Studies on the relationships of *Potato virus Y* and *Potato leaf roll virus* with their aphid vectors in potato

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Abstract

Potato virus Y (PVY) and *Potato leaf roll virus* (PLRV) constrain potato production and are transmitted by many aphid vectors. Green peach aphid (GPA) is the most efficient vector of both viruses. The overall objective of this dissertation was to study the potato-PVY- and PLRV-aphid pathosystems. A recent concern regarding PVY is the emergence of necrotic and recombinant strains (e.g., $PVY^{N:O}$, PVY^{NTN}, $PVY^{NA\text{-}N/NTN}$) and their impacts relative to the ordinary strain (PVY^{O}) in the United States. The first study was aimed at clarifying transmission of virus strains by GPA when multiple virus strains are present in the same source tissue. The apparent primacy of PVY^{O} observed in the study suggests that GPA transmission from mixed infection does not contribute to the increasing prevalence of newer necrotic strains. The second study was aimed at understanding how necrotic PVY isolates are transmitted compared to ordinary isolates by various colonizing (GPA and potato aphid, PA) and non-colonizing aphid (bird cherry-oat aphid, BCOA) species. Although GPA transmitted PVY isolates most efficiently, BCOA transmitted PVY^{NTN} isolates with better efficiency than previously reported. BCOA is one of the most abundant aphids in potato fields in Idaho, suggesting that this species might be a contributing factor to the recent prevalence of necrotic strains. The third study was focused on the characterization of the aphid species complex over time in potato fields adjacent to cereal fields and how their transient flight might contribute to PVY incidence. A diverse fauna of non-colonizing aphid species was captured, including many cereal aphids as well as species from other crops and weeds. PVY incidence in potato increased following peak aphid flight and appeared to be related to aphid abundance. The objectives of the PLRV-aphid pathosystem study were to observe the phenology of aphid vectors and PLRV incidence among Russet Burbank, Russet Norkotah, and Ranger Russet potato varieties over the season. All varieties were found to be at similar risk of PLRV incidence and aphid vector colonization. Findings from these studies contribute to our understanding of the PVY- and PLRV-pathosystems, and their future management for the benefit of potato growers.

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Dedication

I would like to dedicate this work to the potato growers of the United States of America and India.

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Chapter 1

Transmission of single or combinations of *Potato virus Y* strains by the aphid vector *Myzus persicae* (Sulzer) on potato

Abstract

Potato virus Y (PVY) constrains potato production and seed tuber production in the USA and worldwide. PVY is transmitted by many different aphid species in a non-persistent manner, but the green peach aphid (GPA, *Myzus persicae* Sulzer) is the most efficient vector. An evolving concern regarding PVY is the recent emergence of necrotic strains (N, N:O, NTN, NA-NTN) relative to the ordinary strain (O) in the United States. Although rare, mixtures of different PVY strains can be observed in potato fields. GPA-mediated transmission studies were conducted to examine transmission efficiency and strain specificity in single versus mixed-strain inoculum sources. Experimental treatments included three single (O, N:O, NA-NTN), three double (O+N:O, N:O+NA-NTN, O+NA-NTN), one triple (O+N:O+NA-NTN) strain mixture(s), and a sham inoculation. Transmission efficiency was found to vary among strain mixture treatments. Aphids tended to transmit only a single strain when the source mixture included O, but were more likely to transmit multiple strains when the source mixture did not include O. Transmission of O+N:O+NA-NTN and N:O+NA-NTN were not observed from the triple mixture. Regardless of strain combination, the O strain tended to dominate among others. Apparent primacy of O strain transmission from strain mixtures suggests that differences in transmission efficiency for GPA may not be a contributing factor to the recent emergence of necrotic strains and also might suggest adaptation for the O strain by the aphid clone used in this study.

Introduction

Potato (*Solanum tuberosum* L.) is an economically important, high-value crop in the USA, and is a major non-cereal staple food worldwide (FAO 2008). *Potato virus Y* (PVY) (Family *Potyviridae*: Genus *Potyvirus*) is distributed in potato growing areas throughout the world, including the USA (Crosslin et al. 2006, Gray et al. 2010). PVY can severely constrain potato yield and quality, and prevent seed potato certification (Hane and Hamm 1999, Nolte et al. 2004, Whitworth et al. 2006, Gray et al. 2010). An emerging concern regarding PVY is the increasing prevalence of recombinant strains (many strains share genetic information through recombination; e.g., N:O has genetic information for both the O and N strain), some of which cause necrotic symptoms relative to the ordinary strain PVY^{O} (Nie and Singh 2003, Singh et al. 2003, 2008, Piche et al. 2004, Lorenzen et al. 2006). Unlike the O strain, which generally causes mosaic, necrosis, and leaf drop symptoms, necrotic and recombinant strains (e.g., NTN, NA-NTN, N:O, N-Wi) can produce Potato Tuber Necrotic Ringspot Disease (PTNRD) with varying symptoms in many predominant potato cultivars including Yukon Gold, Red Norland, Yukon Gem, Highland Russet, Alturas, and Ranger Russet (van den Heuvel et al. 1994, McDonald and Singh 1996, Boonham et al. 2002, Gray et al. 2010, Cavatorta et al. 2011, Karasev and Gray 2013). PTNRD is a type of tuber defect associated with the infection by necrotic strains; the affected tubers show roughened rings of red or brown skin and necrosis beneath rings extending into the tuber flesh (Beczner et al. 1984, Piche et al. 2004, Gray et al. 2010).

Necrotic PVY strains were first reported in the USA in 2002 (Crosslin et al. 2002), and new recombinant strains were subsequently reported from the Northern USA and Canada (Piche et al. 2004). Beginning in the early 2000s there was a significant increase in

the necrotic N:O/N-Wi strain in the North America (Nie et al. 2004), later characterized as two separate strains (Singh et al. 2008, Gray et al. 2010, Karasev and Gray 2013). Gray et al. (2010) confirmed the increase of necrotic strains and the presence of mixtures of different strains or subgroups in potato growing and seed production areas of the USA. The reasons for the strain shift are unknown, but possible explanations of this phenomenon include differences in relative efficiency in transmission of PVY strains by aphids from their alternative weed host reservoirs to potato, undetected plant material in seed certification programs due to milder symptom expression in some PVY-susceptible potato cultivars, and differences in relative efficiency of insect vectors to transmit necrotic strains versus the ordinary strain.

PVY is transmitted to potato by more than 50 colonizing and non-colonizing aphid species (Ragsdale et al. 2001) in a non-persistent manner (Bradley 1954, Sigvald 1984). Aphids may acquire the virus during very short (<1 min) feeding probes in the epidermal leaf tissue and can transmit the virus to healthy plants by short-duration probes with no latent period (Sigvald 1984, Shrestha et al. 2014). Helper component and aphid salivation play important roles in transmission (Syller 2006, Fereres 2007). Relative transmission efficiency varies among different aphid species, their clones, and life stages, as well as among different PVY strains and isolates (Boiteau et al. 1998, Basky and Almási 2005, Davis et al. 2005, Kaliciak and Syller 2009, Verbeek et al. 2010, Cervantes and Alvarez 2011, Mello et al. 2011). The green peach aphid (*Myzus persicae* Sulzer) is the most efficient vector of PVY in potato (Hoof 1980, Sigvald 1984, Piron 1986, Fernández-Calvino et al. 2006, Kaliciak and Syller 2009, Verbeek et al. 2010), and is, therefore, among the most important vectors from a management perspective.

The literature shows conflicting results regarding whether aphids preferentially transmit ordinary strains or necrotic strains (Harrington and Gibson 1989, Basky and Almási 2005). No significant difference in transmission efficiency of ordinary and necrotic strains was observed in some studies with comparable levels of virus titer (Fereres 1993, Verbeek et al. 2010, Mello et al. 2011). Srinivasan et al. (2012) suggested that strain specificity and simultaneous transmission of closely related strains from a strain mixture by aphids could explain the observed shift in the prevalence of PVY strains. They showed that necrotic strains of PVY were transmitted to tobacco by green peach aphid more often than the ordinary strain when the vector was provided source tissue with two-way mixtures (Srinivasan et al. 2012). The present study was conducted using potato as the strain source and target to test the hypothesis that aphids transmit some strains more efficiently than others from mixtures of two or three strains. The aim of the study was to clarify transmission of virus strains by the green peach aphid when multiple virus strains are present in the same source tissue. To our knowledge, this is the first attempt to examine transmission by aphids of a mixture of PVY strains on potato, and to document transmission efficiency of aphids from a mixture of three virus strains.

Materials and methods

Experimental set up

Eight treatments of the experiment included aphid inoculation of recipient plants from three single strains (O, N:O and NA-NTN), three 2-way (O+N:O, N:O+NA-NTN, O+NA-NTN), one 3-way (O+N:O+NA-NTN) strain mixtures, and a sham inoculation (potato plant

inoculated with non-viruliferous aphids). Twenty recipient plants were used for each treatment and all the treatments were replicated 5 times.

Host plants

Throughout the experiment, virus-free tissue-cultured plantlets of 'Russet Burbank' potato obtained from the tissue culture facility at the University of Idaho, Moscow, were used as virus source and virus recipient plants. Plantlets were potted in 10 × 10 × 15 cm plastic pots in a 2:2:1 sand:peat:vermiculite mix with encapsulated fertilizer of N:P:K at 14:14:14 (Osmocot Scotts Miracle Gro, Marysville, OH). Potted plantlets were placed in the greenhouse at 19-27ºC, 70% relative humidity, and 16:8 h light:dark photoperiod. Plants were used in experiments 15-20 d after potting.

Aphid species

Green peach aphids from a laboratory colony ("*OUR*" clone) were used for the transmission study. The colony, which had been reared on Indian mustard (*Brassica juncea* L.) at the University of Idaho Parma Research & Extension Center for 20 years, was moved to the University of Idaho Aberdeen Research & Extension Center in 2001. Since 2001, the colony has been maintained on Chinese cabbage (*Brassica pekinensis* Ruprecht) in a growth chamber maintained at 21-26°C, 90% relative humidity, and a 14:10 h light:dark photoperiod. Chinese cabbage seeds were obtained from a commercial facility (W. Altee Burpee & Co., Warminster, PA), germinated by scarifying the seeds with gibberellic acid (500 ppm) solution, and then maintained in a greenhouse. Chinese cabbage is used because it is a host for the green peach aphid but not PVY, which facilitates maintaining non-viruliferous aphids.

PVY strains and isolates

One ordinary (O) and two necrotic strains (N:O and NA-NTN) were used in this study. O (*NY31* isolate) and N:O (*NY4* isolate) strains were obtained from Dr. Stewart M. Gray, USDA-ARS, Cornell University. NA-NTN (RRA-1 isolate) strain was provided by Dr. Alex Karasev, University of Idaho. All the strains were maintained on Russet Burbank potato. To maintain a stock of PVY-infected plants, cuttings of the infected plants were dipped in rooting hormone (GREEN LIGHT®, ai = indole-3-butyric acid @ 0.1%), and maintained in separate cages in the greenhouse with the same environmental conditions described above. Infection status of the infected potato plants was confirmed using one-step reverse transcription polymerase chain reaction (RT-PCR) using a multiplex primer (Lorenzen et al. 2006). To maintain aphid transmissibility of the virus, source plants were inoculated mechanically and with aphids in alternate generations. Inoculating plants via an aphid vector ensures that virus-infected plants produce sufficient HC-Pro (helper component), which facilitates subsequent aphid-mediated transmission (Granier et al. 1993, Canto 1995).

Preparation of virus source plants

For the transmission experiment, the following treatments were examined: 3 single strains (O, N:O and NA-NTN), three 2-way mixtures (O+N:O, N:O+NA-NTN, O+NA-NTN), and one 3-way mixture (O+N:O+NA-NTN). For the preparation of the virus source, five potato plants were used for each treatment. Mechanical inoculation was performed following procedures described by Srinivasan and Alvarez (2007). Briefly, PVY-infected leaf tissue (tested with RT-PCR) was ground in 0.1 M phosphate buffer and 1 mL of the sap

was used for the inoculation of 2- to 3-week-old potato plants using a foliar abrasive (carborundum powder, 600 grit, Fisher, Fair Lawn, NJ). To obtain double- and triple-strain mixtures in source plants, foliar sap extracted in phosphate buffer from plants infected with single strains was mixed in equal ratios prior to use as inoculum. The mechanical inoculation was performed using cheesecloth on the adaxial surface of all fully opened leaves of each potato plant. After inoculation, plants were isolated inside insect-proof cages (Megaview Bugdorm® 2400 insect rearing tent, 75 × 75 × 115 cm; MegaView Science Co., Ltd., Taichung, Taiwan) in the greenhouse to prevent contamination by viruliferous aphids, and were observed regularly for symptom development. The greenhouse was fumigated every week with naled at a rate of 0.104 g per m^3 to prevent aphid contamination. Twenty-eight days post-inoculation, the plants were tested with RT-PCR using multiplex-primers (see below; Lorenzen et al. 2006) to confirm presence of the desired strains. From each source plant, an upper leaf with the most characteristic symptoms was used as source material for aphid inoculations. To maintain leaf turgidity, the stem of each detached leaf was placed in a 1.7 ml micro-centrifuge tube containing water.

Detection of PVY strain and strain mixtures

RT-PCR was performed to identify the virus strain or strains present in source plants as well as in recipient plants (see below). Total RNA from the leaf tissue was extracted using the Dellaporta et al. (1983) method, with some modifications. Seven leaf punches from the upper, middle, and lower leaves on each plant were taken and placed in a sterile micro-centrifuge tube. The tube was filled with 800 µl of RNA extraction buffer (containing 100 mM Tris [pH = 8.0], 50 mM EDTA, 50 mM NaCl, and 10 mM 2-mercaptoethanol), and

the leaf tissue was ground with disposable plastic pestles. After grinding, an additional 400 µl of RNA extraction buffer was added and the sap was mixed by vortex and then centrifuged at 13,200 rpm for 10 minutes. One ml of the supernatant was collected in another 1.7 ml micro-centrifuge tube, and 140 µl of 10% sodium dodecyl sulphate (SDS) was added. The solution was mixed by vortex and incubated at 65ºC for 25-30 minutes. Then 250 µl of 8 M potassium acetate solution was added, mixed by inversion (20 times), placed in an ice bath for 12-15 minutes, and centrifuged again at 13,200 rpm for 10 minutes. After centrifugation, 1 ml of the supernatant was collected in a separate 1.7 ml micro-centrifuge tube, and 600 µl of ice-chilled isopropanol was added to it; then the solution was gently mixed by inversion (10 times) and placed in an ice bath for at least 25 minutes or overnight. The tube then was centrifuged at 13,200 rpm for 12 minutes and the supernatant was discarded. The resulting pellet was washed with 70% ethanol and dried in a 37ºC incubator for 10 minutes. The dried RNA pellet was then suspended in 100 µl of nuclease-free sterile water and stored at -20ºC before performing the PCR reaction.

Single step RT-PCR was performed using a multiplex primer as described by Lorenzen et al. (2006). Reaction master mix was prepared using the BIO-RAD iScript one step RT-PCR kit for probes (Bio-Rad, Hercules, CA) and Rediload gel loading dye (Invitrogen, formerly Life Technologies, Inc., Carlsbad, CA). Each 25 µl reaction mix contained 12.5 µl of BIO-RAD PCR reaction mix (0.5 mM of each dNTP [dATP, dCTP, dGTP, dTTP], Mg^{++}, iTaq DNA polymerase, stabilizers), 6.5 µl of nuclease-free (DNase/RNase) water, 1 µl each of both the forward and reverse primer mixture (mixture contained primer pairs of O2172, N2258, N5707, and S5585m for forward and O2439c, N2650c, O6266c, and A6032mc for reverse reaction), 2.5 µl of Rediload gel loading dye

(Invitrogen), 0.5 µl of iScript RT (50X formulation of iScript MMLV reverse transcriptase) and 1 µl of sample RNA extract. The thermocycles for the PCR reaction included 15 min at 50ºC for cDNA synthesis, 5 min at 94ºC for iScript reverse transcriptase inactivation, 30 cycles of 15 sec at 94ºC, 1 min at 58ºC, and 30 sec at 72ºC followed by a final extension at 72ºC for 5 minutes and final storage of PCR product at 4ºC. After the PCR reaction, 15 µl of the final amplified PCR product was analyzed by gel electrophoresis (1.5% agarose gel) by staining the product with ethidium bromide (0.5 mg/ml), and observing the gel under UV light (302 nm).

Overall percent PVY infection was determined for the 20 plants in each treatment (single, double, or triple strain infections).

Aphid inoculation

For each treatment, twenty 2- to 3-week-old potato plants were inoculated using a single aphid per plant. Two hours prior to the aphid inoculation, multiple non-viruliferous aphids were removed from the laboratory colony and placed in a Petri dish together with a piece of moist filter paper for a 2-hour pre-acquisition starvation period. Each aphid was gently moved with a number 2 sable paintbrush to the abaxial surface of the virus source leaf and allowed to feed for 2 minutes (acquisition access period) before being transferred to the abaxial surface of a leaf of the recipient plant. During the acquisition access period, aphids were observed using a magnifying glass to confirm their probes. Each aphid was then confined within a cage on the leaf for the inoculation access period. Caution was taken during this aphid transfer to prevent the aphid from losing its acrostyle (prior to transfer, each aphid was given a gentle poke with the paint brush and was moved only after the stylet

had been removed from the plant). Aphids were allowed to feed on the test plant for 1-2 h (inoculation access period). Feeding was terminated mechanically by physically removing each aphid from the plant and crushing it. Plants were then treated with naled as described above. For the sham inoculation (untreated control), starved non-viruliferous aphids were allowed an "acquisition access period" on healthy potato plants before transfer to test plants.

Post inoculation care and sampling

Upon completion of the aphid inoculation, test plants were transferred to the greenhouse and fogged with naled as described above; fogging was repeated weekly. Four to five weeks post inoculation, plants were assayed with RT-PCR (as described above) to evaluate their infection status.

Statistical analysis

Analysis of variance was used to compare the overall PVY infection percentage in different treatments (single, double, and triple mixtures). Binomial distribution was assumed for assessing the infection status of each test plant. Differences within the treatments were evaluated using PROC-GENMOD with logit link transformation in SAS version 9.2 (SAS Institute, Cary, NC). Differences of presence of single or combination of strains in test plants after inoculation from double mixtures as well as the triple mixture source plants were compared. Pairwise comparisons were carried out within the model by chi-square pairwise contrasts. There were significant interactions in regard to percent infection by specific strains or strain combinations among mixtures, so data were sorted by mixture and analyzed separately within each mixture. Within the double mixtures, there were significant interactions between combinations, so data were analyzed within each combination

separately. Interaction between the combinations within the triple mixture was also significant.

