 1991), and $PVY^{N}$ is known to be better transmitted to progeny tubers than $PVY^{O}$ (Beemster, 1976). In our study we focused our research on secondary infections due to PVY strains which arrived recently in Europe and North America (Karasev & Gray, 2013a, Glais et al., 2002). We showed that the $PVY^{N-Wi}$ strain is very efficient in transmitting the infection to the progeny tubers, and even more efficient than the $PVY^{NTN}$ strain. Note that it may be the case that the ELISA method may have missed some instances of low concentration of virus particles in some of the progeny tubers. However, this has no major implication for our main finding. In fact, while there may be cases where $PVY^{NTN}$ was not detected in progeny tubers, due to a low titer of the virus, $PVY^{N-Wi}$ was detected in almost all progeny tubers, suggesting that the titer of $PVY^{N-Wi}$ virus particles was higher in those tubers. This information might explain why $PVY^{N-Wi}$ strains are increasing in prevalence in some European and North American countries (see Chapter 1).

From our results we can also conclude that the probability of stem infection after inoculation was reduced for older stems. As noted above, this phenomenon is known as mature plant resistance and can be explained mainly by the restriction of cell-to-cell movement (Figure 29) rather than a restriction of the replication of the virus (Draper et al., 2002, Valkonen & Rokka, 1998). This resistance mechanism has important consequences for the epidemiology of PVY. In the case of aphid flights early in the season, young growing plants are particularly vulnerable to infections due to the absence of resistance. On the other hand, in the case of late aphid flights, the risk is reduced due to mature plant resistance, which will be at its maximum before senescence (Sigvald, 1985, Gibson, 1991, Robert et al., 2000).

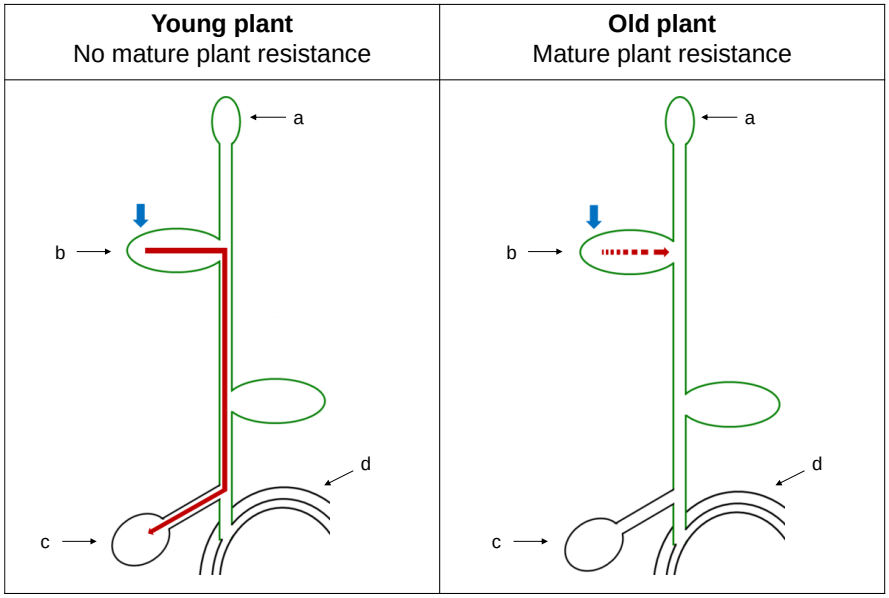


*Figure 29: Movement of PVY in the vascular system of the plant (in red) in a young and in an old plant. The blue arrow shows the location of the inoculation. The solid red lines indicate a rapid movement of the virus in the vascular system of the plant and the dotted lines indicate a slow movement of the virus. Legend: (a) the apical bud, (b) a leaf, (c) a progeny tuber and (d) the mother tuber.*

In the present experiment, mature plant resistance (MPR) appears 56 days after emergence with 50% of inoculation failure of the stems (Figure 26). When compared to the results of other experiments in the literature, MPR starts relatively late. Sigvald (1985) observed 50% of tubers infected with plants of cv. Bintje inoculated with carborundum ($PVY^O$) 37 days after emergence. For plants inoculated by aphids, Gibson (1991) observed that the percentage of tubers infected by PVY was systematically lower than 50% 28 days after emergence, regardless of the cultivar (Désirée, King Edward, Record or Maris Piper, Figure 6) and regardless of the PVY strain ($PVY^O$ or $PVY^N$). The fact that the data of the present experiment are not relative to a percentage of infected tubers but to a percentage of infected stems/plants (Figure 26) could explain the difference. However, Gibson (1991) also tested the percentage of infected plants and obtained the same result as on tubers. Thus, we can assume that both observations give similar results. There is a 28-day delay for the development of MPR between the experiment of Gibson and this experiment. This difference can be

attributed to different factors such as the method of inoculation, the difference of susceptibility of the variety, or the experimental conditions. The method of inoculation probably played an important role, as an inoculation with carborundum facilitates the movement of the virus from the leaf to the vascular system of the plant. An inoculation with carborundum causes important damages to plant tissues and promote the infection of a tremendous quantity of virus particles compared to the 0.5-3.2 virus particles usually transmitted by aphids (Moury et al., 2007). The cultivars Bintje, used by Sigvald (1985) and Charlotte, used in this experiment, are both highly susceptible to PVY (Schwaerzel et al., 2016). Therefore, the delay of 19 days for MPR between both trials cannot be attributed to a difference of susceptibility of the cultivars to PVY. The delay cannot be attributed to the method of inoculation either, seeing as carborundum was used for the inoculation in both experiments. The main difference between these experiments are the experimental conditions, whereby the present trial was undertaken in the greenhouse, whereas Sigvald's trial (1985) was undertaken in open field conditions in Sweden. The temperature of the greenhouse trial was stable at 20°C, while the average temperature in Uppsala (Sweden) varies from 13 to 22°C in July, which is when 50% of MPR was observed (Skålin, 2017). Since high temperature improves potato growth as well as sap flow, the movement of the virus in the plant should be facilitated in high temperature conditions (Burt, 1964, Benoit et al., 1983). All in all, the comparison of our MPR-trial with previous trials stresses the importance of various factors potentially affecting MPR, namely the susceptibility of the cultivar, the method of inoculation and the growing conditions.

Basky and Almasi (2005) previously observed that after primary infection of the sprouts, the virus was able to reach the other stems of the same mother tuber. Our results confirmed this observation, and additionally showed that the probability of stem infection through the vascular system of the mother tuber is significantly reduced with plant age. As mentioned above, the ELISA method used for this experiment may have missed some cases of PVY infections in part of the stems due to virus titers below the sensitivity threshold of the ELISA. However, a post-harvest analysis of progeny tubers grown from non-inoculated stems reveals that the percentage of infected tubers decreased sharply for late inoculations. These results are consistent with the ones obtained for the stems. Thus, our observations suggest a second mechanism of protection associated with mature plant resistance. This mechanism involves the

vascular system of the mother tuber acting as a non-permanent bridge between the growing stems of the plant, and can be seen as natural bottlenecks that lower the transmission of the virus from one stem to the others through the vascular system of the mother tuber: a temporal restriction and a physical restriction (Figure 30).

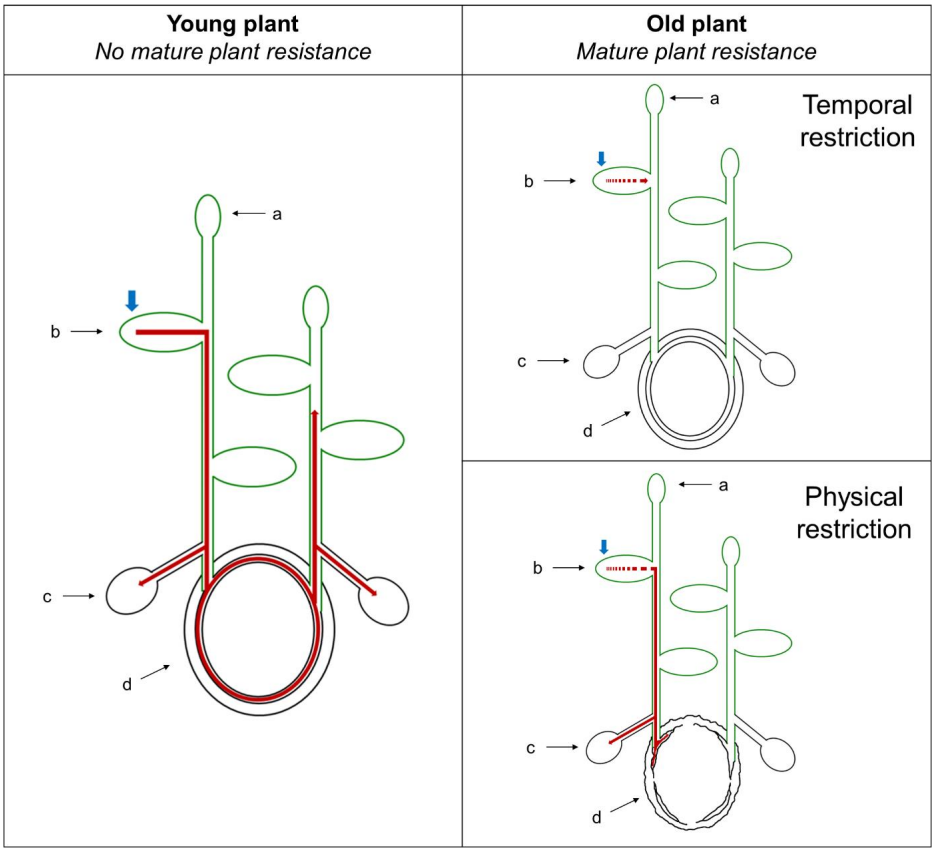


*Figure 30: Movement of PVY in the vascular system of the plant (in red) in a young and in an old plant. The blue arrow shows the location of the inoculation. The solid red lines indicate a rapid movement of the virus in the vascular system of the plant and the dotted lines indicate a slow movement of the virus. Legend: (a) the apical bud, (b) a leaf, (c) a progeny tuber and (d) the mother tuber.*

Temporal restriction occurs when the translocation process occurring late in the growing season is too slow and, hence, the virus does not have “enough time” to reach the mother tuber before haulm killing. As it has been shown previously, cell-to-cell movement is slowed down in mature plants (Draper et al., 2002, Valkonen & Rokka, 1998). In a case of late infection, the virus will thus take more time to reach the phloem parenchyma before being transported on a longer distance by the movement of the sieve elements (Carrington et al., 1996). If the virus particles are not able to reach the phloem before haulm killing and plant desiccation, the virus will not be carried further to the other stems, and will not be able to infect them as well as their related progeny tubers. Our study supports this hypothesis, as for late infection (56 DAE), the probability of a healthy stem to get infected by virus particles drained through the mother tuber is low (a 13% probability in our trials).

On the other hand, *physical restriction* is present when the systemic movement of the sap through the vascular ring of the mother tuber is restricted by the collapsing of the mother tuber. Robert et al. (2000) suggested that this translocation through the mother tuber was slow and probably dependent on the mother tuber’s stage of degeneration. Our results confirm this hypothesis, as it was observed that the probability of virus particles to be transmitted to healthy stems via the mother tuber decreases with plant aging. It was also observed at harvest that most of the mother tubers were decomposed (rotted or mummified), impeding sap transfer from one stem to the other (data not shown).

PVY is efficiently controlled by mineral and vegetable oil sprays (Dupuis et al., 2014b, Powell, 1992). Oil inhibits the acquisition of PVY by aphids and therefore reduces the risk of PVY transmission to healthy plants (Boquel et al., 2013). Oils are usually sprayed in potato fields every seven days. Given the high risk observed of transmitting the virus to all stems of the plant if the infection occurs in the second or third week following emergence, increasing the frequency of sprays during this period could be an option. Two oil sprays a week are carried out by some potato seed growers with success (Jean-Louis Rolot, personal communication), suggesting that more frequent applications might better protect the new leaves of the young, PVY-susceptible, and fast-growing plants. It will be necessary to reduce by half the quantity of oil sprayed for each treatment, in order to avoid any risk of phytotoxicity that an excess of oil may provoke (Boiteau & Singh, 1982, Martin-Lopez et al., 2006).

# CHAPTER 3: EFFICACY OF THREE STRATEGIES BASED ON INSECTICIDE, OIL AND ELICITOR TREATMENTS IN CONTROLLING APHID POPULATIONS AND PVY EPIDEMICS IN POTATO FIELDS

## 1. Preamble

Chapter 2 has shown that the high susceptibility of potato plants soon after their emergence is at least in part due to the lack of mature plant resistance. In this chapter, the efficacy of three PVY and aphids control strategies are compared: i) insecticide spraying (lambda-cyhalothrin), ii) vegetable oil spraying (rapeseed oil) and iii) plant elicitor spraying (acibenzolar-S-methyl). To the best of our knowledge, we are the first to document the role of plant elicitors on the control of PVY in potatoes. All products were sprayed from potato emergence until haulm killing in order to maintain the protection of the crop during the entire season. The efficacy on PVY spread and aphid populations of these control strategies are presented and discussed at the end of this chapter.

This chapter is published in "Journal of Phytopathology" and is referenced as follows: "Dupuis B, Schwaerzel R, Derron J, 2014. *Efficacy of Three Strategies Based on Insecticide, Oil and Elicitor Treatments in Controlling Aphid Populations and Potato virus Y Epidemics in Potato Fields.* Journal of Phytopathology 162, 14-8". The original version of the article is presented in Annex 3, with permission of the "Journal of Phytopathology".

## 2. Introduction

Over the past 50 years, several trials have attempted to identify chemicals that could effectively reduce PVY spread. Such chemicals can be classified into two main groups: insecticides and oils. The majority of insecticides tested were aphicides most of which proved effective in controlling aphid populations but not PVY spread (see Table 8). The only evidence of effective control of PVY spread by insecticides comes from a spray application of imidacloprid on foliage, which achieved on average 36% PVY reduction (Alyokhin et al., 2002), contradicting previous evidence (Collar et al., 1997, Boiteau & Singh, 1999). This lack of effect is mostly due to the non-persistent way in which PVY

is transmitted. When the virus is transmitted non-persistently, the acquisition and inoculation of the virus occur in a matter of seconds, consequently it is difficult to expose the insect vector to a lethal or behaviour-changing dose of insecticide before the virus is transmitted by the aphid (Perring et al., 1999).

As for the second group of chemicals used to limit PVY spread, numerous trials have shown mineral oil to provide some level of protection. Hence, protection is limited to the foliage surface where oil is applied (Simons et al., 1977). In several European countries, the use of mineral oil is prohibited for ecological reasons or due to its phytotoxicity. Phytotoxicity may occur if mineral oil is mixed with fungicides such as captafol (Bell, 1980) or fluazinam (C. Corre, personal communication). In addition, when oils are sprayed under hot weather conditions, the oil heated by the sun in the sprayer pipes may burn potato leaves and stems (J-L. Rolot, personal communication).

We describe three treatment strategies for the control of aphid populations and PVY spread in-field, based respectively on insecticide, oil, and elicitor applications on foliage. The first strategy involved re-investigating the effect of one insecticide, Karate Zeon® (lambda-cyalothrin; Syngenta®, Basel). The quick-acting effect of this pyrethroid could neutralize the aphid before it has time to transmit. Lambda-cyhalothrin has previously been found ineffective in preventing PVY spread when sprayed according to an aphid threshold (van Toor et al., 2009). We adopted a different application modality, by spraying weekly, starting at plant emergence. This insecticide has been found ineffective by spraying weekly until 42 days after plant emergence (Hansen & Nielsen, 2012); however Basky and Almasi (2005) have shown that massive PVY infections can occur up to 45 days after plant emergence. Therefore, we decided to spray de plants until haulm killing. The second strategy involved testing one formulation of rapeseed oil, Telmion® (Omya AG AGRO®, Oftringen). The third strategy consisted of testing the effect of Bion® (acibenzolar-S-methyl; Syngenta®, Basel), which has never previously been tested for PVY spread control in potatoes. This benzothiadiazole is sold commercially as a fungicide. However, it has some insecticidal properties and also activates the general resistance mechanisms of the plant (Green, 2009). Bion® (Figure 17) is a functional analogue of Salicylic Acid (SA) (Petrov & Andonova, 2012) and thus, has the same role as SA in promoting Systemic Acquired Resistance (SAR) of plants. In this experiment we assess the efficacy of Bion® in controlling PVY spread in the field and, we here refer to it as an elicitor.

# 3. Materials and Methods

## 3.1. Field Location and Management

A two-year field experiment was conducted in Switzerland in lowland conditions (425 to 720m a.s.l.). Plots were planted according to a completely randomized block design, with five replications. Each plot was planted with four rows of 25 plants of the PVY-susceptible cultivar Bintje (Schwaerzel et al., 2009), and surrounded by two rows of the same cultivar acting as a buffer zone. The rows were planted every 75 cm, and within a row, tubers were planted every 33 cm. Each plot presented 4% of secondary infected plants resulting from the planting of four tubers infected by $PVY^{N}$605 isolate (Agroscope PVY collection). The experimental field was managed following standard cultural practices, and haulm killing was done 90 days after planting.

## 3.2. Treatments

The mixture volume sprayed on the plots was equivalent to 300 l/ha. Three commercial products were tested: Karate Zeon (Syngenta) as the insecticide, Telmion (Omya Agro®, Oftringen, Switzerland) as the oil, and Bion® (Syngenta) as the elicitor. A description of the products and the application dosage and frequency is given in Table 16. Each year, an untreated control was added to the experimental design.

*Table 16: Description of the treatment strategies tested: trade name in Switzerland, substance group, composition, dosage, and frequency of treatment.*

| Strategy | Trade name | Company | Substance group | AM* | % AM* | Frequency of treatment |
|---|---|---|---|---|---|---|
| Insecticide | Karate Zeon® | Syngenta®, Basel | Pyrethroid | Lambda-cyhalothrin 100g/l | 0.015 | Weekly from emergence until haulm killing |
| Oil | Telmion® | Omya AG AGRO®, Oftringen | Vegetable oil | Rapeseed oil 779g/l | 3 | Weekly from emergence until haulm killing |
| Elicitor | Bion® | Syngenta®, Basel | Benzothiadiazole | Acibenzolar-S-methyl 500g/kg | 0.01 | Weekly from emergence for 3 weeks and then each 14 days until haulm killing |

** AM = active matter*

## 3.3. Observations

A sample of 100 leaves was taken from the five plots of each treatment (20 leaves/replication). Each leaf was collected from a different plant. This sampling was repeated twice during the cropping period; in year 1, the samplings were made 61 and 67 days after planting, and in year 2, the samplings were collected 68 and 75 days after planting. For each sample, we counted the number of apterae adults of the four most important potato colonizing species in Switzerland: *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* (Thomas), *Aulacorthum solani* (Kaltenbach), and *Aphis nasturtii* (Kaltenbach) (Derron & Goy, 1995). In addition, two tubers were manually collected from each plant four weeks after haulm killing and tested by ELISA (Gugerli & Gehriger, 1980). We used monoclonal antibodies specific to serotype N (Bioreba, Reinach, Switzerland).

## 3.4. Statistics

The two-year results were analyzed using Statistica® (Statsofts, Tulsa, USA). For each leaf sampling, we examined the average aphid count (two replications). The analysis was carried out for the different aphid species separately. The number of aphids was converted using the square root transformation method (Dagnelie, 1975). For each plot, we examined the percentage of PVY infected tubers at harvest (five replications). The percentage of tuber infection was converted using the angular data transformation method (Dagnelie, 1975). For both aphids and PVY analysis, we ran a two-factor (year and treatment) analysis of variance (ANOVA) with an α error level of 0.05 (Gomez & Gomez, 1984). A treatment was considered effective in controlling aphid populations or PVY if the following three conditions were met. First, the results of the ANOVA showed statistically significant differences at the 5% level. Second, the treatment and the control belonged to different homogeneity groups, according to the Newman−Keuls post hoc analysis test (Dagnelie, 1975). Third, the treatment had a positive value of efficacy. Treatment efficacy is given by the following formula: $Efficacy = \left[1 - \left(\frac{treatment\ value}{control\ value}\right)\right] * 100$. The efficacy varies from 0% to 100%, 0% signifying that the treatment had no effect and 100% that it was fully effective. A negative efficacy can be obtained when the result of the treatment is higher than the result of the control.

# 4. Results

## 4.1. Control of Aphid Populations

The effect of the treatment strategies could not be assessed on *A. solani*, because the number of captures was too low to detect any differences between treatments ($F(3;6)=1.57$; $p>0.05$, Figure 31). The oil and the elicitor were not effective in controlling *A. nasturtii* populations, and a high variability was observed in plots treated with oil (from nine to 61 insects sampled on 100 leaves). The insecticide was effective in controlling *A. nasturtii*, only one insect having been sampled on 100 leaves in total. This efficacy was not detected by the post-hoc analysis ($F(3;6)=8.36$; $p<0.05$, Figure 31), but was obvious after pairwise comparison with the control (t-test with 6 df: $p<0.05$). The insecticide was the only strategy effective for controlling *M. euphorbiae* populations ($F(3;6)=1.63$; $p<0.05$). Finally, none of the strategies was effective for controlling *M. persicae* populations ($F(3;6)=0.59$; $p>0.05$).

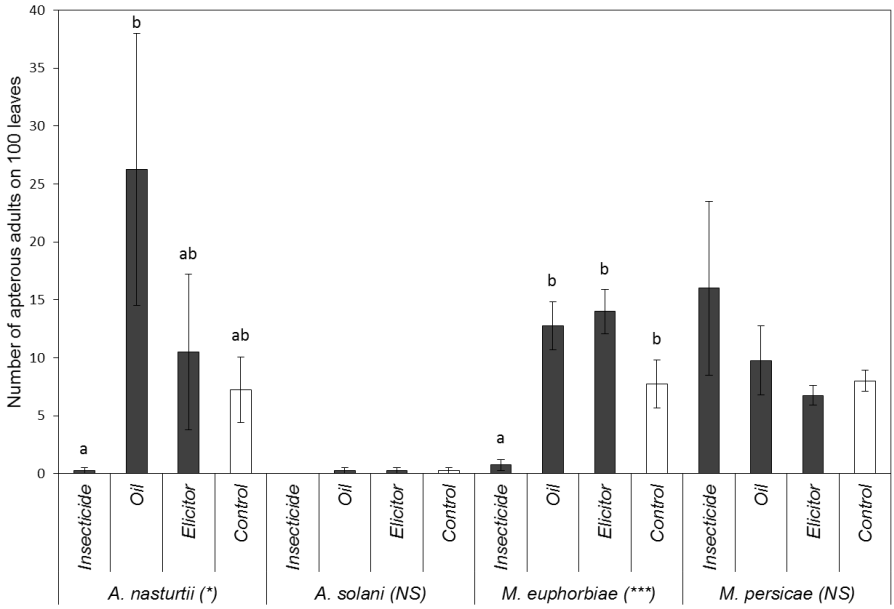


*Figure 31: Average number (years and replication) of adult apterae aphids of major potato virus Y (PVY) vector species on 100 potato leaves. Values with different letters are statistically different according to Newman−Keuls post-hoc analysis (error bars represent standard error of the mean, n=4). The significance of the ANOVA (α=0.05) is given after the name of the aphid species, * if P < 0.05, ** if P < 0.01, *** if P < 0.001 and NS for non-significant.*

### 4.2. Control of PVY Spread

All strategies succeeded in reducing PVY spread (Figure 32). Results suggest that oil effectively prevents PVY spread in the field and that it is significantly more efficient than the elicitor and the insecticide ($F(3;24)=11.78$; $p<0.001$). Oil has an average efficacy of 26% in controlling PVY spread, compared with 14% and 10% for elicitors and insecticide, respectively.

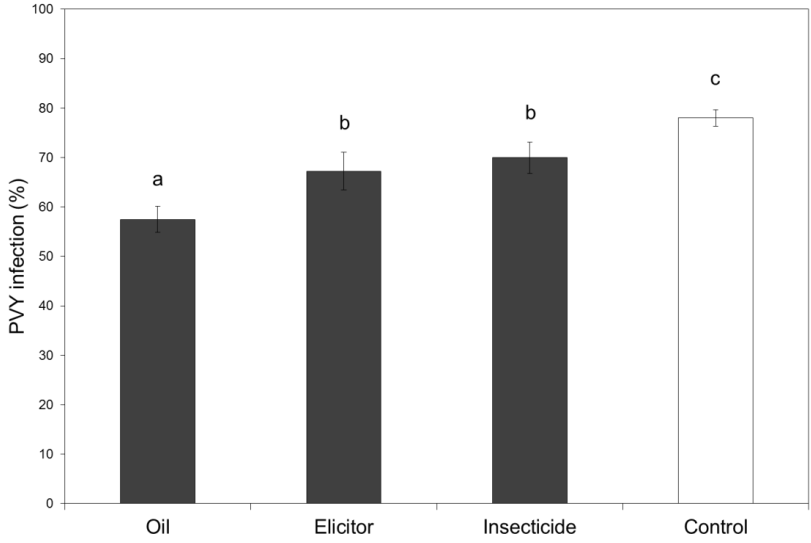


*Figure 32: Average percentage (years and replications) of potato virus Y (PVY) infection of each treatment. Values with different letters are statistically different according to Newman−Keuls post-hoc analysis (error bars represent the standard error of the mean, n=10).*

## 5. Discussion

As expected, Karate Zeon® was effective in controlling aphid populations except for *M. persicae*, the lack of efficacy in this case resulting from a selection of *M. persicae* clones that are probably resistant to the chemical. Many cases of *M. persicae* resistance to pyrethroids have been reported from all parts of Europe (Anstead et al., 2007). No aphicide effect was observed after treatment with the elicitor; hence, the insecticide effect of Bion® mentioned by Green (2009) was not confirmed by this experiment. The failure of rapeseed oil to control aphid populations contradicts

previous studies (Martin-Lopez et al., 2006, Martin et al., 2004). However, this difference may be explained by the fact that those studies were not conducted in open field conditions. In our trial, the persistence of the product may have been reduced because of environmental factors.

All treatments had a statistically significant effect on PVY spread. However, the efficacy of the elicitor (14%) and the insecticide (10%) were too low for them to be considered suitable candidates for use in PVY control. As far as we know, this experiment is the first report of the efficacy of elicitors for the control of PVY spread in potato fields. Acibenzolar-S-methyl, as analogue of salicylic acid (Figure 17) reveals to be able to induce systemic acquired resistance (SAR) of potato against PVY infection. This result is contradictory with previous experiments made with tobacco in which SAR could not be induced against PVY (Pennazio & Roggero, 1988). This result opens new perspectives for PVY control in potato fields using elicitors to promote systemic acquired resistance. Nevertheless, the use of Bion® has some limitations as it reduces the vigour of the plants and may induce yield loss (data not shown). This study showed the efficacy of the vegetable oil to be relatively low, 26%, compared with results obtained by Martin-Lopez et al. (2006), which showed 41% efficacy for refined rapeseed oil. In the same study, the efficacy of rapeseed oil was compared with the efficacy of mineral oil. Mineral oil proved much more effective, with 59% efficacy, similar to the 64% level of protection obtained by Boiteau and Singh (1982). We mentioned above that mineral oil can be phytotoxic; in practice, however, when the application procedures are properly followed, the risk of phytotoxicity is low (Boiteau & Singh, 1982, Martin-Lopez et al., 2006).

In summary, our study confirmed that there is no link between protection against apterae aphids and PVY spread. The elicitor strategy was found to be ineffective for controlling aphid populations and inadequate for controlling PVY spread. Use of insecticide gave incomplete protection from aphid infestations, owing to the selection of aphid-resistant clones. The insecticide gave too little protection against PVY spread for it to be considered a suitable candidate for the purpose. Finally, oil had no effect on aphid populations, but was the best option to reduce PVY spread in potato fields.

# CHAPTER 4: CONTROL OF PVY IN POTATOES BY OIL SPRAYING, STRAW MULCHING, AND INTERCROPPING

## 1. Preamble

Chapter 3 demonstrated that oil spraying was more efficient than insecticides and plant elicitors in controlling PVY spread in the field. However, the efficacy of oil was relatively low (<30%) suggesting the need to improve potatoes protection with more efficient strategies. It was discussed in Chapter 3 that the efficacy of oil could probably be increased by changing the nature of the oil. In this chapter, paraffinic mineral oil will be used instead of rapeseed oil. The objective in this chapter is to compare the efficacy of two other strategies, straw mulching and intercropping, with mineral oil spraying as reference strategy. To our knowledge, we are first to examine the effect of intercropping on PVY spread in a multiyear trial. The combination of straw mulching and mineral oil spraying is also tested in order to identify potential synergies between both strategies.

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## 2. Introduction

Dupuis et al. (2010) hypothesized that potato crops could benefit from the two effects of crop borders, namely the "virus sink" and the "mechanical barrier" effects (Boiteau et al., 2009), by using the intercropping technique. The advantage over border crops is that intercropping can be used on small fields without wasting land. The principle is that the intercrop plants surrounding the potato plants play the role of a mechanical barrier for aphids and also act as a virus sink, with aphids landing on the associated crop. Intercropping may also limit the spread of PVY by reducing gaps in the crop canopy, which are known to favor PVY spread (Davis et al., 2009). The results of a first trial with oat intercropping were reported by Dupuis et al. (2010) and demonstrated that intercropping is efficient in controlling the spread of PVY in the field.

Few studies have examined the combined effect of mineral oil and straw mulching for the control of PVY. In a one-year trial, Dupuis et al. (2010) showed that the association of mineral oil with mulch has beneficial effects in terms of controlling in-field PVY transmission. Another similar one-year trial on the association of mineral oil and straw mulching was reported by Kirchner et al. (2014); however, these authors showed that this combination did not further reduce the incidence of PVY. Given the contradictory results of those two studies, further data are needed in order to clarify the real efficacy of the association of mineral oil with straw mulching in controlling PVY spread. The purpose of the present study is to provide new data on this specific topic. In addition, different amounts of straw were compared in order to define the amount of straw that would be optimal in controlling PVY spread while remaining economically sustainable for the potato seed growers. The study also examines the efficacy of intercropping with oat (*Avena sativa*) and hairy vetch (*Vicia villosa*) as a way to control in-field PVY spread.

# 3. Materials and Methods

## 3.1. Experimental Site and Crop Management

Field trials were conducted at the Agroscope Agricultural Research station in Changins (Nyon, Switzerland) from 2011 to 2014. For each of the four trials, a 0.09- to 0.12-ha field was planted with the cultivar Charlotte, known to be highly susceptible to PVY (Schwaerzel et al., 2014, Steinger et al., 2014), at a seeding rate of one seed piece per 0.33 m with a 0.75 m row spacing. High-grade seed-lots (basic or pre-basic seed) were used for planting in which no PVY was detected in diagnostic tests carried out by the Swiss certification authorities. Individual plots (treatment replicates) were four rows wide and 25 plants long (8.25 m) and were surrounded by a buffer row planted with Lady Christl, a cultivar that is highly resistant to PVY (Schwaerzel et al., 2014). Three seed pieces of cultivar Charlotte infected with strain $PVY^{NTN}$1317 (Agroscope collection) were planted in central locations within each plot (position 12 in row 2, positions 6 and 19 in row 3) to serve as a source of virus inoculum (3% initial infection level). The field was irrigated when necessary but otherwise managed according to usual cultural practices without spraying insecticides. Haulm killing was done with two herbicide treatments; the first treatment was made around 80 days after planting

(Reglone® from Syngenta©; 200 g/l of diquat) and the second treatment one week later (Spotlight Plus® from Syngenta©; 60 g/l of carfentrazone-ethyl).

## 3.2. Oil and Straw Treatments

Two treatments were applied during the four years of trials: mineral oil spraying and mulching with wheat (*Triticum æstivum*) straw. Mineral oil (Zofal D® from Stähler; 830 g/l of mineral oil) was sprayed weekly at a dose of 7l/ha (400l/ha of mixture) with a backpack sprayer (Birchmeier M125) from 80% of emergence until haulm killing. Straw was applied by hand on each row at a quantity of 2.5 t/ha immediately after planting (Figure 33 B). The combination of mineral oil and straw mulching was also tested in each trial except for the one in 2011. Treatments were applied in a randomized complete block design with four replicate plots per trial.

Additional plots were set up in 2013 and 2014 within the same field to evaluate if the amount of straw per unit area (straw quantity) could be reduced without affecting the rate of PVY transmission. Four quantities were tested: 1 t/ha, 1.5 t/ha, 2 t/ha, and 2.5 t/ha.

## 3.3. Intercropping

The effect of intercropping on PVY transmission was evaluated in 2011 and 2012. Hairy vetch (*Vicia villosa*) and oat (*Avena sativa*) were sown by broadcasting the seeds on the rows at the time of potato planting (Figure 33 C and D) (60 kg/ha for oat and 50 kg/ha for hairy vetch). Growth of oat was stopped after three weeks (stage 23 on the BBCH scale) to limit competition with the potato crop for water and nutrients. This was done with two applications of Tetraloxidim (Aramo® from BASF©; 50 g/l of tepraloxydim) at an interval of seven days.

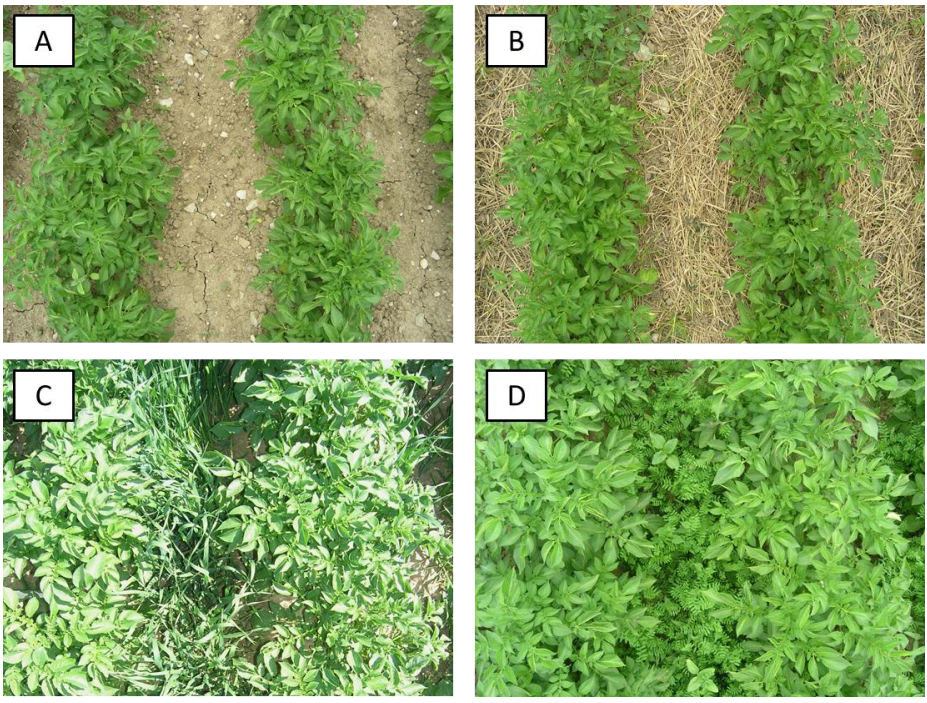


*Figure 33: Details of the experimental plots in 2012, one month after potato emergence: (A) bare soil plot; (B) mulched plot; (C) oat intercropping plot; and (C) vetch intercropping plot.*

## 3.4. Measurement of Crop Cover

The percentage of plot surface covered by the foliage of the growing potato plants as well as the surface covered by the associated crop or mulch was estimated at weekly intervals using the point quadrats method (Wilson, 1963).

## 3.5. PVY Infections in Tubers

Four weeks after haulm killing, two tubers were manually sampled from each plant. PVY infections were tested in sprouting tubers using an enzyme-linked immunosorbent assay (ELISA) as described in Gugerli and Gehriger (1980). Monoclonal antibodies specific for $PVY^N$ isolates (monoclonal PVY IgG from Bioreba AG, Switzerland) were used. PVY incidence was calculated as the percentage of PVY-infected plants per plot.

## 3.6. Tuber Yield

After mechanical harvest of each plot with a one row Samro harvester, the total yield was measured.

## 3.7. Aphid Captures

Aphids were captured using green pan traps (#006400 CSS reference). The traps (26 cm in diameter) were filled with water containing 1-3% detergent, and placed in the center of each plot. Green rather than yellow traps were used because the former may provide more realistic estimates of rates of aphid landings on green potato foliage (Avinent et al., 1991, Boiteau, 1990). Traps were emptied twice a week, and the total number of winged aphids was counted on a weekly basis for 6 weeks. The most prevalent aphid species (>1% of total captures) were identified and counted individually.

## 3.8. Statistical Analysis

A general linear model was used to evaluate the effects of oil spraying (factor with two levels), straw mulching (factor with two levels), and the interaction of the two on the post-harvest PVY incidence in tubers and on total tuber yield. Year and block (nested within year) were included in the model as blocking factors. The interaction term oil x straw was analyzed using trials conducted in 2012-2014 (the experimental design in 2011 omitted the combined applications of oil and straw). Separate general linear models were used to evaluate the effect of straw quantity (factor with five levels) and intercropping (factor with three levels) on post-harvest PVY incidence in tubers and on yield. A Dunnett's multiple comparison procedure was used to compare the straw and intercrop treatments to the control (bare soil plots). Residuals were always checked for normality and homogeneity of variances. Data on PVY incidence and yield were not transformed prior to analysis. Data on total aphid counts was square-root transformed to better satisfy the assumptions of the general linear model. Data on *weekly* aphids captures did not always satisfy model assumptions, and in these cases *p*-values were calculated based on a permutation test. All statistical analyses were performed using R software version 3.2 with the packages *car*, *multcomp, lmPerm*, and *lsmeans*.

# 4. Results

## 4.1. PVY Incidence

Both oil spraying and straw mulching significantly decreased post-harvest PVY incidence, but the strength of the treatment effects varied among years (Table 17). Averaged over the four years of the study, straw reduced PVY incidence by 27%, with a range of 0% to 54% depending on the year (Figure 34). Oil reduced PVY incidence by an average of 43%, with a range of 27% to 67% (Figure 34). The oil x straw interaction evaluated with pooled data from years 2012-2014 was statistically non-significant ($p$=0.83, Table 17). However, the three-way interaction with the year was marginally significant ($p$=0.056, Table 17). A year-wise analysis revealed a relatively complex picture: the combined effects of straw and oil were largely additive in 2013 but not in 2014 (Figure 34). The estimated efficacy of the combined oil-straw treatment (assuming an absence of oil x straw interactions) was an average of 59%, with a range of 43% to 73% depending on the year.

*Table 17: Summary of the ANOVA results of PVY incidence in the harvested tubers, tuber yield, and aphid landings. F-values are given along with stars indicating the significance of the test. Note that ANOVA models also included a term due to block nested within year (results not shown). The interaction terms straw x oil and straw x oil x year were modelled using a subset of the data (excluding the year 2011).*

| Source of variation | PVY incidence | Tuber yield | Aphid captures |
|---|---|---|---|
| Straw mulching | 30.2 *** | 0.2 | 9.1 ** |
| Oil spraying | 108.7 *** | 3 | 0.4 |
| Year | 50.8 *** | 126.7 *** | 82.5 *** |
| Straw x Oil | 0.1 | 0.2 | – |
| Straw x Year | 5.8 ** | 3.1 * | 6.3 ** |
| Oil x Year | 3.5 * | 1.1 | 0.6 |
| Straw x Oil x Year | 3.2 | 0.4 | – |
| Intercropping | 5.6 * | 4.1 * | 0.5 |
| Intercrop x Year | 3.4 | 0.1 | 0.3 |

* p<0.05; ** p<0.01; *** p<0.001

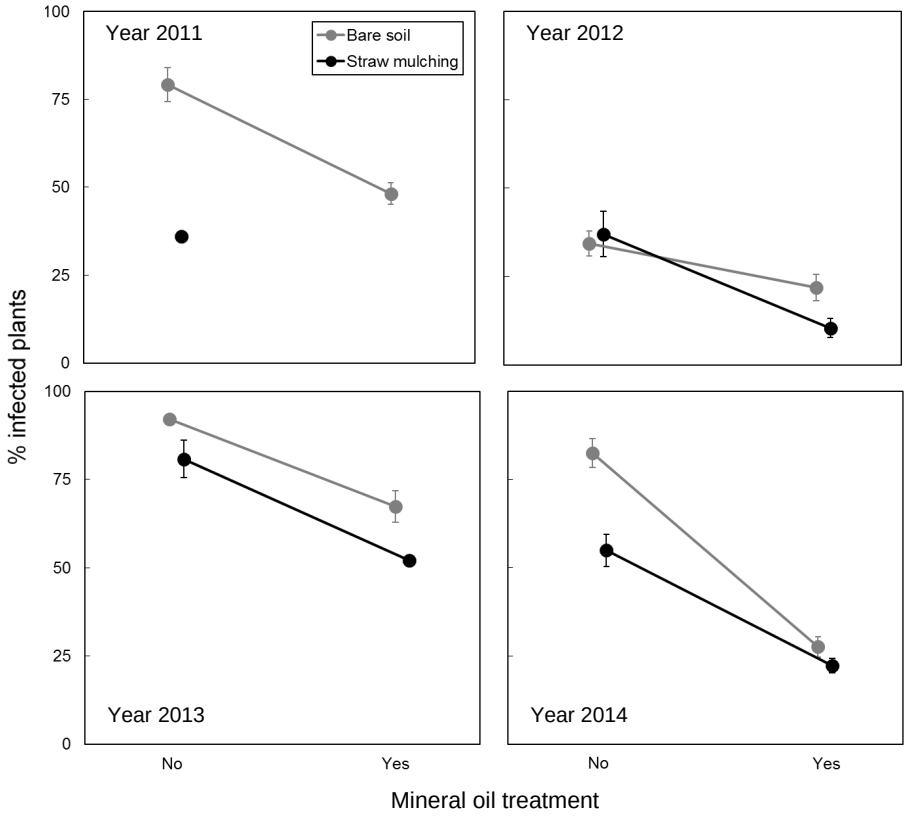


*Figure 34: Effect of oil spraying and straw mulching on the incidence of PVY in potato tubers. Mean values of four years of experiments (n=4 per year). Error bars represent ± 1 SE.*

The effect of different quantities of straw on PVY incidence was evaluated in the 2013 and 2014 trials, which were the years in which high rates of PVY transmission occurred in the untreated control plots. The main effect of the straw quantity was significant ($F_{4,24}$=4.7; $p$<0.01), and there was no significant interaction with year ($F_{4,24}$=1.4; $p$=0.28). When comparing the PVY incidence in plots with different quantity of straw with the incidence of PVY in the bare soil plots (Dunnett test), it appeared that it was significantly lower only for the one with the highest quantity (2.5 t/ha) ($p$<0.01, Figure 35 A).

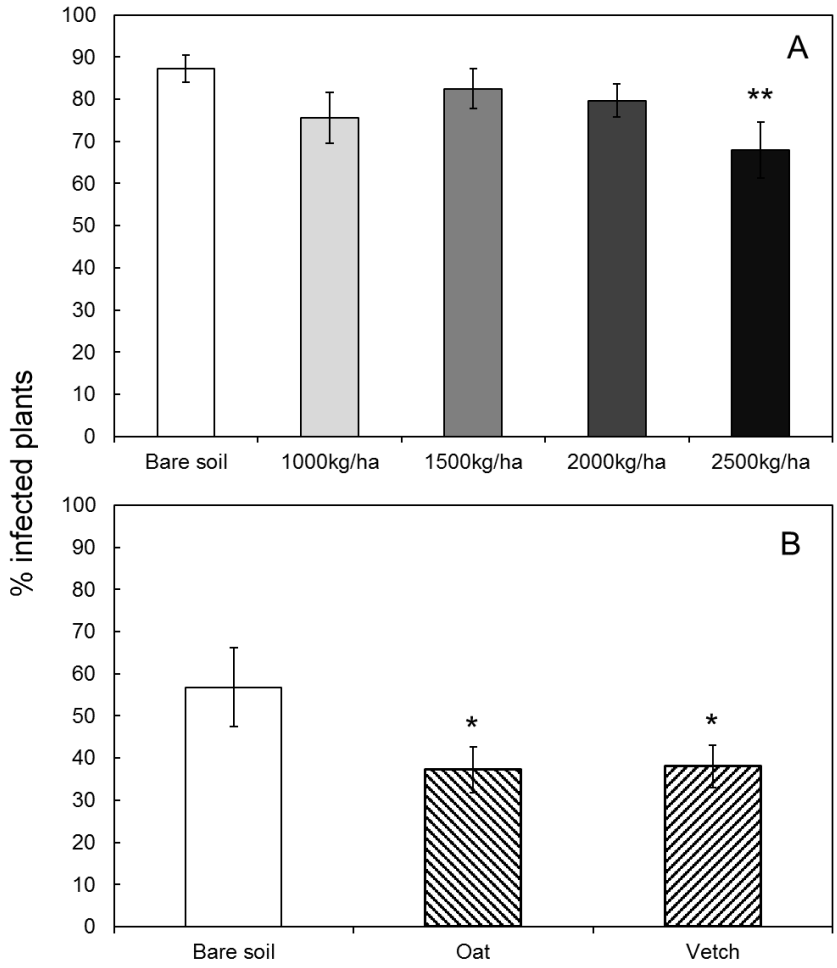


*Figure 35: Mean percentage of PVY-infected tubers (± 1 SE) in each treatment group. Stars indicate significant differences (* p<0.05; ** p<0.01) between the treatment and the bare soil control (Dunnett's multiple comparison procedure): (A) PVY-infected plants in relation to straw quantity, average of the 4 replicates over the two years 2013 and 2014 (n=8); (B) PVY-infected tubers in relation to the method of intercropping, average of the 4 replicates over the two years 2011 and 2012 (n=8).*

Intercropping with oat and vetch was studied in 2011 and 2012. The main effect of intercropping was significant ($p$<0.05), and there was no significant interaction with the year ($p$=0.07). Multiple comparisons of treatment means with the bare soil control (Dunnett test) showed that the effect was significant for both intercropping methods.

Averaged over the two years, the reduction of the PVY incidence was 34% for oat-intercropped plots and 33% for vetch-intercropped plots ($p$<0.05, Figure 35 B).

## 4.2. Tuber Yield

The effect of straw cover on total tuber yield varied among years (Table 17). Plots with straw had a lower yield in 2012 (-18%, $p$<0.05), but a higher yield in 2013 (+25%, $p$=0.07), while no significant difference was observed in 2011 (+1.5%, $p$=0.79) and 2014 (+3%, $p$=0.54) (Figure 36 A).

Oil spraying decreased the total yield in the four years by 10% on average, a non-significant effect ($p$=0.09; Table 17, Figure 36 B).

Averaged over the two years, both intercropping treatments slightly reduced total yield compared with the control plots; a 13% reduction was observed with oats and 11% with vetch (Dunnet test: oat: $p$<0.05; vetch $p$<0.05) (Figure 36 A).

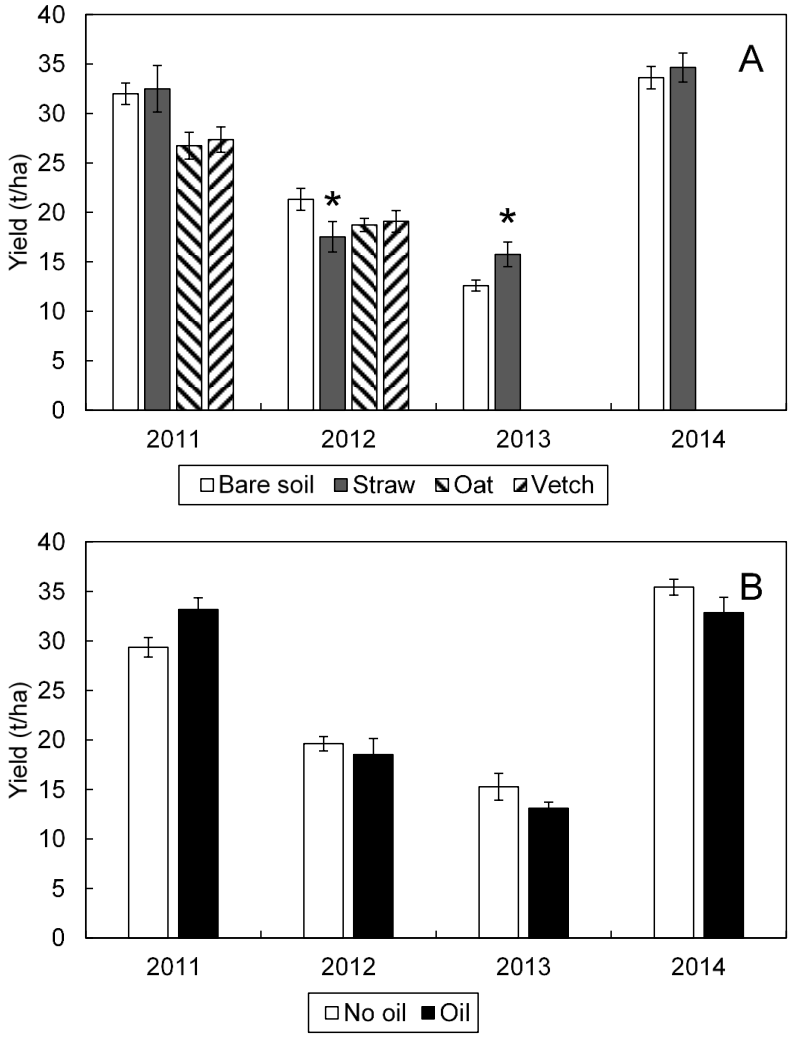


*Figure 36: Mean tuber yield (± 1 SE) in each treatment group. Stars indicate significant differences (* p<0.05) between the treatment and the bare soil/no oil control (Dunnett's multiple comparison procedure): (A) Tuber yield in relation to soil cover, average of the 4 to 8 replicates over the four years 2011 to 2014 (n=8 for bare soil 2011-2014 and for straw 2012-2014; n=4 for the rest) ; (B) Tuber yield in relation to the mineral oil treatment, average of the 4 to 16 replicates over the four years 2011 to 2014 (n=16 for no oil 2011-2016; n=4 for oil 2011 and n=8 for the rest).*

## 4.3. Temporal Dynamics of Soil Surface Cover

Because early-season colonization of potato plants by aphids is thought to be determined by the visual contrast between the crop plants and the surface of the bare soil, the temporal change in soil surface cover was monitored for the different treatments. Figure 37 shows that straw mulch covered a large fraction of the bare soil surface at the time of potato emergence, but the percentage covered by straw rapidly declined in the three weeks after emergence, when the potato foliage is growing and rapidly covering the soil. In contrast, the vetch and oat foliage made only a small contribution to soil surface cover at the time of potato emergence, but their share steadily increased during the course of the growing season.

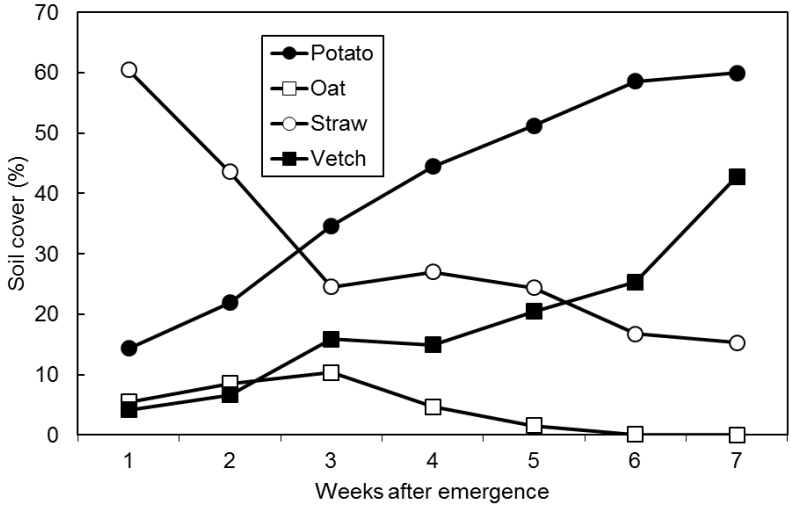


*Figure 37: Temporal change in the percentage of soil covered by the foliage of the potato crop and by the associated straw mulch or intercropped crops (hairy vetch and oat treated with an herbicide three weeks after potato emergence). Average values of 2011 and 2012 trials (n-bare soil=16; n-straw=12; n-oat=8, n-vetch=8).*

## 4.4. Aphid Catches

The number of aphids trapped in each plot was monitored until six weeks after potato emergence. Oil spraying did not influence the total aphid numbers in any year (Table 17). Therefore, the data of oil-sprayed plots and bare soil plots were pooled for subsequent analysis of aphid counts. Similarly, intercropping did not affect the total

aphid numbers when compared with the control plots (Table 17). The cumulative number of all aphids trapped was significantly influenced by straw application, but the effect varied by year (Table 17). Plots with straw mulch had significantly lower aphid counts in 2011 (-45%, $p$<0.001) and 2013 (-30%, $p$<0.05) but not in the other two years (2012: +23%, $p$=0.10; 2014: -9%, $p$=0.44). Analysis of the temporal dynamics within years showed that in the two years in which straw reduced aphid counts, the difference was most pronounced during the four weeks after potato emergence but declined for later trapping dates (Figure 38). The effect of straw also varied considerably among aphid species, although no species was unaffected by straw in all four years (Table 18).

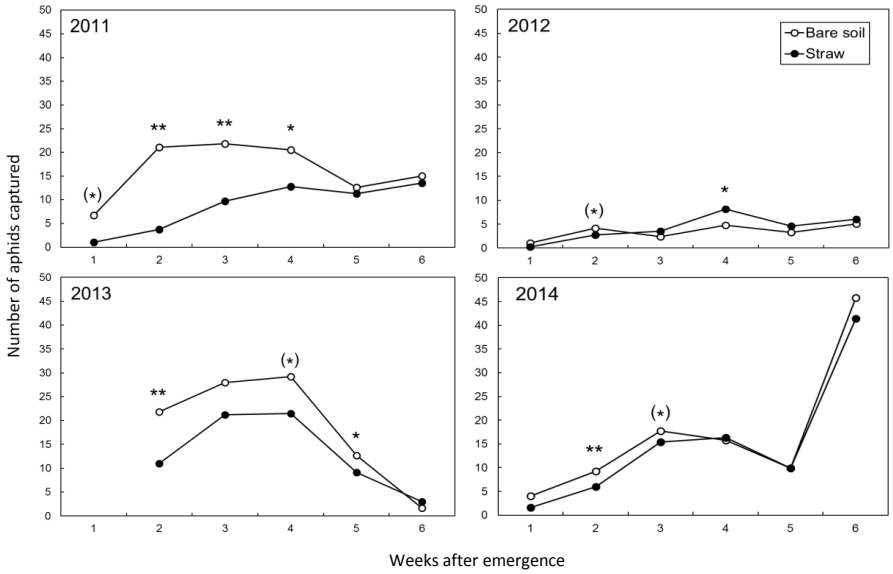


*Figure 38: Mean values of the weekly captures of aphids in plots with straw mulch and in bare soil plots. 2011: n straw=4 and n bare soil=8, 2012-2014: n=8. Stars indicate significant differences of aphids captured between treatment and control (NS: non-significant; (*) p<0.1; * p<0.05; ** p<0.01).*

The main effect of straw quantity on total aphid counts was significant ($p$<0.01), and there was no significant interaction with year ($p$=0.95). Multiple comparisons of treatment means with the bare soil control (Dunnett test) showed that the straw effect was only significant at the highest quantity (Figure 39).

*Table 18: Sum of captures of the eight most prevalent aphid species in plots with straw mulching (2.5 t/ha) and with bare soil six weeks after potato emergence in the four years of trials (n=8 per trial). Significance of the straw effect and the straw x year interaction in the general linear model are indicated.*

| Aphid species | Soil cover | 2011 | 2012 | 2013 | 2014 | Straw effect | Straw x year effect |
|---|---|---|---|---|---|---|---|
| *Acyrthosiphon pisum* | bare soil | 56 | 5 | 30 | 16 | NS | * |
| | straw | 46 | 14 | 26 | 6 | | |
| *Aphis fabae* group | bare soil | 36 | 27 | 74 | 46 | ** | NS |
| | straw | 14 | 28 | 45 | 40 | | |
| *Aphis* spp. | bare soil | 106 | 55 | 107 | 263 | NS | *** |
| | straw | 76 | 97 | 11 | 230 | | |
| *Brachycaudus helichrysi* | bare soil | 38 | 7 | 3 | 17 | *** | NS |
| | straw | 6 | 7 | 1 | 8 | | |
| *Metopolophium dirhodum* | bare soil | 82 | 2 | 61 | 87 | * | * |
| | straw | 46 | 0 | 39 | 84 | | |
| *Myzus persicae* | bare soil | 250 | 0 | 38 | 54 | *** | *** |
| | straw | 135 | 5 | 11 | 48 | | |
| *Rhopalosiphum padi* | bare soil | 45 | 1 | 30 | 256 | NS | * |
| | straw | 8 | 1 | 31 | 207 | | |
| *Sitobion avenae* | bare soil | 41 | 4 | 110 | 22 | * | NS |
| | straw | 20 | 1 | 72 | 19 | | |
| *Total aphids* | bare soil | 756 | 164 | 537 | 829 | *** | ** |
| | straw | 412 | 202 | 367 | 731 | | |

*NS: non-significant; * p<0.05; ** p<0.01; *** p<0.001*

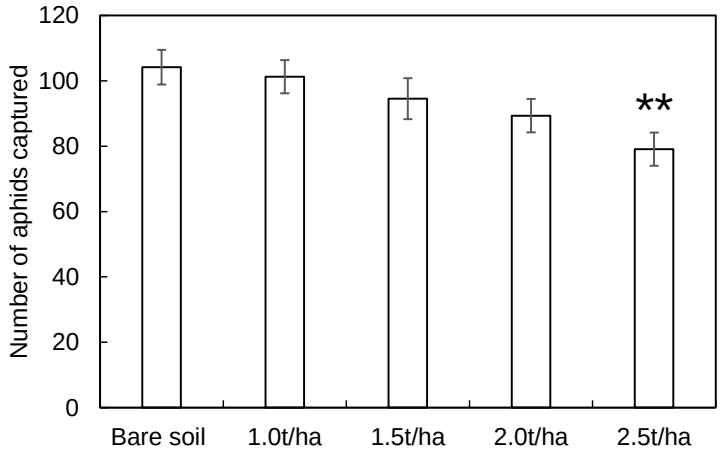


*Figure 39: Mean values (± 1 SE) of 6 weeks of captures of aphids in plots with different quantities of straw mulch and in bare soil plots, average of 2013 and 2014 (n=16 for bare soil and 2.5t/ha and n=8 for the rest). Stars indicate significant differences in the number of aphids captured between treatment and control (** p<0.01).*

## 5. Discussion

Our study evaluated the efficacy of virus control measures, including oil spraying, straw mulching, and intercropping aimed at reducing PVY incidence in a potato crop without affecting tuber yield. There was a particular focus on those potential synergistic effects that may arise from joint applications.

Weekly applications of mineral oil from the time of crop emergence is a common but labor-intensive virus management measure in seed potatoes and is known to reduce PVY transmission by roughly half, albeit with important variation among environmental conditions (Martin-Lopez et al., 2006, Boiteau & Singh, 1982, Kirchner et al., 2014, Hansen & Nielsen, 2012, Steinger et al., 2014, Boiteau et al., 2009, Fageria et al., 2014a). In the present study, mineral oil applications reduced PVY incidence by an average of 43%, with considerable year-to-year variation, which is consistent with the literature. Oil is not thought to interfere with probing and feeding behavior of aphids (Qiu & Pirone, 1989, Vanderveken, 1968), but it is likely to reduce virus acquisition (Boquel et al., 2013, Bradley et al., 1962, Simons & Zitter, 1980) and retention (Wang & Pirone, 1996, Wróbel, 2009) by aphid vectors. Moreover, potato plants treated with

mineral oil were found to produce volatiles that act as a repellent against aphids, at least in the short term (Ameline et al., 2010). In our study, however, we found no difference in aphid landings between oil-treated and untreated plots. This suggests that mineral oil is acting exclusively after aphid landing rather than affecting the aphids' landing behavior.

The observed year-to-year variation in the efficacy of mineral oil may be related to the dynamics of growth of the foliage. The increase in leaf area is the highest after plant emergence and declines until senescence (Shands et al., 1970). As new leaves emerging after oil sprays are susceptible to PVY infection (Fageria et al., 2014a), potato crops may face a relatively higher infection risk during the early stages of crop development compared to later in the season, assuming the common practice of constant (weekly) applications of mineral oil. Consistent with this reasoning, a lower efficacy (27-39%) of oil spraying was found in the years 2011 and 2013, when aphid activity was high early in the season, and a higher efficacy (67%) in the year 2014, when aphid vectors appeared relatively late in the season. Virus control by oil spraying may therefore be optimized by increasing the application frequency during the first few weeks after crop emergence at the expense of later sprayings.

No significant yield reduction was measured in the plots sprayed with mineral oil. Nevertheless, slight phytotoxicity of mineral oil has been observed in other experiments (Boiteau & Singh, 1982, Martin-Lopez et al., 2006), emphasizing the necessity for a cautious use of mineral oil to control PVY (Kirchner et al., 2014).

Straw mulching is less common in practice but proved to be efficient in controlling PVY spread (Dupuis et al., 2010, Heimbach et al., 2004, Saucke & Doring, 2004, Kirchner et al., 2014). The reason that straw mulch scattering is not widely applied is that little information is available on how effective it is at the field scale. The method was previously shown to reduce PVY incidence by roughly 30%, albeit with large year-to-year variation (Kirchner et al., 2014, Saucke & Doring, 2004). The average reduction of PVY incidence in our experiment was 27%, and the efficacy of straw mulching varied substantially among the years of the experiment. This variation may be explained by the fact that straw mulch is not effective during the whole growing period; it is primarily effective from the emergence of the plants until the closure of the canopy (Heimbach et al., 2004, Kirchner et al., 2014, Saucke & Doring, 2004), when straw is visible for winged aphids and thus has an effect on their landing behavior. The results are

consistent with this observation, as lower aphid landings were observed on mulched plots from two to four weeks after potato emergence. Afterwards a relative increase of aphid captures occurred when the crop canopy covered the soil. The ultraviolet reflectance of straw and bare soil are very similar (Döring et al., 2004). Thus, the effect of straw on aphid behavior can not be attributed to UV repellency but to the lower optical contrast between the plant canopy and the straw compared with the contrast between the plant canopy and the bare soil (Döring et al., 2004). This work also emphasizes the effect of straw on the selection of aphid species. This means that the efficacy of the straw may be dependent on the attraction of specific aphid species to the visual stimuli it produces.

Different quantities of straw were tested in this work, and optimum PVY control was obtained with the highest quantity, 2.5 t/ha, with an average efficacy of 27%. It was demonstrated that 2.5 t/ha covered 60% of the soil at potato emergence and that it was the minimum amount needed to reduce aphid captures in the crop. It can be hypothesized that a higher cover of the soil would better control aphid landings early in the season and better protect the plots against PVY spread. This hypothesis is consistent with the results of other trials; Saucke and Doring (2004) used a larger amount of straw, 3.5 t/ha, and obtained an average efficacy of 31% (a 3-year trial in Germany) while Kirchner et al. (2014) used 5.5 t/ha with an efficacy of 44% (a 3-year trial in Finland).

The present work demonstrates the complementarity of mineral oil and straw mulching in controlling PVY spread, as no interaction between the effects of oil and straw mulching was detected. In the experiment, the joint effect of straw mulching and mineral oil spraying resulted in a 59% reduction of PVY incidence compared to 43% and 27% for mineral oil and straw mulching, respectively, when used separately. Additionally, it must be stressed that in the experiments, the efficacy of the association of mineral oil and straw mulching never fell below 43% in any year, compared with a low point of 27% and 0%, respectively, with oil or straw used alone. This complementarity of the two control measures may be explained by the evolution of the crop canopy during the growing season. As outlined above, oil sprayings may provide little protection from PVY infection during periods of rapid foliage expansion at the beginning of the growing season. In contrast, straw mulch is thought to be most effective in preventing aphid landings — and hence virus infections — during early

growth stages (i.e., when the visual contrast between the soil and the foliage is highest in the crops). The results suggest that this complementarity of the mode of action reduces the year-to-year variation observed with oil and straw when used alone as PVY control agents. This combination of methods significantly reduces the annual risk of crop failure caused by massive PVY infection in seed potato production. The cost of straw mulching is approximately half of the price of mineral oil treatments (Table 19; calculation made in the Swiss context in 2016).

The study also showed that the efficacy of intercropping was not due to any reduction in the aphid landings in the crop and may be due to the "virus sink" effect through which the aphids will lose their PVY inoculum after several probes on a non-host plant (Wróbel, 2007, Boiteau et al., 2009). The efficacy of oat and vetch intercropping were very similar and comparable to the efficacy of straw mulching. Nevertheless, intercropping reduced tuber yield, whereas the average effect of straw mulching on yield was low. Therefore, straw mulching is a better candidate to complement the effect of mineral oil for a better protection of potato fields against PVY spread.

In conclusion, this work showed that it was possible to increase the protection of potato fields against PVY spread by combining control strategies with different modes of action that complement each other, such as mulching, oil spraying, and intercropping.

# GENERAL DISCUSSION AND CONCLUSIONS

The objective of this thesis is to provide a better understanding of the epidemiology of PVY and to propose innovative control strategies adapted to seed-potato growers in a given area. The hypothesis is that current strategies can be improved with a better understanding of their mode of action and that new strategies can be found based on a better knowledge of PVY epidemiology. This thesis aims to answer four questions:

(i) What is the role of the resistance status of the potato cultivars against PVY strains in the selection and the biodiversity of PVY strains in the environment?

(ii) Does the vascular movement of PVY strains in the plant influence the epidemiology of PVY in the field?

(iii) Which is the most effective strategy to control PVY spread in the field?

(iv) Can we reduce PVY spread through the combined use of different control strategies?.

## 1. What is the role of the resistance status of the potato cultivars against PVY strains in the selection and the biodiversity of PVY strains in the environment?