Results

Overall infection percentage (regardless of strain or strain combination) differed significantly among treatments (Table 1.01). Overall infection was significantly highest for the triple mixture (41%) followed by N:O+NA-NTN (30%). Infection rate for triple mixture treatment did not differ significantly from the N:O+NA-NTN treatment, but did differ from all other treatments. No significant difference was found in the infection rate between the N:O+NA-NTN and NA-NTN treatments. Infection of the remaining treatments ranged from 16 to 25% and did not differ significantly from each other (Table 1.02, Fig. 1.01).

RT-PCR multiplex results confirmed the presence of viral RNA consistent with all of the treatments in the source plants (Fig. 1.04). The test plants were also checked with multiplex RT-PCR. The numbers of plants with either single or multiple strains present were calculated by examining the bands from those samples. For example, for the O+N:O treatment (Fig. 1.02), 11% test plants had bands present for the O strain (267bp and 689bp), 5% had bands present for N:O (181bp and 689bp), whereas no bands were present for the O+N:O mix in any sample of that set. All the 2-way and 3-way combinations were evaluated in this fashion (Fig. 1.02, Fig. 1.04).

Single-strain treatments did not differ significantly in terms of percent infection (Table 1.03, Fig. 1.02). Aphids that acquired virus from plants infected with the O+N:O 2-way mixture only transmitted one or the other of the strains, and percent infection of O was greater than that of N:O (Table 1.03, Fig. 1.02). From plants with the O+NA-NTN 2-way

mixture, only the O strain was transmitted (Fig. 1.02). From plants with the N:O+NA-NTN 2-way mixture, the combination of the two strains as well as each strain alone were transmitted, with a non-significant trend for a higher transmission rate for N:O than for NA-NTN or the combination of the two strains (Table 1.03, Fig. 1.02). Aphids did not transmit all three strains at once from the 3-way mixture; however, unlike in the 2-way mixtures, combinations of either O+NA-NTN or O+N:O were transmitted whereas N:O+NA-NTN was not (Fig. 1.02). Transmission of O alone from the 3-way mixture was greater than that of O+N:O followed by transmission of O+NA-NTN, N:O, and NA-NTN (Table 1.03, Fig. 1.02).

Infection rates were too low in some of the treatments to permit orthogonal contrasts to compare the treatments statistically. Regardless of combinations, an apparent dominance of the O strain was found; however, statistical comparison of treatments in this way was not possible (Fig. 1.03).

Discussion

The principal objective of this study was to clarify whether differences in transmission rates of different strains of PVY could help explain the emergence of necrotic strains in potato in North America. It was hypothesized that aphid vectors transmit necrotic strains preferentially over the ordinary strain from a strain mixture. However, the results do not support this hypothesis. The apparent primacy of the O strain in transmission from mixed infections suggests that differences among strains in their transmission by the green peach aphid do not contribute to the increasing prevalence of new necrotic strains of PVY.

Srinivasan et al. (2012) found that green peach aphids tended to transmit the necrotic strain over the ordinary strain from double mixtures, which differs from results of the current study; variation in the efficiency of aphids for transmission of different strains might explain this discrepancy. Although green peach aphid has been shown to be the most efficient vector of PVY (Fernández-Calvino et al. 2006, Kaliciak and Syller 2009), the transmission efficiency and relative efficiency factors within an aphid species varies among their clones and life stages, as well as among PVY strains, isolates, and host plants (Boiteau et al. 1998, Basky and Almási 2005, Davis et al. 2005, Kaliciak and Syller 2009, Verbeek et al. 2010, Cervantes and Alvarez 2011, Mello et al. 2011). The virus strains and isolates as well as source and test plants, but not the aphid clone, differed from those used by Srinivasan et al. (2012), which may explain the different results. Virus isolates may react differently when present with different combinations in a host plant, affecting transmission outcomes. Furthermore, although strain mixtures have been reported in the potato growing areas of the USA (Crosslin et al. 2006, Gray et al. 2010), extensive studies on the prevalence of strain mixtures in potato are lacking, and co-infections could be rare. If so, differential transmission of strains may be less important than which single strain or strains predominate in a given area.

Although green peach aphid is the most efficient PVY vector, several other species including floxglove aphid (*Aphis nasturtii*) (Ragsdale et al. 2001), bird cherry-oat aphid (*Rhopalosiphum padi*) (Halbert et al. 2003), and soybean aphid (*Aphis glycines*) (Davis et al. 2005) are important vectors of PVY in potato in the USA. It is possible that differential transmission by these vectors of different virus strains has contributed to the rise of necrotic PVY strains. Mello et al. (2011) found *A. nasturtii* to be a PVY vector with intermediate

efficiency that transmits both the O and N:O strains at similar rates. Furthermore, great variability can be found in the transmission efficiency of *R. padi* including higher transmission of the NTN strain (Sigvald 1984, Katis and Gibson 1985, Halbert et al. 2003, Pelletier et al. 2008, Mello et al. 2011, Mondal et al. in prep.). *Rhopalosiphum padi* is an important vector of PVY in certain potato-growing areas due to its transient flight into potato fields in large numbers (Sigvald 1984, DiFonzo et al. 1997, Ragsdale et al. 2001, Halbert et al. 2003). The O strain was preferentially transmitted by *A. glycines* from a strain mixture (Davis et al. 2005), which also does not support the recent increase in necrotic strains. However, in the present study, higher infection rates were observed from the triple mixture and the mixture of two necrotic strains. Further studies to compare transmission efficiencies from two-way and three-way strain mixtures with other important PVY vectors are merited.

The O strain appeared to dominate in two-way and three-way mixtures; the O+N:O treatment resulted in relatively few N:O infections, and the O+NA-NTN treatment resulted in no infections other than O alone. These patterns suggest an antagonistic interaction between O and the other strains. Facilitative and antagonistic interactions between plant viruses in mixed infections are possible (García-Cano et al. 2006, DaPalma et al. 2010, Syller 2012). Dietrich and Maiss (2003) evaluated the antagonistic interaction of two closely related *Potyviruses* in which two viral populations competed with each other during the colonization of epidermal cells. Interestingly, the O strain dominated in the triple mixture as well; however, the overall transmission rate was higher than in the two-way mixtures with O, which may suggest a synergistic reaction (i.e., a reaction that increased the titer of one or more strains). A similar interaction between PVY and *Potato virus X* (PVX; Family

Alphaflexiviridae: Genus *Potexvirus*) was reported in tobacco plants where the PVX titer was increased 10-fold in the presence of PVY (Rochow and Ross 1955, Vance 1991).

There are several possibilities to explain the apparent dominance by the ordinary strain in mixed infections in this study. One possible explanation may be presence of higher virus titer of the O strain in the mixtures, although this was not measured. Another possibility for the apparent dominance of the O strain may be a wider distribution of this strain among plant cells in the infected host. Pelletier et al. (2008) suggested that the last cell punctured by an aphid probe during acquisition phase in source tissue plays an important role in virus transmission. If the virus particles in the last aphid probe have primacy, then the dominance of O and lack of three-way mixed infections in the results presented here suggest that a single cell may contain two strains but is unlikely to contain three.

Virus particles of non-persistently transmitted viruses attach to a specific receptor-binding site in the aphid stylet, called the acrostyle (Uzest et al. 2007, 2010, Blanc et al. 2011). The results presented here demonstrate that green peach aphid can acquire and transmit multiple PVY strains between potato plants. Whether virus particles attach to specific binding sites in the aphid acrostyle or non-specific binding sites is unclear. Pelletier et al. (2008) concluded that the last cell puncture of the acquisition phase and the first cell puncture of the inoculation phase played the most critical role in PVY transmission. If this is the case, more than one virus strain must be present in a single cell to facilitate multiple strain transmission. Alternately, it may be that a different strain from an infected cell in a double- or triple-infected plant is acquired and transmitted at a lower frequency than the predominant strain that is transmitted. Takeshita et al. (2004) reported that two strains of

Cucumber mosaic virus (Family *Bromoviridae*: Genus *Cucumovirus*) did not occupy the same cells in co-infected cowpea plants; however, it has yet to be determined whether a single plant cell may harbor more than one PVY strain at a time. Further studies into aphid probing behavior in relation to the transmission of different strain types are required to clarify this issue. Vector specificity or receptor specificity of the aphid acrostyle for a particular strain may be affected by several factors that need to be studied further.

In conclusion, aphid transmission of different strains from a mixture of strains is a complex phenomenon. Results from the present study do not support the hypothesis that differential aphid transmission of PVY strains contribute to the observed recent shift toward necrotic strains in the field. Possible alternative explanations include antagonistic and/or synergistic reactions within strain mixtures, differences in virus titer level among strains, varying aphid probing behavior, and the virus inoculum source and/or strain. Some of these questions might be clarified if artificial source tissue could be used with different virus strains of identical titer. All these possibilities warrant further investigation.

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Table 1.01: Comparison of overall PVY infection percentage of potato following transmission by *M. persicae* from single-, double-, and triple-strain mixture source plants.

Treatment[a]	df	$\chi 2$	Pr> $\chi 2$
All treatments	6	25.0	0.0003
Single strains	2	1.7	0.423
O+N:O	2	16.1	0.0003
O+NA-NTN	2	40.2	<0.0001
N:O+NA-NTN	2	4.2	0.122
O+N:O+NA-NTN	6	81.6	<0.0001

[a]Twenty potato plants per treatment were inoculated with viruliferous *M. persicae* from single-, double-, or triple-strain mixture source plants. Infection percentage was determined as the percentage of potato plants within a treatment infected with PVY regardless of strain. Differences in least square means of infection percentage were compared using PROC GENMOD (assuming binomial distribution).

Table 1.02: Pairwise comparison of overall PVY infection percentage in potato plants following transmission by *M. persicae* from source plants with three single PVY strains and their possible 2- and 3-way combinations.

Treatment[a]	Treatment compared with	df	$\chi 2$	Pr> $\chi 2$
N:O	N:O+NA-NTN	1	3.9	0.048
N:O	NA-NTN	1	1.1	0.297
N:O	O	1	1.5	0.228
N:O	O+N:O	1	0.1	0.706
N:O	O+N:O+NA-NTN	1	12.3	0.0004
N:O	O+NA-NTN	1	0.03	0.852
N:O+NA-NTN	NA-NTN	1	0.9	0.337
N:O+NA-NTN	O	1	0.6	0.426
N:O+NA-NTN	O+N:O	1	5.5	0.020
N:O+NA-NTN	O+N:O+NA-NTN	1	2.7	0.102
N:O+NA-NTN	O+NA-NTN	1	4.7	0.031
NA-NTN	O	1	0.03	0.869
NA-NTN	O+N:O	1	2.0	0.158
NA-NTN	O+N:O+NA-NTN	1	6.6	0.010
NA-NTN	O+NA-NTN	1	1.5	0.220
O	O+N:O	1	2.5	0.116
O	O+N:O+NA-NTN	1	5.8	0.016
O	O+NA-NTN	1	1.9	0.164
O+N:O	O+N:O+NA-NTN	1	14.7	0.0001
O+N:O	O+NA-NTN	1	0.04	0.848
O+N:O+NA-NTN	O+NA-NTN	1	13.5	0.0002

[a]Twenty potato plants per treatment were inoculated with viruliferous *M. persicae* from single, double, triple strain mixture source plants. Overall infection percentage was determined as the percentage of potato plants within each treatment infected with PVY regardless of strain. Differences in least square means of infection percentage were compared using PROC GENMOD (assuming binomial distribution).

Table 1.03: Differences in least square means among infection rates in potato plants following transmission of PVY from single strains, double mixture and triple mixtures (O, N:O, NA-NTN and their possible combinations) compared with pairwise contrast.

Treatment name	Treatment[a]	Treatment compared with	df	$\chi 2$	Pr> $\chi 2$
Single strains	N:O	NA-NTN	1	1.1	0.296
	N:O	O	1	1.5	0.227
	NA-NTN	O	1	0.03	0.868
O+N:O	N:O	O	1	2.4	0.123
	N:O	O+N:O	1	2006.5	<0.0001
	O	O+N:O	0	0	-
O+NA-NTN	NA-NTN	O	1	0	0.999
	NA-NTN	O+NA-NTN	1	0	1.00
	O	O+NA-NTN	0	0	-
N:O+NA-NTN	N:O	N:O+NA-NTN	1	2.5	0.117
	N:O	NA-NTN	1	3.3	0.070
	N:O	O	1	0.1	0.785
O+N:O+NA-NTN	N:O	N:O+NA-NTN	1	0	0.999
	N:O	NA-NTN	1	0.9	0.336
	N:O	O	1	14.4	0.0001
	N:O	O+N:O	1	4.3	0.037
	N:O	O+N:O+NA-NTN	1	0	0.999
	N:O	O+NA-NTN	1	0.9	0.336
	N:O+NA-NTN	NA-NTN	1	0	0.999
	N:O+NA-NTN	O	1	0	0.999
	N:O+NA-NTN	O+N:O	1	0	0.999
	N:O+NA-NTN	O+N:O+NA-NTN	1	0	1.00
	N:O+NA-NTN	O+NA-NTN	1	0	0.999
	NA-NTN	O	1	11.6	0.0006
	NA-NTN	O+N:O	1	5.7	0.017
	NA-NTN	O+N:O+NA-NTN	1	0	0.999
	NA-NTN	O+NA-NTN	1	0	1.00
	O	O+N:O	1	6.4	0.011
	O	O+N:O+NA-NTN	1	0	0.999
	O	O+NA-NTN	1	11.6	0.0006

	O+N:O	O+N:O+NA-NTN	1	0	0.999
	O+N:O	O+NA-NTN	1	5.7	0.017
	O+N:O+NA-NTN	O+NA-NTN	1	0	0.999

[a]There were significant interactions between mixtures, so data were sorted by mixture and analyzed separately. Within the double mixtures there were significant interactions between combinations, so data were analyzed within each combination separately. Interaction among the combinations within the triple mixture was also significant, so pairwise comparisons were made among combinations. Differences in least square means of infection percentage were compared using PROC GENMOD (assuming binomial distribution).

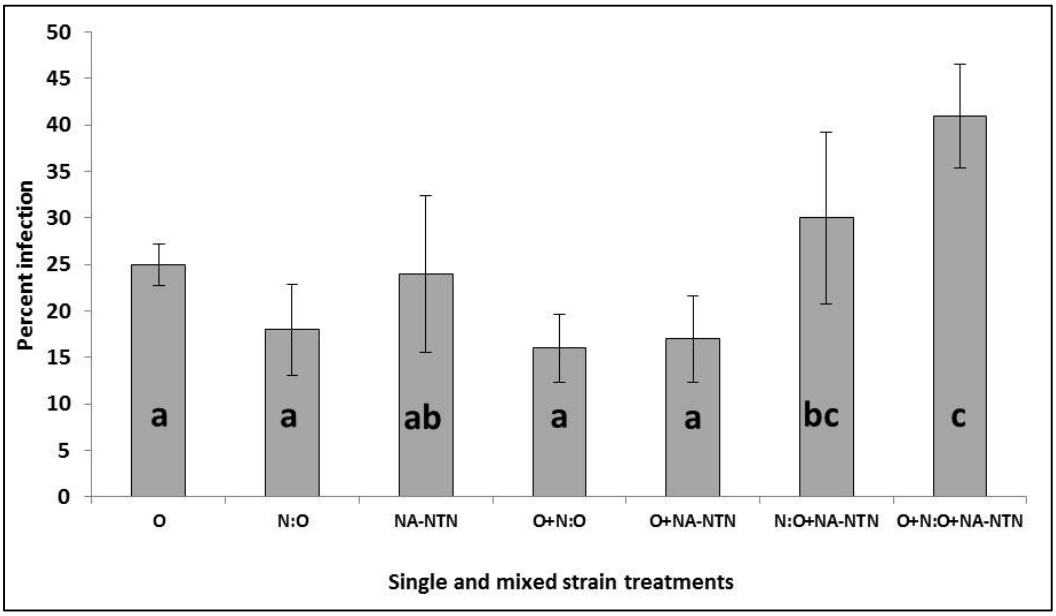

Fig 1.01: Overall infection rates (%) compared among all treatments for plants inoculated with PVY by *M. persicae* from a virus source of O, N:O, or NA-NTN and their possible two-way and three-way combinations. Means that share the same letter are not significantly different. Error bars denote standard error. Statistical inference was based on logit-link transformed data; non-transformed means are shown.

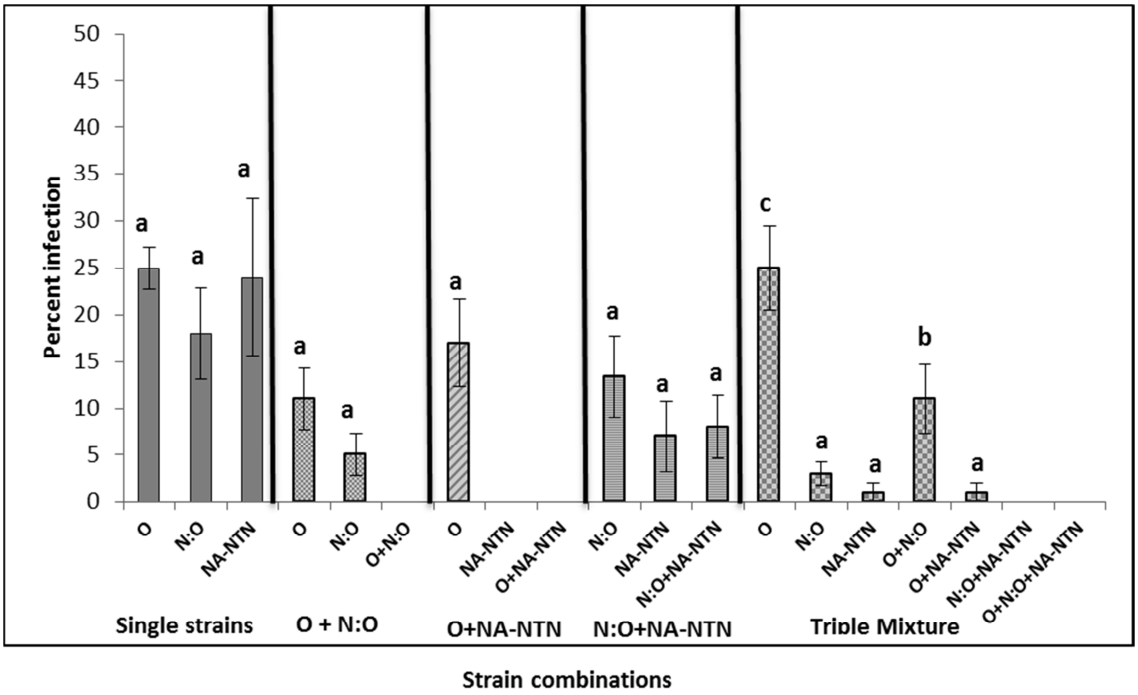

Fig 1.02: Infection rates (%) compared among all possible outcomes of the individual treatments for plants inoculated by *M. persicae* with PVY from a virus source of O, N:O, or NA-NTN and their possible two-way and three-way combinations. Means within each group that share the same letter are not significantly different. Error bars denote standard error. Statistical inference was based on logit-link transformed data; non-transformed means are shown.