In this thesis, we hypothesised that the cultivar resistance/susceptibility levels to PVY influences the diversity of PVY strains in potato crops.

The recent surveys of PVY isolates in Switzerland presented in Chapter 1 revealed an increase of the prevalence of $PVY^{N-Wi}$ strain, and in the meantime, a decrease of the prevalence of $PVY^{NTN}$ strain. In Germany (Lindner et al., 2015), Canada (Gray et al., 2010), and the United States (Stewart Gray, personal communication) an increase of $PVY^{N-Wi}$ is also observed. Various factors may explain this increase of prevalence of $PVY^{N-Wi}$ strain. Chapter 2 showed that $PVY^{N-Wi}$ isolates are more efficient than $PVY^{NTN}$ isolates in translocating to the progeny tubers, which is likely to be a competitive advantage compared to $PVY^{NTN}$ strains. While the vascular spread of $PVY^{N-Wi}$ is more efficient, there is no clear evidence that $PVY^{N-Wi}$ is better transmitted by aphids than $PVY^{NTN}$ (Srinivasan et al., 2012, Mondal et al., 2016, Verbeek et al., 2010). This might explain why the prevalence of $PVY^{N-Wi}$ does not sharply increase in the surveys.

Finally, we showed that the expression of the symptoms of a PVY strain depends on the cultivar infected. In our experiment, plants of cv. Agria infected by $PVY^{N\text{-}Wi}$ displayed less severe symptoms than plants of the same cultivar infected by $PVY^{NTN}$. This result is consistent with other experiments performed with different cultivars. In the experiment of Chrzanowska (1991), cv. Elipsa did not display symptoms when infected by $PVY^{N\text{-}Wi}$. Plants displaying mild symptoms are less likely to be missed during crop inspection, which is a factor that might contribute to the spread of a given strain in the environment. Additional experiments are needed in field conditions i.e. aphid-borne *natural* infection of several varieties with isolates of $PVY^{NTN}$ and $PVY^{N\text{-}Wi}$ to clarify this. The surveys also revealed that the susceptibility of the cultivars varies depending on the PVY strain, as cv. Agria was found to be more frequently infected by $PVY^{N\text{-}Wi}$ (81% of prevalence), whereas this strain only accounts for 19% of the prevalence of PVY strains in Switzerland. This observation was consistent with the results of another survey performed in Germany (Lindner et al., 2015), and with a greenhouse trial presented in Chapter 2, in which $PVY^{NTN}$ elicit an HR on Agria after mechanical inoculation while $PVY^{N\text{-}Wi}$ does not; therefore, Agria is more resistant to $PVY^{NTN}$ compared to $PVY^{N\text{-}Wi}$. This contrasted susceptibility has consequences on the strategy to apply for the detection of the virus for seed-potato certification. To reduce the laboratory costs for the detection of the virus in the potato seed lots, the sampling is generally reduced for resistant cultivars. In addition, either the number of samples (tubers or plants) from each seed lot is reduced or more samples are pooled. This methodology has its limitation for varieties with a distinct susceptibility to PVY strains such as cv. Agria. Indeed, reducing the number of samples for a variety resistant to one PVY strain and susceptible to another increases the risk of spreading the strain for which the variety is susceptible (e.g. $PVY^{N\text{-}Wi}$ for cv. Agria). This could also explain why the prevalence of $PVY^{N\text{-}Wi}$ increased in countries such as Switzerland where, for example, sampling of cv. Agria is done at a lower rate (Peter Frei, personal communication).

## 2. Does the vascular movement of PVY strains in the plant influence the epidemiology of PVY in the field?

In this thesis, we hypothesise that the mother tuber plays an important role as a *vascular bridge* between the different stems of the plant and that this role changes over time during plant development depending on the PVY strains. In Chapter 2, we showed that the changes of the efficacy of this vascular bridge in allowing potato sap to pass from one stem to the other through time plays an important role in the development of the phenomenon of mature plant resistance (MPR) against PVY in potato plants.

MPR is important, because this phenomenon protects the potato plant against late infections by PVY. Late infections can occur, for example, when late aphid flights occur within days before haulm destruction. MPR is described as a phenomenon in which the translocation of the virus from the leaves to the progeny tubers occurs faster in young plants than in old plants (Debokx, 1964). MPR is associated with changes in the metabolism of older plants inducing a reduction of the cell-to-cell movement of the virus and a decrease of the speed of its translocation (Beemster, 1976, Debokx, 1964, Valkonen & Rokka, 1998). In addition, our results showed that the probability of stem infection through the vascular system of the mother tuber was significantly reduced with plant age. Thus, our observations suggest a second mechanism of protection associated with MPR. This mechanism involves the vascular system of the mother tuber acting as a non-permanent bridge between the growing stems of the plant. Thus, there are two independent bottlenecks that lower the transmission of the virus from one stem to the others through the vascular system of the mother tuber: a *temporal restriction* and a *physical restriction* [see details in Chapter 2 and (Dupuis, 2017)]. This finding stresses that MPR is a very complex mechanism in which interacting physiological mechanisms overlap such as the speed of the flow of the sieve in the vascular tissues of the plant and the decay of the vascular system of the mother tuber. This implies that MPR is shaped by numerous physiological cues of the plant, and that the selection of potato cultivars with an early development of MPR is a challenge. First, these genes associated to MPR have not been identified yet. Second, they are likely to be associated with complex multigenic traits. This will require complex breeding strategies for introgressing these traits in the tetraploid potato genome while ensuring efficient protection of older plants against PVY infection.

Given the high risk of transmitting the virus to all the stems when the infection occurs in the second or the third week following emergence, an increase in the frequency of foliar spraying using mineral oils on young developing plants should be considered. This maximises the effectiveness by protecting newly exposed leaves (Fageria et al., 2014a, Fageria et al., 2014b, Demeulemeester, 2013). Some potato seed growers perform two oil sprays a week with significant effects on minimising PVY transmission (Pierre Lebrun, personal communication), suggesting that more frequent applications might be an option to protect new emerging leaves of the young, PVY-susceptible and fast-growing plants.

## 3. Which is the most effective strategy to control PVY spread in the field?

In this thesis, we hypothesise that the adaptation of the existing control methods should increase the protection of potato fields from PVY spread. Figure 40 shows the PVY-control methods tested in this thesis (oil spraying, elicitors spraying, straw mulching and intercropping) alongside the other existing control strategies. The range of efficacy of all methods investigated is also presented in Figure 40, as well as the evolution of the efficacy of the control method over time from potato emergence until haulm killing.

The use of insecticides is the most common PVY-control method used in growing (seed) potato crops (Döring et al., 2007, MacKenzie et al., 2016), despite its relatively low efficacy (Perring et al., 1999). The range of efficacy of insecticide is between 0 and 53% depending on the active compound and the circumstances of the treatment (Figure 40). The main hypothesis for the lack of efficacy of insecticides in controlling PVY spread is that the time needed for virus acquisition and transmission is too short for the insecticides to kill the aphid vector (Radcliffe, 1982). Our field trial described in Chapter 3 confirms this statement, as the efficacy obtained with weekly spraying of insecticide (Lambda-cyalothrin) was the lowest (10%) compared to other treatments tested. We also observed that this insecticide was unable to control *Myzus persicae* in our field experiment, which may indicate a mutation of the voltage-gated sodium channel and the corresponding resistance of this aphid species against this type of insecticide (Bass et al., 2014). Such resistance would also contribute to the low insecticide efficacy observed in our trial.

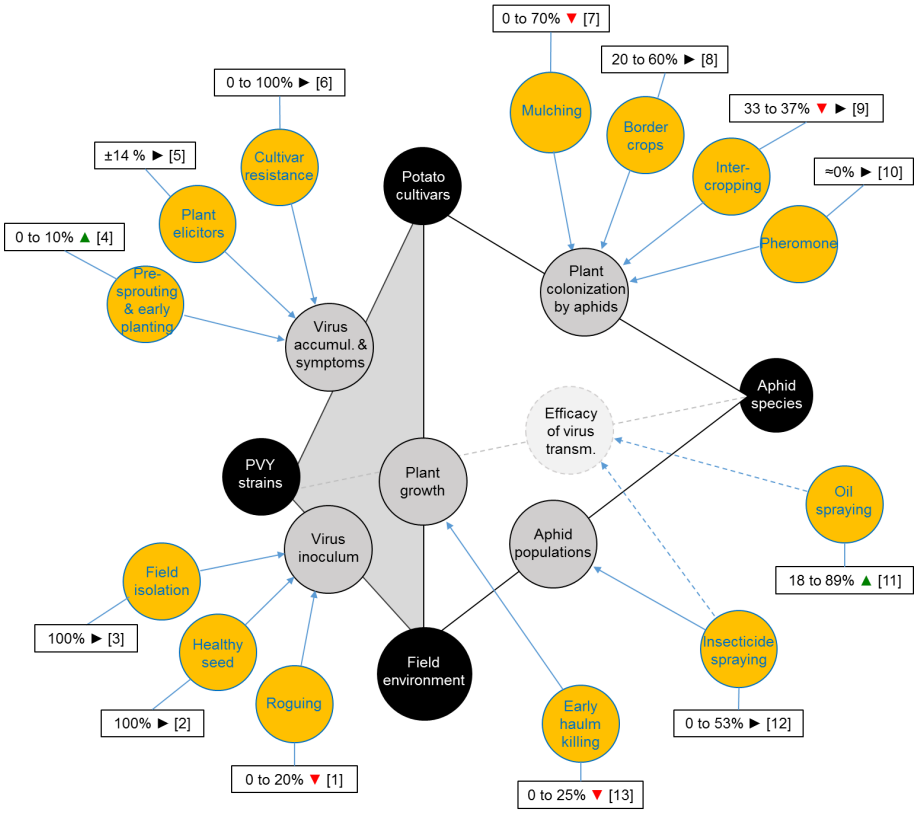


*Figure 40: Diagram presenting the PVY pathosystem with the four components of the system in black, the interactions between each other in grey and with the main control strategies in orange. The blue arrow indicates the location of the main action of each control strategy. The range of efficacy of each control strategy is given in the squares associated to the control strategies. In the same square, control strategies with an increasing efficacy during the cropping period are associated with a green triangle, stable efficacy is associated with a black triangle, and decreasing efficacy is associated with a red triangle. The numbers in brackets are the following references: [1] Broadbent et al. (1950); [2] with no inoculum in the environment; [3] with a clean seed; [4] based on Sigvald (1985) with 10% protection due to mature plant resistance for one week delay of infection; [5] Dupuis et al. (2014b); [6] Schwaerzel et al. (2016); [7] Dupuis et al. (2017) and Kirchner et al. (2014); [8] Difonzo et al. (1996) and Boiteau et al. (2009) ; [9] Dupuis et al. (2010) and Dupuis et al. (2017) ; [10] Crutzen et al. (2014) ; [11] Dupuis et al. (2017) ; [12] Table 8; [13] based on Steinger et al. (2014) with 25% of protection due to an early haulm killing of one week.*

To the best of our knowledge, this was the first time that elicitors were tested to control PVY spread in the field. The elicitor (Acibenzolar-S-methyl) tested in Chapter 3 had a low efficacy of 14% (Figure 40), despite the high frequency of treatments used (weekly from emergence for three weeks and then each 14 days until haulm killing). The same compound was found to be efficient in preventing PVY infection of tomato plants in a greenhouse experiment (Petrov & Andonova, 2012), but its efficacy was not significant under field conditions to be considered a suitable candidate for use in PVY control. Finally, our field experiments presented in Chapter 3 confirmed that oil spraying is the most efficient spraying technique to control PVY. However, the efficacy of the vegetable oil tested (rapeseed oil) was relatively low (26%) compared with the results obtained by Martin-Lopez et al. (2006), which showed 41% efficacy for refined rapeseed oil. In the same study, Martin-Lopez compared the efficacy of rapeseed oil with the efficacy of mineral oil, and mineral oil proved much more effective, with 59% efficacy, which is comparable to the 64% level of protection obtained by Boiteau and Singh (1982). The range of efficacy of mineral oil is between 18 and 89% (Figure 40), and considering our results, mineral oil remains the best spraying technique to control PVY spread in the field.

Chapter 4 provides results of two years of field trials using intercropping as a means to control PVY spread. Oat and hairy vetch were used in this study as associated plants, and both plants were efficient in controlling PVY, with 34% and 33%, respectively, reduction incidence of PVY ($p$<0.05). The range of efficacy of intercropping is narrow compared to most of the existing PVY control strategies (Figure 40). Nevertheless, only one other study using intercropping to control PVY spread in the field is reported in the literature (3 total trial years, 2 years in Chapter 4 and 1 year in Dupuis et al. (2010), see Annex 9). This other study was managed in the same environmental conditions and with the same associated crop (oat). Thus, the range of efficacy of intercropping should be wider than 33 to 37%, as presented in Figure 40, as the efficacy could vary according to the associated plant species and according to the environmental context. Furthermore, shape and colour patterns of plants play an important role in the host-finding process of aphids (Döring, 2014). Therefore, associated plants with distinct colours and shapes differently influence the landings of aphids on the crop and, hence, the PVY spread. The results of Chapter 4 showed that the efficacy of intercropping was not due to a reduction in the aphid landings in the

crop but may be due to a *virus sink* effect through which non-host plants act as *virus traps/aphid's stylets cleansing*; whereby aphids progressively lose their PVY inoculum as they repeatedly probe the plant(s) (Wróbel, 2007, Boiteau et al., 2009). This hypothesis is consistent with the observation made by Mello et al. (2011) in which a single aphid of the specie *Myzus persicae* carrying the virus loses its ability to transmit the virus after probing 8 to 10 plants. The efficacy of oat and vetch intercropping are very similar and comparable to the efficacy of straw mulching that was tested in the same trial. This study highlights one possible drawback of intercropping in reducing tuber yield. The harvested tuber yield was reduced by 13% with oat intercropping (-3.9±2.0 t/ha) and by 11% with vetch intercropping (-3.4±2.4 t/ha), whereas the average effect of straw mulching on yield was low. This yield penalty could be offset by the low cost of the method. At a cost of 166 CHF/ha, intercropping is the cheapest PVY-control strategy when compared to mineral oil spraying, straw mulching and insecticide spraying, with an average cost per hectare of 910 CHF, 465 CHF and 742 CHF, respectively (Table 19). When the average costs of the treatments are compared to their average efficacy, intercropping is the most cost-effective strategy, with only 5 CHF spent to gain 1% of protection against PVY spread, while 21 CHF and 17 CHF are needed to gain 1% of protection with mineral oil spraying and straw mulching, respectively. Insecticide spraying is the least profitable with 74 CHF spent to gain 1% of protection against PVY spread. This cost analysis must not conceal that all these methods have an upper limit of efficacy. Figure 40 shows that the PVY control strategies with the highest efficacy are the use of resistant cultivars (Schwaerzel et al., 2016), planting of healthy seed (Steinger et al., 2015) and field isolation (Kerlan et al., 1987) with up to 100% of protection, and mineral oil spraying, with up to 89% of protection (Dupuis et al., 2017). For example, the upper limits of the efficacy of straw mulching and intercropping are significantly lower with 70% and 37% of efficacy, respectively. Thus, for growers to increase the protection of the fields above these limits, different control strategies with different modes of action may be combined.

*Table 19: Estimation of the average cost of mineral oil spraying, straw mulching, insecticide spraying and hairy vetch intercropping. The product costs were calculated based on Landi (2017) and UFA (2017), while the other costs were calculated based on AGRIDEA (2017b) and (Gazzarin, 2017). The average efficacy of treatments are based on (*) Dupuis et al. (2017) and (**) Dupuis et al. (2014b).*

| **Costs per hectare (CHF/ha)** | **Mineral oil** *Paraffinic oil* *7 L/ha* | **Mulching** *Wheat straw* *2.5t/ha* | **Insecticide** *Lambda-cyhalothrin* *100g/l* | **Intercropping** *Hairy vetch* *50kg/ha* |
|---|---|---|---|---|
| Product | 39 | 400 | 15 | 103 |
| Labor | 14 | 14 | 14 | 14 |
| Fuel | 9 | 9 | 9 | 9 |
| Tractor rental | 24 | 24 | 24 | 24 |
| Rental of Sprayer/Straw blower/Seeder | 44 | 18 | 44 | 16 |
| Total per treatment | 130 | 465 | 106 | 166 |
| Number of treatments | 7 | 1 | 7 | 1 |
| Total | 910 | 465 | 742 | 166 |
| Average efficacy of treatments (%) | 43* | 27* | 10** | 33* |
| Cost for 1% protection | 21 | 17 | 74 | 5 |

## 4. Can we reduce PVY spread through the combined use of different control strategies?

In this thesis, we hypothesise that the combination of control strategies should increase the control of PVY through an additive effect of the efficacy of the combined strategies.

The complementarity of mineral oil and straw mulching in controlling PVY spread was demonstrated in Chapter 4. Furthermore, in our field experiment, no interaction between the effects of oil and straw mulching was detected, showing that the effect of both methods was additive. This complementarity of the two control measures may be explained by the evolution of the crop canopy during the growing season. Indeed, oil sprayings may provide little protection from PVY infection during periods of rapid foliage expansion at the beginning of the growing season, since some new developed leaves are left unprotected (Fageria et al., 2014b, Fageria et al., 2014a). In contrast, straw mulch is thought to be most effective in preventing aphid landings on potato plants—and hence virus infections—during early growth stages (Saucke & Doring,

2004). At this time, the visual contrast between the soil and the foliage is the highest in the crops, and straw might confuse winged aphids in their host-finding process by reducing the contrast between the foliage and the straw (Döring, 2014, Döring et al., 2004). Furthermore, the results suggest that this complementarity of the mode of action reduces the year-to-year variation observed with oil and straw when used alone as PVY control agents. This combination of methods should significantly reduce the risk of crop downgrade caused by massive PVY infection in seed potato production.

We can hypothesise that other combinations of treatments could also increase crop protection in field conditions. This hypothesis is consistent with the results obtained by combining mineral oil with crop borders (Boiteau et al., 2009), mineral oil with oat intercropping [Dupuis et al. (2010), see Annex 9] and mineral oil with insecticides (MacKenzie et al., 2014, Martin-Lopez et al., 2006, Gibson & Rice, 1986, MacKenzie et al., 2016). The quantitative gain in protection against PVY spread has been presented in the introduction of the thesis (section 8).

In Figure 40, three groups of PVY control strategies are presented and are identified with coloured triangles (green, red or black). These strategies have their main efficacy against PVY in distinct periods of the growing season. The red triangles show the PVY control strategies that appear to be more efficient early in the season such as mulching, intercropping (e.g. with oat) and roguing or strategies that are more efficient when implemented early such as haulm killing, reducing the risk of late infection of the crop. In contrast, the green triangles of Figure 40 show the PVY control strategies that are more efficient at a later stage of crop development such as oil spraying or managing mature plant resistance by early planting and/or pre-sprouting. These two groups are complementary, as the efficacy of the control strategies of the first group declines when the efficacy of the control strategies of the second group increases. This hypothesis is confirmed by the results of Chapter 4, as in the experiment, the joint effect of straw mulching (first group) and mineral oil spraying (second group) resulted in a 59% reduction of PVY incidence compared to 43% and 27% for mineral oil and straw mulching, respectively, when used separately.

Finally, the black triangles of Figure 40 show the PVY control strategies for which the efficacy is relatively stable over time such as insecticides, plant elicitors and pheromones spraying, the use of resistant cultivars, border crops, intercropping (e.g. with hairy vetch), healthy seed planting and isolation of the field from other potato

crops. The efficacy of the use of resistant cultivars depends on the level of resistance of the variety and can be very high, up to 100% (0% efficacy for susceptible cultivars to 100% efficacy for fully resistant cultivars). For example, among the cultivars regularly produced in Switzerland, the cultivars Erika, Lady-Christl, Venezia, Lady-Felicia, Laura and Markies have a good resistance to PVY, while the cultivars Celtiane, Charlotte, Gourmandine, Bintje, Victoria, Innovator and Lady Claire are highly susceptible to this virus (Schwaerzel et al., 2016). No additional PVY control method is required for potato fields planted with highly resistant cultivars.

# PERSPECTIVES

The implementation of the recommendations of this thesis allows for an increased sustainability of seed potato production by improving the efficacy of PVY control and by reducing the negative impacts of this control on the environment. Further improvements of the efficacy of the control of PVY are still possible based on the result of this work, and four main research focuses can be identified: (i) Combine resistance of potato cultivars with PVY strains diversity to control outbreaks, (ii) optimise the frequency of treatments with mineral oil, (iii) undertake further research on intercropping, and (iv) assess new combinations of PVY control strategies. Suggestions of methodologies and protocols are detailed in the following paragraphs.

## 1. Combine Resistance of Potato Cultivars with PVY Strains Diversity to Control the Outbreaks

Planting resistant cultivars is the most effective mean to control the spread of PVY. For example, Hansen and Nielsen (2012) have compared the spread of PVY in the field (4% of inoculum) of cv. Spunta, a variety with a good resistance to PVY, with cv. Désirée, a variety highly susceptible to PVY. Without any control method, the PVY incidence of progeny tubers of cv. Désirée were of 97.4%, while the PVY incidence of progeny tubers of cv. Spunta were only 21.4% (i.e. about 5 times less). Our results suggest that the resistance levels of potato cultivars might play an important role in shaping the prevalence of PVY strains in a given area. Improving the efficacy of PVY management using resistant cultivars will require a more detailed description of the resistance of varieties to the whole range of PVY strains, since cultivars were found to be resistant to PVY strains while being susceptible to others. This phenomenon is described for two varieties, cv. Agria is resistant to $PVY^{NTN}$ and susceptible to $PVY^{N\text{-}Wi}$ [see Chapter 1 and Lindner et al. (2015)], while cv. Umatilla Russet is resistant to $PVY^{O}$ and susceptible to $PVY^{NTN}$ and $PVY^{N\text{-}Wi}$ (Funke et al., 2017). To the best of our knowledge, this contrasted susceptibility of cultivars to PVY strains is not formally described for other varieties but is most probably a reality for numerous other cultivars. Indeed, Lindner and Billenkamp (2005) observed through an analysis of the seed certification data of Bayern in Germany that the cultivars Agria, Aula, Exempla and Afra

were systematically infected by the serological group $PVY^{O}$ and never by $PVY^{N}$. Knowing both the susceptibility of the cultivar to all PVY strains and the diversity of strains in a region, it would be possible to allocate varieties to regions according to their susceptibility profiles to PVY strains. Such control strategy is commonly used in the United Kingdom to control cereal rust and mildew and to regularly test the susceptibility of the main commercial winter wheat varieties to the rusts and mildews strains of the environment. These surveys allow the characterisation of the evolution of the resistance of the winter wheat varieties against these fungal diseases, and this information efficiently drives the choice of the growers for cultivars with a good resistance profile (Cooke et al., 2006). This strategy could also be used to control PVY in seed potatoes. For example, cv. Agria can easily be multiplied in Switzerland, as it is a $PVY^{N\text{-}Wi}$-susceptible variety cropped in a region where $PVY^{NTN}$ is predominant (see Chapter 1). Unfortunately, the description of cultivars displayed by breeding companies does not include the differentiation of susceptibility among the main PVY strains. Instead, the assessment of the susceptibility of the cultivars is done by breeders (e.g. Europlant and Norika in Germany) through field trials with a known PVY inoculum level. These trials are managed with only one PVY strain of reference, in general, a $PVY^{NTN}$ strain, to assess the susceptibility of the cultivars to PTNRD (Hans Reinhard Hofferbert, personal communication). Consequently, there is a need to assess the susceptibility of the cultivars to a wider spectrum of PVY strains. This can be done in field trials with distinct inoculum sources of the main PVY strains (for Switzerland, mainly $PVY^{NTN}$ and $PVY^{N\text{-}WI}$ referring to Chapter 1), for which the level of infection of the progeny tubers is measured through analysis, allowing the differentiation of the PVY strain responsible for the infections. Knowing the susceptibility of the cultivar to the main PVY strains of a region, the growers can decide, other things being equal, which varieties to be planted in their farms.

This innovative PVY management strategy would only be efficient when the biodiversity in PVY strains is known with precision. In the past, obtaining this information was difficult and required large and expensive surveys. Currently, however, with the molecular tools used for detecting PVY for seed potato certification, this information can be more easily generated. For example, in Switzerland and the Netherlands, countries where all seed lots are analysed with molecular tools (Eisse de

Haan and Peter Frei, personal communications), allowing differentiation of the strains present in the environment.

## 2. Optimization of the Frequency of Treatments with Mineral Oil

Knowing the evolution of mature plant resistance over the growing season is important, there is a need to schedule the frequency of mineral oil treatments. In Chapter 2, we recommended increasing the frequency of treatments in the days following the emergence. This recommendation is based on the common practices in countries such as Belgium and France, where a higher frequency of spraying is applied following potato emergence (Pierre Lebrun and Yves Le Hingrat, personal communication). This recommendation is also based on the experimental results of Fageria et al. (2014a), who observed less infected tubers for plants of Russet Burbank treated every 3 days with 5 L of mineral oil (superior 70) compared to plants treated every 7 or 10 days. In the same study, the effect of the frequency of treatment is less clear with plants treated with 10 L of mineral oil, as the PVY incidence in the progeny tubers is lower for plants treated every 3 days compared to a 5-day frequency but is equivalent to a 10-day frequency of treatment. Fageria et al. (2014b) mentioned the high variability of the results obtained in this experiment, and this may explain why the effects of a high frequency of treatments on PVY control are not clear in this experiment. The authors (Fageria et al., 2014b) also reported the results of a larger trial performed with three cultivars (Russet Burbank, Shepody and Innovator), two mineral oil products (Superior 70 and Vazyl-Y), two doses (5 and 10 L), and three frequencies of treatments (3, 7 and 10 days). This trial revealed the same complicated picture. In 12 combinations of varieties and products tested, 7 combinations revealed a lower incidence of PVY in the progeny tubers of the plots treated every 3 days compared to a 10-day frequency, 3 combinations showed no difference between frequencies and 2 combinations showed a higher incidence of PVY in the plots treated with the highest frequency. In this second trial as well, a high variability of results was observed. The main tendencies that emerge from the results are clear, and a higher frequency of mineral oil treatments increases the protection, but the efficacy of this protection is subject to high variability. This variability is not discussed by the authors and should be explored more in detail.

A relatively low seasonal spread of PVY is also observed in these experiments, with an average infection rate of the tubers in the untreated control of 44.4%. For the combination with the highest seasonal spread in the control (cv. Shepody treated with Vazyl-Y) with an average of 85.7%, the positive effects of the high frequencies of treatments are clear and statistically significant (see Figure 41). For this sub-trial, the variability of the results is similar to the other sub-trials (a 9.4% confidence interval instead of 7.6% in the other sub-trials), but the differences between treatments and with the control are greater and, hence significant.

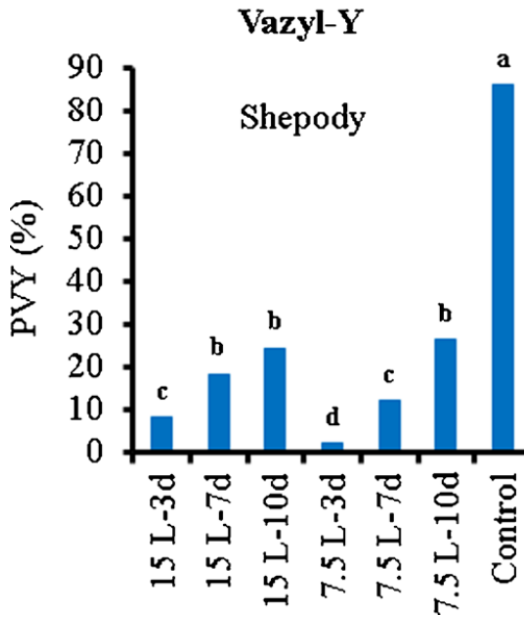


*Figure 41: Effect of the mineral oils Superior 70 and Vazyl-Y applied at different rates (7.5 L ha−1 and 15 L ha−1, respectively) and frequencies (3–4, 7, and 10– 11 days) on seasonal spread of PVY (%) in cv. Shepody. Significant differences between mineral oil treatments are shown with different letters. Adapted from Fageria et al. (2014b).*

Therefore, the efficacy of various spray frequencies scheduled should be examined in relation to the main development stages of the crop. The efficacy of six different programmes of mineral oil (programmes 1; 2; 3; 4; 5 and 10 in Figure 42) should be

assessed on a cultivar very susceptible to PVY (e.g. cv Shepody). The treatment programmes would be the following (see Figure 42): one untreated control (P10), one control treated every 7 days during the entire growing season (P1), and four programmes starting at potato emergence with mineral oil treatments every 3 days and then every 7 days according to the development of the foliage of the plant (P2 to 5). Knowing the high variability of the response of these trials, 6 replicates should be performed to maximise the chances of detecting significant differences between the programmes.

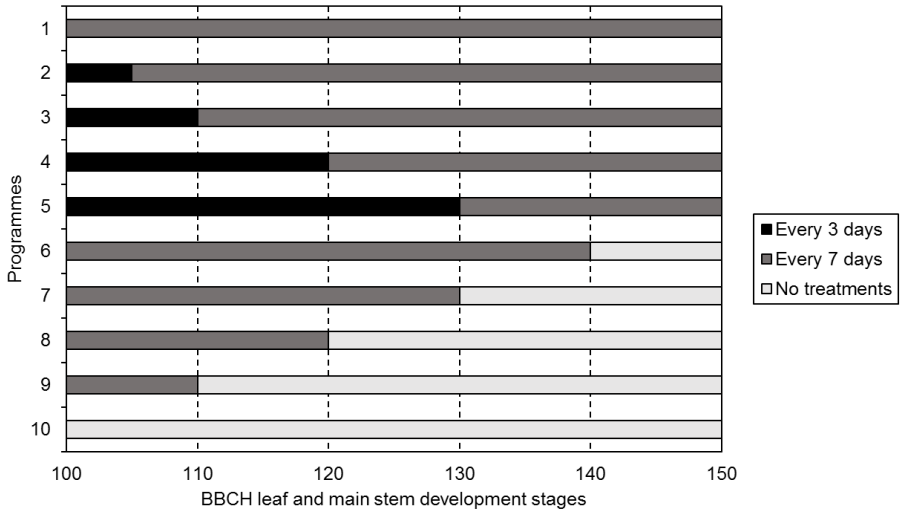


*Figure 42: Programmes of mineral oil treatments according to the development of the foliage [BBCH scale of Hack et al. (1993)]. 100 correspond to the emergence, 110 to the appearance of the first inflorescence, 120 to the appearance of the first flowers, 130 to the appearance of the first fruits, 140 to the early ripening of the fruits and 150 to the beginning of the senescence and to haulm killing. The frequency of mineral oil treatments is provided in the legend on the right side of the chart.*

The increase of the frequency of treatments (from 7 days to 3 days) should be done without an increase in the oil weekly doses to avoid toxicity on young plants (Kirchner et al., 2014). This strategy of increasing the frequency of mineral oil treatments at the early stages of the crop is in line with the fact that mature plant resistance is the lowest at this time (Gibson, 1991, Sigvald, 1985). To be consistent with this strategy, the

frequency of treatments should be gradually reduced until the plant has become fully resistant due to the development of mature plant resistance. This would allow a decrease in the use of mineral oil, reducing the costs for the grower (Table 19) and having positive effects on the environment. As far as the author knows, no experimental data are available, showing the possibility of reducing the treatments with mineral oil at the end of the growing season. Therefore, another trial with a susceptible cultivar is recommended in which the weekly treatments with mineral oil are gradually stopped according to the development of the crop. The treatment programmes would be the following (see Figure 42): one untreated control (P10), one control treated every 7 days during the entire growing season (P1), and four programmes starting at potato emergence, with mineral oil treatments every 7 days and then stopping the treatments as a function of the development of the foliage of the plant (P6 to 9). Sigvald (1985) observed that mature plant resistance to $PVY^{O}$ started 25 days after emergence and then increased until complete protection 10 weeks after emergence. This experiment was done in Sweden with cv. Bintje. Gibson (1991) observed a faster development of mature plant resistance to $PVY^{O}$ than $PVY^{N}$ with cv. King Edward, Record, Maris Piper and Désirée cropped in England. Full resistance appeared four to six weeks after emergence, and faster with cv. Désirée, without differences between strains (Figure 6). Zhang (2014) did not observe mature plant resistance to $PVY^{O}$ and $PVY^{N}$ with cv. Russet Norkotah. These results underline the variability of the response of the potato cultivars for the development of mature plant resistance, as well as the variability of the response depending on the PVY strain responsible for the infection. Consequently, the mature plant resistance mechanisms of the most grown potato cultivars [depending on the country, e.g. cvs. Agria and Charlotte for Switzerland (Swisspatat, 2012)] should be characterised with the most prevalent PVY strains [depending on the country, e.g. $PVY^{NTN}$ and $PVY^{N\text{-}Wi}$ for Switzerland (Rigotti et al., 2011)]. This should be done through inoculation with aphids [e.g. *Myzus persicae*, known as the best vector of PVY (Verbeek et al., 2010)] of plants grown in field conditions but protected by screenhouses from natural infection by wild aphids. The inoculations should be done on a minimum of 20 plants for each inoculation and repeated at the main development stages of the plant [e.g. the BBCH stages 100, 110, 120, 130, 140, 150 and 905 in Hack et al. (1993)].This important information would help establish appropriate mineral oil programmes adapted to the varieties with a progressively decreasing treatment frequency in relation to the growth of the plant.

## 3. Further Research on Intercropping

Intercropping revealed to be efficient in controlling PVY spread in the field. Nevertheless, a significant yield loss was observed with oat and hairy vetch intercropping, and this PVY control strategy was therefore not recommended for practical use despite its low cost (Table 19). However, additional research should be performed to explore the possibility of using other plant species in association with potatoes. Intercropping remains theoretically interesting as it is relatively easier to implement in practice compared to straw mulching. Indeed, straw mulching requires a dedicated equipment to be mechanically spread in the field, and it requires access to straw in high quantity for a reasonable price to be profitable. Thus, additional field experiments should be performed to explore new plants for intercropping. More than 150 cover crops are described in Europe (OSCAR, 2014, Thomas & Archembeaud, 2016) that could potentially be used as associated crop with potatoes. However, not all of them are suitable as associated crop to control PVY in seed potatoes, and eight requirements can be identified for a crop to be a good candidate for that purpose. The first requirement is that the crop should not be a host of PVY, since that could increase the PVY pressure in the crop (Broadbent et al., 1950). Therefore, solanaceous species must be avoided, as many of them are susceptible to PVY (Ali et al., 2008, Kerlan, 2006, Quenouille et al., 2013). Some Asteraceae plant species have been identified as PVY hosts (Kaliciak & Syller, 2009); thus, before using these plants as associated crop, it is important to verify whether they can be infected by the virus (e.g. through inoculation trials). The second requirement is a fast germination and emergence, as the associated plant must be emerged at the early stages of the crop, when the potato is the most susceptible to PVY due to the absence of mature plant resistance (Gibson, 1991, Sigvald, 1985). The third requirement is a good tolerance to cold and frost. As the cover crops are sewed at potato planting, they will emerge in spring (mid-April in Switzerland) when the temperature at night can be below 0°C (e.g. -0.2°C on the 20$^{th}$ of April 2017 and -0.3°C on the 28$^{th}$ of April 2016 in Changins, Switzerland). For example, moha, common millet, Sudan grass, nyger, linen and yellow-lupin do not tolerate frost (Cadillon et al., 2013), and berseem clover does not grow below 6°C (Thomas & Archembeaud, 2016). The fourth requirement is a fast establishment of the associated crops to limit as much as possible the contrast between the potato and the bare soil that helps the aphid vectors in their host-finding process (Döring & Chittka,

2007, Saucke & Doring, 2004). The fifth requirement is a medium canopy height (between 50 and 100 cm) that is at the same canopy level as the potato. The cover crop should be a little bit taller than the potato plant, as it will grow from the bottom of the row, about 20 cm lower than the top of the row (AGRIDEA, 2017a). The sixth requirement is a low competition between the cover crop and the potato. Cover crops with low biomass will compete with the potato for water and nutrients to develop their own biomass without worsening the yield potential of the potato crop (Clark, 2007). Cover crops such as field mustard, vetch and white clover are known to hardly compete for nitrogen (Thomas & Archembeaud, 2016). Some cover crops produce metabolites, limiting the development of other plants, which this phenomenon is called allelopathy (Putnam & Chung-Shih, 1986). Such allelopathic effects have been documented for cereals, including rye and sorghums, and for the brassica family, including rapeseed, mustards, and radishes. These effects were also described for other cover crop species such as buckwheat and nyger and for a legume species, such as the subterranean clover (Rick & Ann, 1995, Putnam & Chung-Shih, 1986, Rice, 1995, Cadillon et al., 2013). The seventh requirement is a life cycle as short as possible to have a senescent cover crop at the time of harvest of the potatoes, which would facilitate the harvest. Cover crops, as well as weeds, can have a serious effect on entangling equipment and slowing the harvest operations (Davies, 2007); thus, it is better to have cover crops decayed at harvest time. This requirement can be replaced by an herbicide treatment to limit the development of the cover crop when it is not needed any more at the time of soil cover by the potato canopy. Nevertheless, the herbicides available for treatments after the emergence of the potatoes allow, with a few exceptions, only the destruction of plants of the *Poaceae* family (AGRIDEA, 2017a); and among which, species such as oat and rye are very susceptible to herbicides (Thomas & Archembeaud, 2016). Finally, the cover crop must not multiply the populations of aphids in his canopy, since that could spread the virus within the crop by walking (apterous) or flying (winged) from plant to plant (Alyokhin & Sewell, 2003, Narayandas & Alyokhin, 2006). Oat, barley and phacelia are known to be susceptible to aphids (Thomas & Archembeaud, 2016), thus the development of aphid populations in these crops needs to be monitored when they are used as associated crops with potatoes. Table 20 presents the descriptions of 38 common cover crops used in Europe for six of the eight requirements listed above, namely speed of germination, cold tolerance, speed of the establishment, canopy height, biomass, and

ripening time. Each cover crop species is scored relative to these requirements. For example, a species for which all the requirements are fulfilled (a fast germination, a high tolerance to frost, a fast establishment, a medium canopy height, a low biomass, and an early ripening time) cumulates 6 points in the score column of Table 20. The cover crops with the best score is camelina (5 points), followed by crimson clover, fenugreek, foxtail millet, Italian ryegrass and white mustard (4 points). These six species should be tested in field trials to assess their efficacy in controlling the spread of PVY when associated with the potato. This can be done using the same protocol proposed in Chapter 4 and used to test the efficacy of hairy vetch as an associated crop.

# 4. Assessment of new Combinations of PVY Control Strategies

The combination of mineral oil and straw mulching revealed to be synergetic for the control of PVY by providing a higher and more stable protection compared to the control methods used separately. This higher protection was demonstrated to be due to the complementarity and the difference of mode of action between straw mulching and mineral oil. This result opens numerous new perspectives to improve the control of PVY spread in potato fields, and the idea is to combine PVY control techniques with distinct modes of action and to use them when they are the most efficient to improve the protection. Figure 40 shows the evolution of all the PVY control methods over time: the methods with an increasing efficacy (green triangle), the methods with a decreasing efficacy (red triangle) and the methods with a relatively stable efficacy (black triangle). Therefore, this figure is a good tool to imagine new combinations of complementary control strategies that could be tested in the future. For example, the insecticides may be used with mineral oil to complement the low efficacy of mineral oil at the early stages of the development of the crop. MacKenzie et al. (2016) showed through a recent on-farm survey that there is a good correlation between PVY control and the spraying of insecticides combined with mineral oils. However, it is difficult from this study to determine the relative efficacy of the control strategies, because the insecticide sprays were always mixed into the oil sprays, thus representing a combination mineral oil-insecticide spray rather than an insecticide spray alone.

*Table 20: Description of 38 cover crops for their characteristics to be used as an associated crop for the control of PVY spread. The characteristics corresponding to this use are shown in bold, and the number of these characteristics for each crop is given in the "score" column. References: [1] Cadillon et al. (2013); [2] OSCAR (2014); [3] Thomas and Archembeaud (2016); [4] Clark (2007).*

| Family | Common name* | Latin name | Germination | Cold tolerance | Establishment | Canopy height | Biomass | Ripening time | Score | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| *Fabaceae* | Crimson clover | *Trifolium incarnatum* L. | **Fast** | **High** | **Fast** | **Medium** | High | Medium | 4 | [1; 2] |
| | Fenugreek | *Trigonella faenum-graecum* L. | **Fast** | Low | Medium | **Medium** | **Low** | **Early** | 4 | [1; 3] |
| | Bitter vetch | *Lens nigricans* Godr. | **Fast** | Low | **Fast** | Low | **Low** | Medium | 3 | [1; 3] |
| | Black medick | *Medicago lupulina* L. | **Fast** | **High** | Medium | **Medium** | Medium | Medium | 3 | [1; 2] |
| | Field pea | *Pisum arvense* L. | **Fast** | **High** | Medium | **Medium** | Medium | Medium | 3 | [1] |
| | Pigeon pea | *Pisum sativum* L. | **Fast** | **High** | Medium | **Medium** | Medium | Medium | 3 | [1; 3] |
| | Yellow-lupin | *Lupinus luteus* L. | Slow | Low | **Fast** | **Medium** | Medium | Medium | 2 | [1] |
| | Berseem clover | *Trifolium alexandrinum* L. | Medium | **High** | **Fast** | Low | Medium | Medium | 2 | [1; 2] |
| | Birdsfoot trefoil | *Lotus corniculatus* L. | Slow | **High** | Slow | **Medium** | Medium | Medium | 2 | [1; 2; 3] |
| | Coastal medick | *Medicago littoralis* Lois. | **Fast** | Low | Medium | **Medium** | Medium | Medium | 2 | [1; 2] |
| | Hairy vetch | *Vicia villosa* Roth | **Fast** | **High** | Low | High | High | Medium | 2 | 1; 2; 3 |
| | Red clover | *Trifolium pratense* L. | **Fast** | **High** | Medium | High | High | Medium | 2 | [1; 2] |
| | White clover | *Trifolium repens* L. | Slow | **High** | Medium | **Medium** | High | Medium | 2 | [1; 2] |
| | Faba bean | *Vicia faba* L. | Slow | Medium | **Fast** | High | High | Medium | 1 | [1; 2] |
| | Lucerne | *Medicago sativa* L. | **Fast** | Medium | Medium | High | High | Medium | 1 | [1; 2] |
| | Common vetch | *Vicia sativa* L. | **Fast** | Medium | Medium | High | High | Medium | 1 | [1; 2; 3] |
| | Persian clover | *Trifolium resupinatum* L. | Medium | **High** | Medium | Low | High | Medium | 1 | [1; 3] |
| | White sweet clover | *Melilotus alba* Medik. | Medium | **High** | Medium | High | Medium | Medium | 1 | [1; 2; 4] |
| | French serradella | *Hedysarum coronarium* L. | Medium | Medium | Medium | High | Medium | Medium | 0 | [1; 2] |
| | White vetch | *Lathyrus sativus* L. | Medium | Medium | Medium | High | Medium | Medium | 0 | [1; 2] |

** One common name is given as exemple*

| Family | Common name* | Latin name | Germination | Cold tolerance | Establishment | Canopy height | Biomass | Ripening time | Score | Ref. |
|---|---|---|---|---|---|---|---|---|---|---|
| *Poaceae* | Foxtale millet | *Panicum germanicum* L. | **Fast** | Low | **Fast** | High | **Low** | **Early** | 4 | [1; 3] |
| | Italian ryegrass | *Lolium multiforum* Lam. | **Fast** | **High** | Slow | **Medium** | **Low** | Late | 4 | [1; 3] |
| | Barley | *Hordeum vulgare* L. | **Fast** | Medium | **Fast** | High | Medium | **Early** | 3 | [1; 3] |
| | Cereal rye | *Secale cereale* L. | **Fast** | **High** | **Fast** | High | High | Medium | 3 | [1; 3] |
| | Common millet | *Panicum miliaceum* L. | **Fast** | Low | **Fast** | High | **Low** | Late | 3 | [1; 3] |
| | Common oat | *Avena sativa* L. | **Fast** | **High** | **Fast** | High | Medium | Late | 3 | [1; 3] |
| | Black oat | *Avena strigosa* Schreb. | **Fast** | Low | **Fast** | High | Medium | Late | 2 | [1; 3] |
| | Sudan grass | *Sorghum sudanense Nees ex. Steud.* | **Fast** | Low | **Fast** | High | Medium | Medium | 2 | [1; 3] |
| *Asteraceae* | Nyger | *Guizota abyssinica* L. F. | **Fast** | Low | Medium | High | **Low** | **Early** | 3 | [1; 3] |
| | Common sunflower | *Helianthus annuus* L. | Medium | Low | **Fast** | High | High | **Early** | 2 | [1; 3] |
| *Linaceae* | Linen | *Linum usitatissimum* L. | **Fast** | Low | Medium | **Medium** | **Low** | Medium | 3 | [1; 3] |
| *Brassicaceae* | Camelina | *Camelina sativa* L. | **Fast** | **High** | **Fast** | Low | **Low** | **Early** | 5 | [1; 3] |
| | White mustard | *Sinapsis alba* L. | **Fast** | Medium | **Fast** | **Medium** | Medium | **Early** | 4 | [1; 3] |
| | Field mustard | *Brassica rapa oleifera* L. | **Fast** | Medium | **Fast** | Low | Medium | **Early** | 3 | [1; 3] |
| | Oilseed rape | *Brassica napus* L. | **Fast** | Low | **Fast** | High | Medium | **Early** | 3 | [1; 2; 3] |
| | Brown mustard | *Brassica juncea* L. | **Fast** | Medium | Medium | High | Medium | **Early** | 2 | [1; 3] |
| *Hydrophyllaceae* | Phacelia | *Phacelia tanacetifolia* Benth. | Slow | Medium | **Fast** | High | High | **Early** | 2 | [1; 2; 3] |
| *Polygonaceae* | Buckwheat | *Fagopyrum esculentum* M. | Medium | Low | **Fast** | High | Medium | **Early** | 2 | [1; 2; 3] |

** One common name is given as exemple*

This observation should then be further explored with dedicated field experiments in which the same insecticides, mainly lambda-cyhalothrin (pyrethroid class) or flonicamid (pyridine class), are tested. Chapter 3 revealed that lambda-cyhalothrin has a low efficacy in controlling PVY spread; consequently, the research efforts should then focus on flonicamid and other systemic insecticides that have never been tested, such as spirotetramat (keto-enol class) and thiaclopride (neonicotinoid class). The number of treatments allowed with one insecticide during the growing season is generally limited [e.g. two treatments with flonicamid, spirotetramat and thiaclopride are allowed for potatoes in Switzerland (OFAG, 2017)]. Therefore, the new insecticides should be tested by alternating the molecules every two weeks, which is the recommended frequency of treatment for these products (OFAG, 2017). For example, the insecticide treatments may start at 100% emergence with Flonicamide (0.16 kg/ha), followed by a spraying of Thiaclopride (0.3 L/ha) two weeks later, then again Flonicamide two weeks later and finally Thiaclopride with the same time interval. It would be interesting to compare the insecticides sprayed alone and in combination with mineral oil (7 L weekly), straw mulching alone and combined with mineral oil. Of course, an untreated control is also required for this experiment. Four replicates gave clear and interpretable results in the experiments of Chapter 4, and it is a minimum for PVY control trials in the field.

Prior to the implementing combined strategies into practice, the costs resulting from these combinations need to be evaluated. Two types of costs can be identified, financial costs and environmental costs. The financial costs are related to the intrinsic costs of each strategy (Table 19) and to the possibility in combining the treatments. For example, mineral oil and insecticide control strategies can easily be combined, as they are both spraying strategies and because oil and insecticides can be mixed in the same sprayer. Based on Table 19, the average costs of this combination are about 1’015 CHF/ha. The combination of mineral oil treatments with straw mulching is costlier, as both treatments are done with distinct material and need to be implemented separately. Based on Table 19, the corresponding cost is 1’375 CHF/ha. Nevertheless, these costs must be compared to the level of the protection and the possible gain related to a higher quality of the potato seed-tubers to know whether they are justified. The combination of the treatments also has an environmental cost due to the side effect of the treatments on non-target organisms. Given the low efficacy of insecticides,

it is needed to determine whether the combination of mineral oil and insecticide is justified, considering the side effects of insecticides, such as their negative effects on beneficial arthropods. Oils are also known to have side effects on non-targeted organisms. For example, oils have mortality inducing effects on the cuticle of Coleoptera, Lepidoptera and Hemiptera. They also have effects on the respiratory system of Hemiptera and Blattodea, also inducing mortality, as well as other negative effects (on receptors, tissues, nervous system and metabolism) on the same insect orders and on Thysanoptera and Diptera (Buteler & Stadler, 2011). Due to their toxicity for non-target organisms, the use of mineral oils for the control of PVY is prohibited in Germany (Kerstin Lindner, personal communication). Therefore, it is recommended to reduce their use as much as possible to avoid the negative side-effects on the environment. The management of PVY also evolves with the introduction of new control methods. All techniques must be assessed for their efficacy, their risk assessed with respect to the environment and their impact on the quality of the crop. The combination of control methods with partial effects is a solution to increase the protection of the potato fields against PVY spread.

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# ANNEX I

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# Chapter 7
# *Potato virus Y*: Control, Management and Seed Certification Programmes

**Brice Dupuis, Claude Bragard, Stuart Carnegie, John Kerr, Laurent Glais, Mathuresh Singh, Phillip Nolte, Jean-Louis Rolot, Kürt Demeulemeester, and Christophe Lacomme**

**Abstract** The management of *Potato virus Y* (PVY) in potato crops poses a continual challenge due to the non-persistent mode of transmission of the virus and the propagation of seed potato tubers over several generations in the field. While PVY-resistant cultivars remain the most efficient way to protect potato crops against PVY, a vast majority of cultivars grown do not display significant resistance to PVY. Due to the short time period for PVY transmission by non-colonising aphid vectors, efficient control of PVY relies on preventing aphids landing on a crop and on adopting precautionary measures by ensuring that crops are grown in areas of

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low aphid and low virus pressure and limiting field generation. Prophylactic measures such as roguing and early haulm destruction limit PVY spread but are not efficient alone. Among all existing control methods, spraying potato crops with mineral oils can offer significant protection against PVY spread, but their efficacy do vary in field conditions. The combination of several control methods such as mineral oil treatments, crop borders, intercropping, straw mulching or insecticide treatments can increase protection. These emphasise the importance of controlling virus through appropriate monitoring methods and crop management enforced by seed certification schemes through the use of 'clean' input seed and, when possible, the segregation of seed and ware crops to minimise the risk of virus transmission. This chapter presents and discusses the most widely used techniques of control and management of PVY, their effectiveness and their mode of action. This chapter also presents the history, objectives and principles of seed potato certification schemes and their role in minimising the spread of viruses within potato crops worldwide.

## 1 Introduction

The management of *Potato virus Y* (PVY) in potato crops poses a continual challenge due to the non-persistent mode of transmission of PVY and the propagation of seed potato tubers over several generations in the field, which presents a risk for primary and secondary infections of plants by PVY.

Aphids transmit the virus by flying from plant to plant within or between crops (Sigvald 1984; Boiteau 1997). An aphid will probe an infected plant, acquire the virus and then fly to a healthy plant, probe again and transmit the virus. The length of time required by an aphid for acquisition and subsequent transmission of PVY is very short, with each step generally accomplished in seconds (Fereres and Moreno 2009; Robert et al. 2000; Bragard et al. 2013) (see Chap. 6). The challenge in controlling PVY efficiently lies in either preventing aphids landing on a crop or in promoting a rapid (almost instantaneous) deleterious effect on them after landing to prevent any further transmission. These aspects will be discussed in the following sections.

Potatoes are vegetatively multiplied by seed potato growers in order to maintain the characteristics of a cultivar and to bulk up sufficient quantities of certified seed potatoes to meet the requirements of the market (Frost et al. 2013). This method of propagation enables PVY to be transmitted from one generation of a crop to the next through its translocation from an infected seed tuber into growing plant and daughter tubers (Basky and Almasi 2005). While it is still not possible to prevent translocation of the virus, it is, however, possible to minimise the multiplication of an infected seed lot by a systematic control of its quality. This can be done by the implementation of seed potato certification programmes and appropriate virus testing regimes.

A number of methods have been developed to control the spread of PVY in potato crops, and some of them, either individually or in combination, are now used by seed potato producers. This chapter will present and discuss the most widely used techniques of control and management of PVY, their effectiveness and their mode of action. This chapter will also present the history, objectives and principles of seed potato certification programmes and the role of these programmes in minimising the spread of viruses within potato crops worldwide.

## 2 Cultural Methods

### *2.1 Prophylactic Measures*

#### 2.1.1 Use of Virus-Free Seed Potatoes

The risk of PVY spread can be reduced by planting seed potatoes which are virus free or have a very low incidence of tuber infection by PVY, thus minimising the number of inoculum sources within a crop (Kerlan et al. 1987; Rolot 2005; Steinger et al. 2014). This will be discussed in the paragraph relating to seed potato certification programmes.

#### 2.1.2 Use of Virus-Resistant Cultivars

The most effective means of controlling virus in potato crops is to grow resistant potato cultivars. This strategy allows potatoes to be produced in areas where aphid pressure is high and hence unsuitable for potato production because of disease. There are several breeding programmes worldwide whose goal is to breed new potato cultivars that are resistant to PVY (see Chap. 8). In summary, there are several different types of resistance (see Chaps. 2 and 8): (i) extreme resistance which does not allow a virus to multiply in a plant, (ii) hypersensitive response is defined as a mechanism of resistance against pathogen invasion by the rapid death of cells at the infection site preventing/reducing significantly the spread of pathogens to other parts of the plant and (iii) tolerance which allows a virus to multiply and spread within a plant without it expressing visible symptoms. However, breeders develop their selection strategy independently depending on market requirements, and the specific disease and pest pressures in the countries intended for marketing. In the USA and Canada, breeders have developed a number of widely grown cultivars which are tolerant to PVY, such as cv. Russet Norkotah, which shows mild or 'latent' symptoms of PVY (Whitworth et al. 2010), and cv. Red LaSoda, which is fully susceptible to infection by PVY but does not express symptoms (Draper et al. 2002). This could be one of the reasons for the re-emergence of PVY in these potato production areas (Gray et al. 2010; Schramm et al. 2011). In Germany and France, the selection programmes for new cultivars are different. Breeders mainly aim to

develop potato cultivars resistant to the multiplication of PVY and avoid the selection of cultivars susceptible to potato tuber necrotic ringspot disease (PTNRD) (Le Romancer and Kerlan 1991). However, it is widely believed that breeding for cultivars displaying high level of PVY resistance might often not be seen as a major goal due to poor economic return on investments (Ruedi Schwaerzel, personal communication).

### 2.1.3 Isolation from Virus Sources

Location of crops is important in determining the risk of PVY transmission by aphids (Kerlan et al. 1987). Low-risk areas are usually those with low aphid populations, often as a consequence of low mean temperatures (Robert et al. 2000; Gabriel 1965). Klueken et al. (2009) studied the flight behaviour of alate cereal aphid *Sitobion avenae* (Fabricius) and demonstrated that, for a 16-h period, no aphid flight occurred at 10°C, while the proportion of aphids flying increased to 70% at 15°C to reach 100% at 20°C. They also showed that the temperature threshold was 3°C higher for *Metopolophium dirhodum* (Walker) (bird cherry oat aphid), another cereal aphid. This implies that, for cold temperature, these aphid species are likely to stay on their 'winter host' instead of colonising crops ('summer hosts'). Under these conditions, vector pressure and aphid transmission of PVY are minimised. In Switzerland, it was shown that an increase in altitude from 400 m to 800 m resulted in a decrease in infection of potatoes by 57%, mainly due to an average colder temperature at the higher altitude (Steinger et al. 2014). In addition, strong wind speeds (> 0.8 km/h) are not favourable for alate aphid displacement but also for aphid feeding and development, so windy areas tend to be more suited to seed potato production because there is less opportunity for aphids to transmit virus (CIP 1979; Walters and Dixon 1984). The presence of plants infected by PVY in neighbouring crops can also pose a risk with regard to inoculum sources of PVY, especially if the seed potatoes planted are not certified and the crop is being grown for consumption (ware) without management measures to control aphids. Virus is likely to be more prevalent in farm-saved seed potatoes than in certified seed potatoes (Kerlan et al. 1987).

### 2.1.4 Time of Planting and Haulm Killing

The physiological state of a plant may affect the transmission of PVY by aphids because older plants appear to be less susceptible to PVY infection than younger plants (Gibson 1991; Robert et al. 2000; Sigvald 1985; Dupuis 2016), a phenomenon termed mature plant resistance (Beemster 1972). Sigvald (1985) reported that potato plants are at their most susceptible state up to 25 days post-emergence, becoming more resistant thereafter at the rate of 10% every week. Managing the physiology of plants could, therefore, be integrated into a programme to control PVY in potato crops. Advancing the time of planting and haulm destruction could be important in reducing the risk of PVY spread. Pre-sprouting seed potatoes can

enable plants to emerge earlier and daughter tubers to bulk earlier, thus allowing earlier haulm destruction than with unsprouted tubers. However, the effectiveness of pre-sprouting in controlling PVY spread will depend to a great extent on the timing of aphid flights. If aphid flights are late in the growing season, pre-sprouting could be an effective measure. However, if spring aphid flights are earlier than normal, this could pose a greater risk to crops which have been planted with sprouted seed tubers because the plants will have emerged when aphids are flying, whereas those from unsprouted seed potatoes will not (Saucke and Doring 2004). Early haulm destruction will reduce the time of exposure of the crop to aphid flights, thereby reducing the risk of infection (Basky 2003; Kerlan et al. 1987; Steinger et al. 2014). In some conditions, it has been reported that delaying haulm destruction can increase the incidence of PVY infection by 3.5% per day (Steinger et al. 2014). For mature crops, the choice of herbicide or the use of mechanical haulm destruction has a limited impact on reducing virus transmission (Dupuis and Schwaerzel 2011). Nevertheless, rapid and total haulm destruction is required to reduce the risk of late PVY transmission. If new foliage develops on desiccated stems, transmission of PVY by aphids could be possible as the relatively immature leaves are potentially susceptible to PVY transmission (Sigvald 1985). New growth that develops after haulm destruction can be susceptible to aphid-borne infection especially when significant aphid pressure is still observed late in the growing season. However, quantitative data are still lacking to assess accurately the impact of late infection of new growth and PVY incidence at post-harvest.

## *2.2 Roguing and Weed Control*

For effective control of PVY in crops, it is essential to eliminate any sources of inoculum. These can be plants from infected seed tubers, from weeds or from volunteer plants, *i.e.* potato plants derived from tubers or parts of a tuber left in the soil after harvesting a previous crop (Jones et al. 1996). The use of certified seed potatoes according to official tolerances can provide assurance regarding the maximum amount of virus disease which could develop in a crop, but cannot rule out the absence of viruses in a crop. When a crop is being grown for marketing as seed potatoes, roguing is a key component in maintaining the health of a crop. Roguing is the removal of potato plants which are atypical of the cultivar in appearance or diseased including virus (Kerlan et al. 1987). For virus diseases, roguing is efficient in reducing PVY spread, but the effect can vary from none to a 20% reduction of PVY spread (Broadbent et al. 1950). To be effective, roguing should be conducted as soon as possible after plant emergence to minimise the opportunity for aphids to acquire virus from infected plants within a crop. However, roguing will be ineffective if a cultivar is tolerant to a virus because infected plants will be symptomless and, therefore, not recognised and removed. There is a risk that excessive amounts of virus can build up in crops of such cultivars cultivated over several generations and pose a serious threat to the health of crops of susceptible cultivars from aphid

transmission (Ragsdale et al. 2011). It is also possible that roguing could enhance the transmission of PVY by aphids if too many plants are removed in a small area creating bare patches. Aphids land preferentially in areas where there is a contrast between bare soil and potato plants (reviewed in Döring (2014)). Excessive gaps might promote the landing of aphids and, consequently, increase the risk of infection of plants surrounding a gap. The risk of infection increases with the size of the gap (Davis et al. 2009). The incidence of PVY infection was 13% around gaps of $\leq$ 0.6 $m^2$ and 29% around gaps of $\geq$ 0.6 $m^2$.

Volunteer plants are a major concern in potato-growing areas. It has been estimated that in 1 hectare, 20,000–300,000 tubers could remain in a potato field following harvest because a significant proportion of tubers are too small to be collected by harvesters and will remain in the soil over the winter (Yves Le Hingrat, personal communication). Some of these tubers will survive the winter if soil temperatures are not sufficiently cold enough. The extent of tuber overwintering will vary with their depth in soil, the severity and length of cold periods (Lutman 1977; Boydston et al. 2006; Cooke et al. 2011). The growth of plants from surviving tubers (volunteer potatoes) even after several years of crop rotation could be impaired by the foliar growth of the newly planted crop, depending on its capacity to cover and shade volunteer potato plants. In France between 2007 and 2011, the density of volunteer plants in fields in the Brittany region was estimated to range from none to six stems per $m^2$ depending on cultivar, cropping practices and succeeding crop (Rakotonindraina et al. 2011). In addition to volunteer potatoes being a source of varietal mixtures in potato crops and posing weed control issues in other field crops, they can also act as a reservoir for potato diseases and could impact on the phytosanitary status of seed potatoes. A survey was conducted in the UK in 1996 in which volunteer plants were collected from three different sites and the percentage of volunteer plants infected by PVY was found to range from 2 to 54% (Jones et al. 1996). Inoculum from within or near a crop can also originate from weeds. In the late 1990s, 36 weed species belonging to 13 different botanical families were identified as potential host plants for PVY (in natural and/or artificial conditions) (Edwardson and Christie 1997). More recently, seven additional weed species have been identified as potential hosts (Kaliciak and Syller 2009; Kazinczi et al. 2004) (see Chap. 6). It is almost certain that other weed hosts for PVY will be reported in the future. However, the epidemiological role and impact of those weeds in the field are not fully understood, and it is unclear whether the presence of weed species will contribute significantly to the spread and prevalence of PVY in potato crops.

## *2.3 Crop Borders*

Crop borders can be planted in order to limit the amount of virus introduced into a crop (Boiteau et al. 2009; Difonzo et al. 1996). Crop borders display two distinct modes of action, serving as a ‘virus barrier’ and also as a ‘virus sink’ (Boiteau et al. 2009). Viruliferous aphids landing on a border crop will probe the plants and shed

any virus particles that they might be carrying into the barrier plant. In this case, the border crop serves the role of 'virus sink' by retaining the virus. To be effective, border crops must be planted with a plant species which is not susceptible to PVY. Border plants can also act as a physical barrier interrupting aphid flight (Simons 1957). To provide this type of protection, the border plants have to be taller than the potato plants at all stages of growth. A border must also be wide enough to maximise the probability of aphids landing on it (Boiteau et al. 2009; Difonzo et al. 1996). With a soybean border of 24 m, Difonzo et al. (1996) obtained an efficacy of PVY control of 27 and 60% over the 2 years of an experiment, while Boiteau et al. (2009) obtained 32% efficacy for an experiment with a narrower border of 4 m sown with grass. This technique has the advantage of being effective for the entire cropping season, whatever the environmental conditions. Nevertheless, the use of crop borders requires relatively large areas of a field to be removed from potato production with no commercial return so the method may not be practical, especially if fields are relatively small. To solve this issue, a potato cultivar resistant to PVY can be used as a border. A border of 4 m of cv. Kennebec (known to be relatively resistant to PVY) was used in a 3-year field trial in Canada to protect a central plot of cv. Russet Burbank, susceptible to PVY, and its effectiveness was compared with applying mineral oil (Boiteau et al. 2009). The border crop alone reduced PVY transmission by about 20% in the first 2 years of the trial and 60% in the third year, while mineral oil application alone reduced PVY transmission by 20% in the first 2 years and by 70% in the third year.

## *2.4 Mulching and Intercropping*

Straw mulching (Fig. 7.1) is effective in controlling PVY spread (Heimbach et al. 2004; Saucke and Doring 2004; Kirchner et al. 2014; Dupuis et al. 2010). The mode of action of straw mulching is not well understood, but the main hypothesis is that straw impacts on the visual perception of a crop by an aphid (Döring 2014). The contrast between potato foliage and the yellow straw is considerably lower than potato foliage and bare soil, so that potato plants in mulched plots are less easily seen by aphids (Döring 2014; Döring and Schmidt 2007). This was confirmed when fewer aphids were captured in mulched plots compared with those in bare soil plots (Heimbach et al. 2004; Saucke and Doring 2004). In the study reported by Saucke and Doring (2004), aphids were counted on the foliage of potato plants in straw-mulched and bare soil plots. An average reduction of 54% in aphid numbers was recorded in the mulched plots. This reduction of aphid populations on foliage was 18% in an independent study (Heimbach et al. 2004), while in the same experiment, 85% fewer winged aphids were captured by sticky net traps within the mulched plots. Thus, the lower number of winged aphids landing on potato plants in mulched plots might be the main factor in controlling PVY spread by mulching. PVY control by mulching is more effective in the early stages of crop development, declining as the crop canopy develops over the mulch (Heimbach et al. 2004). This was shown

**Fig. 7.1** Wheat straw mulch in potatoes, 2500 kg/ha of straw in cv. Charlotte (Photo: Maud Tallant)

**Fig. 7.2** Oat intercropping (*Avena sativa*, 60kg/ha) in potatoes (cv. Charlotte), 7 days after spraying oats with tepraloxydim (200g/l) to stop their development (Photo: Maud Tallant)

clearly in a field experiment undertaken over a 3-year period (Saucke and Doring 2004) in which the efficacy of PVY control recorded in the mulched plots was about 48% in the first year, 33% in the second year and 27% in the third year. In the first year of the trial, aphids were most prevalent a few days after potato emergence in mid-May, whereas in the third year of the trial, aphids were most active 2 weeks later. In the second year of the trial, aphid activity remained low during the entire growing period. Intercropping was also tested for its effectiveness in reducing PVY spread in potato crops (Dupuis et al. (2010); Fig. 7.2). Oats sown between rows of potatoes but killed before becoming large enough to provide unwanted competition for the potatoes resulted in a significant reduction in PVY spread (Fig. 7.3). The mode of action of intercropping is comparable to the 'sink effect' of a border crop

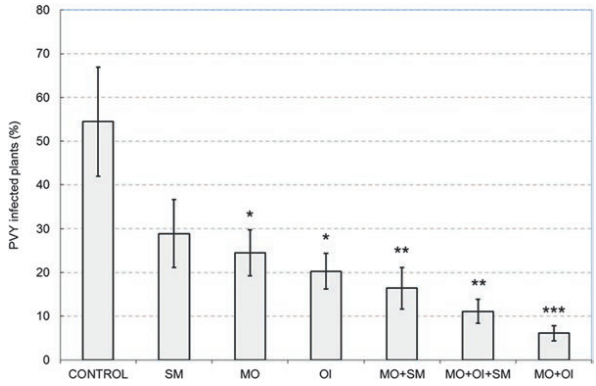


**Fig. 7.3** Percentage of PVY-infected plants assessed by testing daughter tubers for six treatments and untreated (1 year; four replications of 100 plants per plot). The acronyms for the treatments are *SM* = straw mulching, *MO* = mineral oil, *OI* = oat intercropping. Error bars show the standard error, and stars show the treatments with a percentage of infection significantly lower than untreated (Dunnett's test; * for $p<0.05$ and ** for $p<0.01$) (Dupuis et al. 2010)

in which inoculum being carried by aphids is lost during probing on the associated crop (Boiteau et al. 2009). Intercropping offers an additional mode of action called the 'dilution effect' by significantly decreasing the probability of an aphid landing on potato plants. Given the same plot surface, plots containing an associated crop present many more plants to potential vectors, thereby reducing the risk of potatoes being infected.