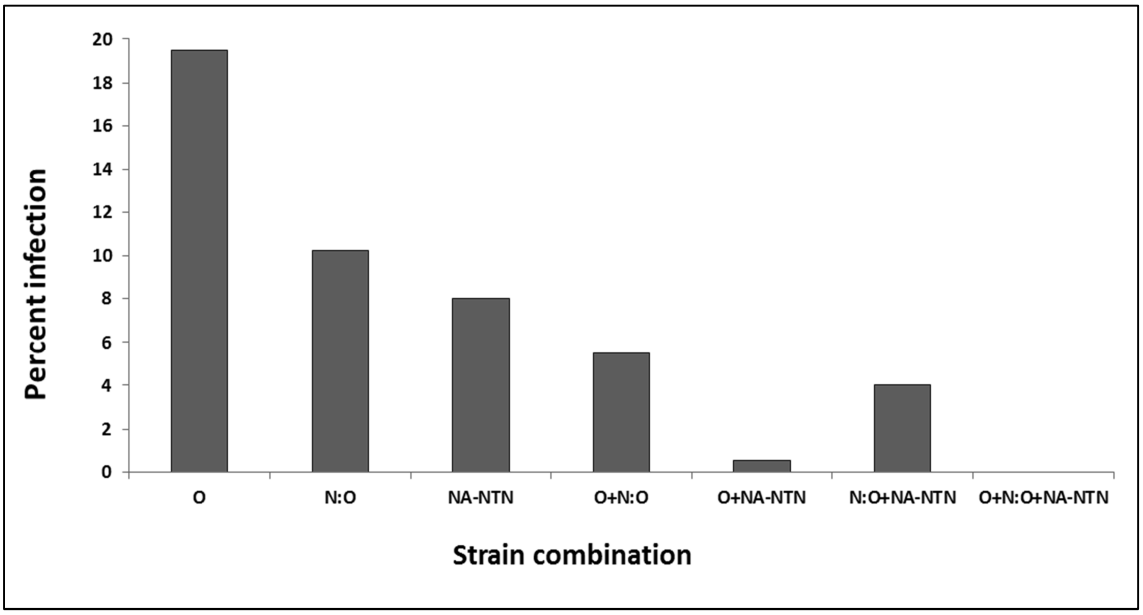

Fig 1.03: A comparison of overall infection rate (%) among all possible combinations of strain outcomes of all treatments regardless of combinations. Infection rates were too low in some cases to allow statistical analysis for orthogonal contrasts.

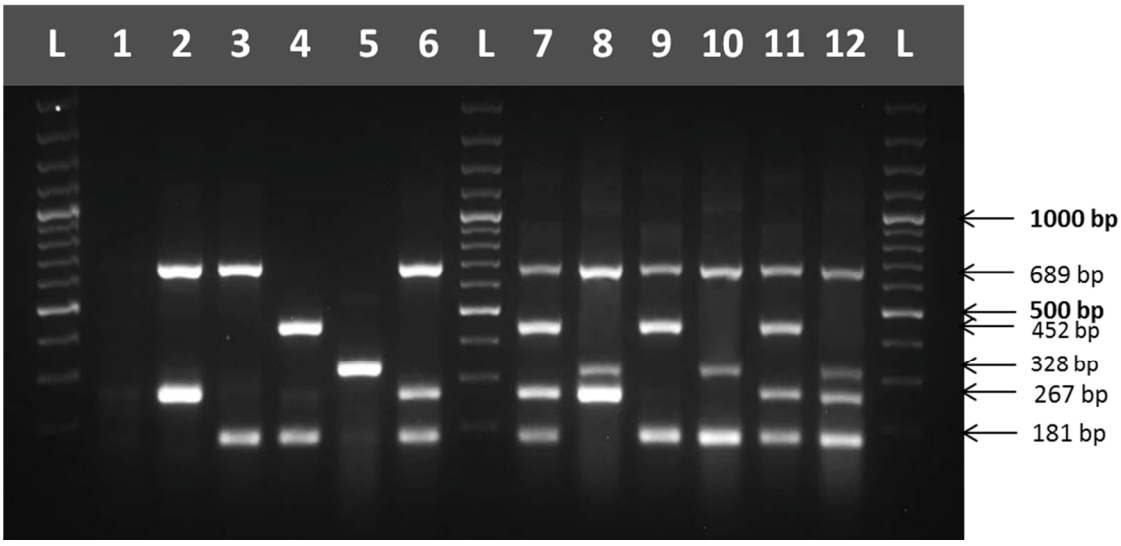

Fig 1.04: Detection of strains and their mixtures in aphids by RT-PCR in ethidium bromide-stained agarose gel (2%). RT-PCR product of RNAs of Lane 1 – Healthy; Lane 2 – O strain; Lane 3 – N:O strain; Lane 4 – NTN strain; Lane 5 – NA-NTN strain; Lane 6 – O+N:O; Lane 7 – O+NTN; Lane 8 – O+NA-NTN; Lane 9 – N:O+NTN; Lane 10 – N:O+NA-NTN; Lane 11 – O+N:O+NTN; Lane 12 – O+N:O+NA-NTN; Lane L – 100 bp ladder.

Chapter 2

Comparison of transmission efficiency of different isolates of *Potato virus Y* among three aphid vectors

Abstract

Potato virus Y (PVY) strains are transmitted by different aphid species in a non-persistent, non-circulative manner. Green peach aphid (GPA, *Myzus persicae* Sulzer) is the most efficient vector but bird cherry-oat aphid (BCOA, *Rhopalosiphum padi* L.) and potato aphid (PA, *Macrosiphum euphorbiae* Thomas) also contribute to PVY transmission. Studies were conducted with GPA, PA, and BCOA to assess transmission efficiency for different isolates of the same strain. Treatments included three PVY strains (PVY^{O}, $PVY^{N:O}$, PVY^{NTN}) and two isolates of each strain (*Oz* and *NY31* for O; *Alt* and *NY4* for N:O; *N4* and *NY29* for NTN), using each of 3 aphid species as well as a sham inoculation. Virus-free tissue-cultured plantlets of potato cv. Russet Burbank were used as virus source and recipient plants. Ten test plants per treatment and 10 aphids per plant were used, and the experiment was replicated five times. Five weeks post inoculation, recipient plants were tested with quantitative DAS-ELISA to assess infection percentage and virus titer. ELISA-positive recipient plants were assayed with RT-PCR to confirm presence of the desired strains. Transmission efficiency (percent infection of plants) was highest for GPA. However, transmission efficiency did not differ significantly between isolates within each strain. For both GPA and BCOA, isolates of NTN were transmitted with greatest efficiency followed by isolates of O and N:O. Correlations among source plant titer, infection percentage, and recipient plant titer were not observed. BCOA transmitted PVY with higher

efficiency than previously reported, suggesting that this species is more important to PVY epidemiology than originally considered. This result might explain the increasing prevalence of necrotic strains in potato growing regions.

Introduction

Potato is a globally important crop. Potato production is severely constrained by many viral pathogens, among which *Potato virus Y* (PVY) may be the most destructive (Gray et al. 2010, Karasev and Gray 2013). PVY can cause considerable economic losses by reducing potato yield and quality, as well as preventing seed certification (Hane and Hamm 1999, Nolte et al. 2004, Whitworth et al. 2006, Gray et al. 2010). An emerging concern regarding PVY is the increasing prevalence of necrotic and recombinant strains, many of which produce milder symptoms than the ordinary PVY^{O} strain (Gray et al. 2010, Karasev and Gray 2013).

Singh et al. (2008) classified PVY isolates into seven strain groups based on host responses, serological properties, and genomic sequencing. Historically, PVY^{O} (ordinary strain) was the most common PVY strain in Europe (Sigvald 1985). In the late 1980s and the beginning of the 1990s, the spread of PVY^{N} (necrotic strain) was reported in both Europe and Canada (Weidemann 1988, Mcdonald and Kristjansson 1993, Nie and Singh 2002) followed by many other countries, including the USA (Crosslin et al. 2002, 2006). PVY^{NTN} and $PVY^{N\text{-}Wi}$ are relatively newer strains and are type members of the PVY^{N} group that emerged via recombination phenomena (Beczner et al. 1984, Blanco-Urgoiti et al. 1998, Glais et al. 2002, Nie et al. 2004). Unlike PVY^{O}, which generally causes foliar mosaic, necrosis, and leaf drop symptoms, the recombinant strains (PVY^{NTN}, $PVY^{NA\text{-}NTN}$, $PVY^{N:O}$, $PVY^{N\text{-}Wi}$) often induce mild foliar mosaic symptoms, but also can induce Potato Tuber

Necrotic Ringspot Disease (PTNRD) of varying severity in many important potato cultivars including Yukon Gold, Norland Red, Yukon Gem, Highland Russet, Alturas, and Ranger Russet (van den Heuvel et al. 1994, McDonald and Singh 1996, Boonham et al. 2002, Cavatorta et al. 2011, Karasev and Gray 2013). The affected tubers show roughened rings of red or brown skins and necrosis beneath rings extending into the tuber flesh (Beczner et al. 1984, Piche et al. 2004, Gray et al. 2010). Despite the historic prevalence of the ordinary strains, recombinant and necrotic strains appear to be replacing PVY^{O} as the dominant strains in many potato growing regions (Chikh Ali et al. 2010, Gray et al. 2010). The underlying reasons behind these strain shifts remain largely unknown (Mello et al. 2011).

Numerous aphid species efficiently transmit PVY in a non-persistent manner (Sigvald 1984, Bokx and Piron 1990, Radcliffe and Ragsdale 2002). Although green peach aphid (GPA, *Myzus persicae* Sulzer) is the most efficient vector of PVY in potato (Hoof 1980, Sigvald 1984, Fernández-Calvino et al. 2006), several other colonizing, migrant, and non-colonizing species also contribute to the epidemiology of PVY (Ragsdale et al. 2001). Potato aphid (PA, *Macrosiphum euphorbiae* Thomas) is a colonizing aphid species in potato growing areas of the Pacific Northwest (PNW) in the USA, and can transmit PVY strains with 4-29% transmission efficiency in terms of percent infection (Piron 1986, Harrington and Gibson 1989). GPA and PA contribute to the field spread of PVY from different inoculum sources including hairy nightshade (*Solanum sarrachoides* Sendt.), an alternative PVY host (Cervantes and Alvarez 2011). Non-colonizing aphids might occur in very large numbers making their effect on virus spread disproportionately large relative to their lower virus transmission efficiency. For example, bird cherry-oat aphid (BCOA, *Rhopalosiphum padi* L.) is abundant in many potato growing areas (Sigvald 1992, Halbert et al. 2003) and

may contribute to PVY spread during the aphid's migration in large numbers from cereal fields (Katis and Gibson 1985, DiFonzo et al. 1997, Halbert et al. 2003, Pelletier et al. 2008).

Virulence and aggressiveness vary among isolates of PVY strains (Blanchard et al. 2008, Quenouille et al. 2013). The transmission efficiency and relative efficiency factors also vary among different aphid species, clones, and biotypes within a species, different aphid life stages, as well as among PVY strains and isolates and their host plants (Boiteau et al. 1998, Basky and Almási 2005, Davis et al. 2005, Kaliciak and Syller 2009, Verbeek et al. 2010, Cervantes and Alvarez 2011, Mello et al. 2011, Shrestha et al. 2014). GPA is the most efficient vector of PVY^{O} (Sigvald 1984) and PVY^{N} (Hoof 1980, Piron 1986). However, floxglove aphid (*Aphis nasturtii*; Harrington and Gibson 1989), BCOA (Halbert et al. 2003), and soybean aphid (*Aphis glycines*; Davis et al. 2005) have been reported to transmit O, N:O, and NTN strains with varying transmission efficiency (Katis and Gibson 1985, Pelletier et al. 2008, Verbeek et al. 2010, Mello et al. 2011). Necrotic and recombinant strains of PVY have been reportedly transmitted with higher efficiency than the ordinary strains by several aphid species including GPA (Basky and Almási 2005, Davis et al. 2005, Kaliciak and Syller 2009). Furthermore, Verbeek et al. (2010) found slight differences in transmission efficiency of various PVY isolates among GPA biotypes and several other species. However, it remains to be determined whether this holds for all PVY strains and isolates and aphid species (Kaczmarek and Hnat 1998, Mello et al. 2011).

In the present study, aphid transmission efficiency of two potato-colonizing aphids (GPA and PA), and a non-colonizing aphid (BCOA) were compared between two different isolates within each of three PVY strains (the ordinary PVY^{O} strain and the necrotic $PVY^{N:O}$

and PVY^{NTN} strains) in potato plants. GPA and PA were chosen because they are the only known potato-colonizing PVY vectors in the PNW. Additionally, BCOA was chosen because of its relatively high abundance in cereal fields over other cereal aphid species in potato growing regions of Southern Idaho. As PVY is transmitted by several aphid species, and the abundance of aphid species varies in different regions, it would be useful to know which aphid species are transmitting PVY isolates with greater efficiency in any given region (as isolates are collected from a particular region). This information may also be helpful to understand the recent prevalence of necrotic strains in potato growing regions of the PNW.

Materials and Methods

Host plants (source and recipient plants)

Virus-free tissue-cultured plantlets of 'Russet Burbank' potato were used from the tissue culture facility at the University of Idaho, Moscow, ID. The plantlets were potted in 10 × 10 × 15 cm plastic pots in a 2:2:1 sand:peat:vermiculite mix with encapsulated fertilizer of N:P:K (14:14:14) (Osmocot Scotts Miracle Gro, Marysville, OH). The potted plantlets were placed in the greenhouse and maintained in controlled environmental conditions of 19-27ºC, 70% relative humidity, and 16:8 h light:dark photoperiod. Plants were used in experiments 15-20 d after potting.

Aphid species and biotype

GPA were obtained from a laboratory colony ("*OUR*" clone; see Chapter 2 for detailed description). PA were previously collected in 2005 from potato fields in Aberdeen, Idaho. Field-collected aphids were initially maintained on rose plants (var. Rosa Burway)

and then transferred to seed-raised hairy nightshade plants and maintained in growth chambers (as described above). BCOA were obtained from Dr. Nilsa A. Bosque-Pérez, University of Idaho, Moscow, ID and have been maintained on barley plants, *Hordeum vulgare* (L.) cv. Sprinter, in growth chambers (as described above) since 2005.

PVY strains and isolates

PVY^{O} (*NY31* isolate), $PVY^{N:O}$ (*NY4* isolate), and PVY^{NTN} (*NY29* isolate) were obtained from Dr. Stewart M. Gray, USDA-ARS, Cornell University. PVY^{O} (*Oz* isolate), $PVY^{N:O}$ (*Alt* isolate), and PVY^{NTN} (*N4* isolate) were obtained from Dr. Alex Karasev, University of Idaho, Moscow, ID. All the PVY isolates were maintained on Russet Burbank. To maintain a stock of PVY-infected plants, cuttings of the infected plants were dipped into a rooting hormone (0.1% indole-3-butyric acid) and maintained in separate cages in the greenhouse. Infection status of the infected potato plants was confirmed using one-step reverse transcription polymerase chain reaction (RT-PCR) using a multiplex primer (Lorenzen et al. 2006). To maintain aphid transmissibility of the virus, source plants were inoculated mechanically and via aphids in alternate generations.

Preparation of virus source plants

Five potato plants were used for each treatment as virus source material. Mechanical inoculation was performed following procedures described by Srinivasan and Alvarez 2007. Briefly, PVY-infected leaf tissue (tested with RT-PCR) was ground in 0.1 M phosphate buffer and 1 mL of the sap was used for the inoculation of 2-3-week-old Russet Burbank potato plants using a foliar abrasive (carborundum powder, 600 grit, Fisher, Fair Lawn, NJ). Mechanical inoculation was performed using cheese cloth on the adaxial surface of all fully

opened potato leaves. After the inoculation, plants were isolated in insect-proof cages (MegaView Bugdorm® 2400 insect rearing tent, 75 × 75 × 115 cm; MegaView Science Co., Ltd., Taichung, Taiwan) in the greenhouse to prevent cross contamination. The greenhouse was fumigated weekly with naled (Dibrom®8 Emulsive, Amvac Chemical Corporation, Los Angeles, CA) at a rate of 0.105 g ai/m^3 to prevent aphid contamination. Twenty-eight days after inoculation, the plants were tested with RT-PCR using multiplex-primers (see below; Lorenzen et al. 2006) to confirm presence of the desired strains. From each source plant, an upper leaf with the most characteristic symptoms was used as source material for aphid inoculations. To maintain leaf turgidity, the stem of each detached leaf was placed in a 1.7 ml micro-centrifuge tube containing water.

Experimental design

This experiment included eighteen treatment combinations. Recipient plants were inoculated with one of two isolates of one of three virus strains (PVY^{O}, $PVY^{N:O}$, or PVY^{NTN}) using one of three aphid species (GPA, PA, or BCOA) and a sham inoculation (potato plant with no virus) as a control. Ten recipient plants were used in each treatment and all treatments were replicated 5 times.

Aphid inoculation

For each treatment, ten 2- to 3-week-old potato plants were inoculated using 10 aphids per plant. Two hours prior to the aphid inoculation, multiple non-viruliferous aphids were removed from the laboratory colony and placed in a Petri plate together with a piece of moist filter paper for a 2-h pre-acquisition starvation period. Each aphid was gently moved with a number 2 sable hair paint brush to the abaxial surface of the virus source leaf and

allowed to feed for 120 seconds (acquisition access period) before being transferred to the abaxial surface of a leaf of the recipient plant. During the acquisition access period, aphids were observed using a magnifying glass to confirm that they were probing. Ten aphids that had been observed probing were then transferred to a test plant leaf and confined within a cage on the leaf. Caution was taken during this aphid transfer to prevent any aphid from losing its acrostyle by giving each aphid a gentle poke with the paint brush and moving only after removal of their stylet from the plant. Aphids were allowed to feed on the test plant for 1-2 h (inoculation access period). Feeding was terminated mechanically by removing each aphid from the plant and crushing it. Plants were then treated with naled as described above. Starved non-viruliferous aphids were used for the sham inoculation.

Post inoculation care and sampling

Upon completion of the aphid inoculation, test plants were transferred to the greenhouse and fogged weekly with naled as described above. Four to five weeks after inoculation, plants were assayed with DAS-ELISA and RT-PCR.

Infection rates and titer estimation

Twenty eight days after aphid inoculation, a 1-g composite leaf sample was taken from the top, middle, and bottom portion of each test plant. Composite leaf samples were ground using an electric leaf press, sap was collected and mixed with 5 ml sample buffer (1× phosphate-buffered saline [pH 7.4] containing 3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, 0.05% Tween 20, and 2 g/liter of powdered non-fat dry milk). The samples were tested with DAS-ELISA (Clark and Adams 1977) using a monoclonal cocktail (Anti PVY monoclonal cocktail and Anti PVY conjugated with AP; BIOREBA, Reinach,

Switzerland) to determine infection rates and virus titers. All the ELISA-positive plants were tested with multiplex RT-PCR (Lorenzen et al. 2006) to confirm the presence of desired strains.

Relative titer for each isolate of the three PVY strains was determined by comparison of absorbance values at 405 nm (after allowing substrate development in ELISA for 45 min) with standard curves constructed with purified virus of each virus strain. Virus concentrations of 1600, 800, 400, 200, 100, 50, 25, 12.5, and 6.25 ng/100 µl were used to construct the standard curves. Purified virus samples of PVY^{O}, PVY^{NTN}, and $PVY^{N:O}$ were obtained from Dr. James Crosslin, USDA-ARS, Prosser, WA.

Calculation of relative efficiency factor

The relative efficiency factor (REF) for each aphid species was determined for each isolate. The REF of GPA was set to 1.00 for each isolate and used as an internal control to determine the REF of other aphid species. The REF of each aphid species was calculated by dividing the number of infected plants of those inoculated by that species by the number of infected plants by GPA.

$$\text{REF (PA or BCOA)} = \frac{\text{Number of infected plants (PA or BCOA)}}{\text{Number of infected plants (GPA)}}$$

Statistical analysis

Analysis of variance was used to compare the infection percentage in different treatments. Binomial distribution was assumed for assessing the infection status of each test plant. Differences within the treatments were evaluated using PROC-GENMOD with logit link transformation in SAS version 9.2 (SAS Institute, Cary, NC). In the model, treatments

were sorted by strains to maintain the power of statistical analysis. Pairwise comparisons were carried out within the model by chi-square pairwise contrasts. Analysis of variance was also used to examine treatment effects on titer in the test plants; differences within the treatments were evaluated using PROC-GENMOD with logit link transformation. Correlations among source plant titer, percent infection, and test plant titer were evaluated using PROC-CORR (Spearman).

Results

Percent infection in plants

Regardless of PVY strain, the aphid species by isolate interaction and the isolate effect for infection percent were not significant (Table 2.01). When isolates were pooled across aphid species, the infection percentages of the PVY^{O}, $PVY^{N:O}$, and PVY^{NTN} strains differed significantly among the three species. GPA transmitted all three strains with greater efficiency than the other two aphids whereas transmission by BCOA was greater than that by PA (Table 2.02).

GPA tended to transmit the *NY31* isolate of the O strain with greater efficiency than the Oz isolate and the *NY4* (N:O) with greater efficiency than the *Alt* (N:O) isolate (Fig. 2.01), although the differences were not statistically significant in either case. GPA transmitted the two NTN isolates (*NY29* and *N4*) with numerically and statistically similar efficiency (Fig. 2.01).

Virus titer in test plants

The virus titer in the test plants infected with PVY^{O} did not differ between isolates, among aphid species, or among isolate by aphid interactions (Table 2.03; Fig. 2.02). For $PVY^{N:O}$, virus titer in the test plants differed significantly between isolates, but not among

aphid species or isolate by aphid interactions (Table 2.03; Fig. 2.02;). Virus titer in the test plants infected with PVY^{NTN} did not differ among aphid species, but did differ significantly between isolates and among isolate by aphid interactions (Table 2.03; Fig. 2.02).

The isolate effect was significant for the $PVY^{N:O}$ strain; when data were pooled across aphid species, virus titer was greater for Alt than for the *Oz* isolate (pooled data not shown). The aphid species × isolate interaction for virus titer level was not significant for the O and N:O strains, but was significant for the NTN strain (Table 2.03). Neither aphid species, isolate, nor their interaction was significant for titer level in test plants infected with the isolate of the O strain; however, compared with *Oz* isolate, *NY31* had relatively greater titer levels regardless of the aphid species transmitting (Fig. 2.02).

When data were analyzed separately by aphid species for the NTN strain, virus titer level in the test plants challenged with BCOA was greater for the *N4* isolate than the NY29 isolate (Fig. 2.02). Although not significantly different, virus titer level in test plants challenged with GPA tended to be higher for the *N4* isolate than for the NY29 isolate. PA transmission resulted in similar virus titer levels between the *N4* and NY29 isolates (Fig. 2.02).

Correlation between source plant titer and both percent infection in test plants and test plant titer

The only significant correlation found among source plant virus titer, infection percent, and test plant titer was a negative correlation between source plant titer and test plant titer for the NTN isolates transmitted by PA (Table 2.04).

Relative efficiency factor (REF)

Among the three aphid species tested, GPA was usually found to be the most efficient vector of all strains and isolates, followed by BCOA and then PA (Table 2.05). Within the O strain, BCOA tended to transmit the *Oz* isolate with relatively higher efficiency than the *NY31* isolate; however, GPA and PA transmitted *NY31* with numerically higher efficiency than *Oz*. Within the N:O strain, BCOA and PA tended to transmit *Alt* with relatively higher efficiency than *NY4*, but GPA tended to transmit *NY4* with relatively higher efficiency than *Alt*. For the NTN strain, transmission percentage of the *N4* isolate by all three aphid species was numerically higher than that of *NY29* (Table 2.05).

Discussion

The primary aim of this study was to examine whether differences in transmission efficiency among aphid species and PVY strains and isolates might contribute to the recent shift in prevalence of necrotic and recombinant strains over the ordinary strains in the USA (Gray et al. 2010). The species complex of potato-colonizing and non-colonizing aphid vectors of PVY and the PVY isolates themselves may vary on a regional basis (Piche et al. 2004). Aphid biotypes can differ in transmitting different isolates of PVY strains (Verbeek et al. 2010). Several studies have addressed vector efficiency in the transmission of different PVY strains (Sigvald 1984, Verbeek et al. 2010, Mello et al. 2011). However, information is lacking about the efficiency of local aphid biotypes to transmit diverse PVY isolates as efficiency varies with both aphid biotype and isolate. In the present study, transmission by two colonizing (GPA and PA) and one non-colonizing aphid (BCOA) vectors using two isolates of three PVY strains was considered. GPA was found to be the most efficient PVY

vector regardless of strain as well as isolate. BCOA was found to transmit all of the strains with higher relative efficiency than PA. Furthermore, although many vector transmission efficiency studies have been reported for PVY, few have considered newer necrotic strains.

GPA was the most efficient vector compared with PA and BCOA, which is consistent with other studies (Sigvald 1984, Verbeek et al. 2010, Mello et al. 2011). In our study, BCOA was found to be more efficient than PA in transmitting all strains, which is at odds with previous reports that showed lower transmission efficiency by this species (Ragsdale et al. 2001, Halbert et al. 2003, Verbeek et al. 2010, Cervantes and Alvarez 2011). BCOA was reported to show a transmission efficiency of 0.5-11.5% (Hoof 1980, Sigvald 1984, Harrington and Gibson 1989) in terms of percent infection and 0-0.04 in terms of relative efficiency factor (REF) of GPA (Verbeek et al. 2010, Boquel et al. 2011). In contrast, in the present study BCOA transmitted PVY with 10-40 % efficiency in terms of percent infection and was found to be exceptionally efficient at transmitting NTN. This efficiency may be due to the aphid clone used in this study or other transmission factors including potato cultivar, aphid feeding behavior in the source plants infected with particular isolates, altered aphid-specific HC-Pro (helper component essential for aphid transmission of PVY) level due to different PVY isolates present in the test plants, or a combination of these effects.

In the present study no differences in percent transmission efficiency were observed among isolates within strains, but there was a difference in virus titer between isolates regardless of aphid species used for transmission. GPA and PA tended to transmit *NY31* with greater efficiency than *Oz* but BCOA transmitted *Oz* slightly better than *NY31*,

although this difference was not statistically significant. All the aphid species transmitted the *NY4* isolate of N:O strain with numerically higher transmission efficiency than the *Alt* isolate. Furthermore, compared to other strains and isolates NTN isolates tended to be transmitted with greatest efficiency by all the aphid species, which might explain the recent prevalence of the NTN strain in potato fields (Crosslin et al. 2006, Karasev et al. 2009, Gray et al. 2010, Karasev and Gray 2013).

Although GPA, PA, and BCOA were used in this study, many other species can transmit PVY with some level of efficiency (Ragsdale et al. 2001); *Schizaphis graminum, Rhopalosiphum maidis, Diuraphis noxia, Capitophorus elaeagni* are other PVY vectors along with GPA, PA and BCOA in Idaho (Halbert et al. 2003). Even though *Aphis fabae* and several other species of *Aphis* are able to transmit PVY with low efficiency, a high number of these aphids moving from sugar beet fields also could contribute to the PVY transmission in adjacent potato fields (Fereres 1993, Perez et al. 1995, DiFonzo et al. 1997). Since aphid species transmit PVY with varying efficiency, further transmission studies with other species are necessary for the newer necrotic strains. Other aphid vectors might be more efficient in transmitting the newer necrotic strains than previously thought and may be a contributing factor in the recent emergence of necrotic strains.

Another aim of this research was to examine whether virus titer in the source plant was related to aphid transmission efficiency. Verbeek et al. (2010) compared aphid transmission efficiencies for N, NTN, and N-Wi isolates and did not find significant differences when source tissue contained comparable virus titers. In the present study, no correlation was found between source plant titer and either infection percentage or recipient

plant titer. This suggests that virus titer in the source plant may not be a good predictor of infection status or titer of an infected recipient plant. Although very low titer was detected for the *NY4* isolate of N:O strain, the infection percent tended to be greater than for the *Alt* isolate. Furthermore, although a very small percentage of plants was infected after transmission using PA, the recipient plant titer tended to be high in those infected plants. These results suggest that virus titer may be a result of individual host reaction (plant cell-virus interaction), while factors other than source plant titer influence the percent infection and recipient plant titer. Cervantes and Alvarez (2011) found that titer build up in a plant varies among plant tissues, with virus titer tending to accumulate more in the lower plant parts than the upper portions. However, virus may replicate in particular areas of a plant; virus particles may gather in a particular area of a leaf and possibly particular cells within an area of a leaf, thus, virus titer measurements may not be an accurate estimation of the possibilities of virus transmission.

Although one non-colonizing cereal aphid was considered in this study, many other cereal aphid species including *Diuraphis noxia*, *Metopolophium dirhodum*, *Rhopalosiphum insertum*, *R. maidis*, and *Sitobion avenae* can transmit PVY with varying efficiency (Sigvald 1984, Katis and Gibson 1985, Harrington and Gibson 1989, Perez 1995, Ragsdale et al. 2001, Halbert et al. 2003). BCOA is one of the most abundant cereal aphid species in the potato growing regions of the PNW (Feng and Nowierski 1992, Ragsdale et al. 2001, Halbert et al. 2003, Ragsdale et al. 2001. Halbert et al. 2003, J.M Alvarez unpublished data; see Chapter 4). In the present study BCOA tended to transmit NTN isolates with higher efficiency than O and N:O isolates. Despite relatively low transmission efficiency compared to GPA, the sheer numbers of BCOA (7 to 20 times more depending on field locations)

might make this species a more important vector of PVY than GPA (J.M. Alvarez unpublished data; see Chapter 4). The greater transmission efficiency for BCOA observed in this study, as compared with previous reports, suggests BCOA is a more important PVY vector than previously considered. Studies on the characterization of the aphid complex in potato growing areas and their dispersal pattern are needed to clarify the extent to which cereal aphids are contributing to PVY incidence during their dispersal from drying cereal fields to potato fields. Higher titer build up in the field together with sheer numbers of the cereal aphids and their higher transmission efficiency for necrotic strains may facilitate the secondary spread of the virus and can contribute to PVY incidence in potato growing regions of the PNW.

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Table 2.01: Chi-square results comparing infection percentages among two different isolates of three PVY strains by three aphid species.

PVY strain [a]	Effects and interactions	Infection Rate		
		df	$\chi 2$	Pr> $\chi 2$
O	Isolate	1	2.9	0.091
	Aphid	2	68.6	<0.0001
	Isolate*Aphid	2	3.3	0.195
N:O	Isolate	1	2.0	0.153
	Aphid	2	49.3	<0.0001
	Isolate*Aphid	2	0.2	0.887
NTN	Isolate	1	0.2	0.671
	Aphid	2	57.6	<0.0001
	Isolate*Aphid	2	3.9	0.141

Infection was determined as the percentage of potato plants infected with PVY that were inoculated by viruliferous *M. persicae* (GPA), *M. euphorbiae* (PA), and *R. padi* (BCOA) from the virus source (*NY31* and *Oz* isolates of PVY^{O}; *NY4* and *Alt* isolates of $PVY^{N:O}$; *NY29* and *N4* isolates of PVY^{NTN}). Data were sorted by PVY strain for statistical analyses. Differences in least square means of infection percentage were compared using PROC GENMOD (assuming binomial distribution).

Table 2.02: Pairwise comparisons of percent transmission of three PVY strains by three aphid species.

PVY strain[a]	Aphid	Compared with Aphid	df	$\chi 2$	Pr> $\chi 2$
O	GPA	BCOA	1	19.4	<0.0001
	GPA	PA	1	11.3	0.001
	BCOA	PA	1	38.6	<0.0001
N:O	GPA	BCOA	1	14.5	0.0001
	GPA	PA	1	21.7	<0.0001
	BCOA	PA	1	7.4	0.007
NTN	GPA	BCOA	1	51.2	<0.0001
	GPA	PA	1	67.4	<0.0001
	BCOA	PA	1	20.0	<0.0001

[a]Infection was determined as the percentage of Russet Burbank potato recipient plants infected from a virus source (*NY31* and *Oz* isolates of PVY^{O}; *NY4* and *Alt* isolates of $PVY^{N:O}$; *NY29* and *N4* isolates of PVY^{NTN}) by viruliferous *M. persicae* (GPA), *M. euphorbiae* (PA), and *R. padi* (BCOA). The aphid species by isolate interaction was not significant so isolates were pooled for pairwise comparisons of species transmission. Differences in least square means of infection percentage were compared using PROC GENMOD (assuming binomial distribution). Data were sorted by PVY strain to maintain the power of statistical analyses.

Table 2.03: Effect of isolate, inoculating aphid species, and their interaction on infected plant virus titer for three PVY strains.

Treatment[a]	Interactions	Infection Rate		
		df	$\chi 2$	Pr> $\chi 2$
O	Isolate	1	0.7	0.645
	Aphid	1	0.7	0.666
	Isolate*Aphid	1	0.4	0.386
N:O	Isolate	1	15.1	0.0001
	Aphid	1	0.4	0.514
	Isolate*Aphid	1	0.3	0.616
NTN	Isolate	1	9.9	0.002
	Aphid	1	0.3	0.58
	Isolate*Aphid	1	3.9	0.048

[a]Virus titer was measured as µg virus/100 µl plant sap of potato test plants infected with *NY31* and *Oz* isolates of PVY^{O} strain; *NY4* and *Alt* isolates of $PVY^{N:O}$ strain; *NY29* and *N4* isolates of PVY^{NTN} strain that were inoculated by viruliferous *M. persicae* (GPA), *M. euphorbiae* (PA), and *R. padi* (BCOA). Data were sorted by PVY strain for the statistical analysis. Differences in least square means of infection percentage were compared using PROC GENMOD (assuming binomial distribution). Statistical analysis was performed separately for different strains to maintain the power of statistical analyses.

Table 2.04: Correlation between source plant titer and percent infection in test plants as well as source plant titer and test plant titer for three aphid species and three PVY strains.

PVY strain	Aphid species	Spearman correlation coefficient of source plant titer and infection	Spearman correlation coefficient of source plant titer and test plant titer
O	GPA	0.09 (0.81)	0.03 (0.93)
	PA	0.37 (0.28)	0.02 (0.95)
	BCOA	-0.19 (0.61)	-0.45 (0.18)
N:O	GPA	-0.12 (0.74)	0.33 (0.34)
	PA	-0.35 (0.32)	-0.34 (0.34)
	BCOA	0.04 (0.92)	0.39 (0.26)
NTN	GPA	-0.34 (0.34)	0.11 (0.76)
	PA	-0.57 (0.08)	**-0.70** **(0.02)**
	BCOA	0.01 (0.97)	0.11 (0.76)

Test plants were inoculated with *NY31* or *Oz* isolates of the PVY^{O} strain, *NY4* or *Alt* isolates of the $PVY^{N:O}$ strain, or *NY29* or *N4* isolates of the PVY^{NTN} strain using viruliferous *M. persicae* (GPA), *M. euphorbiae* (PA), or *R. padi* (BCOA). The titer of source and recipient plants was measured by DAS-ELISA and expressed as µg virus/100 µl plant sap. Spearman correlation analysis was performed using PROC-CORR method separately for different strains and aphid species. The values in parenthesis are the P-values associated with the respective correlation coefficient. Data were sorted by PVY strain to maintain the power of statistical analyses, and aphid species were pooled across isolates for comparisons.

Table 2.05: Relative efficiency factors (REFs) of three aphid species transmitting three PVY strains and two isolates within each strain.