## 3 Chemical Methods

### *3.1 Oil Treatments*

Spraying oil on potato plants has been reported to provide a means of controlling the spread of PVY (Boiteau et al. 2009, Bell 1980, Bell 1989, Boiteau and Wood 1982, Bradley et al. 1962, 1966, Dewijs 1980, Dupuis et al. 2014, Hansen and Nielsen 2012, Kirchner et al. 2014, Martin-Lopez et al. 2006, Milosevic 1996, Steinger et al. 2014, Rolot 2005). Mineral oil was found to be more effective than vegetable oil (Martin-Lopez et al. 2006; Rolot 2005; Wrobel 2012), although among the latter, refined oils were more effective than raw oils (Martin-Lopez et al. 2006). The mechanism of action of the oils is not fully understood. It has been suggested

that oil on aphid mouthparts reduces acquisition and retention of PVY by aphids. Oil may impede binding of virus particles on to the stylet (Bradley 1963; Boquel et al. 2013; Loebenstein et al. 1964; Powell 1992) and/or shorten the duration of virus retention in the stylets (Wróbel 2009). Mineral oil has also been shown to reduce virus replication and accumulation in inoculated plants, possibly due to the general activation of defence mechanisms in a plant (Loebenstein et al. 1964; Peters and Lebbink 1973; Martoub 2010; Al-Daoud et al. 2014). Oil also has the ability to kill aphids by interfering physically with their respiration (Martin-Lopez et al. 2006; Hesler and Plapp 1986). Studies have investigated the effects of mineral oil treatment of potato plants on selection of host plant, growth and reproduction of the colonising potato aphid, *Macrosiphum euphorbiae* (Thomas) (Ameline et al. 2009). Olfactometry experiments showed that mineral oil treatment induced a transient repellent effect shortly after spraying that lasted a day. While probing behaviour was not drastically affected in oil-treated plants, the treatment resulted in antagonistic effects with a significant reduction in nymph survival shortly after treatment and concomitantly a higher fitness and fecundity rate in adult aphids (Ameline et al. 2009). Other studies have reported antagonistic effects of mineral oil on *M. euphorbiae* that were dose dependent; topical contact at the highest concentration of oil resulted in complete mortality, while lower concentrations and exposure to oil volatiles enhanced aphid fecundity but had no effect on aphid survival (Martoub 2010). Tan et al. (2005) suggested that, after spraying, oil is 'absorbed' in various plant tissues and cells and transported throughout the plant, resulting in a decrease in local concentrations and the induction of physiological changes (Tan et al. 2005), altering photosynthesis (Helson and Minshall 1951; Wedding et al. 1952), and triggering the expression of pathogenesis-related proteins (Kachroo et al. 2001; Lin et al. 1996).

In practice, the protection achieved with mineral oil spraying varies greatly depending on the time of application (Figs. 7.3, 7.5 and 7.6). Mineral oils are more effective on older plants in reducing the speed with which PVY moves in the vascular system (Al-Daoud et al. 2014). The reduction in the transmission of PVY by oil treatments is likely to vary among different potato cultivars and with different aphid and virus pressures. Steinger et al. (2014) reported that oil treatments reduced the average incidence of PVY by 39% ($p<0.001$) over a 4-year period. The protective effect of mineral oil was slightly greater in the year in which the incidence of PVY was greatest (50% decrease in infection $N_{treated} = 432$, $N_{non\text{-}treated} = 86$) than in other years and with the susceptible cvs Bintje and Charlotte (54% decrease in infection, $N_{treated} = 819$, $N_{non\text{-}treated} = 79$) compared with more resistant cultivars. In a separate study over 3 years of field trials in the UK, mineral oil reduced PVY infection two- to threefold in cvs King Edward and Maris Piper ($N = 160$ plants per cultivar per treatment per year) during a year of relatively high virus pressure (overall 30% incidence of plants infected by PVY) (Dawson et al. 2015). However, there was no significant effect of mineral oil treatments on the incidence of PVY in years of low virus pressure (3–13% PVY incidence). In this study, significant variation in the effectiveness of the oil treatment in controlling PVY suggests that local differences in aphid phenology and aphid vector pressure strongly influenced the effectiveness of the treatment. These results are consistent with the expected reduction in the

incidence of PVY for oil-based treatments compared with untreated plants, usually between 30 and 60% (reviewed by Al-Mrabeh et al. (2010)).

Mineral oils are usually applied weekly to a seed potato crop. The protection provided by oil is generally limited to the leaf surfaces exposed to the spray being applied (Simons et al. 1977). Effective coverage of foliage is easier to achieve on older plants than on younger plants on which new leaves are constantly developing. The rate of foliar development should be considered when determining the optimum interval between sprays for effective application of oil. An increase in the frequency of foliar applications on young plants should be considered for maximum effectiveness in order to protect recently opened leaves (Fageria et al. 2014a, b; Demeulemeester 2013). The usual practice in France to protect high-grade seed is to spray mineral oil three times a week starting at 30% plant emergence and continuing until complete emergence followed by weekly applications thereafter. In Belgium, the practice is very similar except that only two applications are recommended during the early period of crop growth followed by one each week until haulm destruction is complete (Yves Le Hingrat and Pierre Lebrun, personal communication).

Mineral and vegetable oils must be used with caution to avoid plant phytotoxicity (Kirchner et al. 2014). It is essential to use a labelled product (Dewijs 1980). Paraffinic oils are preferably used for the treatment of plants instead of naphthenic oils (phytotoxic) and aromatic oils (phytotoxic and unstable) (Rolot 2005). The linear structure of saturated paraffinic oils is relatively stable and not phytotoxic. Dewijs et al. (1979) demonstrated that the viscosity of the oil, which is related to the number of carbon atoms in the molecular chain, is an important character determining the efficacy of the oil. Nevertheless, when the chains have more than 25 carbon atoms, phytotoxicity can be observed (Walsh 2000). The phytotoxicity of the paraffinic oil also depends on its degree of refining. Less than 8% of sulphonated residues (residues reacting with sulphuric acid) are required for plant treatments (Walsh 2000). For treatments with paraffinic oils, it is also required to not exceed the maximum rate and to avoid application during hot weather because oil can become so hot that it can burn potato foliage on application after being heated in the sprayer pipes during prolonged sun exposure. Mineral oil treatment can alter plant physiology and, in some cases, be phytotoxic. This can have an adverse effect on the appearance of plants and potentially might affect crop inspection by reducing a seed potato inspector's ability to identify cultivars and virus symptoms visually in a growing crop. A recent study in the UK of the effect of oil treatments on a range of virus-infected and healthy plants of various potato cultivars concluded that the ability of inspectors to identify both cultivars and virus symptoms was not diminished by applications of mineral oil to foliage (Dawson et al. 2015), even though phytotoxic symptoms (localised necrotic spots) were occasionally observed (Fig.7.4). However, some loss of tuber yield was reported for some cultivars after mineral oil treatment, emphasising the necessity for a cautious use of mineral oil to control PVY (Kirchner et al. 2014). Delaying haulm destruction by several days could compensate for this reduction in yield (Dawson et al. 2015).


**Fig. 7.4** Phytotoxic symptoms (localised necrotic spots and leaf midrib necrosis) developing on leaves of potato cv. Desiree following mineral oil spraying (Dawson et al. 2015). Note the beading of rainwater on the foliage of sprayed plants (Photo: Courtesy of SASA Crown copyright)

### *3.2 Insecticide Treatments*

The acquisition and inoculation periods of non-persistent viruses like PVY by their aphid vectors are extremely short (seconds to minutes) (Bragard et al. 2013). To be effective, an insecticide has to kill or incapacitate an aphid very quickly to limit the infection of a plant. However, the effect of an insecticide may impair the capacity of a viruliferous aphid to fly from a treated plant to another plant. Various insecticides and their formulations have been tested for their effectiveness in controlling PVY transmission in the field (Table 7.1). Pyrethroids have a near-instantaneous 'knock-down' effect and can provide a reduction in PVY transmission in controlled conditions (Perring et al. 1999; Gibson et al. 1982; Collar et al. 1997). Unfortunately, results obtained in field-grown potato crops with the same products have proven to be variable (Table 7.1). Other group of insecticides with different modes of action can potentially interfere with the transmission of viruses either by repelling aphids (deltamethrin, Rice et al. (1983)), altering feeding behaviour (flonicamid, imidacloprid, pymetrozine and thiamethoxam, Morita et al. (2007), Cho et al. (2011), Boquel et al. (2014)) and reducing aphid's movement (aldicarb, Boiteau et al. (1985)). However, no significant reduction of PVY transmission was reported in field trials for imidacloprid, pymetrozine (Table 7.1) and flonicamid (Fig. 7.5). Pyrethroids such as deltamethrin (Gibson et al. 1982) and cypermethrin (Collar et al. 1997) can provide a significant degree of PVY protection in controlled conditions, but it is not known whether virus acquisition or transmission is affected. Lambda-cyhalothrin, dimethoate and pymetrozine reduce PVY acquisition by aphids (Boquel et al. 2014;

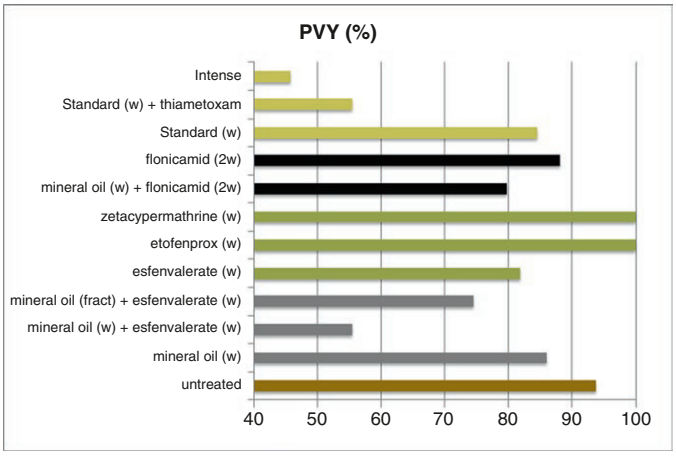


**Fig. 7.5** Percentage of PVY-infected daughter tubers produced by plants treated with a range of insecticides and mineral oil. The insecticides used belong to different chemical groups such as pyrethroids (esfenvalerate, etofenprox, zeta-cypermethrin), neonicotinoids (thiacloprid, thiamethoxam) and pyridine (flonicamid, pymetrozine). The 'standard' programme refers to a weekly application of mineral oil, together with a pyrethroid in the first week after emergence (esfenvalerate) and a systemic insecticide every 2 weeks (one treatment with flonicamid followed by pymetrozine and thiacloprid). The 'intense' programme is similar to the 'standard' programme with, in addition, two applications of mineral oil in the first week after emergence. The letter (w) means weekly application, (2w) means one application every 2 weeks and (fract) means two applications per week with half dose for the first 3 weeks after emergence and thereafter one application per week (Demeulemeester 2013)

Margaritopoulos et al. 2010), but the effect of these insecticides is too slow to prevent PVY spread in the field (Table 7.1). In summary, for most of the field trial studies reported, the efficacy of an insecticide in controlling PVY spread was either not significant, of a limited impact or highly variable between independent experiments. One of the reasons that insecticides have been demonstrated to be either ineffective or only of limited effectiveness is because many aphids which transmit PVY do not colonise plants in a crop and thus only come in contact with the insecticides for a very limited period (reviewed in Perring et al. (1999)).

**Table 7.1** Insecticides tested in field trials to control the spread of Potato virus Y (PVY)

| Active compound | Chemical group | Application | Protection | Authors |
|---|---|---|---|---|
| Cypermethrin | Pyrethroid | Foliage | 7%; | Bell (1989) |
| | | | 29%; | Gibson and Cayley (1984) |
| | | | NS | Martin-Lopez et al. (2006) |
| Demeton-S-methyl | Organophosphate | Foliage | NS | Milosevic (1996) |
| Esfenvalerate | Pyrethroid | Foliage | 29%; NS; NS (Years 1, 2 and 3) | Kirchner et al. (2014) |
| Imidacloprid | Neonicotinoid | In furrow at planting | NS | Boiteau and Singh (1999), Alyokhin et al. (2002) |
| | | Seed tubers | NS | van Toor et al. (2009) |
| | | Foliage | 53%; | Alyokhin et al. (2002) |
| | | | NS | Boiteau and Singh (1999) |
| Lambda-cyhalothrin | Pyrethroid | Foliage | 10% | Dupuis et al. (2014) |
| | | | NS | Hansen and Nielsen (2012) |
| | | | NS | van Toor et al. (2009) |
| Permethrin | Pyrethroid | Foliage | 22% | Bell (1989) |
| Pymetrozine | Pyridine | Foliage | NS | van Toor et al. (2009) |
| | | | | Rolot et al. (2006) |
| Thiomethon and tau-fluvalinate | Organophosphorus and pyrethroid | Foliage | 8% | Rolot (2005) |
| Tau-fluvalinate and thiacloprid | Pyrethroid and neonicotinoid | Foliage | NS | Kirchner et al. (2014) |
| Thiamethoxam | Neonicotinoid | Foliage | NS | Kirchner et al. (2014) |

The table shows the name of the active compound and its chemical group, the type of treatment applied in the trial, the authors and the corresponding protective efficiency expressed as a percentage of reduction in the incidence of tuber infection in comparison with that for untreated plants (NS = test not significant or no protective effect)

### *3.3 Limitation of Chemical Control: Insecticide Resistance in Aphids*

The (intensive) use of insecticides has led to the development of different mechanisms of resistance in some aphid populations. The main aphid vectors displaying various levels of resistances to insecticides worldwide are *Myzus persicae* (Sulzer), *Aphis nasturtii* (Kaltenbach), *Macrosiphum euphorbiae* (Thomas) and *Sitobion avenae* (Fabricius) (Foster et al. 2014, 2002). Different types of biochemical and molecular mechanisms of insecticide resistance can be found with *Myzus persicae* (Bass et al. 2014), which includes (i) the overproduction of carboxylesterases (conferring broad-spectrum resistance to members of the organophosphate (OP), (monomethyl) carbamate and, to a much lesser extent, pyrethroid classes) that hydrolyse the active compound before it affects the nervous system of the insect; (ii) mutation of the acetylcholinesterase enzyme (MACE – modified acetylcholinesterase) resulting in the insensitivity of target site of the insecticides, the acetylcholinesterase enzyme; (iii) mutation of the voltage-gated sodium channel, also named 'knock-down resistance' (kdr or super-kdr for the enhanced allelic form), which is conferred by a mutation of a transmembrane ion channel that plays an essential role in the initiation and propagation of action potentials in neurons; and (iv) the mutation of the nicotinic acetylcholine receptor (nAChR), a neurotransmitter-gated ion channel that plays an important role in nerve signalling (Bass et al. 2014). In addition, there are new types of resistance that are currently being identified (such as resistance to pyridine compounds) (Table 7.2; Bass et al. (2014)). Table 7.2 presents the chemical groups of insecticides frequently used in potato production and the corresponding resistance mechanism displayed by *Myzus persicae*. The incidence of *Myzus persicae* susceptible to the insecticides listed in Table 7.2 is highly variable from one year to another. For example, in aphid samples collected in the UK in 2004, about 60% of the sampled aphids were genotypically associated with carboxylesterases-mediated resistance, 70% with MACE and 80% with super-kdr, while 5 years before, this proportion was about 20%–5%–80% respectively (IRAG

**Table 7.2** Examples of chemical groups and active compounds available for aphid control in potatoes and types of resistance developed by *Myzus persicae* (IRAG 2014; Bass et al. 2014)

| Chemical group | Active compound(s) | Resistance mechanism (period of first reported resistance) |
|---|---|---|
| Dimethyl carbamate | Pirimicarb | MACE (1940s) and/or carboxylesterase (1950s) |
| Neonicotinoid | Acetamiprid, thiacloprid, thiamethoxam | Alteration of nAChR (1990s) |
| Pyrethroid | Esfenvalerate, λ-cyhalothrin, etofenprox, cypermethrin | Super-kdr (1970s) |
| Pyridine azomethine | Pymethrozine | Action on the chordotonal organ TRPV channel modulators (1990s) |
| Pyridine carboxamide | Flonicamid | Action on the chordotonal organ TRPV channel modulators (2010) |

2008). *Myzus persicae* individuals resistant to neonicotinoids (with an alteration of the nAChR) have not yet been found in the UK; however, a survey performed in Southern France and Northern Spain in 2010 revealed that resistance to neonicotinoids is relatively widespread in these regions, with an average of 76% of resistance to thiamethoxam (Slater et al. 2012). Combinations of the three main resistance mechanisms (MACE, carboxylesterase and kdr) can be found in individuals of *Myzus persicae*, but the most common association of resistances found in the field in the UK are MACE with super-kdr (IRAG 2008). Consequently, growers are recommended to minimise whenever possible the use of insecticide mixtures in order to prevent the outbreak of *Myzus persicae* populations displaying cross-resistances. Populations of *Aphis nasturtii* (Buckthorn-potato aphid) sampled in Belgium and northern France in the 1990s were found to be resistant to insecticide; however, resistance in *Aphis nasturtii* seems to be a relatively rare event (Duvauchelle et al. 1997). *Macrosiphum euphorbiae* has developed a resistance mechanism based on overproduction of an esterase after treatment with pirimicarb and λ-cyhalothrin. This mechanism of resistance is analogous to the one found in *M. persicae*. However, it is not known if this mode of resistance is effective when treatments are applied at the recommended rate (Foster et al. 2002). Kdr resistance to pyrethroids (λ-cyhalothrin) was found in *Sitobion avenae* in the UK (Foster et al. 2014), and the prevalence of resistant individuals in 2015 varied from 45 to 75% depending on the location of sampling (Malloch et al. 2016).

### *3.4 Synthetic Pheromones*

Numerous aphid species secrete pheromones as a behavioural mechanism when attacked by natural enemies (Kislow and Edwards 1972; Nault et al. 1973). (E)-β-farnesene (EβF) is the main alarm pheromone (Pickett et al. 1992; Zhang et al. 1997). EβF has a repellent effect on aphid colonies and affects the development and fecundity of the aphid populations (Su et al. 2006; Gibson et al. 1984). Additionally, it was shown in the laboratory that EβF inhibits the acquisition and subsequent inoculation of PVY by *Myzus persicae* (Sulzer) (Gibson et al. 1984). Another laboratory experiment performed with tobacco plantlets using apterous aphids (*Myzus persicae* (Sulzer) and *Macrosiphum euphorbiae* (Thomas)) revealed a higher spread of PVY when the aphids are in contact with EβF (Lin et al. 2016). This result suggests an increase in the movement of apterous aphids from plant to plant in the presence of EβF. Apterous aphids are known to be able to move from plant to plant even without the overlapping of crop canopy, by walking on the soil surface (Alyokhin and Sewell 2003; Narayandas and Alyokhin 2006). Nevertheless, winged aphids are more motile in the crop by flying from plant to plant and, hence, have a higher impact on PVY spread in the field (Boiteau 1997). A 1-year experiment undertaken in field conditions showed that PVY spread was identical with or without the diffusion of EβF (Crutzen et al. 2014). While pheromone treatment might have a limited impact on the aphid transmission of PVY, the efficacy of insecticide

in controlling aphid populations can be improved by using EβF treatments, as reported in Chinese cabbage fields treated simultaneously with imidacloprid and EβF (Cui et al. 2012). The strategy of combining EβF diffusion with insecticide treatments to control PVY spread has yet to be tested and might represent an interesting approach to control aphid populations and consequently virus transmission.

### *3.5 Elicitors*

Several chemicals are able to induce systemic resistance of plants to pathogens (Kessmann et al. 1994). Among those chemicals, salicylic acid (SA) is known to induce resistance to a wide range of pathogens including viruses (Nakayama et al. 1996; Vasyukova and Ozeretskovskaya 2007). It was shown that *Potato virus X* (PVX) accumulation in tobacco leaves is reduced after SA treatment (Naylor et al. 1998). This effect was also observed in tomato leaves inoculated with PVY and treated with acibenzolar-S-methyl (Bion®), a functional analogue of SA (Petrov and Andonova 2012). Bion® was also tested in a field trial to control PVY spread in potatoes, which resulted in a relatively low (14%) but nevertheless significant reduction of PVY transmission (Dupuis et al. 2014).

## 4 Combined Methods

Many of the techniques cited above have often been tested in combination. Crop borders and mineral oil treatments have been tested together, and the combination of those two techniques was more effective than when they were applied individually. In the field trials of Boiteau et al. (2009), the combination of applying oil sprays to potato crops and a border crop improved the efficacy of PVY control compared with oil treatment and borders used alone: 47–59% reduction in the incidence of PVY in the first year, 57–63% in the second year and 79–97% in the third year of field trials. Insecticide treatment of a border crop did not produce a reduction in the incidence of tuber infection by PVY (Difonzo et al. 1996). A synergistic effect of applying straw mulching and mineral oil together was recorded in a 1-year trial in Switzerland (Fig. 7.3), but no synergy was found in a similar trial in Finland (Kirchner et al. 2014). In the Swiss trial, the efficacy of PVY control with straw mulching was about 47%, while spraying mineral oil produced a similar efficacy of a 55% reduction in PVY. However, an efficacy of 70% was achieved when both techniques were used together. In the Finnish trial, straw mulching produced a reduction of 26% in PVY infection, but a combined mineral oil spraying and straw mulching treatment gave only a 19% reduction. It is likely that the extent of any synergistic effect of mineral oil with straw mulch may depend upon the experimental conditions of a trial. As explained above, the efficacy of mineral oil treatment increases the older the plants are when they are sprayed, while the efficacy of straw

mulching is greatest early in the season before the soil surface becomes covered by the crop canopy. Thus, the timing and location of the peak of aphid activity (data not shown) could explain the differences between those two trials. Oat intercropping was also tested with mineral oil applications, and some synergy was recorded (Fig. 7.3). Oat intercropping resulted in 63% reduction in PVY infection in the potato crop, and the efficacy of PVY reduction reached 89% when oat intercropping was used together with mineral oil spraying. The combination of insecticides and mineral oil treatments can also provide some synergy, increasing the protection of treated crops against PVY. A Canadian survey of 56 crops of seed potatoes being multiplied using distinct cropping techniques revealed that mineral oil was more effective when combined with insecticide applications, particularly when used early in the season (Mackenzie et al. 2014; 2016). This synergy was not found in all circumstances. A weekly treatment with esfenvalerate together with mineral oil gave better protection (42%) of a crop against PVY spread than a weekly spray of mineral oil alone (9%; $p<0.05$) (Fig 7.5). Contrastingly, the combination of a weekly treatment of thiomethon + fluvalinate and a twice-weekly treatment with mineral oil did not improve the protection of the crop against PVY infection ($p > 0.05$ for all years of trial) than when treatments were applied individually (Fig. 7.6). Additional studies conducted in a greenhouse have shown a significant reduction in the transmission of PVY in plants treated with mineral oil and insecticides (Martin-Lopez et al. 2006; Gibson and Rice 1986). This synergistic effect of insecticides and oil may be influenced by the particular chemistry of an insecticide, *e.g.* synthetic pyrethroids (Fig. 7.5), and by the time of application because mineral oil alone is less efficient when applied early in the season (Mackenzie et al. 2014; Gibson and Cayley 1984; Demeulemeester 2013).

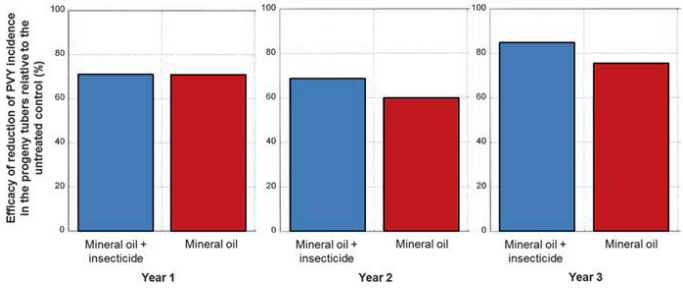


**Fig. 7.6** Efficacy in the reduction of the incidence of PVY in progeny tubers in response to foliar treatments with either mineral oil (3 l twice a week) sprayed alone or in combination with a pyrethroid insecticide (0.6 l of thiomethon (200g/l) +fluvalinate (72g/l) once a week) over a 3-year period. Each year, the trials were conducted in three different locations with three replications of each treatment (Rolot 2005)

## 5 Seed Certification Schemes

### *5.1 History and Evolution*

Potato is a vegetatively propagated crop, and consequently tubers can potentially be contaminated with a large range of organisms (Franc 2001). These pathogens can then be transmitted from the seed tuber to the new plant in the next generation of multiplication and also to healthy plants in the crop. The pathogens can range from fungi to viroids and can be carried within and/or on the surface of a tuber. The diseases caused by the pathogens can have a serious effect on the yield and the quality of a crop. Seed potatoes are potatoes intended for planting, unlike those intended for end uses such as consumption and processing. As commercial trade in seed potatoes developed, a perceived need arose for some form of independent verification of the quality of seed potatoes being marketed. This resulted in the establishment of seed potato certification schemes under official control with tolerances for quality aspects of seed potatoes being applied at each year of propagation (Rousselle et al. 1996; EPPO 1999). The first potato seed certification scheme was established in Europe (Germany) in the first years of the nineteenth century and about 10 years later in the USA (Shepard and Claflin 1975). Similar schemes were introduced in New Zealand in the late 1920s and in Australia in the 1930s (Maunder 2005; Crump 2008). Until early 1970s, production of the initial planting material was largely by 'clonal selection' (or 'positive selection'), which involved selecting disease-free tubers from apparently healthy mother plants (Gildemacher et al. 2011). As far as possible, testing was conducted on mother plants and tubers to ensure freedom from viruses known to be present in a country. However, appropriate testing was not available to confirm freedom from bacterial and fungal tuber pathogens. Subsequently, tissue culture techniques were developed for producing initial planting material in which greater security of plant health could be achieved through an extensive testing programme (Espinoza et al. 1984; Dodds 1988). In Scotland, the first technique used was 'stem cuttings' starting in the late 1960s with the aim of eliminating latent tuber-borne pathogens, especially those causing blackleg, from the initial planting material (Jeffries 1986; Hall 1993), but this was superseded by micropropagation to produce 'nuclear stock'. Nowadays, this method is used as the starting point in almost all certification schemes worldwide because it allows material to be tested comprehensively for quality and notifiable pathogens and pests (EPPO 1999; Donnelly et al. 2003; Frost et al. 2013). One exception is the Netherlands, where clonal selection is also used, together with micropropagation, to produce the initial planting material (NIVAP 2016). Tubers produced from the initial planting material are then multiplied for a number of generations in the field as seed potatoes under certification control until finally marketed for end use (Frost et al. 2013). In Asia, Africa and South America, 'informal' seed potato production systems account for 94% of the market (Thomas-Sharma et al. 2016). In informal seed potato production systems, seed tubers are sourced on farm, neighbouring farms, local markets and unofficial specialist producers (Hirpa et al. 2010). The health status of this type of

seed potatoes can be very variable, and consequently crop yields fall far short of their potential. This is largely due to the inherently high amounts of virus in the potatoes and greater aphid pressure in more favourable environments for spread. Gildemacher et al. (2011) assessed the economic return of three different seed potato schemes: (i) the use of the farmer seed stock, (ii) the use of the 'positive selection' technique and (iii) the use of certified seed from the market. This showed that buying high-quality seed is generally more economically effective than the other schemes because the yield obtained with certified seed is usually greater. This does assume that farmers can afford buying high-quality seed potatoes and that the required cultivar is available for planting.

## *5.2 Objectives of Seed Potato Certification Schemes*

The main goal of a potato certification scheme is to assure the quality of seed potatoes being marketed through an independent process of verification and testing conducted by a designated certifying authority. The aspects covered by certification are identity of cultivar, purity of crop, diseases and pests affecting quality or yield, external quality, physiology, size and labelling (UNECE 2015). Seed potatoes are propagated over a number of years so genealogy and traceability are key elements of a well-developed certification scheme. In order to maintain the health of seed potatoes during propagation, tolerances for disease and quality characteristics are set for each generation, being strictest for first generation and becoming slightly more relaxed for subsequent generations in the field (EPPO 1999). Verification of tolerances is largely conducted by visual inspection of plants or tubers supported by testing as appropriate (Franc 2001; Frost et al. 2013).

## *5.3 Principles of Certification*

Although varying degrees of complexity exist, the basic principles of seed certification remain the same (EPPO 1999). Currently, the first step is the multiplication of an in vitro pathogen-free plant (nuclear stock) which can then be multiplied in an approved facility to produce large numbers of in vitro plantlets or microtubers (*i.e.* tubers produced in vitro by a micropropagated plantlet). Nuclear stock is normally subject to an extensive testing programme to ensure its freedom from a range of specified pathogens before being used to produce minitubers in insect-free greenhouses or screenhouses (Frost et al. 2013; EPPO 1999). These tubers are then planted in the field the following year to produce the first generation of seed potatoes (Franc 2001). The import of healthy plant material or clonal selection can be used as an alternative to tissue culture (Franc 2001; Schulte-Geldermann et al. 2012). Several years of field multiplication will ensue before being used by ware potato producers. In European countries, most certification schemes classify seed

potatoes into three categories: pre-basic, basic and certified in accordance with United Nations Economic Commission for Europe (UNECE) Standard for Seed Potatoes (UNECE 2015). At least two classes within each category are normally set nationally although the possibility of agreed common classes is being explored by EU (2002). The number of years that seed potatoes can be multiplied is also limited in a scheme, normally less than nine (Frost et al. 2013), and with a maximum of nine generations allowed in the EU since 2016 (directives 2014/20/CE and 2014/21/CE). Some schemes, especially in North America, are based solely on generation number although the number does not always correspond to the number of generations in the field from initiation (Willem Schrage, personal communication). Each year, crops are visually examined by seed potato inspectors for varietal purity and a range of faults including virus diseases around the time when plants are flowering. Inspected crops have to comply with specific tolerances for faults for the class for which they have been entered; otherwise the crop is downgraded to the appropriate class or, in extreme circumstances, not accepted as being suitable for marketing as seed potatoes (Frost et al. 2013). The UNECE has developed an international standard for certification and marketing of seed potato that sets out a common terminology and minimum commercial quality requirements for the certification of high-quality seed intended for marketing internationally (UNECE 2015). This standard is a useful blueprint to facilitate seed potato trade between countries that already have their own certification schemes in accordance with the UNECE standard. It is also a good model for countries aiming to develop their own seed potato certification scheme.

## *5.4 Virus Diagnostic Methods Used in Certification Schemes*

Historically, the incidence of viruses, including PVY, in seed potato crops was assessed visually at two or more inspections according to disease symptoms reported as severe mosaic or mild mosaic (see Chap. 5). Knowledge of the virus causing symptoms was not essential for certification purposes but was nevertheless collected for future analysis. If any tolerance is exceeded at inspection, a crop is downgraded as appropriate or rejected (Shepard and Claflin 1975). This method is still used in seed potato production areas where PVY pressure is low (*e.g.* Scotland). Diseased plants seen at inspection are generally the result of secondary transmission from infected tubers although symptoms of primary infection can sometimes be observed, but this can underestimate the true extent of such infection. The incidence of symptomatic plants derived from primary infection varies with environment and time of infection. Late-season infection of a susceptible potato plant will probably result in no development of foliar symptoms although virus may be translocated to daughter tubers, highlighting the necessity for post-harvest testing (Franc 2001; Frost et al. 2013). Therefore, in some environments, relying on visual inspection may be insufficient to provide assurance that seed potatoes will meet the expectations of customers (Lindner et al. 2015). Additional measures such as a targeted

post-harvest assessment were introduced in some certification schemes to check for late-season virus infection or, in some cases, symptomless infection of tolerant cultivars.