Aphid species	REF per *Potato virus Y* (PVY) isolate					
	PVY^{O} (*NY31*)	PVY^{O} (*Oz*)	$PVY^{N:O}$ (*NY4*)	$PVY^{N:O}$ (*Alt*)	PVY^{NTN} (*NY29*)	PVY^{NTN} (*N4*)
Myzus persicae (Control)[a]	1.00 (68%)	1.00 (44%)	1.00 (60%)	1.00 (28%)	1.00 (86%)	1.00 (88%)
Macrosiphum euphorbiae	0.12	0.05	0.03	0.07	0.02	0.11
Rhopalosiphum padi	0.32	0.55	0.30	0.36	0.35	0.41

[a]*Myzus persicae* was used as the internal control in all experiment and the REF of this species was set to 1.00 as a reference for calculation. The percentage of infected test plants is indicated in parentheses. REF of each aphid species was calculated by dividing the number of infected plants inoculated by that species by the number of infected plants by the internal control, *M. persicae*.

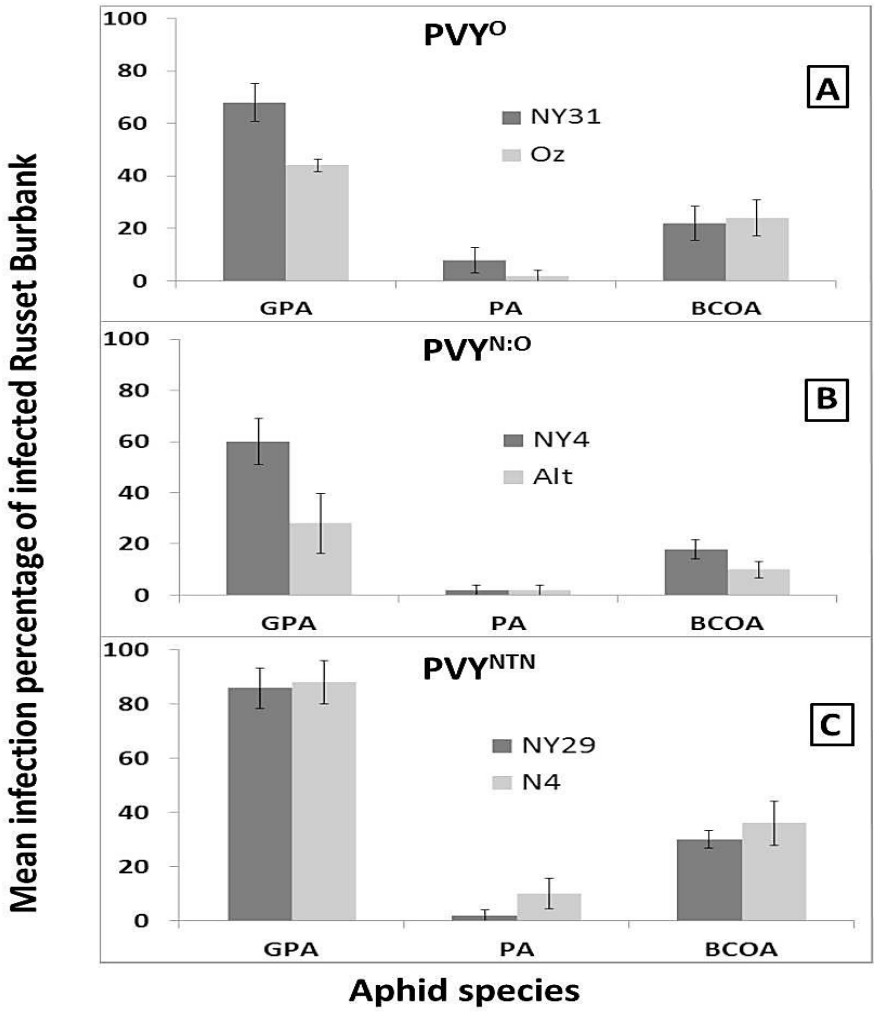

Fig. 2.01: Infection rates (%) for Russet Burbank potato plants inoculated by *M. persicae* (GPA), *M. euphorbiae* (PA), or *R. padi* (BCOA) from virus source plants infected with *NY31* or *Oz* isolates of PVY^{O} (**A**); *NY4* or *Alt* isolates of $PVY^{N:O}$ (**B**); or *NY29* or *N4* isolates of PVY^{NTN} (**C**). Error bars denote standard error. Statistical inference was based on logit-link transformed data; non-transformed means are shown. Data were sorted by PVY strain to maintain the power of statistical analyses.

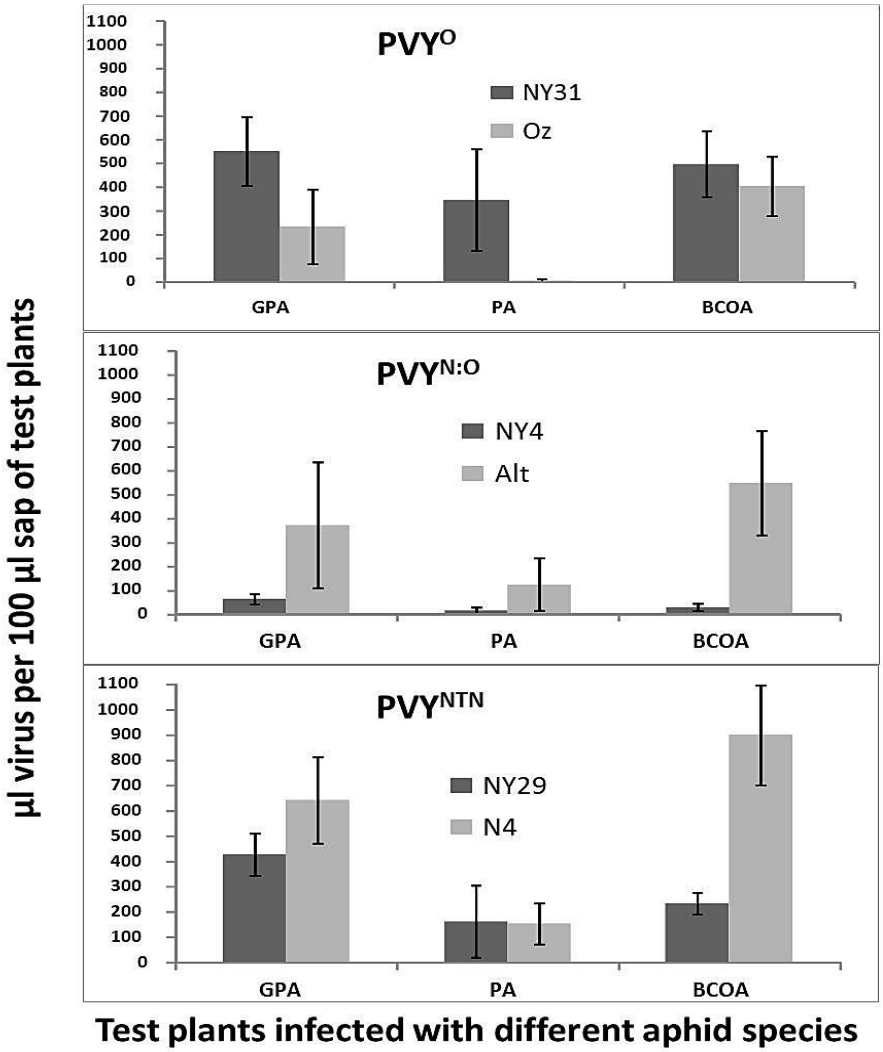

Fig 2.02: Virus titer (µg virus/100 µl plant sap) compared among all treatments for plants inoculated by *M. persicae* (GPA), *M. euphorbiae* (PA), or *R. padi* (BCOA) after acquisition from the virus source: *NY31* or *Oz* isolates of the PVY^{O} strain; *NY4* or *Alt* isolates of the $PVY^{N:O}$ strain; *NY29* or *N4* isolates of the PVY^{NTN} strain. Error bars denote standard error. Statistical inference was based on logit-link transformed data; non-transformed means are shown. Data were analyzed separately by PVY strain.

Chapter 3

Contribution of non-colonizing aphids to PVY incidence in potato in Southern Idaho

Abstract

Potato Virus Y (PVY) is a major concern for potato production in the USA given its impact on both crop quality and yield. Many aphid species transmit PVY in a non-persistent manner. Although green peach aphid (GPA), *Myzus persicae*, is the most efficient PVY vector, it is less abundant in potato growing areas of Idaho compared to several non-potato-colonizing aphids that can also transmit this pathogen. Even though these aphids transmit PVY less efficiently than GPA, they may contribute to spread of PVY in adjacent potato fields due to their abundance. A field study was conducted during 2012-2013 to examine if cereal aphids disperse to nearby potato fields as cereal crops dry down before harvest. Aphid fauna in four different commercial potato fields in south-central and south-eastern Idaho were sampled weekly using yellow sticky traps and yellow pan traps. Potato fields were chosen with an adjacent cereal field such that the prevailing westerly wind would facilitate aphid dispersal from cereal fields to potato. The most abundant cereal aphid species from pan trap samples both years were *Rhopalosiphum padi* and *Metopolophium dirhodum*; a diverse fauna of species from other hosts was found as well. GPA abundance was relatively low, ranging from 0.5-2.5% of the total aphid capture. Aphid abundance peaked in mid- to late-July (cereal ripening stage) and decreased after early August (when cereals had dried). Occurrence of PVY in the potato fields, confirmed via DAS-ELISA on potato leaves sampled three times per season, increased in all the locations following mid-

summer increases in aphid abundance in both years. This study suggests that cereal aphids and other non-colonizing aphids are important contributors to PVY incidence in potato in southern Idaho.

Introduction

Potato is an economically important crop that is produced worldwide (FAO 2008). Potato production is severely constrained by *Potato virus Y* (PVY, Family: *Potyviridae*; Genus: *Potyvirus*), with potential to cause considerable economic impact including yield and quality reduction as well as prevention of seed certification (Sigvald 1992, Hane and Hamm 1999, Nolte et al. 2004, Whitworth et al. 2006, Gray et al. 2010, Karasev and Gray 2013). PVY strains differ in serological, molecular, and biological properties and produce different symptoms in different potato cultivars including mosaic, necrosis, and leaf drop. Necrotic strains cause Potato Tuber Necrotic Ringspot Disease (PTNRD) with varying symptoms (van den Heuvel et al. 1994, McDonald and Singh 1996, Boonham et al. 2002, Baldauf et al. 2006, Singh et al. 2008, Gray et al. 2010).

Numerous aphid species efficiently transmit PVY in a non-persistent manner (Sigvald 1984, Piron 1986, Ragsdale et al. 2001). Although green peach aphid is the most efficient vector of PVY in potato (Hoof 1980, Sigvald 1984, Singh et al. 1996, Fernández-Calvino et al. 2006), several other colonizing, migrant, and non-colonizing species also contribute to PVY incidence (Ragsdale et al. 2001). Non-colonizing aphid species that are important vectors of PVY in the USA include floxglove aphid (*Aphis nasturtii*) (Ragsdale et al. 2001), bird cherry-oat aphid (*Rhopalosiphum padi*) (Halbert et al. 2003), and soybean aphid (*Aphis glycines*) (Davis et al. 2005). The non-colonizing aphids might occur in very

large numbers making their effect on virus spread disproportionately large relative to their lower virus transmission efficiency.

Idaho contributes significantly to the national cereal crop acreage and production, which includes winter and spring wheat as well as spring barley (NASS 2013). Various aphid species colonize cereal crops, including spring wheat and spring barley (Halbert et al. 2003, Robertson and Stark 2003). Historically, the most abundant aphid species reported on cereals in Idaho were rose grass aphid (*Metopolophium dirhodum*), English grain aphid (*Sitobion avenae*), greenbug (*Schizaphis graminum*), Russian wheat aphid (*Diuraphis noxia*), and bird cherry-oat aphid (*Rhopalosiphum padi*) (Feng et al. 1991, Feng and Nowierski 1992, Schotzko and Bosque-Pérez 2000, Bosque-Pérez et al. 2002). Recently, another cereal aphid, *Metopolophium festucae cerealium*, has been reported from Idaho with diverse graminaceous hosts and a preference for wheat and barley (Halbert et al. 2013, Davis et al. 2014).

Many cereal aphids have been reported to transmit PVY with varying efficiency (Ragsdale et al. 2001). Some aphid species for which transmission efficiency is known include *Metopolophium dirhordum* (0.5-10% efficiency), *M. albidum* (11%), *M. festucae* (0.4-0.5%), *R. insertum* (0.8-50%), *R. padi* (0.5-11.5%), *S. avenae* (0.06-1.8%); these species may contribute to PVY incidence in potato (Hoof 1980, Piron 1986, Harrington and Gibson 1989, Halbert et al. 2003). *Rophalosiphum padi* was found to transmit necrotic strains of PVY with greater efficiency than previously reported (Mondal et al. in prep; See Chapter 2). These cereal aphids, specifically, *R. padi,* are abundant in many potato-growing areas, including southern Idaho (Sigvald 1984, Ragsdale et al. 2001, Halbert et al. 2003),

and may contribute to PVY spread during their dispersal in large numbers from cereal fields (Katis and Gibson 1985, Sigvald 1992, DiFonzo et al. 1997, Pelletier et al. 2008a).

Robert et al. (2000) listed potential causes of differential PVY incidence that include initial virus inoculum, vector behavior (feeding and flight) and status (colonizing versus transient), and interaction between aphid and crop phenology. Several strategies practiced widely to manage PVY include use of timely insecticide application, and behavioral manipulation of aphid feeding by alternative application of mineral oil and/or insecticide, application of antifeedants (Powell et al. 1998, Ucko et al. 1998, Saucke and Döring 2004, Martín-López et al. 2006, Boiteau et al. 2009, Gray et al. 2010, Hansen and Nielsen 2012, Kirchner et al. 2014). However, the dynamic PVY pathosystem, and its complex interactions among host, vector, pathogen, and environment limit the effectiveness of the above mentioned control options (Döring 2011, Davidson et al. 2013).

For the aphid / PVY pathosystem, aphid monitoring within not only potato, but the entire cropping system may contribute to development of a disease prediction model that would improve PVY management (Kirchner et al. 2011, Vučetić et al. 2013). The present study was conducted to examine if cereal aphids disperse to nearby potato fields as cereal crops dry down before harvesting, and if during this transient flight the aphids transmit PVY to adjacent potato crops. It is not economical for cereal growers to control aphids late season, but it may be economical to keep aphids from moving into potato. The main objectives of this study were to: 1) characterize the aphid species complex over time in potato fields adjacent to cereal fields, 2) investigate how transient aphids may be contributing to PVY spread, and 3) gain insight into the timing of aphid dispersal and PVY incidence in potato fields in relation to cereal crop maturation. Results from this work could

inform management practices for aphids on cereals to prevent their movement to potato. To our knowledge, this is the first attempt to characterize aphid dispersal from cereal to potato.

Materials and methods

Field sites and trap layout

Four different commercial potato fields—two in south-central and two in south-eastern Idaho—were selected for sampling aphid fauna weekly using yellow sticky traps and yellow pan traps during 2012 and 2013 (see below for trap details). In 2012, the Eden (42°59'64"N; 114°26'97"W) and Hazelton (42°31'60"N; 114°3'34"W) sites represented the south-central cites; Idaho Falls (43°20'68"N; 112°5'10"W) and Newdale (43°52'34"N; 111°35'01"W) represented the south-eastern sites. In 2013, the Burley (42°47'45"N; 113°97'09"W) and Paul (42°38'67"N; 113°53'14"W) sites represented the south-central cites; Idaho Falls (43°20'05"N; 112°5'87"W) and Rexburg (43°43'68"N; 111°41'11"W) represented the south-eastern sites. Potato fields were chosen with an adjacent cereal field such that the prevailing westerly wind would facilitate aphid dispersal from cereal fields to potato. At all the sites, the cereal field was separated from the potato field by a road except the Hazelton site. In 2012, forty yellow sticky traps (see below for trap details) per field were arranged in four rows of 10 traps spaced 15 m apart and 15 m within each row. Row placement of sticky traps was as follows: along the edge of the cereal field, along the edge of the adjacent potato field, and 15 and 30 m inside the potato field. Additionally, 12 yellow pan traps (see below for trap details) charged with 35% propylene glycol were arranged in three rows of four traps spaced 15 m apart and 30 m within each row. Pan traps were placed on the cereal field edge, the potato field edge, and 15 m inside the potato field. In 2013, all

the sticky traps were replaced with pan traps, and all the pan traps with sticky traps (Fig. 3.01); this was done to because aphid species identification was more reliable from pan trap samples.

In 2012, both sites in south-central Idaho (Eden and Hazelton) and the Idaho Falls site were planted with 'Russet Burbank' potato; the Newdale site was planted with 'Ranger Russet.' In 2013, both sites in south-central Idaho (Burley and Paul) were planted with Ranger Russet, and both sites in south-eastern Idaho (Idaho Falls and Rexburg) with Russet Burbank. In all sites during both years, the cereal field was spring-planted wheat. Cereal growth stage (University of Idaho cereal extension 1999, Miller 1999) was recorded each week at each site. In all sites, both the cereal and potato fields were cultivated with standard agronomic practices utilized by commercial growers.

Sticky traps

Yellow sticky traps (EPA Est. No. 047362-CA-001; Seabright Laboratories, Emeryville, CA) of 10.2 cm × 35.6 cm size with gridded surface were installed at all sites for sampling aphids. Each trap was clipped to a 1.2 m long × 0.9 cm thick wooden stake using a binder clip. The height of the traps was adjusted with the height of the crop such that traps were positioned just above the crop canopy each week. Sticky traps were collected and replaced every week and collected traps were stored in -20°C for later identification of aphids.

Pan traps

Yellow pan traps were made by gluing a square yellow ceramic floor tile (sunburst yellow with natural hues, model no. QH92; Daltile Tile Corporation, Dallas, TX) of 10.8 × 10.8 × 0.64 cm size in a square-shaped plastic container (16.5 × 16.5 × 6.0 cm) and attaching the container to a rectangular flat wooden plate (30.5 × 19.7 × 0.7 cm) with thick rubber bands. For trap installation, two 0.9 cm thick wooden stakes were each threaded through holes in the wooden plate and inserted into the ground. A binder clip on each wooden stake held the trap in position just above the crop canopy. Trap height was adjusted weekly as plants grew. Each trap was charged with 35% propylene glycol (Bulk Apothecary Co., Streetsboro, OH) and the trap contents were collected and replaced every week. Contents from each trap were stored separately in glass jars for later identification of aphids.