There are numerous post-harvest diagnostic methods for viruses. The first method to be developed was termed ‘post-harvest grow-out assay’ or ‘growing-on assay’. A sample of a prescribed number of tubers is collected at random from a crop before harvest or shortly after harvest. Seed pieces consisting of a single eye are removed from each tuber and planted (with or without a treatment to break dormancy) in a greenhouse or outdoors if the climate of a country is suitable. When these tests were first conducted, the plants grown from each seed piece were then examined visually for symptoms of virus infection (Franc 2001). Microscopy (Igel-Lange test) or serological (radial-immunodiffusion) techniques were used to support these assessments (Shepard and Claflin 1975; Gugerli and Fries 1983). Visual observation was later replaced by an enzyme-linked immunosorbent assay (ELISA) for the detection of virus (see Chap. 5). France and Belgium still use ELISA for this type of testing.

A variation of this method is the so-called winter test. After breaking tuber dormancy, a standard sample from a crop is shipped to a ‘warmer’ location for planting. After emergence, each plant is visually inspected, and/or a leaf sample is taken for further testing by serological or molecular methods. The northern states of the USA use this approach and ship seed samples to southern states for a ‘winter test’. These programmes are expensive and are gradually being replaced by laboratory-based tuber tests (Shepard and Claflin 1975; Franc 2001). Laboratory-based tests of tubers were first developed in Switzerland in the early 1980s. After dormancy of the tubers is broken by fumigation with Rindite (a mixture of 2-chloroethanol, 1,2-dichloroethane and carbon tetrachloride 7:3:1), sap from the basis of the sprouts of each tested tuber is tested by ELISA (Gugerli and Gehriger 1980). In Europe, this product is banned due to its toxicity and is replaced by a solution of gibberellic acid. In this case, ELISA test is performed on recently emerged potato leaflets. ELISA is gradually being replaced by molecular techniques such as real-time RT-PCR, which has been employed in seed potato certification schemes in the Netherlands and Scotland since 2013 and in Switzerland and France since 2015 (this list of countries is not exhaustive).

## *5.5 The Role of Seed Certification Schemes for PVY Control and Future Challenges*

Seed certification schemes are essential in managing and controlling non-persistent viruses such as PVY in seed potato production through inspection of seed potato crops and statutory virus testing. They provide a purchaser with assurance that seed potatoes have been produced according to scheme requirements and standards, particularly with regard to health and cultivar purity. A post-harvest test should provide

a reasonable estimate of the incidence of PVY in the daughter crop, provided the sample collected is representative of the crop (Frost et al. 2013). Certification, therefore, offers the opportunity to discard or 'flush through' crops, which pose a risk to the health of subsequent crops. The population dynamics of PVY are changing (see references in Chap. 3). Ordinary and veinal necrotic PVY strains (*i.e.* $PVY^{O}$ and $PVY^{N}$) are gradually becoming less prevalent in Europe and are being replaced by recombinant strains $PVY^{NTN}$ and $PVY^{N\text{-}Wi}$. $PVY^{O}$ and $PVY^{N}$ are generally believed to be more aggressive than the now-prevalent above-mentioned recombinant strains and elicit severe foliar symptoms on potato plants, although this does vary between cultivars. On certain cultivars, the recombinant $PVY^{NTN}$ and $PVY^{N\text{-}Wi}$ strains tend to produce milder symptoms, and in some occasions, higher copy numbers of viral RNA were found in leaves displaying milder symptoms (Lindner et al. 2015; Karasev and Gray 2013). This suggests an apparent lack of correlation between symptom severity, virus concentration and virus strains; therefore, adopting different tolerances with respect to the severity of virus symptoms might no longer be appropriate in certification schemes when it comes to control PVY (Lindner et al. 2015). Consequently, the Specialised Section on Standardisation of Seed Potatoes at UNECE has revised the UNECE Standard (S-1) Seed Potatoes and adopted new virus tolerances irrespective of the severity of viral symptoms observed in growing crops (UNECE 2014). As mild mosaic/viral symptoms may be missed during crop inspection, particularly if conditions are less favourable for visual inspection of plants (*e.g.* bright sunlight or water on foliage) or, as stated above, when plants are infected by less aggressive virus strains or if a cultivar is tolerant to some virus species, visual inspections should be complemented by post-harvest virus testing, as undertaken by most developed certification schemes worldwide.

## 6 Conclusion

Controlling the spread of PVY remains a challenge to the potato industry worldwide because of its non-persistent mode of transmission and the evolution of new strains and variants. Prophylactic measures such as roguing and early haulm destruction are required to limit PVY spread but are not efficient alone; the implementation of additional control strategies is needed to protect the susceptible potato cultivars. The control strategies presented in this chapter can help to reduce PVY transmission by aphids; however, each individual control strategy has its own limitations. Border crops can reduce the introduction of virus from outside the field but will not reduce the risk of transmission within a crop. While displaying variable levels of protection, mineral oil is generally used by seed potato growers to reduce aphid transmission of non-persistent viruses. Nevertheless, the effectiveness of mineral oil is dependent on the timing of aphid flights. For 'early' aphid flights that occur during the first weeks after plant emergence when plants are at their most susceptible stage, frequent oil application (two to three times per week to maximise coverage of new leaves) might provide some level of protection by reducing PVY transmission if

inoculum is present. For ‘late’ aphid flights (3–4 weeks after emergence), most leaves on a potato plant should be more effectively covered with a film of oil, which reduces the risk of infection. The efficacy of insecticides in controlling PVY spread is highly variable and at best of a limited impact. The effectiveness of control of PVY transmission through combined insecticide and mineral oil treatment may be more effective than individual insecticide or mineral oil treatment. Due to the rapid development of leaves and the difficulty of forecasting aphid flights during early crop development, it is advisable to combine mineral oil treatments with other control techniques such as intercropping, straw mulching and insecticide treatments. These emphasise the importance of controlling virus through appropriate monitoring methods and crop management enforced by seed certification schemes through the use of ‘clean’ input seed (ideally virus free or seed potatoes with a low incidence of virus) and, when possible, the segregation of seed and ware crops to minimise the risk of virus transmission between them.

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# ANNEX II

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# The movement of potato virus Y (PVY) in the vascular system of potato plants

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**Abstract** *Potato virus Y* (PVY) is responsible for major viral diseases in most potato seed areas. It is transmitted by aphids in a non-persistent manner, and it is spread in potato fields by the winged aphids flying from an infected source plant to a healthy one. Six different PVY strains groups affect potato crops: $PVY^{C}$, $PVY^{N}$, $PVY^{O}$, $PVY^{N:O}$, $PVY^{NTN}$, and $PVY^{N\text{-}Wi}$. Nowadays, $PVY^{NTN}$ and $PVY^{N\text{-}Wi}$ are the predominant strains in Europe and the USA. After the infection of the leaf and accumulation of the virus, the virus is translocated to the progeny tubers. It is known that $PVY^{N}$ is better translocated than $PVY^{O}$, but little is known about the translocation of the other PVY strains. The translocation of PVY occurs faster in young plants than in old plants; this mature plant resistance is generally explained by a restriction of the cell-to-cell movement of the virus in the leaves. The mother tuber may play an important role in explaining mature plant resistance. PVY is able to pass from one stem to the other stems of the same plant through the vascular system of the mother tuber, but it is unknown whether this vascular link between stems is permanent during the whole life of the plant. Two greenhouse trials were set up to study the spread of PVY in the vascular system of the potato plant. The PVY-susceptible cultivar Charlotte was used for both trials. It was demonstrated that all stems growing from a PVY-infected tuber will become infected sooner or later, and that $PVY^{N\text{-}Wi}$ translocates more efficiently to progeny tubers than $PVY^{NTN}$. It was also demonstrated that the progressive decay of the mother tuber in the soil reduces the possibility for virus particles to infect healthy stems through the vascular system of the mother tuber. This new element contributes to a better understanding of the mechanism of mature plant resistance.

**Keywords** Potato · PVY · Mature plant resistance · Translocation · Primary infection · Secondary infection

## Introduction

*Potato virus Y* (PVY, family *Potyviridae,* genus *Potyvirus*) is considered the most economically damaging potato virus in most countries that produce seed potatoes (Rolot 2005; Nolte et al. 2004). The virus is responsible for decreased yield and quality around the world. Most importantly, it is the main reason for rejection of seed lots for certification (Gray et al. 2010; Nolte et al. 2004). Six different PVY strains affect these potato crops. $PVY^{O}$, $PVY^{C}$, and $PVY^{N}$ are the historical strains. They were gradually replaced in Europe and North America by the strains $PVY^{NTN}$, $PVY^{N:O}$, and $PVY^{N\text{-}Wi}$, which are new recombinants of the historical strains (Karasev and Gray 2013b; Glais et al. 2002). PVY is transmitted by aphids in a non-persistent manner

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from an infected source plant to a healthy plant, giving rise to a so-called *primary infected plant* (Fereres and Moreno 2009). Later on, the virus particles migrate down to the progeny tubers; this migration is called the *translocation* of the virus (Debokx 1964). The following year, seed potatoes harvested from the primary infected plant will give rise to infected plants, usually called *secondary infected plants* (Malnoe et al. 1994).

The breaking of the tuber's dormancy activates transport mechanisms through the phloem of the young tissues of the growing sprouts, which are actively draining sap out of the mother tuber. In PVY-infected tubers, this sap is loaded with virus particles that will concentrate at the rose end of the tuber where most of the sprouts are located (Basky and Almasi 2005; Gugerli and Gehriger 1980). Roots will grow horizontally from the base of the sprout while its apex will grow vertically, emerge from the soil, and give rise to a stem with branches and leaves. It is unknown whether all the stems growing from the same tuber will be infected through an upward systemic movement of PVY particles from the mother tuber.

The descending flow of sap from the stems to the progeny tubers, which allows the translocation of the virus, is better documented. Debokx (1964) was the first to study PVY translocation in potato plants, and to identify differences between varieties with respect to the rate of tuber infection in primary infected plants. Beemster (1976) confirmed those results and demonstrated that the efficacy of the translocation varied according to the PVY strain. He observed that $PVY^{N}$ primary infected plants presented a higher rate of infected progeny tubers compared to $PVY^{O}$ strains. Debokx (1964) described for the first time the mature plant resistance of potatoes to PVY. Mature plant resistance is a phenomenon in which the translocation of the virus from the leaves to the progeny tubers occurs faster in young plants than in old plants. In Debokx's experiment, mature plant resistance was more pronounced against the $PVY^{O}$ strain than against $PVY^{N}$ strains.

Resistance of mature plants to PVY appears about four weeks after emergence. The protection increases with plant age, reaching complete protection of the progeny tubers against infections at about 8–10 weeks after emergence (Sigvald 1985; Gibson 1991). Basky and Almasi (2005) investigated within-plant translocation further and observed that $PVY^{N}$ migrates faster than $PVY^{O}$ from infected leaves to other plant organs. After a cell-to-cell movement from the infected leaf cells to the vascular system, the virus was detected either in the upper or the lower segments of the stem, meaning that it can be transported by the flow of sap up and down the stem. It was also demonstrated that when a sprout is inoculated, the virus can systemically migrate to the other sprouts and develop symptoms in the stems later on (Basky and Almasi 2005; Valkonen and Rokka 1998; Draper et al. 2002).

Taken together, these results show that the virus is able to migrate through the phloem of the infected stem, and after reaching the vascular ring of the mother tuber it is able to colonize the vascular system of the other growing stems. This physical continuity of the vascular ring of the mother tuber with the vascular systems of the growing sprouts is clearly visible in Fig. 1. As each stem has its own set of progeny tubers, if PVY succeeds in colonizing all the stems of the plant, it may also be capable of infecting all the plant's progeny tubers as well.

The first objective of this research is to track the spread of two PVY strains ($PVY^{NTN}$ and $PVY^{N\text{-}Wilga}$) from an infected mother tuber to the above-ground parts of the plant, and then from the haulms to the progeny tubers. The second objective of this research is to establish whether the earliness/moment of primary infection in one stem may influence the infection of the other stems of the same plant. This result will contribute to a better understanding of the resistance mechanisms implemented in mature plants. To achieve these two objectives, two greenhouse trials were managed in Changins (Switzerland): a movement and translocation trial, and a mature plant resistance trial.

## Materials and methods

### Plant material

For both greenhouse trials, the PVY-susceptible cultivar Charlotte was used (Schwaerzel et al. 2014). For the spread and translocation trial, 30 tubers infected by the $PVY^{NTN}$ 1317 strain (Agroscope collection) and 30 tubers infected by the $PVY^{N\text{-}Wilga}$ 1315 strain (Agroscope collection) were used. Those PVY infected tubers were produced the previous year in the field in separated plots of 100 plants grown from healthy tubers. In each plot 4 % of plants were inoculated with aforementioned PVY strains. The virus was then naturally transmitted to the neighboring plants through aphids.


**Fig. 1** Longitudinal section of a potato tuber with two growing stems. Legend: (a) vascular system of the first stem; (b) junction of the stems and mother tuber vascular systems; (c) vascular ring of the mother tuber; (d) vascular system of the second stem

The identity of the inoculum was confirmed using a triplex RT-PCR as described by Rigotti and Gugerli (2007) and the identity of the strains in the 60 progeny tubers was confirmed using an enzyme-linked immunosorbent assay (ELISA). For the mature plant resistance trial, healthy tubers of cultivar Charlotte were used. They were analyzed using the same ELISA method to verify the presence/absence of the virus. Details of the ELISA method are presented below.

## PVY detection method

PVY infections were tested in sprouting tubers using an ELISA method as described in Gugerli and Gehriger (1980). After harvest, a Rindite treatment was used to break dormancy and the treated tubers were then stored in the dark for 5 weeks at 22 °C and 80–90 % of relative humidity to promote sprouting. After this storage period, sap is extracted from the vascular tissue underneath the sprouts with a drill (Bioreba AG, Reinach, Switzerland) and analyzed by ELISA. Two sets of monoclonal antibodies were used to verify the identity of the serotype, on the one hand an anti-PVY-N (191,073, Bioreba, specific for N strain) and on the other hand an anti-PVY-NOC (210,369, Bioreba). The samples reacting positively to the anti-PVY-NOC and negatively to the anti-PVY-N were declared infected by $PVY^{N\text{-}Wilga}$ 1315 strain whereas the samples reacting positively to both antibodies were declared to be infected by $PVY^{NTN}$ 1317 strain. For PVY detection in the stems, three leaflets were collected from the same stem: one in the upper, one in the medium, and one in the lower part of each stem. The three leaflets were ground together using Homex 6® (Bioreba AG, Reinach, Switzerland) and the sap was analyzed by ELISA. The same sets of antibodies as for tubers analysis were used to verify the identity of the PVY serotype in the leaflets (Bioreba AG, Reinach, Switzerland). A sample was considered positive when the optical density value was three times higher than the optical density of sap extracted from a healthy potato tuber.

## Management of trials

For the movement and translocation trial, sprouts of the infected tubers were removed in order to maintain only three sprouts per tuber: one close to the rose end, one close to the heel end, and one in the middle. Following PVY detection underneath each sprout, the tubers were half-buried in a perlite bed in plastic pots (19x19x18 cm). The pots were then dispatched in four distinct insect-proof greenhouses (20 °C, 70 % relative humidity, and 12 h photoperiod) following a randomized block design. After development of the three stems (from the three sprouts), the stems were accordingly numbered and the pots were filled with compost. The plants were then grown until flowering and a leaf sample was taken for PVY detection. At the beginning of natural senescence, a sample of progeny tubers was analyzed by ELISA.

For the mature plant resistance trial, the heathy tubers were planted in plastic pots (19x19x18cm) filled with compost. The pots were randomized and maintained in an insect-proof greenhouse (20 °C, 70 % relative humidity, and 12 h photoperiod). The plants were distributed into three groups. The first group was inoculated seven days after plant emergence (DAE). In order to do that, three leaves from one stem—one in the upper, one in the medium, and one in the lower part of each stem—were mechanically inoculated with the $PVY^{NTN}$ 1317 strain (Agroscope collection) using carborundum (Perez 2011), and the inoculated stem was tagged. The other stems were numbered for traceability. The second group of plants was inoculated 21 DAE and the last group 56 DAE. After inoculation, leaf samples were collected on each potato stem one, two, four, and six weeks after inoculation, in order to determine whether the stems were infected by PVY. The plants were grown until the beginning of natural senescence, around 90 days after emergence. This trial was repeated with an eight-month interval between trials. For the first trial, 11 plants were allocated to each inoculation group, and for the second trial 15 plants were allocated to each group. For the replication of the trial, the tubers of each plant were counted and weighted. A sample of progeny tubers was analyzed by ELISA and it was establish whether the tubers were coming from the inoculated stem or from any other stems of the plant.

### Statistical analysis

The data on infection percentages were transformed using the angular data transformation method (Dagnelie 1975) prior to running an analysis of variance (ANOVA). The software STATISTICA® (StatSoft, Tulsa, USA) was used for all analysis. For the movement and translocation trial, another two-factor ANOVA was run to compare the percentage of infected sprouts, stems, and tubers between the two PVY strains. The effects of the factors "PVY strain" and "sprout location" were tested as well as the interaction between those factors, and the "greenhouse" effect was used as a replication factor. For the mature plant resistance trial, a Cochran Q test (Cochran 1950) was used to test the effect of inoculation dates (7, 21, or 56 DAE) on the probability of infection of stems after inoculation, and the probability of a new stem to be infected through the vascular system of the plant. A simple regression analysis was used to evaluate the effect of the infection date on the yield. Finally, a two-factor ANOVA was run to compare the percentage of infected stems. The effects of the factors "day of inoculation" and "replication of the trial" were tested, as well as the interaction between these factors.

## Results

### Movement and translocation trial

We did not observe any effect of the insertion site of the sprout on the probability of sprout becoming infected ($p > 0.05$). However, all the sprouts growing from infected tubers finally became stems in which the virus was detected (Table 1), proving that even if the infection was not present in the vascular system of the sprout itself, it may have arrived later from other parts of the mother tuber. Part of the sprouts never grew up (Table 1), meaning that the de-sprouting of the tuber before planting was not sufficient to break the apical dominance of the main sprout. As presented in Table 1, the rate of PVY-infected sprouts was significantly higher ($p < 0.001$) for $PVY^{N\text{-}Wilga}$ infected tubers (84 %) than for $PVY^{NTN}$ infected tubers (69 %). The $PVY^{N\text{-}Wilga}$ stem infection was efficiently transmitted to the progeny tubers, as 99 % of them were infected (Table 1). The transmission of $PVY^{NTN}$ was significantly lower than $PVY^{N\text{-}Wilga}$, with no more than 43 % of the progeny tubers infected ($p < 0.001$).

### Mature plant resistance trial

The results indicate that not all the inoculated stems developed infections: the later the inoculation was done, the fewer infected stems were observed ($p < 0.01$). At early plant senescence times, PVY was detected in 92 % and 81 % of the stems inoculated at 7 and at 21 days after emergence, respectively. In contrast, it was detected in only 50 % of the stems inoculated at 56 days after emergence (Fig. 2). However, all the progeny tubers ($n = 36$) harvested from the inoculated stems were infected by PVY, whatever the date of inoculation.

The probability for a non-inoculated stem to get infected through the movement of the virus particle along the vascular system of the mother tuber also varied as a function of the inoculation date ($p < 0.001$). The results of the last ELISA analysis done before plant senescence show that 92 % of the stems of

**Table 1** Evolution of the PVY infection percentage of potato plants during the different phases of their development from sprouts to stems and progeny tubers. Two groups are presented: the plants infected by $PVY^{N\text{-}Wilga}$ and by $PVY^{NTN}$ strains. For each group, the location of the sprout on the mother-tuber is specified

| PVY strain | Percentage of infeceted sprouts | | Percentage of infected stems | | Percentage of infected tubers | |
|---|---|---|---|---|---|---|
| $PVY^{NTN}$ | 69 % | ($n$ = 90) | 100 % | ($n$ = 51) | 43 % | ($n$ = 202) |
| Rose end sprout | 63 % | ($n$ = 30) | 100 % | ($n$ = 22) | 35 % | ($n$ = 106) |
| Middle sprout | 73 % | ($n$ = 30) | 100 % | ($n$ = 15) | 42 % | ($n$ = 56) |
| Heel end sprout | 70 % | ($n$ = 30) | 100 % | ($n$ = 14) | 59 % | ($n$ = 40) |
| $PVY^{N\text{-}Wilga}$ | 84 % | ($n$ = 90) | 100 % | ($n$ = 57) | 99 % | ($n$ = 225) |
| Rose end sprout | 83 % | ($n$ = 30) | 100 % | ($n$ = 23) | 100 % | ($n$ = 95) |
| Middle sprout | 93 % | ($n$ = 30) | 100 % | ($n$ = 14) | 98 % | ($n$ = 72) |
| Heel end sprout | 77 % | ($n$ = 30) | 100 % | ($n$ = 20) | 99 % | ($n$ = 58) |
| Total | 77 % | ($n$ = 180) | 100 % | ($n$ = 108) | 74 % | ($n$ = 427) |

early-inoculated plants (7 DAE) were infected (Fig. 3). This percentage was less than half for the plants inoculated 21 days after emergence (39 %), and even lower (13 %) for the late-inoculated plants (56 DAE). The percentage of infected progeny tubers harvested from non-inoculated stems of early-inoculated plants (7 DAE) was 89 % ($n$ = 35), 58 % ($n$ = 12) from plants inoculated 14 days later, and 10 % ($n$ = 10) from plants inoculated 56 DAE.

After inoculation of one stem, the virus migrated faster to the other stems of the plant when the inoculation was done shortly after emergence (7 DAE) compared to later inoculations (21 DAE). As shown in Fig. 4, the percentage of infected stems in plants inoculated 7 and 21 DAE is low, and not significantly different at one week and two weeks after inoculation ($p > 0.05$). Four weeks after inoculation, the percentage of infected stems in early-inoculated plants increased sharply, reaching around 71 %, while this percentage remained low (10 %) for the plants inoculated 21 DAE ($p < 0.001$). The percentage of infected stems continued to increase 6 weeks after inoculation to reach 83 % for the early-inoculated plants (7 DAE) and 30 % for the late-inoculated plants (21 DAE). Due to the decay of the plants inoculated 56 days after emergence, the evolution of the percentage of infected stems could not be monitored properly until 6 weeks after inoculation, and therefore is not presented in Fig. 4. However, the evolution was slow, with no stems infected one and two weeks

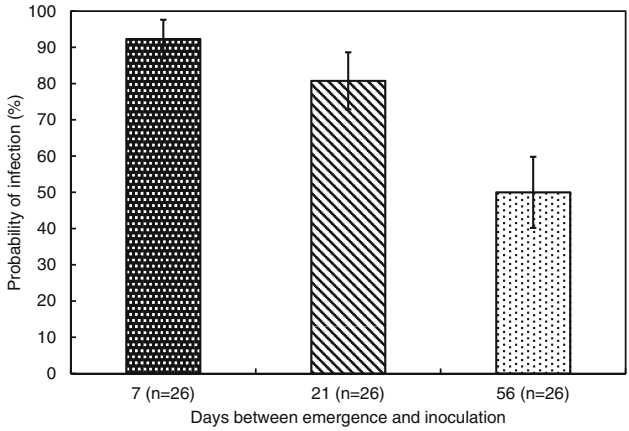


**Fig. 2** Probability of stem infection after inoculation, function of the inoculation schedule. Error bars present the standard error of the mean and "n" values indicate the number of plants considered in the statistical analysis

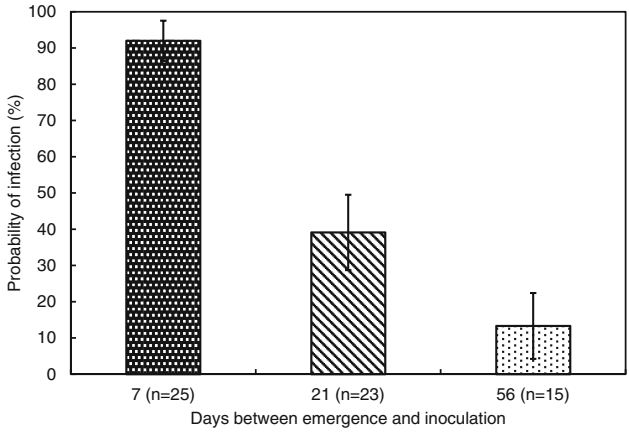


**Fig. 3** Probability for non-inoculated stems to get infected, function of the inoculation schedule. Error bars present the standard error of the mean and "n" values indicate the number of plants considered in the statistical analysis

after inoculation and only 10 % infected 4 weeks after inoculation.

From our results we could observe that the later the inoculation was done, the higher the yield was ($p$ = 0.001). Nevertheless, the model's coefficient of determination is low ($R^2$ = 0.3). Finally, no significant linear correspondence was found between the inoculation schedule and the number of progeny tubers harvested ($p > 0.05$; $R^2$ = 0.042).

## Discussion

The initial source of inoculum in the field is known to be the main risk factor for the subsequent spread of PVY to the healthy plants of the field (Steinger et al. 2014). The in-field source of inoculum comes from the infected seed tubers planted that offer PVY-infected foliage to the flying aphids, the vectors of the virus. Our results showed that all the stems of these tubers will become infected sooner or later. However, we also observed that not all the sprouts of an infected tuber are infected by PVY. We can hypothesize that the ELISA test was not sensitive enough to detect latent PVY infections in some of the sprouts, or that the virus came from other parts of the tuber and infected all the stems after the ELISA test. This suggests that all stems growing from an infected tuber will, eventually, become a source of inoculum in the field. In order to reduce the in-field sources of inoculum, it is generally recommended to remove the

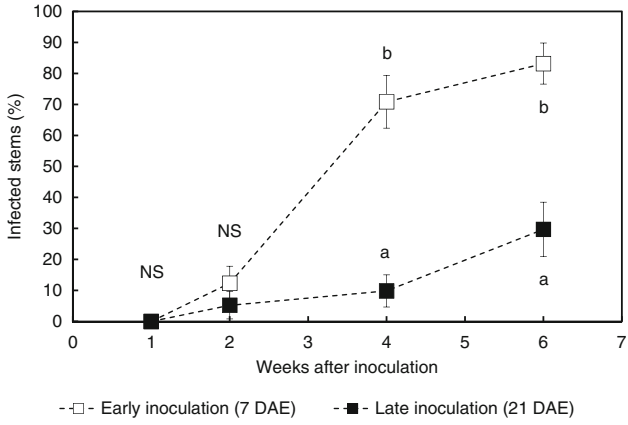


**Fig. 4** Evolution of the percentage of infected stems: 1, 2, 4, and 6 weeks after inoculation for plants inoculated 7 and 20 days after emergence (DAE). Error bars present the standard error of the mean. Different letters indicate that the percentage of stems are different among the groups of plants inoculated at different dates ($p < 0.05$ after analysis of variance ANOVA). "NS" indicates that the ANOVA results were not significant ($p > 0.05$)

PVY-symptomatic plants as well as their progeny tubers (Davis et al. 2009). Some plants may, however, escape the vigilance of the seed grower and produce progeny tubers, which will present a risk for the following year. We also observed that the infection rate of these progeny tubers varied depending on the PVY strain. This variation of the infection rate is well-documented for primary infections (Sigvald 1985; Beemster 1976; Gibson 1991), and $PVY^{N}$ is known to be better transmitted to progeny tubers than $PVY^{O}$ (Beemster 1976). In our study we focused our research on secondary infections due to PVY strains which arrived recently in Europe and North America (Karasev and Gray 2013a; Glais et al. 2002). We showed that the $PVY^{N\text{-}Wilga}$ strain is very efficient in transmitting the infection to the progeny tubers, and even more efficient than the $PVY^{NTN}$ strain. Note that it may be the case that the ELISA method may have missed some instances of low concentration of virus particles in some of the progeny tubers. However, this has no major implication for our main finding. In fact, while there may be cases where $PVY^{NTN}$ was not detected in progeny tubers, due to a low titer of the virus, $PVY^{N\text{-}Wilga}$ was detected in almost all progeny tubers, suggesting that the titer of $PVY^{N\text{-}Wilga}$ virus particles was higher in those tubers. This information might explain why $PVY^{N\text{-}Wilga}$ strains are increasing in prevalence in some European countries (Dupuis et al. 2013; Dupuis and Schwaerzel 2011; Rigotti et al. 2011; Lindner et al. 2015).

From our results we can also conclude that the probability of stem infection after inoculation was reduced for older stems. As noted above, this phenomenon is known as mature plant resistance and can be explained mainly by the restriction of cell-to-cell movement rather than a restriction of the replication of the virus (Draper et al. 2002; Valkonen and Rokka 1998). This resistance mechanism has important consequences for the epidemiology of PVY. In the case of aphid flights early in the season, young growing plants are particularly vulnerable to infections due to the absence of resistance. On the other hand, in the case of late aphid flights, the risk is reduced due to mature plant resistance, which will be at its maximum before senescence (Sigvald 1985; Gibson 1991; Robert et al. 2000).

Basky and Almasi (2005) previously observed that after primary infection of the sprouts, the virus was able to reach the other stems of the same mother tuber. Our results confirmed this observation, and additionally showed that the probability of stem infection through the vascular system of the mother tuber is significantly reduced with plant age. As mentioned above, the ELISA method used for this experiment may have missed some cases of PVY infections in part of the stems due to virus titers below the sensitivity threshold of the ELISA. However, a post-harvest analysis of progeny tubers grown from non-inoculated stems reveals that the percentage of infected tubers decreased sharply for late inoculations. These results are consistent with the ones obtained for the stems. Thus, our observations suggest a second mechanism of protection associated with mature plant resistance. This mechanism involves the vascular system of the mother tuber acting as a non-permanent bridge between the growing stems of the plant, and can be decomposed into two restriction elements that lower the transmission of the virus from one stem to the others through the vascular system of the mother tuber: a *temporal restriction* and a *physical restriction*.

*Temporal restriction* occurs when the virus does not have enough time to reach the mother tuber before haulm killing. As has been shown previously, cell-to-cell movement is slowed down in mature plants (Draper et al. 2002; Valkonen and Rokka 1998). In a case of late infection, the virus will thus take more time to reach the phloem parenchyma before being transported a longer distance by the movement on the sieve elements (Carrington et al. 1996). If the virus particles are not able to reach the phloem before haulm killing and plant desiccation, the virus will not be carried further to the other stems, and will not be able to infect them as well as their related progeny tubers. Our study supports this hypothesis, as for late infection (56 DAE), the probability for a healthy stem to get infected by virus particles drained through the mother tuber is low (a 13 % chance in our trials).

On the other hand, *physical restriction* is present when the systemic movement of the sap through the vascular ring of the mother tuber is restricted by the collapsing of the mother tuber. Robert et al. (2000) suggested that this translocation through the mother tuber was slow and probably dependent on the mother tuber's stage of degeneration. Our results confirm this hypothesis, as it was observed that the probability for virus particles to be transmitted to healthy stems via the mother tuber decreases with plant aging. It was also observed at harvest that most of the mother tubers were decomposed (rotted or mummified), impeding sap transfer from one stem to the other (data not shown).

PVY is efficiently controlled by mineral and vegetable oil sprays (Dupuis et al. 2014; Powell 1992). Oil inhibits the acquisition of PVY by aphids and therefore

reduces the risk of PVY transmission to healthy plants (Boquel et al. 2013). Oils are usually sprayed in potato fields every seven days. Given the high risk observed of transmitting the virus to all stems of the plant if the infection occurs in the second or third week following emergence, increasing the frequency of sprays during this period could be an option. Two oil sprays a week are carried out by some potato seed growers with success (Jean-Louis Rolot, personal communication), suggesting that more frequent applications might better protect the new leaves of the young, PVY-susceptible, and fast-growing plants. It will be necessary to reduce by half the quantity of oil sprayed for each treatment, in order to avoid any risk of phytotoxicity that an excess of oil may provoke (Boiteau and Singh 1982; Martin-Lopez et al. 2006).

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# ANNEX III

Dupuis B, Schwaerzel R, Derron J, 2014. Efficacy of Three Strategies Based on Insecticide, Oil and Elicitor Treatments in Controlling Aphid Populations and Potato virus Y Epidemics in Potato Fields. *Journal of Phytopathology* **162**, 14-8.

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ORIGINAL ARTICLE

# Efficacy of Three Strategies Based on Insecticide, Oil and Elicitor Treatments in Controlling Aphid Populations and *Potato virus Y* Epidemics in Potato Fields

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**Keywords**
aphids, elicitor, insecticide, oil, potato, *Potato virus Y*, Switzerland

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## Abstract

*Potato virus Y* (PVY), the potato virus with the highest economic impact in Europe, is transmitted by aphids in a non-persistent manner. A two-year field experiment was conducted in Switzerland to evaluate the efficacy of three strategies for controlling aphid populations and the spread of PVY, consisting of treatment with one insecticide (Karate Zeon®), one elicitor (Bion®) and one oil (Telmion®), respectively. The elicitor strategy proved to be ineffective for controlling aphid populations and inadequate for controlling PVY spread. The insecticide strategy gave incomplete protection from aphid infestations, owing to the selection of aphid-resistant clones. The insecticide gave too little protection against PVY spread for it to be considered a suitable candidate for the purpose. The oil strategy had no effect on aphid populations, but was the best option to reduce PVY spread.