Identification of aphids

Aphids were identified using a binocular microscope based on morphological identification keys (Taylor and Robert 1981, Blackman and Eastop 1984, 1994, van Emden and Harrington 2007). All aphid species from yellow pan traps were identified and counted. All aphids from sticky traps were counted, but due to the difficulty in identifying aphids from sticky trap samples, only two aphid species, *R. padi* and *S, avenae*, were identified. Cereal aphids and other aphids were grouped separately for analyses.

Virus incidence in potato plants

PVY incidence in potato plants in each field was determined three times throughout the growing season: early season, mid-season, and just before vine kill. Sampling date

varied depending on field location, but occurred during the last week of June, last week of July, and last week of August, respectively, each year. Leaf samples were taken from the upper portion of 60 potato plants within each sticky trap and pan trap row (totaling 840 leaf samples per field per date). Leaf samples were ground using an electric leaf press. Sap was collected and mixed with 0.5 ml sample buffer (1× phosphate-buffered saline [pH 7.4] containing 3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, 0.05% Tween 20, and 2 g / liter of powdered non-fat dry milk). The samples were tested via double-antibody sandwich enzyme linked immuno-sorbent assay (DAS-ELISA; Clark and Adams 1977) using monoclonal coating IgG and conjugate (Anti PVY monoclonal cocktail and Anti PVY conjugated with AP; BIOREBA, Reinach, Switzerland) to determine infection status of the plants.

Statistical analysis

Because the sampling methods differed each year, statistical analyses were conducted separately for each year and trap type. Furthermore, preliminary statistical analysis yielded significant trap row distance × sampling site as well as a date × trap row distance × sampling site interactions (P-values not shown) for each sampling year. Therefore, statistical analysis was conducted separately for each sampling site of each year. Binomial distribution was assumed for aphid counts per trap. Pairwise comparisons of mean aphid captures among trap rows were conducted using PROC-GENMOD with logit-link transformation to compare the mean aphid captures among trap rows (cereal field edge trap row and three subsequent potato field trap rows) at each sampling date. For sticky trap data, analyses were conducted separately for total aphid captures and captures of bird cherry-oat

aphid. For the pan traps, analyses were conducted separately for total aphids and total cereal aphids and separately for the two most abundant aphid species: bird cherry-oat aphid, and rose grass aphid. PVY incidence in plants was also compared among sampling dates using PROC-GENMOD with logit-link transformation assuming a binomial distribution. All analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA).

Results

Aphid species

More than 40 different aphid species and species clusters were captured; a preponderance of those aphids were cereal aphids (67% in 2012 and 34.4% in 2013) (Table 3.01). In 2012, the most abundant cereal aphid was *Metopolophium dirhordum* followed by *R. padi*, *D. noxia*, and *S. avenae* (Table 3.01). However in 2013, *R. padi* was more abundant than *M. dirhordum* (Table 3.01). Among other cereal aphid species, *S. graminum, D. noxia* were relatively abundant (Table 3.01). Several other species of diverse host range were captured; the *Aphis fabae* species complex [many subspecies can co-occur over much of their natural geographical range (Raymond et al. 2001, Tosh et al. 2001, van Emden and Harrington 2007)] being the most important among them. Other abundant species included *Acyrthosiphon lactucae*, *Theoraphis trifoli*, *Aphis* spp., and *Capitophorous elaeagni* (Table 3.01). Additionally, overall abundance of the most efficient PVY vector, *M. persicae*, as well as another important potato colonizing aphid species, *Macrosiphum euphorbiae*, was relatively low (Table 3.01).

Sticky trap captures

Overall captures of aphids started increasing around mid-July and decreased after the

first week of August, peaking during the last week of July or first week of August. In 2012, mean aphid capture (all aphid species together) per trap was higher in the cereal field edge than within the potato field until the second week of July in the Eden and Hazelton sites. Aphid captures were relatively low through 2 July and then increased for the following 2-3 weeks (Fig. 3.02). At both sites, no differences in aphid captures among trap rows were found after July. For the other two sites (Idaho Falls and Newdale), aphid captures were relatively low (Fig. 3.02). In the Idaho Falls site, mean total aphid captures were higher in the trap row along the cereal field edge for at least the first four weeks of sampling; for most dates, aphid captures tended to decline for trap rows that were further into the potato field (Fig. 3.02). At the Newdale site, the differences among rows tended to vary for each sampling date, without any discernable pattern (Fig. 3.02). At the Burley and Paul sites, mean BCOA aphid captures per trap were higher in the trap row along the cereal field edge for the first to third week of July in 2012 (Fig. 3.03). At the Idaho Falls site, no regular pattern of BCOA capture was found among trap rows; however, captures began in mid-July and peaked around the last week of July (Fig. 3.03). At the Newdale site, mean BCOA captures in the trap row along the cereal field edge were higher than the other three trap rows in potato fields in the last week of July, but no such differences were found at the beginning of August in 2012 (Fig. 3.03).

In 2013, overall captures of aphids started increasing around mid-July and decreased after the second week of August, peaking during the last week of July or first week of August for the south-central sites; however, in the south-eastern sites, captures peaked around the last week of July and decreased in the first week of August (Fig. 3.04). At the Burley and Paul sites, mean aphid captures (all aphid species together) per trap were lower

in the cereal field edge than in one or both of the trap rows in potato fields in mid-July; however, aphid captures were either significantly higher than the potato traps rows or not different between the cereal field edge and the first potato row in the third week of July (Fig. 3.04). At the Idaho Falls site, mean aphid captures (all aphid species together) were significantly higher in the cereal field edge than in the potato field during mid- to late-July (Fig. 3.04). At the Rexburg site, aphid captures were higher in the potato than cereal field edge during the last week of July when the overall aphid abundance also peaked (Fig. 3.04). BCOA captures started increasing around mid-late July and decreased after the first or second week of August, peaking during the last week of July or first week of August (Fig. 3.05). At the Burley site, BCOA captures were lower in the cereal field edge than the potato field during most sampling dates between mid-July to mid-August (Fig. 3.05). At the Paul site, BCOA density started to increase during late July, and peaked in the beginning of August, when aphid captures in the cereal field edge were higher than the trap rows in the potato field (Fig. 3.05). For the Idaho Falls and Rexburg sites, BCOA captures were relatively low, though higher than during 2012 (Fig. 3.05). BCOA captures started increasing by the second week of July and decreased after the last week of July (Fig. 3.05). During late July, BCOA captures in the cereal field edge either did not differ from or were lower than those in the potato field at both sites except for the last sampling date in Idaho Falls (Fig. 3.05).

Pan traps

During both years, overall aphid captures started increasing around mid-July and decreased after the first week of August, peaking during the last week of July or first week

of August. Aphid captures during 2012 (data not shown) were lower than during 2013. In 2013, overall aphid captures (all species together) started increasing by the second week of July in the Burley and Paul sites; mean aphid captures were significantly higher along the edge of the potato field than along the cereal field edge during the second and third week of July (Fig. 3.06). In the beginning of August, mean aphid captures were significantly lower along the cereal edge at the Burley site. However, in Paul, trap captures showed the opposite pattern relative to Burley for the beginning of August (Fig. 3.06). Aphid abundance in pan traps was much lower at the Idaho Falls and Rexburg sites in 2013, and no clear patterns were found (Fig. 3.06). In all four field sites, aphid captures decreased after the second week of August.

When considering captures of just cereal aphids, similar trends in trap captures were observed relative to total aphid captures for all the field locations during 2013 (Fig. 3.07). Captures of cereal aphids also started increasing around mid-July and decreased after the first week of August, peaking during the last week of July or first week of August (Fig. 3.07). Specifically for BCOA, at the Burley and Paul sites, aphid captures were highest in the last week of July and first week of August (Fig. 3.08). At the Burley site, although BCOA captures in the trap row along the cereal field edge were higher in the last week of July, they tended to decrease in subsequent weeks (Fig. 3.08). At the Paul site, higher BCOA captures were recorded in the first two weeks of August relative to late July along the cereal field edge (Fig. 3.08). However, at the Idaho Falls and Rexburg sites, BCOA aphid densities was lower than the two south-central sites; captures did not differ significantly between the cereal field edge and potato field, although a numerical trend of low aphid capture was recorded at the Rexburg site in the last week of July and first week of

July (Fig. 3.08). For another cereal aphid that was relatively abundant during 2013, *M. dirhorum*, captures increased earlier than for BCOA. Trap captures along the cereal field edge were lower than in the potato field from the third week of July to the first week of August for the Burley and Paul sites (Fig. 3.09).

PVY Incidence

In 2013, PVY incidence was numerically higher than for 2012 for all field sites (Fig. 3.10). South-central Idaho sites generally had higher PVY incidence than south-eastern sites, especially during 2013 (Fig. 3.10). During both 2012 and 2013, percent infection in plants on the third sampling was numerically higher for the third sampling date than for the first (Fig. 3.10).

Aphid capture in relation to cereal crop maturing stages

In 2012, aphid captures in the south-central Idaho sites peaked after the first week of July and decreased after third week of July (Fig. 3.11). However, in 2013, cumulative aphid captures peaked after the first week of July, but decreased after the end of July at the south-central sites (Fig. 3.11). However, at one site (Paul) aphid captures peaked again in the beginning of Aug of 2013 and decreased after two weeks (Fig. 3.11).

In 2012 and 2013, cumulative aphid captures in the south-eastern sites peaked after the second week of July and decreased after the end of July (Fig. 3.11). In both years and in all locations, aphid captures peaked after the cereal fields started drying down after the ripening stage, and started to decrease after the cereal fields dried out, with the exception of the late-season increase in captures in Paul during 2013 (Fig. 3.11).

Discussion

The principal aims of this study were to characterize the aphid species complex over time in potato fields adjacent to cereal fields and to investigate how aphid movement to potato may be contributing to PVY spread. Over forty different aphid species were found, and a preponderance of them were cereal aphids. Aphids were presumably dispersing from cereal fields through potato fields to colonize weeds or grassy hosts. PVY incidence in each field increased following peak aphid flight and appeared to be related to aphid abundance, suggesting that flight of transient aphids contributed to the secondary spread of PVY in the potato fields.

Several aphid species have been reported to colonize and damage the major cereal crops in the Great Plains and the Pacific Northwest of the United States of America (Feng and Nowierski 1992, Kieckhefer and Gellner 1992, Schotzko and Bosque-Pérez 2000, Bosque-Pérez et al. 2002, Halbert et al. 2003, Brewer and Elliott 2004). The most abundant cereal aphids sampled in the present study included *R. padi*, *M. dirhordum*, *S. avenae*, *D. noxia*, and *Schizaphis graminum*. Although in 2012 a majority of the aphids were cereal aphids, in 2013 many other non-cereal aphid species were found, including *A. lactucae*, *Aphis fabae* species complex, *C. elaeagni*, and various species of the genus *Aphis*. This complex of aphid species exhibit diverse host ranges, different settling preferences, diverse flight activity patterns, different biologies, and different potentials as PVY vectors. The non-colonizing, non-cereal aphid species might have arrived from nearby fields of other predominant field crops such as sugar beet (e.g., *A. fabae*), alfalfa (e.g., *T. trifoli*), or other crops, as well as from wild plants and weeds. The present study sites were chosen such that the predominant westerly winds facilitated dispersal of aphids from cereal to potato. Aphid

species compositions in traps might have differed if study sites had instead featured sugar beet or alfalfa crops adjacent to potato. The relative abundance of aphids from alfalfa and sugar beet host underscores the need to conduct similar studies that consider proximity of potato to alfalfa or sugar beet fields.

Aphids use visual and volatile cues for choosing hosts (Hildebrand et al. 1993, Chittka and Döring 2007). In southern Idaho, cereal crops mature before most potato crops and lose green color well before potato crops. Furthermore, as cereal crops mature, their volatile profiles likely change considerably (Rotz and Muck 1994). In the current experiment, trap captures for cereal aphids started to increase after the second week of July, and aphids were captured subsequently in the potato field traps. This timing coincided with the ripening and drying of cereal crops during both years of the study. This dispersal from cereal fields may represent the migrant phase of many of the cereal aphid species as they disperse to other summer hosts and weeds. In the present experiment it was found that trap captures in the cereal field often declined during the first week of August while trap captures in the potato field increased. This may suggest that as soon as the cereals dry, aphids migrate to other hosts through potato fields. One exception to this pattern occurred during 2013 at the field site in Paul, which had high captures of BCOA at the beginning of August. This might have been due to a large flight from another neighboring, highly colonized cereal field. This underscores that looking at the whole crop landscape rather just the one adjacent field would be important for a thorough understanding of aphid movement and colonization patterns. Further research over a landscape scale could clarify if non-colonizing aphids migrating from distant fields could also contribute to PVY incidence.

In the present study, patterns of aphid captures tended to differ between the south-central and south-eastern sites, the former of which generally exhibited greater overall aphid abundance. Trap capture patterns tended to follow the trend of cereal aphid captures in many of the sites. In many cases similar capture patterns were observed for sticky trap and pan trap data; however, captures in pan traps generally were lower than captures in sticky traps. With the exception of a few dates, similar capture patterns were observed for all the south-central sites for both years.

The observed high PVY incidence in southern Idaho might be due to an abundance of non-colonizing aphids including cereal aphids. Although various insecticides are effective for managing potato-colonizing aphid species, those insecticides are not expected to control transient winged aphids that can transmit and spread PVY within seconds of probing. Other than green peach aphid, several other species have been reported to transmit PVY with varying levels of efficiency, a majority of them being cereal aphid species (Ragsdale et al. 2001). In the present study, many cereal aphids as well as potato non-colonizing non-cereal aphid species were captured during the sampling period which suggests that they might be contributing to PVY incidence in southern Idaho. Among the important cereal aphids, *R. padi*, *M. dirhordum*, and *D. noxia* have been reported to transmit PVY with 0.5-40%, 0.5-10% , and 4-7% relative transmission efficiency, respectively (Hoof 1980, Sigvald 1984, Piron 1986, Harrington and Gibson 1989, Perez et al. 1995, Halbert et al. 2003, Mondal et al. in prep). Among other non-cereal aphids that do not colonize potato, pea aphid (*Acyrthosiphon pisum*) (3.8-14%), *Aphis fabae* (7.6-24%), and artichoke aphid (*C. elaeagni*) were reported to transmit PVY with varying transmission efficiency (Sigvald 1984, Katis

and Gibson 1985, Piron 1986, Harrington and Gibson 1989, Bokx and Piron 1990, Fereres 1993, Halbert et al. 2003, Verbeek et al. 2010).

Abundance of the two potato-colonizing species in Idaho, green peach aphid and potato aphid, was negligible relative to non-colonizing species. Further scouting of potato fields during this study revealed no colonization of green peach aphid or potato aphid (data not shown). This suggests that the primary contribution for the PVY incidence in these fields may be from cereal aphids and other non-colonizing aphid species rather than the two potato-colonizing species in Idaho.

The transient non-colonizing aphid flight has epidemiological significance for PVY. PVY is a major concern in seed potato as well as commercial potato growing areas and can be spread by primary or secondary means (Gray et al. 2010, Karasev and Gray 2013). In both years of the study, PVY incidence initially was low, but increased after peak capture of aphids in July. This suggests that the non-colonizing aphid vectors contributed to the secondary spread of PVY. Also, PVY incidence at the beginning of the season tended to be higher in 2013 than in 2012; this may suggest that the primary inoculum was higher in 2013 than the 2012, which might have contributed to higher PVY incidence in combination with the higher aphid population in 2013. Despite the presence of relatively high PVY incidence during the beginning of season at the Rexburg site than the Paul site during 2013, PVY incidence did not increase at the Paul site. This suggests a relationship between higher PVY incidence and higher aphid abundance. In the present study only overall PVY incidence in potato was determined; the strains of PVY were not identified. Previous studies by our group revealed that BCOA is able to transmit necrotic PVY strains with better efficiency

than previously reported (see Chapter 2). Thus, greater abundance of BCOA in the potato growing areas might contribute to the prevalence of the necrotic strains.

PVY management options have had limited success due to the dynamic pathosystem, non-persistent transmission of the virus by a wide range of aphid vectors, and limitations of seed certification programs (Powell et al. 1998, Ucko et al. 1998, Saucke and Döring 2004, Martín-López et al. 2006, Boiteau et al. 2009, Gray et al. 2010, Döring 2011, Hansen and Nielsen 2012, Karasev and Gray 2013, Kirchner et al. 2014). One of the aims of this study was to gain insight into optimal timing for implementing management efforts for aphids in cereal to prevent their movement to potato. Current efforts to manage aphid vectors of PVY are concentrated only in potato fields and feature frequent foliar sprays of aphidicides. In the present study, a diverse complex of cereal aphid species and other non-potato-colonizing aphid species was found in potato fields following maturation of the cereal crop. To prevent the transient flight in the potato crop by non-colonizing aphids, management tactics might be implemented in the cereal fields in the first week of July to reduce cereal aphid populations. However, this would only be feasible if growers own and manage both the cereal and the potato fields.

Aphis fabae species complex and *T. trifoli* (for which the primary cultivated host plants are sugar beet and alfalfa, respectively) were captured in some south-central locations, which may suggest that management means might be taken to reduce their populations in neighboring sugar beet or alfalfa fields. These findings warrant further research. *Therioaphis trifoli* is not reported to transmit PVY; it would be important to determine if they transmit PVY and contribute to PVY incidence, and if they need to be

managed. Additionally, it is not clear if all the observed non-colonizing aphids originated from the adjacent field or the other hosts or crops in distant fields. If such is the case, managing aphids in the adjacent cereal field might not be sufficient to reduce PVY incidence. All these questions need to be investigated further.

The results presented here show evidence that cereal aphids along with other non-colonizing aphids may be important contributors to PVY incidence in the potato growing regions of southern Idaho. The non-colonizing, non-cereal aphids presumably did not come from cereals, but might have come from other hosts including sugar beet, alfalfa, or weed hosts. Their importance should not be underestimated considering the diverse landscape of different crops in southern Idaho. Adoption of aphid management means in those adjacent crops might restrict the transient aphid flight to potato fields, but this warrants further research. More research also is required on the importance of other crop interfaces (such as sugar beet / potato and alfalfa / potato) along with the cereal / potato interface, given the abundance of alfalfa and sugar beet aphids observed in this study. Further study also is required regarding the dispersal distances of non-colonizing aphids; it is unknown, for example, whether cereal aphids sampled in this study dispersed from the adjacent cereal field or other nearby fields. In addition, although the present study assessed the overall PVY incidence, identification of different strains was not conducted. Future research should be directed towards characterizing the distribution of necrotic and recombinant strains present in the field and determining to what extent the non-colonizing aphids are contributing to their prevalence. Furthermore, climate change may drive dramatic changes in the abundance, distribution, and life cycles of various aphid vectors of viruses in plants

including aphid vectors of PVY (Newman 2005, Roos et al. 2011). Such changes could similarly affect the aphid / PVY pathosystem in the future.

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Table 3.01: Aphid species captured in pan traps in southern Idaho potato fields in 2012 and 2013.

[a]**Aphid species captured in pan traps**	Pan traps 2012	% relative abundance in 2012	Pan traps 2013	% relative abundance in 2013
Cereal aphid species:	**67 % of total abundance**		**34.4% of total abundance**	
Diuraphis noxia (Russian wheat aphid)	×	9.7	×	2.4
Metopolophium dirhodum (Rose grass aphid)	×	22.4	×	9.8
Pemphigus sp.	×	0.3	×	0.5
Rhopalosiphum maidis			×	0.04
Rhopalosiphum nymphaeae			×	0.01
Rhopalosiphum padi (Bird cherry-oat aphid)	×	15.0	×	19.3
Rhopalosiphum spp.			×	0.03
Rhopolomyzys poae	×	0.3	×	0.01
Schizaphis graminum (Greenbug)	×	0.8	×	2.1
Sipha elegans (Spiny grass aphid)	×	10.2	×	0.01
Sitobion avenae (English grain aphid)	×	8.6	×	0.2
Other aphid species:	**33 % of total abundance**		**65.6 % of total abundance**	
Acyrthosiphon kondoi (Blue alfalfa aphid)	×	0.5		
Acyrthosiphon lactucae	×	6.4	×	9.6
Acyrthosiphon malvae			×	0.6
Acrythosiphon pisum	×	0.8	×	0.5
Acrythosiphon spp.	×	4.4	×	1.7
Aphis craccivora			×	0.5
Aphis fabae group	×	0.8	×	12.2
Aphis helianthi	×	0.5	×	0.8
Aphis spiraecola			×	0.01
Aphis spp.	×	3.0	×	10.2
Brachycaudus cardui	×	1.9	×	0.5
Brachycaudus helichrysi			×	0.1
Brevicoryne brassicae	×	0.3	×	0.9
Capitophorous elaeagni (Artichoke aphid)	×	2.5	×	3.5
Capitophorous hippophaes	×	0.8	×	0.5
Capitophorous spp.			×	0.1
Caveriella aegopodii	×	1.4	×	0.8

Dysaphis aucuparie	×	0.3		
Dysaphis spp.			×	0.2
Eriosoma spp.	×	0.5	×	3.2
Hydaphis foeniculi			×	0.1
Hyalopterpus pruni			×	0.1
Illinioa spp.	×	0.3	×	0.8
Lipaphis erysimi			×	1.0
Macrosiphum euphorbiae (Potato aphid)	×	0.3	×	0.05
Macrosiphum sp.			×	0.03
Myzus certus	×	0.3	×	0.1
Myzus persicae (Green peach aphid)	×	0.3	×	2.5
Myzus spp.			×	0.1
Prociphilus americanus			×	0.1
Therioaphis trifoli (Spotted alfalfa aphid)			×	10.1
Therioaphis riehmi			×	2.1
Unknown species	×	3.8	×	1.8
Uroleucon sonchi	×	2.2	×	0.6
Uroleucon tenaceti	×	0.3	×	0.1
Uroleucon spp.			×	0.1

[a] Four different commercial potato fields in south-central and south-eastern Idaho were selected for sampling aphid fauna. In 2012, twelve yellow pan traps charged with 35% propylene glycol were arranged in three rows 15 m apart and four traps spaced 30 m within each row per field. In 2013, forty yellow pan traps charged with 35% propylene glycol were arranged in four rows 15 m apart and 10 traps spaced 15 m within each row per field. Pan traps were collected weekly and aphids were identified on the basis of morphological keys.

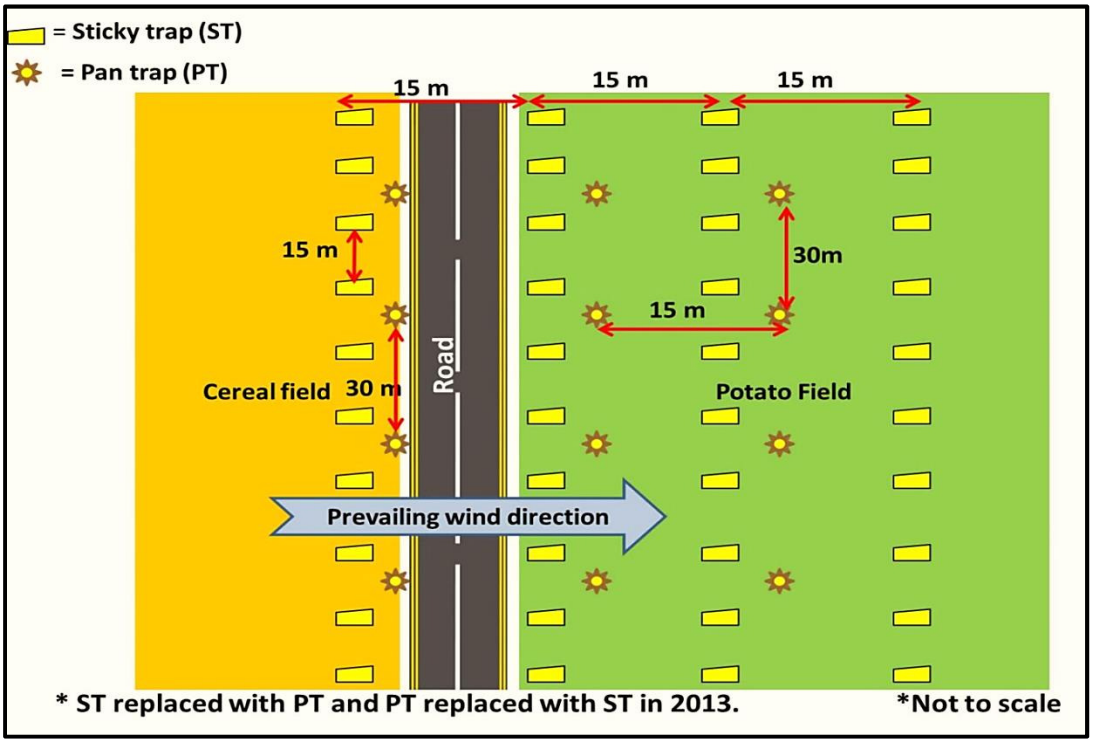

Fig. 3.01: Four different commercial potato fields in south-central and south-eastern Idaho were selected for sampling aphid fauna. Potato fields were chosen with an adjacent cereal field such that the prevailing wind direction was from the cereal to the potato field. The cereal field was separated from the potato field by a road except at the Hazelton site. In 2012, forty yellow sticky traps per field were arranged in four rows of 10 traps spaced 15 m apart and 15 m within each row. Row placement of sticky traps was as follows: edge of the cereal field, edge of the adjacent potato field, and 15 and 30 m into the potato field. Additionally, 12 yellow pan traps charged with 35% propylene glycol were arranged in three rows of four traps spaced 15 m apart and 30 m within each row. Pan traps were placed on the cereal field edge, the potato field edge, and 15 m inside the potato field. In 2013, all the sticky traps were replaced with pan traps, and all the pan traps with sticky traps.

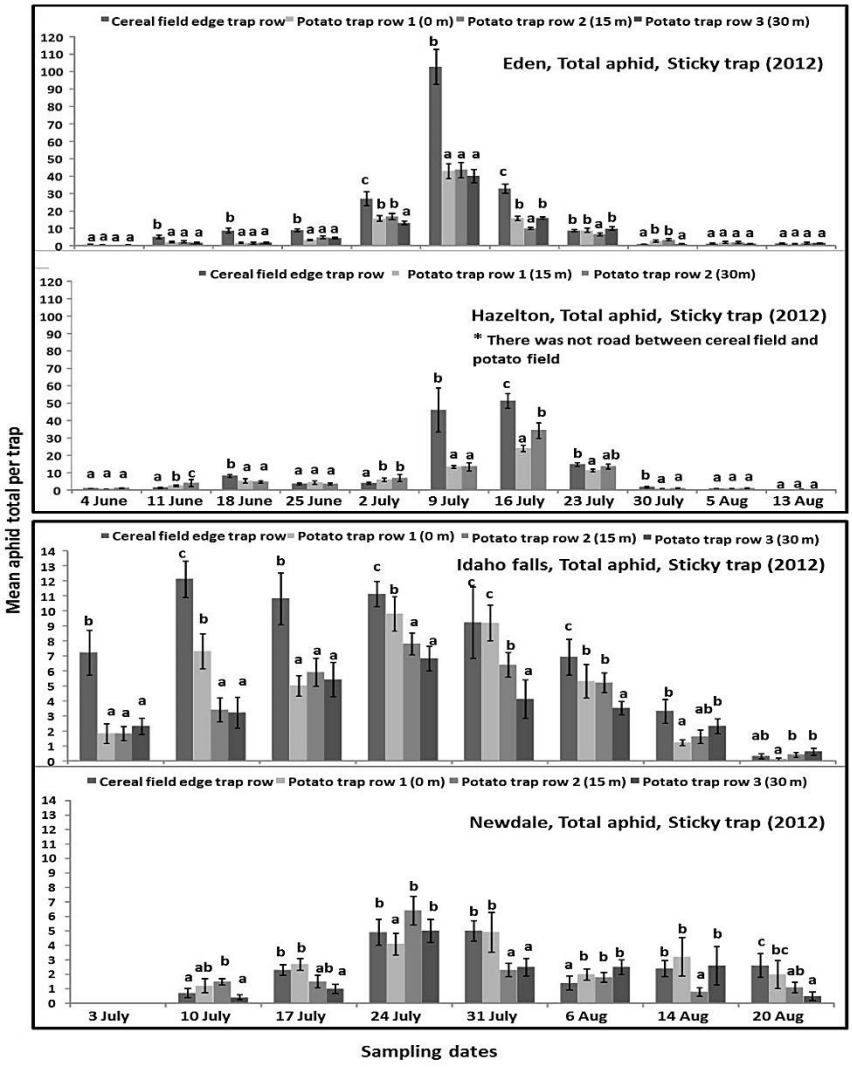

Fig. 3.02: Mean aphid total (all species together) per trap compared among sticky trap rows for each date at four locations in 2012. The upper panel represents the fields from south-central Idaho and lower panel represent the south-eastern Idaho. Cereal and potato fields in the Hazelton site were adjacent to each other, not separated by a road; hence, three rows of traps were installed starting from the potato field edge. Differences in least square means of mean aphid total per trap were compared using PROC GENMOD (assuming binomial distribution). Means within each date that share the same letter are not significantly different. Error bars represent SEM.

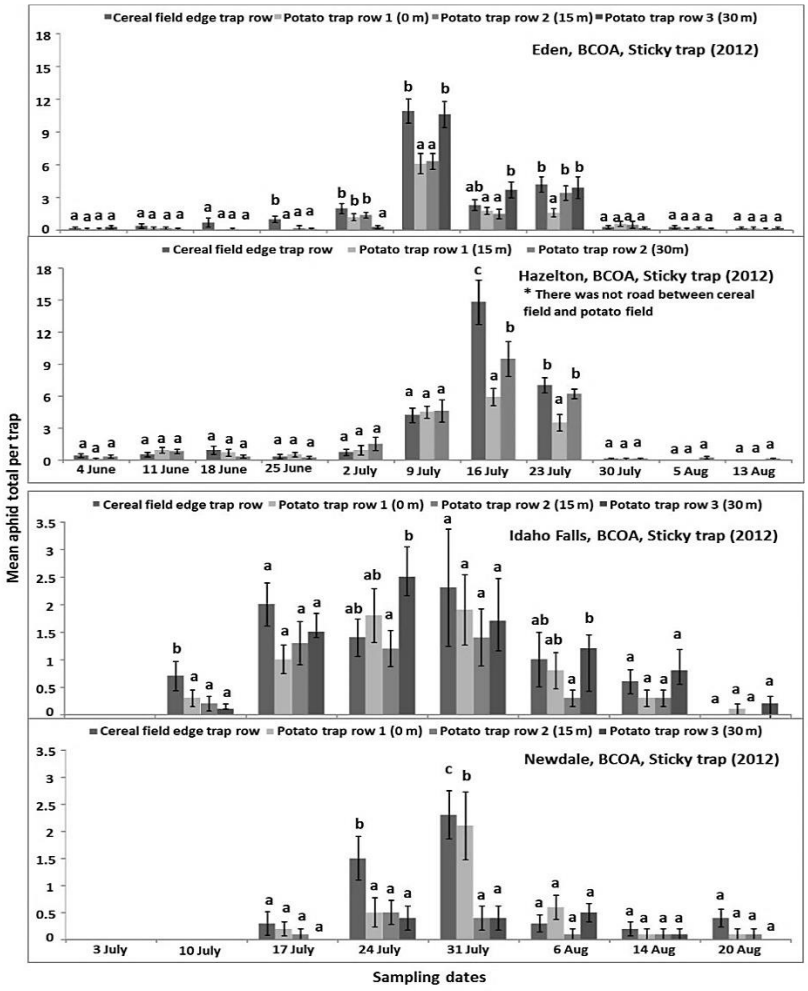

Fig. 3.03: Mean aphid total bird cherry-oat aphid (*R. padi*) per trap was compared among sticky trap rows for each date at four locations in 2012. The upper panel represents the fields from south-central Idaho and lower panel represent the south-eastern Idaho. Cereal and potato fields in the Hazelton site were adjacent to each other, not separated by a road; hence three rows of traps were installed starting from the potato field edge. Differences in least square means of mean aphid total per trap were compared using PROC GENMOD (assuming binomial distribution). Means within each date that share the same letter are not significantly different. Error bars represent SEM.

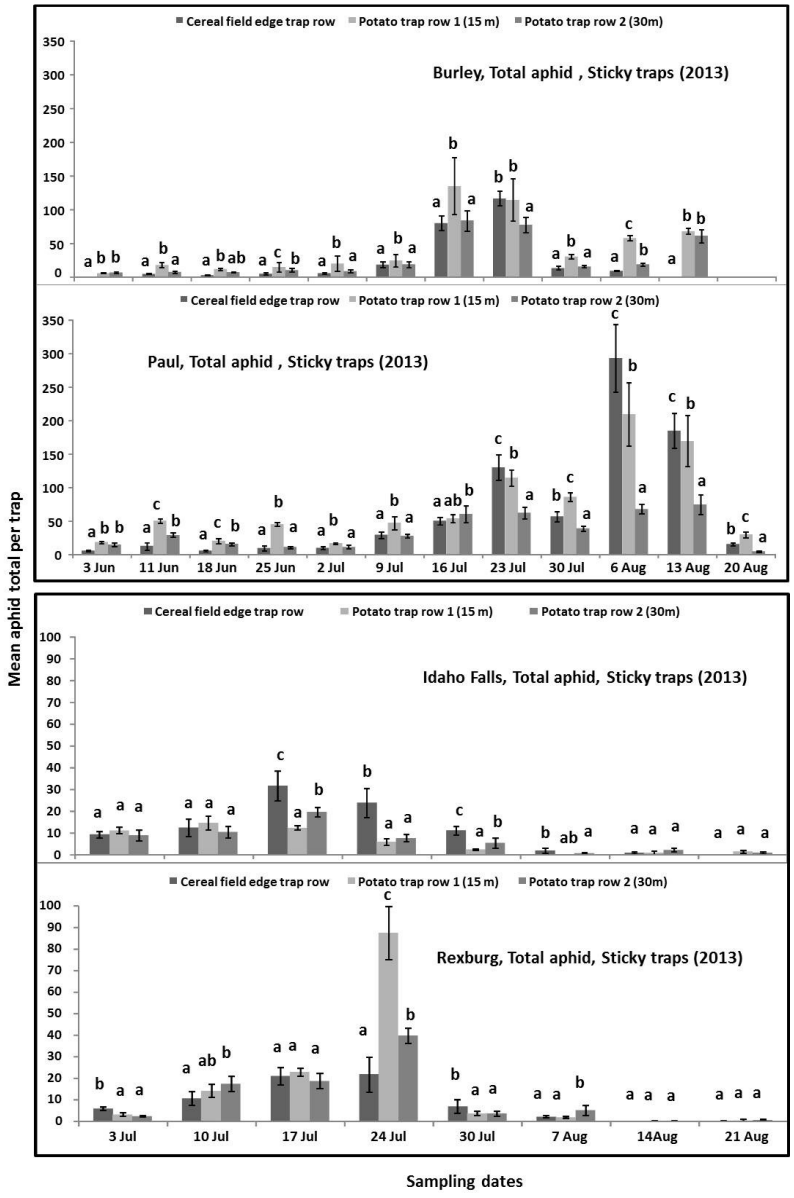

Fig. 3.04: Mean aphid total (all species together) per trap compared among sticky trap rows for each date at four locations in 2013. The upper panel represents the fields from south-central Idaho and lower panel represent the south-eastern Idaho. Differences in least square means of mean aphid total per trap were compared using PROC GENMOD (assuming binomial distribution). Means within each date that share the same letter are not significantly different. Error bars represent SEM.