## Introduction

*Potato virus Y* (PVY; genus *Potyvirus,* family *Potyviridae*), the potato virus with the highest economic impact in Europe (Rolot 2005), is transmitted in a non-persistent manner. It is both acquired and inoculated during brief probing by aphids of several species that do not necessarily colonize potatoes (see Woodford 1992). Over the last 50 years, several trials have attempted to identify chemicals that effectively reduce PVY spread. Such chemicals can be broadly classified into two groups: insecticides and oils. The majority of insecticides tested were aphicides most of which proved effective in controlling aphid populations but not PVY spread. This was the case for pirimicarb (Collar et al. 1997), permethrin (Bell 1989), Cypermethrin (Bell 1989; Collar et al. 1997; Martin-Lopez et al. 2006), demeton-S-methyl (Milosevic 1996), methamidophos (van Toor et al. 2009), lambda-cyhalothrin (van Toor et al. 2009; Hansen and Nielsen 2012), pymetrozine (van Toor et al. 2009), imidacloprid in furrow at plantation (Boiteau and Singh 1999; Alyokhin et al. 2002), imidacloprid on seed tubers (van Toor et al. 2009) and imidacloprid on foliage (Collar et al. 1997; Boiteau and Singh 1999). The only evidence of effective control of PVY spread by insecticides comes from a spray application of imidacloprid on foliage, which achieved on average 36% PVY reduction (Alyokhin et al. 2002), contradicting previous evidence. This lack of effect is mostly due to the non-persistent manner in which PVY is transmitted. When a virus is transmitted non-persistently, the acquisition and inoculation of the virus occur in a matter of seconds, so it is difficult to expose the vector to a lethal or behaviour-changing dose of insecticide before the virus is inoculated by the aphid (see Perring et al. 1999). Alatae aphids are more important than apterae in transmitting PVY in potato fields because alatae can easily fly from plant to plant (Woodford 1992). Therefore, the control of viruses transmitted in a non-persistent manner is more complicated when non-colonizing viruliferous alatae fly into the field. In the light of such results, it is surprising that insecticides are still widely used for potato seed production in Europe, although they are mostly used to control the spread of *Potato leaf roll virus* (PLRV; genus *Polerovirus*, family *Luteoviridae*) rather than PVY (Klostermeyer

1959; Milosevic 1996; Boiteau and Singh 1999; Perring et al. 1999).

As for the second group of chemicals used to limit PVY spread, many trials have shown mineral oil to be effective (Bradley et al. 1966; Loebenstein et al. 1970; Shands 1977; Bell 1980; Boiteau and Singh 1982; Powell 1992; Powell et al. 1998; Martin-Lopez et al. 2006; Boiteau et al. 2009). Both the nature and the quality of the oil are important. Among the vegetable oils tested, rapeseed oil was more effective than soya oil, and raw oils were less effective than refined ones (Martin et al. 2004; Martin-Lopez et al. 2006). The oil creates a physical barrier to the aphid's stylet penetration and reduces PVY transmission after momentary contact with the aphid labium (Bradley 1963; Simons et al. 1977). Hence, protection is limited to the foliage surface where oil is applied (Simons et al. 1977). In several European countries, the use of mineral oil is prohibited for ecological reasons or due to phytotoxicity. Damage due to phytotoxicity may occur if mineral oil is mixed with fungicides such as captafol (Bell 1980) or fluazinam (C. Corre, personal communication). In addition, when oils are sprayed under hot weather conditions, the oil heated by the sun in the sprayer pipes may burn potato leaves and stems (J.L. Rolot, personal communication).

We describe three treatment strategies for the control of aphid populations and PVY spread in field, based respectively on insecticide, oil and elicitor application on foliage. The first strategy involved re-investigating the effect of one insecticide, Karate Zeon® (lambda-cyhalothrin; Syngenta®, Basel, Switzerland). The quick-acting effect of this pyrethroid could neutralize the aphid before it has time to transmit. Lambda-cyhalothrin has previously been found ineffective in preventing PVY spread when sprayed according to an aphid threshold (van Toor et al. 2009). We adopted a different application modality, by spraying weekly, starting at plant emergence. This insecticide has been found ineffective by spraying weekly until 42 days after plant emergence (Hansen and Nielsen 2012); however, Basky and Almasi (2005) have shown that massive PVY infections can occur up to 45 days after plant emergence. Therefore, we decided to spray the plants until haulm killing. The second strategy involved testing one formulation of rapeseed oil, Telmion® (Omya AG AGRO®, Oftringen). The third strategy consisted of testing the effect of Bion® (acibenzolar-S-methyl; Syngenta®, Basel), which has never previously been tested for PVY spread control. This benzothiadiazole is sold commercially as a fungicide. However, it has some insecticidal properties and also activates the general resistance mechanisms of the plant (Green 2009), and we here refer to it as an elicitor.

## Material and Methods

### Field location and management

A two-year field experiment was conducted in Switzerland in lowland conditions (425 to 720 m a.s.l.). Plots were planted according to a completely randomized block design, with five replications. Each plot was planted with four rows of 25 plants of the PVY-susceptible cv. Bintje (Schwaerzel et al. 2009) and surrounded by two rows of the same cultivar acting as a buffer zone. The rows were planted every 75 cm, and within a row, tubers were planted every 33 cm. Each plot presented 4% of secondary infected plants resulting from the planting of four tubers infected by $PVY^{N}$605 isolate (Agroscope PVY collection). The experimental field was managed following standard cultural practices, and haulm killing was done 90 days after planting.

### Treatments

The mixture volume sprayed on the plots was equivalent to 300 l/ha. Three commercial products were tested: Karate Zeon® (Syngenta®) as the insecticide, Telmion® (Omya AG AGRO(R), Oftringen, Switzerland) as the oil and Bion® (Syngenta®) as the elicitor. A description of the products and the application dosage and frequency is given in Table 1. Each year, an untreated control was added to the experimental design.

### Observations

A sample of 100 leaves was taken from the five plots of each treatment (20 leaves/replication). Each leaf was collected from a different plant. This sampling was repeated twice during the cropping period; in year 1, the samplings were made 61 and 67 days after planting, and in year 2, the samplings were collected 68 and 75 days after planting. For each sample, we counted the number of apterae adults of the four most abundant potato colonizing species in Switzerland: *Myzus persicae* (Sulzer), *Macrosiphum euphorbiae* (Thomas), *Aulacorthum solani* (Kaltenbach) and *Aphis nasturtii* (Kaltenbach) (Derron and Goy 1995). In addition, two tubers were manually collected from each plant 4 weeks after haulm killing and tested by ELISA (Gugerli and Gehriger 1980)]. We used monoclonal antibodies specific to serotype N (Bioreba, Reinach, Switzerland).

**Table 1** Description of the treatment strategies tested: trade name in Switzerland, substance group, composition, dosage and frequency of treatment

| Strategy | Trade name | Company | Substance group | Composition | Percentage of active matter in the spray liquid | Frequency of treatment |
|---|---|---|---|---|---|---|
| Insecticide | Karate Zeon® | Syngenta®, Basel | Pyrethroid | Lambda-cyhalothrin 100 g/l | 0.015 | Weekly from emergence until haulm killing |
| Oil | Telmion® | Omya AG AGRO®, Oftringen | Vegetable oil | Rapeseed oil 779 g/l | 3 | Weekly from emergence until haulm killing |
| Elicitor | Bion® | Syngenta®, Basel | Benzothiadiazole | Acibenzolar-S-methyl 500 g/kg | 0.01 | Weekly from emergence for 3 weeks and then each 14 days until haulm killing |

### Statistics

The two-year results were analysed using Statistica® (Statsofts, Tulsa, OK, USA). For each leaf sampling, we examined the average aphid count (two replications). The analysis was carried out for the different aphid species separately. The number of aphids was converted using the square root transformation method (Dagnelie 1975). For each plot, we examined the percentage of PVY-infected tubers at harvest (five replications). The percentage of tuber infection was converted using the angular data transformation method (Dagnelie 1975). For both aphids and PVY analysis, we ran a two-factor (year and treatment) analysis of variance (ANOVA) with an $\alpha$ error level of 0.05 (Gomez and Gomez 1984).

A treatment was considered effective in controlling aphid populations or PVY if the following three conditions were met. First, the results of the ANOVA showed statistically significant differences at the 5% level. Second, the treatment and the control belonged to different homogeneity groups, according to the Newman–Keuls post hoc analysis test (Dagnelie 1975). Third, the treatment had a positive value of efficacy. Treatment efficacy is given by the following formula: $\text{Efficacy} = \left[1 - \left(\frac{\text{treatement value}}{\text{control value}}\right)\right] \times 100$. The efficacy varies from 0% to 100%, 0% signifying that the treatment had no effect and 100% that it was fully effective. A negative efficacy can be obtained when the result of the treatment is higher than the result of the control.

## Results

### Control of aphid populations

The effect of the treatment strategies could not be assessed on *A. solani*, because the number of captures was too low to detect any differences among treatments ($F(3;6) = 1.57$; $P > 0.05$, Fig. 1). The oil and the elicitor were not effective in controlling *A. nasturtii* populations, and a high variability was observed in plots treated with oil (from 9 to 61 insects sampled on 100 leaves). The insecticide was effective in controlling *A. nasturtii*, only one insect having been sampled on 100 leaves in total. This efficacy was not detected by the post hoc analysis ($F(3;6) = 8.36$; $P < 0.05$, Fig. 1), but was obvious after pairwise comparison with the control (t-test with 6 df: $P < 0.05$). The insecticide was the only effective strategy for controlling *M. euphorbiae* populations ($F(3;6) = 1.63$; $P < 0.05$). Finally, none of the strategies were effective for controlling *M. persicae* populations ($F(3;6) = 0.59$; $P > 0.05$).

### Control of PVY spread

All strategies succeeded in reducing PVY spread (Fig. 2). Results suggest that oil effectively prevents PVY spread in the field and that it is significantly more efficient than the elicitor and the insecticide ($F(3;24) = 11.78$; $P < 0.001$). Oil has an average efficacy of 26% in controlling PVY spread, compared with 14% and 10% for elicitor and insecticide, respectively.

## Discussion

As expected, Karate Zeon® was effective in controlling aphid populations except for *M. persicae*, the lack of efficacy in this case resulting from a selection of *M. persicae* clones that are probably resistant to the chemical. Many cases of *M. persicae* resistance to pyrethroids have been reported from all parts of Europe (Anstead et al. 2007). No aphicide effect was observed after treatment with the elicitor; hence, the insecticide effect of Bion® mentioned by Green (2009) was not confirmed by this experiment. The failure of

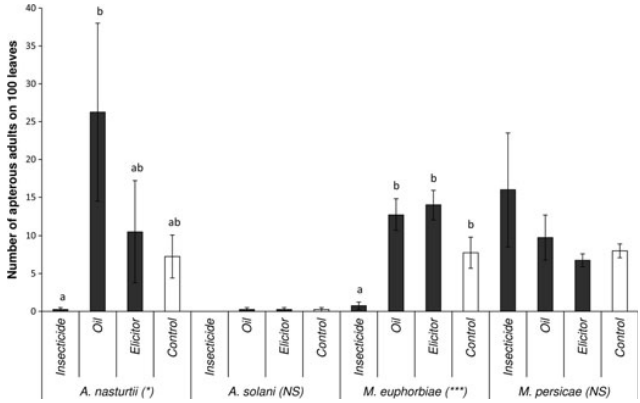


**Fig. 1** Average number of adult apterae aphids of major potato virus Y (PVY) vector species on 100 potato leaves. Values with different letters are statistically different according to Newman–Keuls post hoc analysis (error bars represent standard error of the mean, $n = 4$). The significance of the ANOVA ($\alpha = 0.05$) is given after the name of the aphid species, * if $P < 0.05$, ** if $P < 0.01$, *** if $P < 0.001$ and NS for non-significant.

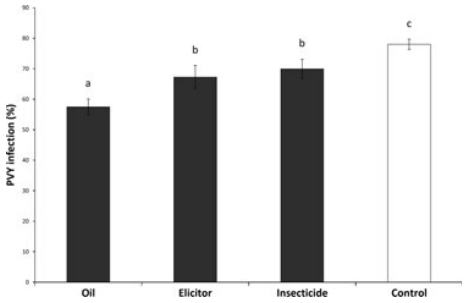


**Fig. 2** Average percentage of potato virus Y (PVY) infection of each treatment. Values with different letters are statistically different according to Newman–Keuls post hoc analysis (error bars represent standard error of the mean, $n = 10$).

rapeseed oil to control aphid populations contradicts previous studies (Martin et al. 2004; Martin-Lopez et al. 2006). However, this difference may be explained by the fact that those studies were not conducted under open field conditions. In our trial, the persistence of the product may have been reduced because of environmental factors.

All treatments had a statistically significant effect on PVY spread. However, the efficacy of the elicitor (14%) and the insecticide (10%) was too low for them to be considered suitable candidates for use in PVY control. This study showed the efficacy of the vegetable oil to be relatively low, 26%, compared with results obtained by Martin-Lopez et al. (2006), which showed 41% efficacy for refined rapeseed oil. In the same study, the efficacy of rapeseed oil was compared with the efficacy of mineral oil. Mineral oil proved much more effective, with 59% efficacy, similar to the 64% level of protection obtained by Boiteau and Singh (1982). We mentioned above that mineral oil can be phytotoxic; in practice, however, when the application procedures are properly followed, the risk of phytotoxicity is low (Boiteau and Singh 1982; Martin-Lopez et al. 2006).

In summary, our study confirmed that there is no link between protection against apterae aphids and PVY spread, because alatae aphids are more important transmitters of PVY in the field than apterae. The elicitor strategy was found to be ineffective for controlling aphid populations and inadequate for controlling PVY spread. Use of insecticide gave incomplete protection from aphid infestations, owing to the selection of aphid-resistant clones. The insecticide gave too little protection against PVY spread for it to be considered a suitable candidate for the purpose. Finally, oil had no effect on aphid populations, but was the best option to reduce PVY spread in potato fields.

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# ANNEX IV

Dupuis B, Cadby J, Goy G, et al., 2017. Control of potato virus Y (PVY) in seed potatoes by oil spraying, straw mulching, and intercropping. *Plant Pathology* **66**, 960-9.

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# Control of potato virus Y (PVY) in seed potatoes by oil spraying, straw mulching and intercropping

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*Potato virus Y* (PVY) is the potato virus with the highest economic impact on seed potato production. Insecticides are efficient in controlling aphids, which are the vectors of this virus, but rarely limit virus spread in the field. Straw mulching and mineral oil spraying are known as alternatives to insecticides to reduce PVY incidence, but important year-to-year variation in efficacy has been observed with both of these techniques. Preliminary studies revealed the efficacy of intercropping in controlling PVY spread, but more data are needed to validate this observation. A four-year field trial was conducted in Switzerland to assess the potential synergistic effect of combining mineral oil spraying with straw mulching to increase the protection of seed potato crops against PVY spread. Furthermore, the efficacy of intercropping with oat and hairy vetch was examined as a novel way to control in-field PVY spread. The present work demonstrates that the modes of action of mineral oil and straw mulching are complementary and reduce the year-to-year variation observed with oil and straw when used alone as PVY control agents. The results also demonstrate the efficacy of intercropping for the control of PVY, and the mode of action of this novel control method is discussed. Overall, this work shows that it is possible to increase the protection of potato fields against PVY spread by combining control strategies with different modes of action that complement each other, such as mulching, oil spraying and intercropping.

*Keywords:* aphids, IPM, *Potyvirus*, vector

## Introduction

*Potato virus Y* (PVY, genus *Potyvirus*, family *Potyviridae*) is the potato virus with the highest economic impact on seed potato production worldwide (Rolot, 2005; Valkonen, 2007). It is transmitted in a nonpersistent manner by various species of aphids, including those that do not necessarily colonize potatoes (Woodford, 1992). Non-winged morphs of aphids are relatively unimportant vectors of PVY, because of their limited dispersal capacity (Nemecek, 1993; Radcliffe & Ragsdale, 2002). Winged aphids, by contrast, are responsible for spreading the virus as they fly from plant to plant and field to field (Broadbent, 1957a,b; Woodford, 1992; Robert *et al.*, 2000). Use of insecticides, while effective in controlling the vectors, seems to have a low impact on the spread of PVY because of the short time needed to transmit the nonpersistent virus (Shanks & Chapman, 1965; Gibson *et al.*, 1982; Boiteau *et al.*, 1985; Lowery & Boiteau, 1988; Boquel *et al.*, 2014). Aphids often transmit PVY prior to being killed by the insecticide (Perring *et al.*, 1999).

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It has been shown that the initial colonization of potato fields by aphids tends to be concentrated on the margins of the fields (Difonzo *et al.*, 1996; Carroll *et al.*, 2009). It was postulated that the strong visual contrast provided by the fallow ground and the crop canopy might be more attractive to aphids than a homogeneous surface with relatively little contrast (Döring & Chittka, 2007). Two types of PVY control techniques make use of this phenomenon by manipulating the contrast between the bare soil and the crop canopy: mulching (Wyman *et al.*, 1979; Basky, 1984; Heimbach *et al.*, 2004; Saucke & Döring, 2004) and crop borders (Difonzo *et al.*, 1996; Fereres, 2000; Boiteau *et al.*, 2009). Straw mulching is known to reduce PVY incidence by roughly 30% (Saucke & Döring, 2004; Kirchner *et al.*, 2014). Nevertheless, important year-to-year variation in efficacy was observed in all multiyear trials of straw mulching, ranging from 6% (Saucke & Döring, 2004) to 70% (Kirchner *et al.*, 2014). Empirical studies showed that fewer winged aphids were captured in mulched plots compared to non-mulched ones (Heimbach *et al.*, 2004; Saucke & Döring, 2004). According to a hypothesis proposed by Döring *et al.* (2004), aphids land indiscriminately on either the straw or the plant as a consequence of the low contrast between the plant and the straw background. However, once an aphid has landed on the straw and probed on it, it will take off again and leave the plot, starting the so-called 'rejection flight' (Kring, 1972; Döring *et al.*, 2004).





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Olfactory stimuli could also play a role in this rejection flight process, as it was demonstrated that aphids could respond to olfactory stimuli (Döring, 2014). This effect of straw on the behaviour of aphids has been shown to be stronger early in crop development and to decline later in the season (Saucke & Döring, 2004).

In the case of crop borders, two different mechanisms may operate. It is thought that by the time an aphid has probed the plants of the crop border, it will have lost its charge of virus particles and be unable to transmit PVY any longer (Boiteau *et al.*, 2009). This first effect of border crops is named the 'virus sink' effect. Secondly, tall barrier crops create a physical obstacle around the field, impeding the colonization of the potato crop by aphids. This second effect of barrier crops is named the 'mechanical barrier' effect (Difonzo *et al.*, 1996; Fereres, 2000; Boiteau *et al.*, 2009). In practice, however, crop borders are not easy to implement effectively, especially in small fields. Therefore, this technique is mainly used in the USA (Davis *et al.*, 2009) but remains rare in European countries where the average field surface is significantly smaller. For example, the average surface of a seed potato field in Switzerland is about 1.4 ha (H. Gilliand, Agroscope, Nyon, Switzerland, personal communication), compared to North Dakota, where the average surface of a seed potato field is about 11.7 ha (W. Schrage, North Dakota State Seed Dept, Fargo, USA, personal communication).

Dupuis *et al.* (2010) hypothesized that potato crops could benefit from the same two effects of crop borders by using the intercropping technique. The advantage over border crops is that intercropping can be used on small fields without wasting land. The principle is that the intercrop plants surrounding the potato plants play the role of a mechanical barrier for aphids and also act as a virus sink, with aphids landing on the associated crop. Intercropping may also limit the spread of PVY by reducing gaps in the crop canopy, which are known to favour PVY spread (Davis *et al.*, 2009). The results of a first trial with oat intercropping were reported by Dupuis *et al.* (2010) and demonstrated that intercropping is efficient in controlling the spread of PVY in the field.

A third PVY control option is the spraying of mineral oil on potato foliage (Bradley *et al.*, 1966; Boiteau & Singh, 1982; Powell, 1992; Powell *et al.*, 1998; Martin-Lopez *et al.*, 2006; Boiteau *et al.*, 2009). Mineral oil can reduce the acquisition and retention of PVY by aphids (Wróbel, 2009; Boquel *et al.*, 2013). Nevertheless, the efficacy of mineral oil in controlling the spread of PVY varies widely, from 18% to 89% (with an average of 49%) depending on the study (Boiteau & Singh, 1982; Martin-Lopez *et al.*, 2006; Boiteau *et al.*, 2009; Hansen & Nielsen, 2012; Fageria *et al.*, 2014; Kirchner *et al.*, 2014; Mackenzie *et al.*, 2014; Steinger *et al.*, 2014). There is a profound need to increase the efficacy and stability of mineral oil treatments, e.g. by combining it with a complementary control method.

Few studies have examined the combined effect of mineral oil and straw mulching for the control of PVY. In a one-year trial, Dupuis *et al.* (2010) showed that the association of mineral oil with mulch has beneficial effects in terms of controlling in-field PVY transmission. Another similar one-year trial on the association of mineral oil and straw mulching was reported by Kirchner *et al.* (2014); however, these authors showed that this combination did not further reduce the incidence of PVY. Given the contradictory results of those two studies, further data are needed in order to clarify the real efficacy of the association of mineral oil with straw mulching in controlling PVY spread. The purpose of the present study is to provide new data on this specific topic. In addition, different amounts of straw were compared in order to define the amount of straw that would be optimal in controlling PVY spread while remaining economically sustainable for the potato seed growers. The study also examines the efficacy of intercropping with oat (*Avena sativa*) and hairy vetch (*Vicia villosa*) as a way to control in-field PVY spread.

## Materials and methods

### Experimental site and crop management

Field trials were conducted at the Agroscope Agricultural Research station in Changins (Nyon, Switzerland) from 2011 to 2014. For each of the four trials, a 0.09–0.12 ha field was planted with the cultivar Charlotte, known to be highly susceptible to PVY (Schwaerzel *et al.*, 2014; Steinger *et al.*, 2014), at a seeding rate of one seed piece per 0.33 m with a 0.75 m row spacing. High-grade seed-lots (basic or pre-basic seed) were used for planting, in which no PVY was detected in diagnostic tests carried out by the Swiss certification authorities. Individual plots (treatment replicates) were four rows wide (3 m) and 25 plants long (8.25 m) and were surrounded by a buffer row planted with Lady Christl, a cultivar that is highly resistant to PVY (Schwaerzel *et al.*, 2014). Three seed pieces of cultivar Charlotte infected with strain $PVY^{NTN}$1317 (Agroscope collection) were planted in central locations within each plot (position 12 in row 2, positions 6 and 19 in row 3) to serve as a source of virus inoculum (3% initial infection level). The field was irrigated when necessary but otherwise managed according to usual cultural practices, without spraying of insecticides. Haulm killing was performed with two herbicide treatments; the first treatment was carried out around 80 days after planting (Reglone; Syngenta; 200 g $L^{-1}$ diquat) and the second treatment 1 week later (Spotlight Plus; Syngenta; 60 g $L^{-1}$ carfentrazone-ethyl).

### Oil and straw treatments

Two treatments were applied during the 4 years of trials: mineral oil spraying and mulching with wheat (*Triticum aestivum*) straw. Mineral oil (Zofal D®; Stähler; 830 g $L^{-1}$ mineral oil) was sprayed weekly as a 1.75% solution in water at a rate of 400 L $ha^{-1}$ with a backpack sprayer (Birchmeier M125) from 80% of emergence until haulm killing. Straw was applied by hand on each row at a quantity of 2.5 t $ha^{-1}$ immediately after planting (Fig. 1b). The combination of mineral oil and straw mulching was also tested in each trial except for the one in 2011. Treatments were applied in a randomized complete block design with four replicate plots per trial.

Additional plots were set up in 2013 and 2014 within the same field to evaluate whether the amount of straw per unit

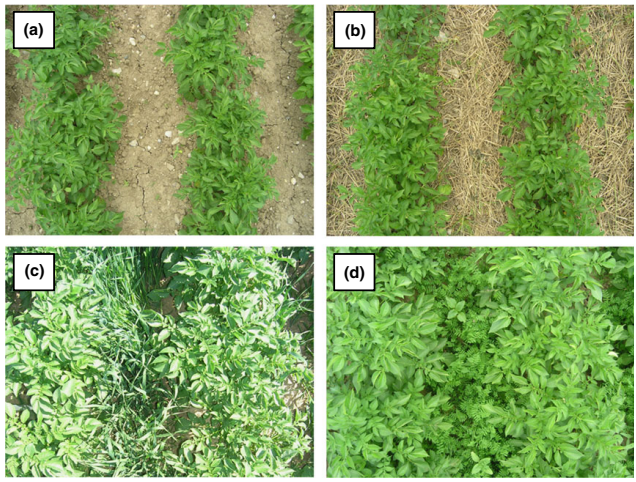


Figure 1 Details of the experimental plots in 2012, one month after potato emergence: (a) bare soil plot; (b) mulched plot; (c) oat intercropping plot; and (d) vetch intercropping plot. [Colour figure can be viewed at wileyonlinelibrary.com]

area (straw quantity) could be reduced without affecting the rate of PVY transmission. Four quantities were tested: 1, 1.5, 2 and 2.5 t $ha^{-1}$.

### Intercropping

The effect of intercropping on PVY transmission was evaluated in 2011 and 2012. Hairy vetch (*V. villosa*) and oat (*A. sativa*) were sown by broadcasting the seeds on the rows at the time of potato planting (Fig. 1c,d) (60 kg $ha^{-1}$ for oat and 50 kg $ha^{-1}$ for hairy vetch). Growth of oat was stopped after 3 weeks (stage 23 on the BBCH scale) to limit competition with the potato crop for water and nutrients. This was achieved with two applications of Aramo (BASF; 50 g $L^{-1}$ tepraloxydim) at an interval of 7 days.

### Measurement of crop cover

The percentage of plot surface covered by the foliage of the growing potato plants as well as the surface covered by the associated crop or mulch was estimated at weekly intervals using the point quadrats method (Wilson, 1963).

### PVY infections in tubers

Four weeks after haulm killing, two tubers were manually sampled from each plant. PVY infections were tested in sprouting tubers using an enzyme-linked immunosorbent assay (ELISA) as described in Gugerli & Gehriger (1980). Monoclonal antibodies specific for $PVY^{N}$ isolates (monoclonal PVY IgG; Bioreba AG) were used. PVY incidence was calculated as the percentage of PVY-infected plants per plot.

### Tuber yield

After mechanical harvest of each plot with a one-row Samro harvester, the total yield was measured.

### Aphid captures

Aphids were captured using green pan traps (colour #006400 CSS reference). The traps (26 cm in diameter) were filled with water containing 1–3% detergent, and placed in the centre of each plot. Green rather than yellow traps were used because the former may provide more realistic estimates of rates of aphid landings on green potato foliage (Boiteau, 1990; Avinent *et al.*, 1991). Traps were emptied twice a week, and the total number of winged aphids was counted on a weekly basis during 6 weeks. The most prevalent aphid species (>1% of total captures) were identified and counted individually.

### Statistical analysis

A general linear model was used to evaluate the effects of oil spraying (factor with two levels), straw mulching (factor with two levels), and the interaction of the two on post-harvest PVY incidence in tubers and on total tuber yield. Year and block (nested within year) were included in the model as blocking factors. The interaction term oil × straw was analysed using trials conducted in 2012–14 (the experimental design in 2011 omitted the combined applications of oil and straw). Separate general linear models were used to evaluate the effect of straw quantity (factor with five levels) and intercropping (factor with three levels) on post-harvest PVY incidence in tubers and on yield. A Dunnett's multiple comparison procedure was used to compare the straw and intercrop treatments to the control (bare soil plots). Residuals were always checked for normality and homogeneity of variances. Data on PVY incidence and yield were not transformed prior to analysis. Data on total aphid counts was square-root transformed to better satisfy the assumptions of the general linear model. Data on weekly aphid captures did not always satisfy model assumptions, and in these cases *P*-values were calculated based on a permutation test. All statistical analyses were performed using R v. 3.2 software with the packages CAR, MULTCOMP, LMPERM and LSMEANS.

## Results

### PVY incidence

Both oil spraying and straw mulching significantly decreased postharvest PVY incidence, but the strength of the treatment effects varied among years (Table 1). Averaged over the 4 years of the study, straw reduced PVY incidence by 27%, with a range of 0–54% depending on the year (Fig. 2). Oil reduced PVY incidence by an average of 43%, with a range of 27–67% (Fig. 2). The oil × straw interaction evaluated with pooled data from years 2012–14 was statistically nonsignificant ($P = 0.83$, Table 1). However, the three-way interaction with year was marginally significant ($P = 0.056$, Table 1). A year-wise analysis revealed a relatively complex picture: the combined effects of straw and oil were largely additive in 2013 but slightly antagonistic in 2014 (Fig. 2). The estimated efficacy of the combined oil–straw treatment (assuming an absence of oil × straw interactions) was an average of 59%, with a range of 43–73% depending on the year.

The effect of different quantities of straw on PVY incidence was evaluated in the 2013 and 2014 trials, which were the years in which high rates of PVY transmission occurred in the untreated control plots. The main effect of the straw quantity was significant ($F_{4,24} = 4.7$; $P < 0.01$), and there was no significant interaction with year ($F_{4,24} = 1.4$; $P = 0.28$). When comparing the PVY incidence in plots using different quantities of straw with the incidence of PVY in the bare soil plots (Dunnett test), it appeared that incidence was significantly lower only for the plot with the highest quantity (2.5 t ha$^{-1}$; $P < 0.01$; Fig. 3a).

Intercropping with oat and vetch was studied in 2011 and 2012. The main effect of intercropping was significant ($P < 0.05$), and there was no significant interaction with the year ($P = 0.07$). Multiple comparisons of treatment means with the bare soil control (Dunnett test) showed that the effect was significant for both intercropping methods. Averaged over the two years, the reduction of the PVY incidence was 34% for oat-intercropped plots and 33% for vetch-intercropped plots ($P < 0.05$; Fig. 3b).

Table 1 Summary of the ANOVA results of PVY incidence in harvested potato tubers, tuber yield and aphid landings

| Source of variation | PVY incidence | Tuber yield | Aphid captures |
|---|---|---|---|
| Straw mulching | 30.2*** | 0.2 | 9.1** |
| Oil spraying | 108.7*** | 3.0 | 0.4 |
| Year | 50.8*** | 126.7*** | 82.5*** |
| Straw × oil | 0.1 | 0.2 | – |
| Straw × year | 5.8** | 3.1* | 6.3** |
| Oil × year | 3.5* | 1.1 | 0.6 |
| Straw × oil × year | 3.2 | 0.4 | – |
| Intercropping | 5.6* | 4.1* | 0.5 |
| Intercrop × year | 3.4 | 0.1 | 0.3 |

*F*-values are given along with asterisks indicating the significance of the test. Note that ANOVA models also included a term due to block nested within year (results not shown). The interaction terms straw × oil and straw × oil × year were modelled using a subset of the data (excluding the year 2011). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

### Tuber yield

The effect of straw cover on total tuber yield varied among years (Table 1). Plots with straw had a lower yield in 2012 (−18%, $P < 0.05$), but a higher yield in 2013 (+25%, $P = 0.07$), while no significant difference was observed in 2011 (+1.5%, $P = 0.79$) and 2014 (+3%, $P = 0.54$; Fig. 4a).

Oil spraying decreased the total yield in the four years by 10% on average, a nonsignificant effect ($P = 0.09$; Table 1, Fig. 4b).

Averaged over the two years, both intercropping treatments slightly reduced total yield compared with the control plots; a 13% reduction was observed with oat and 11% with vetch (Dunnet test: oat: $P < 0.05$; vetch $P < 0.05$; Fig. 4a).

### Temporal dynamics of soil surface cover

Because early-season colonization of potato plants by aphids is thought to be determined by the visual contrast between the crop plants and the surface of the bare soil, the temporal change in soil surface cover was monitored for the different treatments. Figure 5 shows that straw mulch covered a large fraction of the bare soil surface at the time of potato emergence, but the percentage covered by straw rapidly declined in the 3 weeks after emergence, when the potato foliage was growing and rapidly covering the soil. In contrast, the vetch and oat foliage made only a small contribution to soil surface cover at the time of potato emergence, but their share steadily increased during the course of the growing season.

### Aphid captures

The number of aphids trapped in each plot was monitored until 6 weeks after potato emergence. Oil spraying did not influence the total aphid numbers in any year (Table 1). Therefore, the data of oil-sprayed plots and bare soil plots were pooled for subsequent analysis of aphid counts. Similarly, intercropping did not affect the total aphid numbers when compared with the control plots (Table 1). The cumulative number of all aphids trapped was significantly influenced by straw application, but the effect varied by year (Table 1). Plots with straw mulch had significantly lower aphid counts in 2011 (−45%, $P < 0.001$) and 2013 (−30%, $P < 0.05$) but not in the other two years (2012: +23%, $P = 0.10$; 2014: −9%, $P = 0.44$). Analysis of the temporal dynamics within years showed that in the two years in which straw reduced aphid counts, the difference was most pronounced during the 4 weeks after potato emergence but

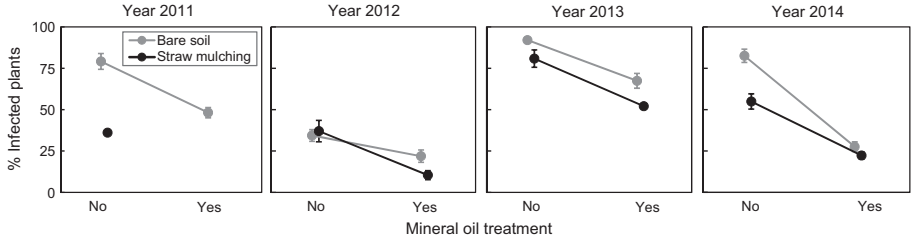


Figure 2 Effect of oil spraying and straw mulching on the incidence of PVY in potato tubers. Mean values of four years of experiments ($n = 4$ per year). Error bars represent ± 1 SE.

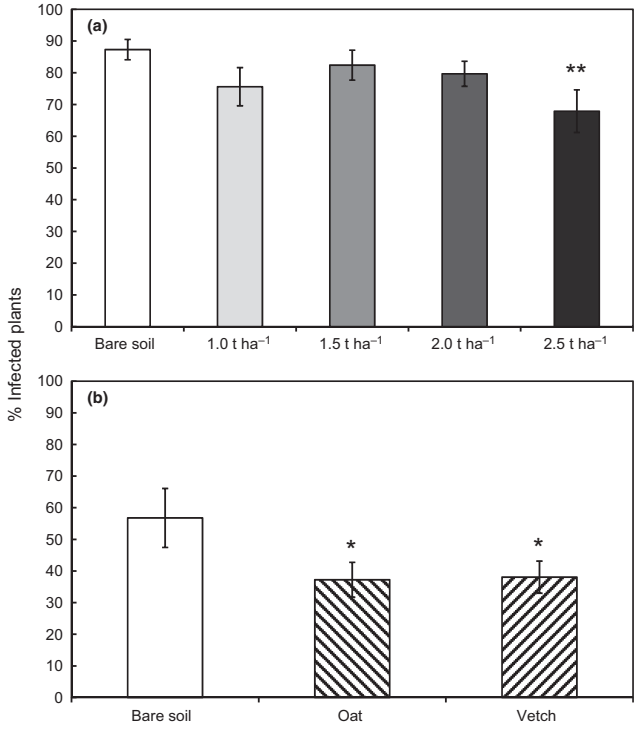


Figure 3 Mean percentage of PVY-infected tubers (±1 SE) in each treatment group. Asterisks indicate significant differences (*$P < 0.05$, **$P < 0.01$) between the treatment and the bare soil control (Dunnett's multiple comparison procedure): (a) PVY-infected plants in relation to quantity of straw, average of the four replicates over the two years 2013 and 2014 ($n = 8$); (b) PVY-infected tubers in relation to the method of intercropping, average of the four replicates over the two years 2011 and 2012 ($n = 8$).

declined for later trapping dates (Fig. 6). The effect of straw also varied considerably among aphid species, although no species was unaffected by straw in all four years (Table 2).

The main effect of straw quantity on total aphid counts was significant ($P < 0.01$), and there was no significant interaction with year ($P = 0.95$). Multiple comparisons of treatment means with the bare soil control (Dunnett test) showed that the straw effect was only significant at the highest quantity (Fig. 7).

## Discussion

This study evaluated the efficacy of various virus control measures, including oil spraying, straw mulching and intercropping, aimed at reducing PVY incidence in a

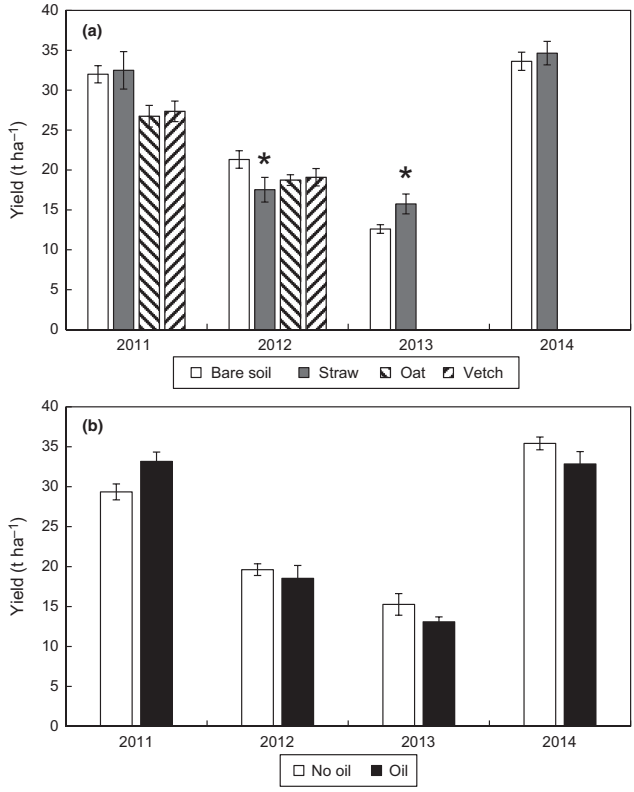


Figure 4 Mean tuber yield (±1 SE) in each treatment group. Asterisks indicate significant differences (*$P < 0.05$) between the treatment and the bare soil/no oil control (Dunnett's multiple comparison procedure): (a) tuber yield in relation to soil cover, average of the 4–8 replicates over the four years 2011–2014 ($n = 8$ for bare soil 2011–14 and for straw 2012–14; $n = 4$ for the rest); (b) tuber yield in relation to the mineral oil treatment, average of the 4–16 replicates over the four years 2011 to 2014 ($n = 16$ for 'no oil' 2011–16; $n = 4$ for 'oil' 2011 and $n = 8$ for the rest).

potato crop without affecting tuber yield. There was a particular focus on those potential synergistic effects that might arise due to joint applications of virus control measures.

Weekly applications of mineral oil from the time of crop emergence is a common but labour-intensive virus management measure in seed potatoes and is known to reduce PVY transmission by roughly half, albeit with important variation among environmental conditions (Boiteau & Singh, 1982; Martin-Lopez *et al.*, 2006; Boiteau *et al.*, 2009; Hansen & Nielsen, 2012; Fageria *et al.*, 2014; Kirchner *et al.*, 2014; Steinger *et al.*, 2014). In the present study, mineral oil applications reduced PVY incidence by an average of 43%, with considerable year-to-year variation, which is consistent with the literature. Oil is not thought to interfere with probing and feeding behaviour of aphids (Vanderveken, 1968; Qiu & Pirone, 1989), but it is likely to reduce virus acquisition (Bradley *et al.*, 1962; Simons & Zitter, 1980; Boquel *et al.*, 2013) and retention (Wang & Pirone, 1996; Wróbel, 2009) by aphid vectors. Moreover, potato plants treated with mineral oil were found to produce volatiles that act as a repellent against aphids, at least in the short term (Ameline *et al.*, 2010). In the present study, however, no difference was found in aphid landings between oil-treated and untreated plots. This suggests that mineral oil is acting exclusively after aphid landing rather than affecting the aphids' landing behaviour.

The observed year-to-year variation in the efficacy of mineral oil may be related to the dynamics of growth of the foliage. The increase in leaf area is the highest after plant emergence and declines until senescence (Shands *et al.*, 1970). As new leaves emerging after oil sprays are susceptible to PVY infection (Fageria *et al.*, 2014), potato crops may face a relatively higher risk of infection during the early stages of crop development compared to later in the season, assuming the common practice of constant (weekly) applications of mineral oil. Consistent with this reasoning, a lower efficacy (27–39%) of oil spraying was found in the years 2011 and 2013, when aphid activity was high early in the season, and a higher efficacy (67%) in the year 2014, when aphid vectors appeared relatively late in the season. Virus control by oil spraying may therefore be optimized by increasing

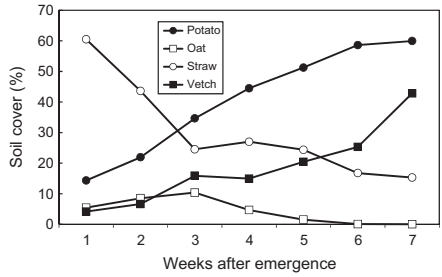


Figure 5 Temporal change in the percentage of soil covered by the foliage of the potato crop and by the associated straw mulch or intercrops (hairy vetch and oat, treated with an herbicide 3 weeks after potato emergence). Average values of 2011 and 2012 trials (*n* 'bare soil' = 16; *n* 'straw' = 12; *n* 'oat' = 8, *n* 'vetch' = 8).

the application frequency during the first few weeks after crop emergence at the expense of later sprayings.

No significant reduction in yield was measured in the plots sprayed with mineral oil. Nevertheless, slight phytotoxicity of mineral oil has been observed in other experiments (Boiteau & Singh, 1982; Martin-Lopez *et al.*, 2006), emphasizing the necessity for a cautious use of mineral oil to control PVY (Kirchner *et al.*, 2014).

Straw mulching is less common in practice but proved to be efficient in controlling PVY spread (Heimbach *et al.*, 2004; Saucke & Döring, 2004; Dupuis *et al.*, 2010; Kirchner *et al.*, 2014). The reason that straw mulch scattering is not widely applied is that little information is available on how effective it is at the field scale. The method was previously shown to reduce PVY incidence by roughly 30%, albeit with large year-to-year variation (Saucke & Döring, 2004; Kirchner *et al.*, 2014). The average reduction of PVY incidence in the present investigation was 27%, and the efficacy of straw mulching varied substantially among the years of the experiment. This variation may be explained by the fact that straw mulch is not effective during the whole growing period; it is primarily effective from the emergence of the plants until the closure of the canopy (Heimbach *et al.*, 2004; Saucke & Döring, 2004; Kirchner *et al.*, 2014), as during this time the straw is visible for winged aphids and thus has an effect on their landing behaviour. The results are consistent with this observation, as lower aphid landings were observed on mulched plots from 2 to 4 weeks after potato emergence. Afterwards, when the crop canopy covered the soil, a relative increase of aphid

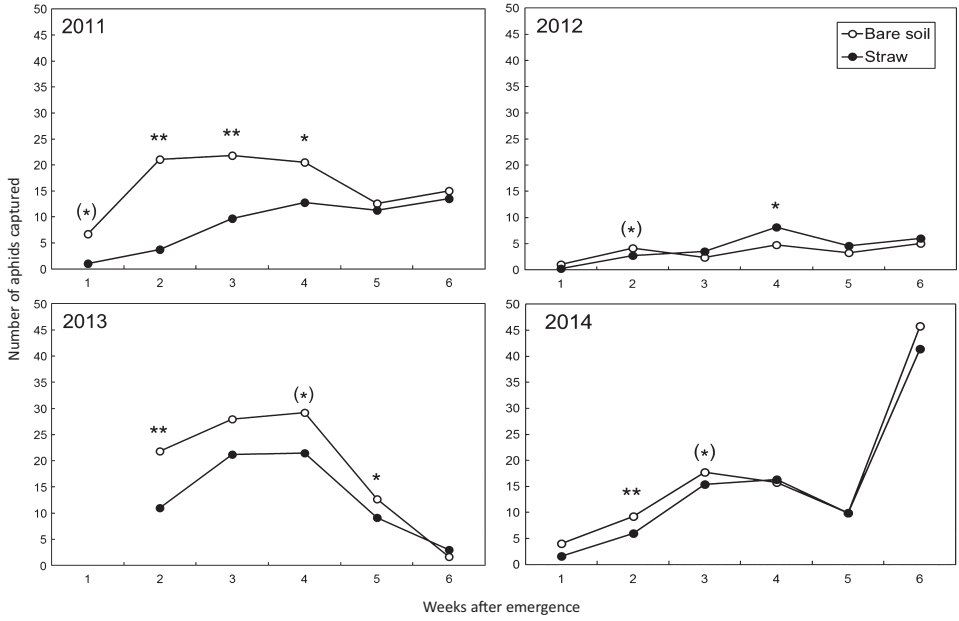


Figure 6 Mean values of the weekly captures of aphids in potato plots with straw mulch and in bare soil plots. 2011: *n* 'straw' = 4 and *n* 'bare soil' = 8, 2012–14: *n* = 8. Asterisks indicate significant differences in aphids captured between treatment and control. NS: nonsignificant; (*)$P < 0.1$; *$P < 0.05$; **$P < 0.01$.

Table 2 Sum of captures of the eight most prevalent aphid species in plots with straw mulching (2.5 t ha$^{-1}$) and with bare soil 6 weeks after potato emergence in the four years of trials ($n$ = 8 per trial)

| Aphid species | Soil cover | 2011 | 2012 | 2013 | 2014 | Straw effect | Straw × year effect |
|---|---|---|---|---|---|---|---|
| *Acyrthosiphon pisum* | Bare soil | 56 | 5 | 30 | 16 | NS | * |
| | Straw | 46 | 14 | 26 | 6 | | |
| *Aphis fabae* group | Bare soil | 36 | 27 | 74 | 46 | ** | NS |
| | Straw | 14 | 28 | 45 | 40 | | |
| *Aphis* spp. | Bare soil | 106 | 55 | 107 | 263 | NS | *** |
| | Straw | 76 | 97 | 11 | 230 | | |
| *Brachycaudus helichrysi* | Bare soil | 38 | 7 | 3 | 17 | *** | NS |
| | Straw | 6 | 7 | 1 | 8 | | |
| *Metopolophium dirhodum* | Bare soil | 82 | 2 | 61 | 87 | * | * |
| | Straw | 46 | 0 | 39 | 84 | | |
| *Myzus persicae* | Bare soil | 250 | 0 | 38 | 54 | *** | *** |
| | Straw | 135 | 5 | 11 | 48 | | |
| *Rhopalosiphum padi* | Bare soil | 45 | 1 | 30 | 256 | NS | * |
| | Straw | 8 | 1 | 31 | 207 | | |
| *Sitobion avenae* | Bare soil | 41 | 4 | 110 | 22 | * | NS |
| | Straw | 20 | 1 | 72 | 19 | | |
| Total aphids | Bare soil | 756 | 164 | 537 | 829 | *** | ** |
| | Straw | 412 | 202 | 367 | 731 | | |

Significance of the straw effect and the straw × year interaction in the general linear model are indicated. NS: nonsignificant, *$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001.

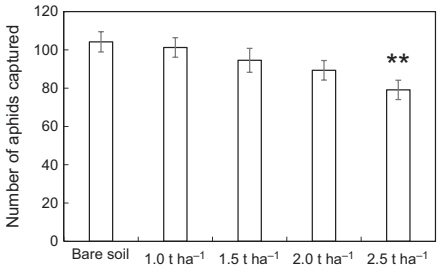


Figure 7 Mean values (±1 SE) of 6 weeks of captures of aphids in potato plots with different quantities of straw mulch and in bare soil plots, average of 2013 and 2014 ($n$ = 16 for bare soil and 2.5 t ha$^{-1}$ and $n$ = 8 for the rest). Asterisks indicate significant differences in the number of aphids captured between treatment and control, **$P$ < 0.01.

captures occurred. The ultraviolet reflectance of straw and bare soil are very similar (Döring *et al.*, 2004). Thus, the effect of straw on aphid behaviour cannot be attributed to UV repellency but to the lower optical contrast between the plant canopy and the straw compared with the contrast between the plant canopy and the bare soil (Döring *et al.*, 2004). This work also emphasizes the effect of straw on the selection of aphid species. This means that the efficacy of the straw may be dependent on the attraction of specific aphid species to the visual stimuli it produces.

Different quantities of straw were tested in this work, and optimum PVY control was obtained with the highest quantity, 2.5 t ha$^{-1}$, with an average efficacy of 27%. It was demonstrated that 2.5 t ha$^{-1}$ covered 60% of the soil at potato emergence and that it was the minimum amount needed to reduce aphid captures in the crop. It can be hypothesized that a higher cover of the soil would better control aphid landings early in the season and better protect the plots against PVY spread. This hypothesis is consistent with the results of other trials: Saucke & Döring (2004) used a larger amount of straw, 3.5 t ha$^{-1}$, and obtained an average efficacy of 31% (a 3-year trial in Germany) while Kirchner *et al.* (2014) used 5.5 t ha$^{-1}$ with an efficacy of 44% (a 3-year trial in Finland).

The present work demonstrates the complementarity of mineral oil and straw mulching in controlling PVY spread, as no interaction between the effects of oil and straw mulching was detected. In the experiment, the joint effect of straw mulching and mineral oil spraying resulted in a 59% reduction of PVY incidence compared to 43% and 27% for mineral oil and straw mulching, respectively, when used separately. Additionally, it must be stressed that, in the experiments, the efficacy of the association of mineral oil and straw mulching never fell below 43% in any year, compared with a low point of 27% and 0%, respectively, with oil or straw used alone. This complementarity of the two control measures may be explained by the evolution of the crop canopy during the growing season. As outlined above, oil sprayings may provide little protection from PVY infection during periods of rapid foliage expansion at the beginning of the growing season. In contrast, straw mulch is thought to be most effective in preventing aphid landings – and hence virus infections – during early growth stages (i.e. when the visual contrast between the soil and the foliage is highest in the crops). The results suggest that this complementarity of the mode of action reduces the year-to-year variation observed with oil and straw when used

alone as PVY control agents. This combination of methods significantly reduces the annual risk of crop failure caused by massive PVY infection in seed potato production. The cost of straw mulching is approximately half the price of mineral oil treatments (calculation made in the Swiss context in 2016).

The study also showed that the efficacy of intercropping was not due to any reduction in the aphid landings in the crop and may have been due to the 'virus sink' effect, through which the aphids lose their PVY inoculum after several probes on a non-host plant (Wróbel, 2007; Boiteau *et al.*, 2009). The efficacies of oat and vetch intercropping were very similar and comparable to the efficacy of straw mulching. Nevertheless, intercropping reduced tuber yield, whereas the average effect of straw mulching on yield was low. Therefore, straw mulching is a better candidate to complement the effect of mineral oil for a better protection of potato fields against PVY spread.

In conclusion, this work showed that it was possible to increase the protection of potato fields against PVY spread by combining control strategies with different modes of action that complement each other, such as mulching, oil spraying and intercropping.

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# ANNEX V

Survey 2017 of the prevalence of potato viruses in Europe and North America – Survey questions

SURVEY 2017 OF THE PREVALENCE OF POTATO VIRUSES IN EUROPE AND NORTH AMERICA – SURVEY QUESTIONS

Please answer the below questions directly in the text.

**1) In general, what risk do potato viruses (any of them) pose to seed-potato production in your country? Please select one of the following:**

- **High risk:** the risk due to viruses is considered very serious by growers; Important and systematic measures are undertaken by them to reduce the spread of the viruses in the field, irrespective of potato varieties nor field location.

- **Medium risk:** the risk due to viruses is well known to growers; Measures adapted to potato varieties and field location are undertaken to reduce the risk of spread in the field.

- **Low risk**: growers know that under specific circumstances (for example massive aphids flights in the case of PVY) there is a risk of virus spread in their fields but think that outside of these situations no particular measures are needed to control for it.

- **No risk**: viruses are not a problem; nothing particular is done by growers to control for them.

**2) What is the relative occurrence in your country of each of the potato viruses listed in the table* below?**

**Please fill in the last two columns in the table* below providing for each virus:**

a) A qualitative appreciation of the occurrence of the virus in your country: Please select one of the following:

- **Very important**: the virus is very often detected/observed in potato seed lots and this is the case every year.

- **Important**: the virus is regularly detected/observed in the potato seed lots and this is the case every year.

- **Not important**: the virus is rarely detected/observed in potato seed lots and it is not detected every year.

- **Not present:** as far as I know, this virus is not present in my country

b) A quantitative estimate of the occurrence of these potato viruses in your country:

Based on your experience, can you please provide what you think is a good estimate of the average percentage of potato seed lots infected by each type of viruses. If your estimate is not recent (more than 5 years) please specify. Please report 0 if the virus is not present in your country and report “NA” if you do not have the information.

| Species (acronym) | Genus & family | Vector | Qualitative estimate of virus occurrence | Quantitative estimate of virus occurrence |
|---|---|---|---|---|
| *Alfalfa mosaic virus* (AMV) | Alfamovirus, Bromoviridae | Aphids | | |
| *Beet curly top virus* (BCTV) | Curtovirus, Geminiviridae | Leafhoppers | | |
| *Cucumber mosaic virus* (CMV) | Cucumovirus, Bromoviridae | Aphids | | |
| *Potato aucuba mosaic virus* (PAMV) | Potexvirus, Flexiviridae | Contact, aphids | | |
| *Potato latent virus* (PotLV) | Carlavirus, Flexiviridae | Aphids | | |
| *Potato leafroll virus* (PLRV) | Polerovirus, Luteoviridae | Aphids | | |
| *Potato mop-top virus* (PMTV) | Pomovirus, – | *Spongospora subterranea* | | |
| *Potato virus A* (PVA) | Potyvirus, Potyviridae | Aphids | | |
| *Potato virus M* (PVM) | Carlavirus, Flexiviridae | Aphids | | |
| *Potato virus S* (PVS) | Carlavirus, Flexiviridae | Aphids | | |
| *Potato virus V* (PVV) | Potyvirus, Potyviridae | Aphids | | |
| *Potato virus X* (PVX) | Potexvirus, Flexiviridae | Contact | | |
| *Potato virus Y* (PVY) | Potyvirus, Potyviridae | Aphids | | |
| *Potato yellowdwarf virus* (PYDV) | Nucleorhabdovirus, Rhabdoviridae | Leafhoppers | | |
| *Sowbane mosaic virus* (SoMV) | Sobemovirus, – | ? | | |
| *Tobacco mosaic virus* (TMV) | Tobamovirus, – | Contact | | |
| *Tobacco necrosis virus* (TNV) | Necrovirus, Tombusviridae | Olpidium brassicae | | |
| *Tobacco rattle virus* (TRV) | Tobravirus, – | Nematodes | | |
| *Tomato black ring virus* (TBRV) | Nepovirus, Comoviridae | Nematodes, TPS | | |
| *Tomato mosaic virus* (ToMV) | Tobamovirus, – | Contact | | |
| *Tomato spotted wilt virus* (TSWV) | Tospovirus, Bunyaviridae | Thrips | | |
| Other?... | | | | |
| | | | | |
| | | | | |

* This table is inspired from “Valkonen JPT, 2007. Chapter 28—viruses: economical losses and biotechnological potential. In: Vreugdenhil D, Bradshaw NJ, Gebhardt C, et al., eds. Potato Biology and Biotechnology. Amsterdam: Elsevier Science B.V., 619–41.”

# ANNEX VI

Survey 2017 of the prevalence of potato viruses in Europe and North America – Contact persons

| Country | Name | Position | Institution |
|---|---|---|---|
| Belgium | Jean-Louis ROLOT | Scientist | Walloon Agricultural Research Centre |
| Canada | Mathuresh SINGH | Director | Agricultural Certification Services Inc. |
| Czech Republic | Petr DĚDIČ | Scientist - plant virology | Potato Research Institute |
| Finland | Hanna RANTA | Scientist - plant analytics | The Finnish food safety authority Evira |
| France | Laurent GLAIS | In charge of the virology program | La Fédération Nationale des Producteurs de Plants de Pomme de Terre |
| Germany | Lisa HERTEL | Scientist - official seed control | Bavarian State Agency for Agriculture, Institute of Crop Production and Plant Breeding |
| | Friedhilde TRAUTWEIN | Senior adviser Seed potatoes | Federal variety office |
| | Peter STEINBACH | Scientist - plant protection service | State office for agriculture of Mecklenburg-Vorpommern |
| | Volker ZAHN | Group leader, virology and bacteriology | Agricultural chamber of Lower Saxony |
| Italy | Laura TOMASSOLI | Scientist - Plant virology | Council for Agricultural Research and Agricultural Economy Analysis |
| The Netherlands | Eisse DE HAAN | Scientist - Research laboratory methods and diagnostics | Nederlandse Algemene Keuringsdienst |
| Norway | Ola NØREN JOHANSEN | Senior adviser Seed potatoes | Norwegian Food Safety Authority |
| Poland | Ewa ZIMNOCH-GUZOWSKA | Professor | Plant breeding and aclimatization institute |
| Scotland | Christophe Lacomme | Senior Virologist | Science and Advice for Scottish Agriculture |
| Slovenia | Peter DOLNIČAR | Scientist - Plant Breeding, Genetics and Physiology potatoes, potato cultivation | Agricultural Institute of Slovenia |
| Switzerland | Peter FREI | Senior adviser Seed potatoes | Agroscope |
| | Olivier SCHUMPP | Scientist - Plant virology | Agroscope |
| United States of America | Stewart GRAY | Professor, Plant Pathology & Plant-Microbe Biology | Cornell University / U.S. Department of Agriculture, Agricultural Research Service |

# ANNEX VII

Dupuis B, Hebeisen T, Steinger T. The influence of aphid flights on the transmission of the potato virus Y (PVY) in the field. In: Nia, ed. *Proceedings of the 16th triennial meeting of the Virology Section of the European Association of Potato Research (EAPR)*, 2016. Ljubljana, Slovenia, 25.

# The influence of aphids flights on the transmission of the potato virus Y (PVY) in the field

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PVY is transmitted in a non-persistent manner by various aphid species. The efficiency of the transmission of the virus is generally expressed as a relative efficiency factor (REF) using the green peach aphid (*Myzus persicae*) as a reference. *Myzus persicae* is considered as the most efficient PVY vector. These REFs are calculated using data of transmission trials performed in the laboratory in which individuals carrying the virus are put into contact with a healthy plant known as a host plant of the virus. A recent study relating the flight activities of aphid species in Switzerland to the prevalence of PVY in the national seed potato production showed that the abundance of *Myzus persicae* was not correlated with the incidence of the virus. That study also showed that the counts of leaf curl plum aphid (*Brachycaudus helichrysi*) was a good predictor variable for PVY prevalence. It is surprising to observe that the aphid species with the highest REF has no or little influence on the spread of PVY in the field. To clarify this further, a 14 years field trial was setup in Switzerland to study the influence of aphid flights on PVY spread. Tubers of the susceptible cultivar Bintje were planted in two locations 200 km apart from each other. Aphids were captured with a 'Rothamsted suction-trap' during the entire growing season in both locations and the species were identified. Each year, 100 potato tubers were analyzed by ELISA to determine PVY incidence. A simple linear regression analysis was used to assess the relationship between the total captures of each aphid specie the first 7 weeks after emergence with the percentage of PVY infected tubers after harvest. Our results revealed that the capture data of *Brachycaudus helichrysi* as well as the capture data of aphids of the genus *Aphis* were the best predictors of PVY incidence in the field. In contrast, the captures of *Myzus persicae* revealed to be a poor predictor of the PVY incidence in the field, since the coefficient of determination was lower than the mean of the other species. These results are consistent with previous findings and highlight the limits of PVY transmission trials performed in the laboratory to predict the risk of PVY spread in the field. REF values need to be complemented with data of vector behavior in the field to get a more complete understanding of the relative importance of different aphid species as vectors of PVY.

# ANNEX VIII

Dupuis B, Schwaerzel R. Impact of Haulm killing on Potato Virus Y (PVY) spread. *Proceedings of the The 18th Triennial Conference of the European Association for Potato Research*, 2011. Oulu, 167.

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## Impact of Haulm killing on Potato Virus Y (PVY) spread

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Potato Virus Y (PVY) is a Potyvirus transmitted by aphids in potato fields. Haulm killing is a major cultural practice used to stop tuber growth in order to advance tuber maturation, control tuber size and starch content and induce proper skin set. Haulm killing has an indirect phytosanitary effect by reducing crop exposure to diseases and pests. When haulm killing is carried out, crop is old and potato plants are known to have a mature plant resistance to PVY infections. Nevertheless, this resistance is not absolute and a risk of transmission still exists. The first objective of this experiment is to evaluate the risk of PVY transmission after haulm killing. The second objective is to compare the efficiency of different herbicides and cropping techniques in reducing PVY spread. In 2003, 2004 and 2005, field experiments were carried out in Changins, Switzerland (430m above sea level) in high PVY pressure conditions but without inoculation. During those three years of experiment, two herbicides were compared, one with a quick contact effect on plant desiccation (Dinoseb) and the other with a longer term effect on desiccation and presenting limited translocation (Carfentrazone-ethy). Those herbicides were tested on four different potato cultivars in association with haulm topping. Different haulm killing techniques combining haulm topping and herbicides applications for different periods of time were compared. The first technique involved complete haulm killing (herbicide+topping) compared to complete haul killing carried out five days earlier. The second technique involved haulm topping five days prior to the herbicide application. After haulm killing, one part of the field trial was covered with a polymer web which was removed just before harvest. This polymer web was used to avoid aphids contact with potato plants and prevent any virus transmission. This device will allow evaluation of virus transmission after haulm killing comparing the infection percentage of the protected and unprotected part of the field. Percentage of infected plants was recorded by ELISA testing on progeny tubers. Results suggest there was no significant difference in virus transmission when using Dinoseb or Carfentrazone-ethyl. This could be explained by the fact that, whatever the haulm killing technique, the transmission of PVY after haul killing is low. This low transmission have been highlighted by the use of polymer covers. Early haulm topping or early haulm killing did not allow to reduce significantly the virus transmission. One possible explanation is the fact that the five days period was not sufficient to significantly reduce crop exposure to aphid pressure particularly for mature plants less susceptible to PVY. All in all, haulm killing management procedures seems to have a limited impact on PVY spread. This is likely to be because haulm killing is carried out when potato crops show a high degree of mature plant resistance to PVY.

# ANNEX IX

Dupuis B, Schwaerzel R, Goy G, Tallant M, Derron J. Stepwise development of an efficient method to control Potato Virus Y spread in seed potato fields. In: Focus B, ed. *Proceedings of the EAPR virology*, 2010. Hamar: Bioforsk Focus, 22.

# Stepwise development of an efficient method to control *Potato Virus Y* spread in seed potato fields

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*Potato Virus Y* (PVY) is a Potyvirus transmitted by aphids in potato fields. For susceptible cultivars, the association of mineral oil and aphicide is generally used to control PVY spread in seed potato fields. Mulching and border crops are also reported as alternative control methods. Four years of field trials have been carried out in Switzerland to compare common and alternative methods to control PVY spread. Four aphicides have been tested with foliage application: Lambda-Cyhalothrine, Triazamate, Pymetrozine and Imidacloprid. One commercial mineral oil and one commercial plant oils have been evaluated for virus control. Acibenzolar-S-methyl has been tested as elicitor. To reduce PVY transmission by aphid behaviour disruption, mulching, oat intercropping and film covering were tested eventually associated with mineral oil. Each control method has been tested at least one year. The results indicate that aphicides are effective to control the aphid populations in the field but inefficient to control PVY spread. Acibenzolar-S-methyl has no effect. Mineral oil has been shown to be efficient to control PVY spread but has no effect on aphid populations. Plant oils were less efficient than mineral oil in virus spread limitation. Mulching, oat intercropping and film cover reduce aphid populations on potato plants and PVY transmission. We concluded that the association of mineral oil with mulch or oat strengthens the control of aphid populations and PVY transmission on seed potato plants.

# ANNEX X

Dupuis B, Balmelli C, Schumpp O, Steinger T. Within-field spread of two strains of potato virus Y (PVY) In: Research EaFP, ed. *Proceedings of the 19th Triennial Conference of the European Association for Potato Research*, 2014. Brussels: European Association for Potato Research, 65.

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## WITHIN-FIELD SPREAD OF TWO STRAINS OF POTATO VIRUS Y (PVY)

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### Introduction

Potato virus Y (PVY) is economically the most detrimental viral disease affecting seed potato production in Europe [1] PVY is transmitted in a non-persistant manner by several species of aphids [2]. Within-field PVY spread is affected by different factors such as cultivar susceptibility [3]; vectors pressure [4]; mineral oil treatments [5]; mature plant resistance [6] and haulm killing date [7]. This study will focus on plant-pathogen interactions affecting within-field PVY spread.

### Material and Methods

A two-years field trial was conducted in Switzerland to assess the spread of two PVY isolates from two different strains (PVYNTN and PVYN-WI) on two different cultivars (Charlotte and Nicola). No mineral oil and no insecticide was sprayed during the season and flying aphids were captured at weekly intervals to evaluate the risk of PVY transmission. Leaf samples taken at two time points during the growing season as well as pre-haulm killing and pre-harvest samples were analyzed by DAS-ELISA to evaluate the rate of PVY spread from emergence until harvest.

### Results

Peak of aphid flight arrived around 20 days later the second year of experiment but the main PVY infections appeared at the same time for both year. Over time aphid species distribution could explain this phenomenon. Overall, cv. Nicola was more susceptible than cv. Charlotte and the PVYN-WI isolate was spreading at a higher rate than the PVYNTN isolate. Nevertheless, many interactions between year and isolate and year and cultivar were statistically significant.

### Conclusions and perspectives

This study shows that two PVY isolates from different PVY strains can spread at different rates under the same agro-climatic conditions. The next step would be to determine if, within the same PVY strain, different isolates could spread differently.

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