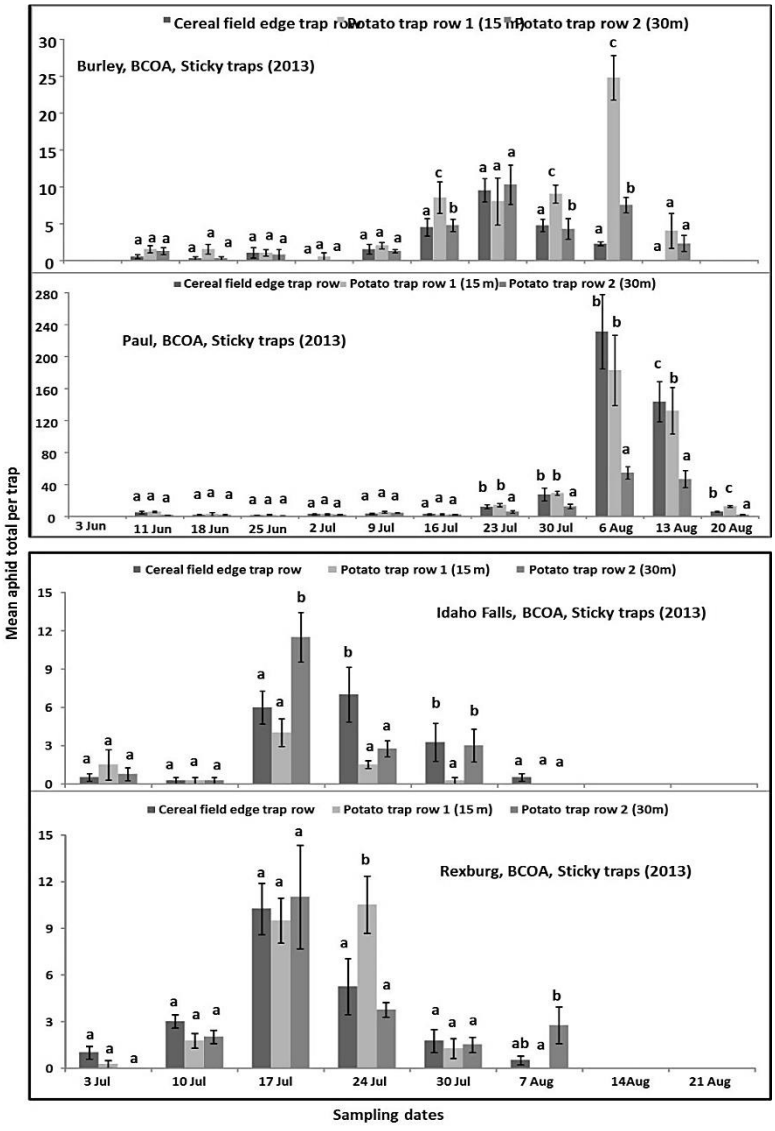

Fig. 3.05: Mean aphid total bird cherry-oat aphid (*R. padi*) per trap was compared among sticky trap rows for each date at four locations in 2013. The upper panel represents the fields from south-central Idaho and lower panel represent the south-eastern Idaho. Differences in least square means of mean aphid total per trap were compared using PROC GENMOD (assuming binomial distribution). Means within each date that share the same letter are not significantly different. Error bars represent SEM.

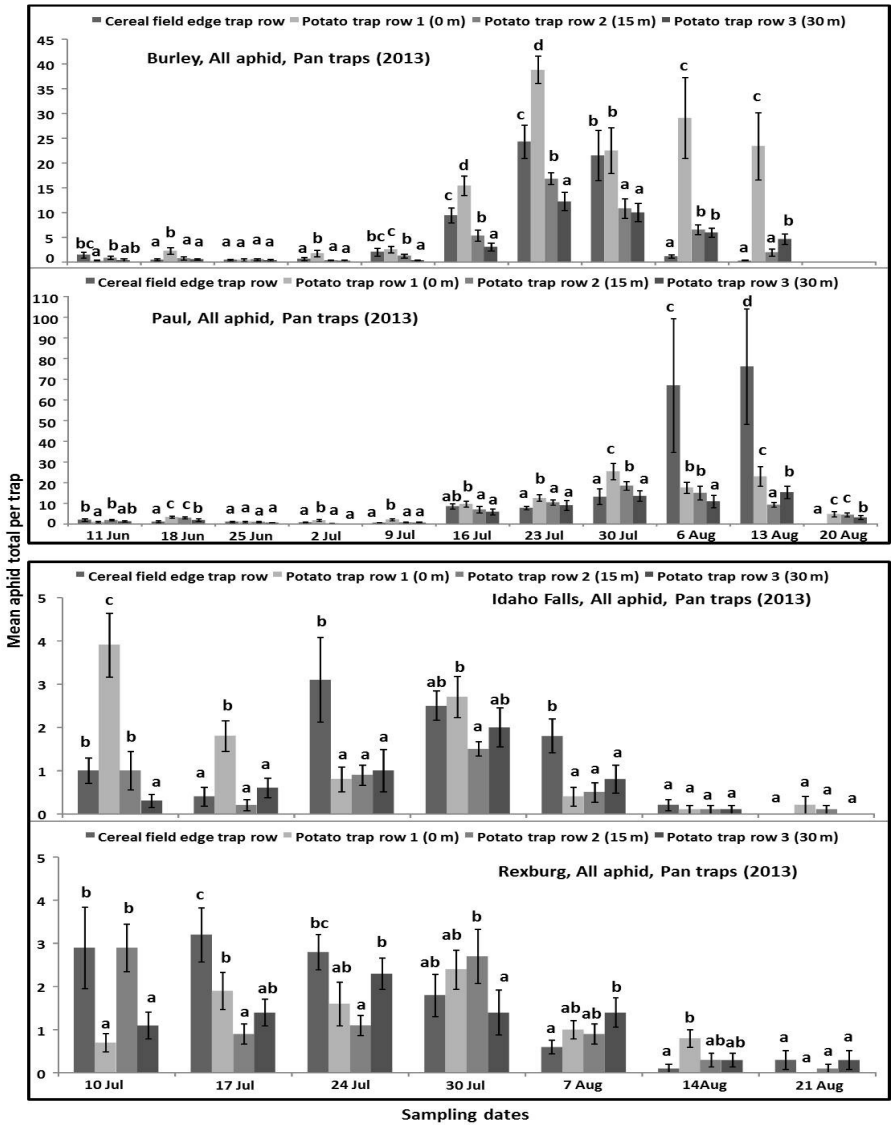

Fig. 3.06: Mean aphid total (all species together) per trap compared among pan trap rows for each date at four locations in 2013. The upper panel represents the fields from south-central Idaho and lower panel represent the south-eastern Idaho. Differences in least square means of mean aphid total per trap were compared using PROC GENMOD (assuming binomial distribution). Means within each date that share the same letter are not significantly different. Error bars represent SEM.

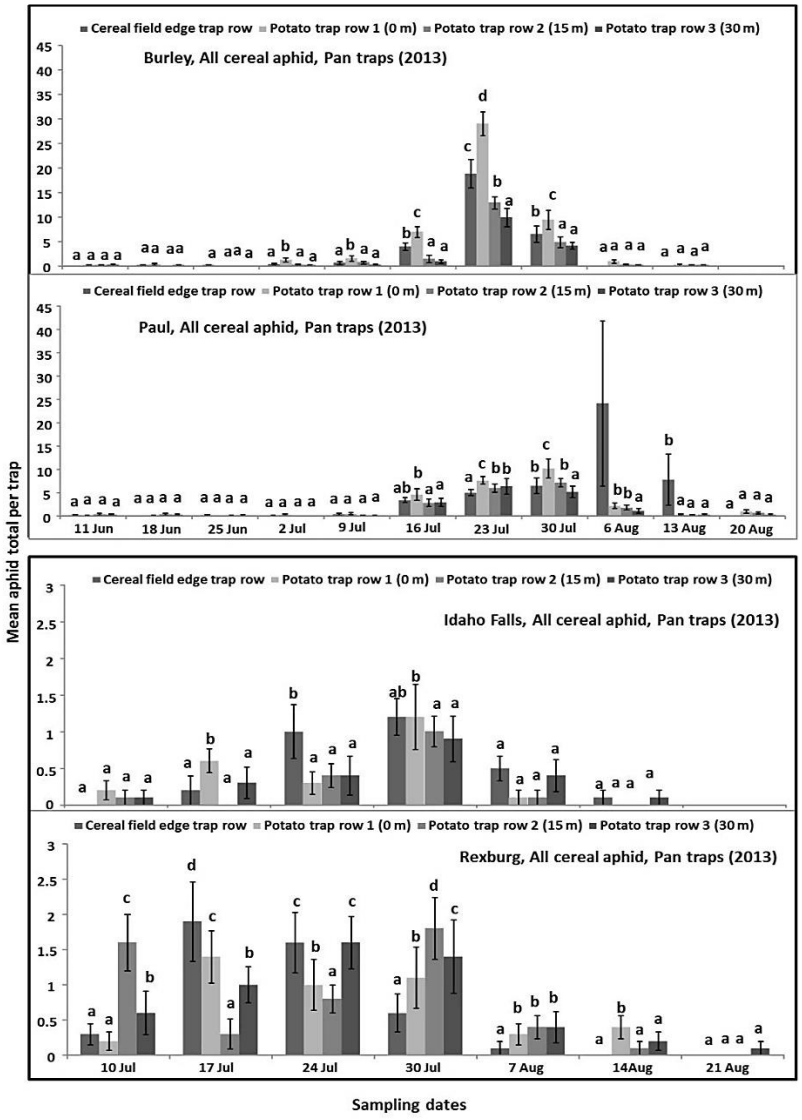

Fig. 3.07: Mean aphid total (all cereal aphid species together) per trap compared among pan trap rows for each date at four locations in 2013. The upper panel represents the fields from south-central Idaho and lower panel represent the south-eastern Idaho. Differences in least square means of mean aphid total per trap were compared using PROC GENMOD (assuming binomial distribution). Means within each date that share the same letter are not significantly different. Error bars represent SEM.

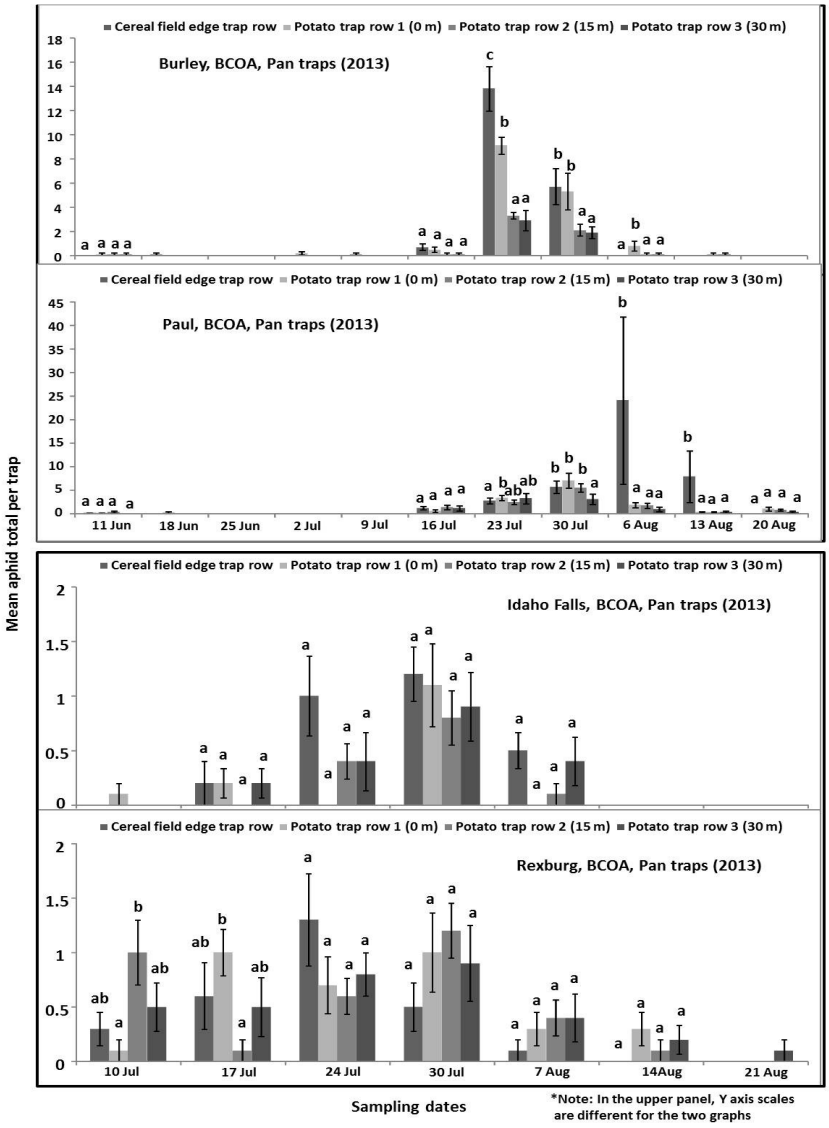

Fig. 3.08: Mean aphid total bird cherry-oat aphid (*R. padi*) per trap was compared among pan trap rows for each date at four locations in 2013. The upper panel represents the fields from south-central Idaho and lower panel represent the south-eastern Idaho. Differences in least square means of mean aphid total per trap were compared using PROC GENMOD (assuming binomial distribution). Means within each date that share the same letter are not significantly different. Error bars represent SEM.

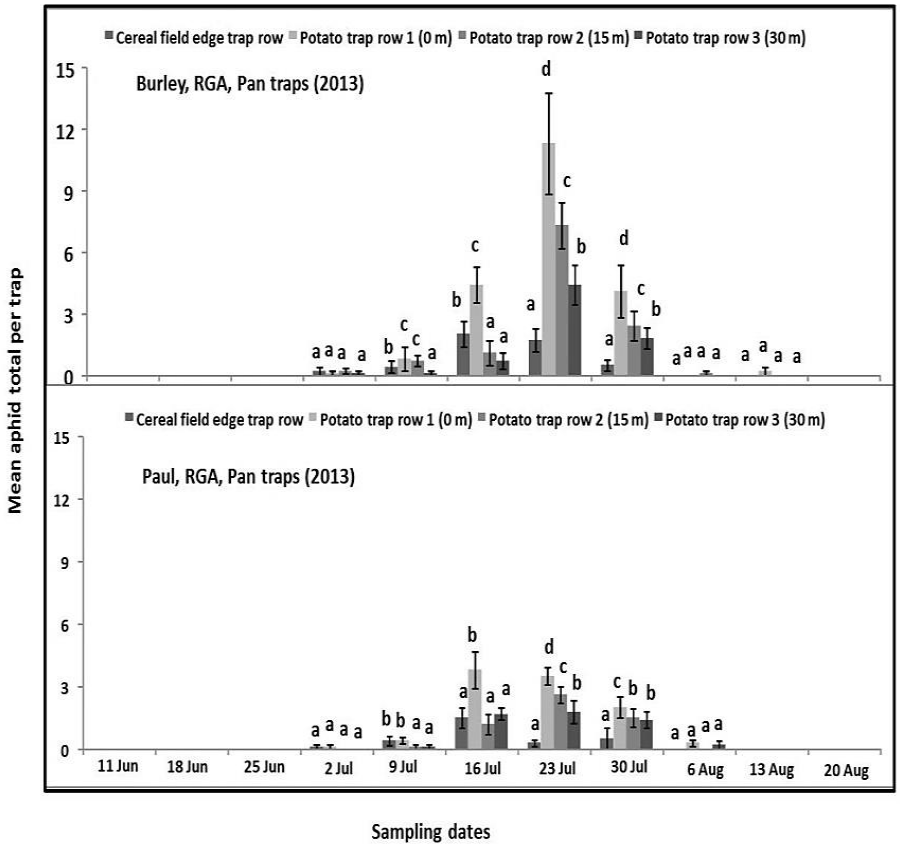

Fig. 3.09: Mean aphid total rose grass aphid (*M. dirhordum*) per trap compared among pan trap rows for each date at four locations in 2013. The upper panel represents the fields from south-central Idaho and lower panel represent the south-eastern Idaho. Differences in least square means of mean aphid total per trap were compared using PROC GENMOD (assuming binomial distribution). Means within each date that share the same letter are not significantly different. Error bars represent SEM.

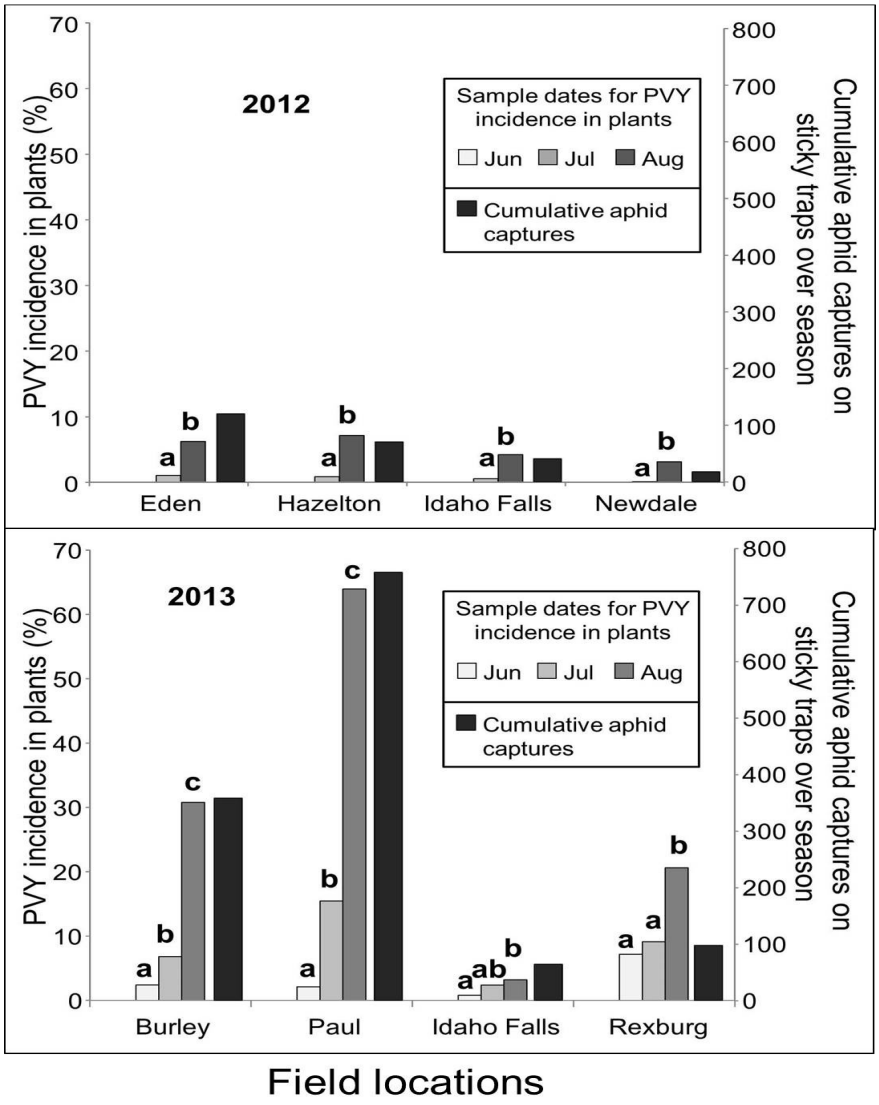

Fig. 3.10: PVY incidence in potato plants was measured three times throughout the growing season (last week of June, July, and Aug during both years) using DAS-ELISA. Means within each location that share the same letter are not significantly different. Cumulative aphid captures were not statistically compared.

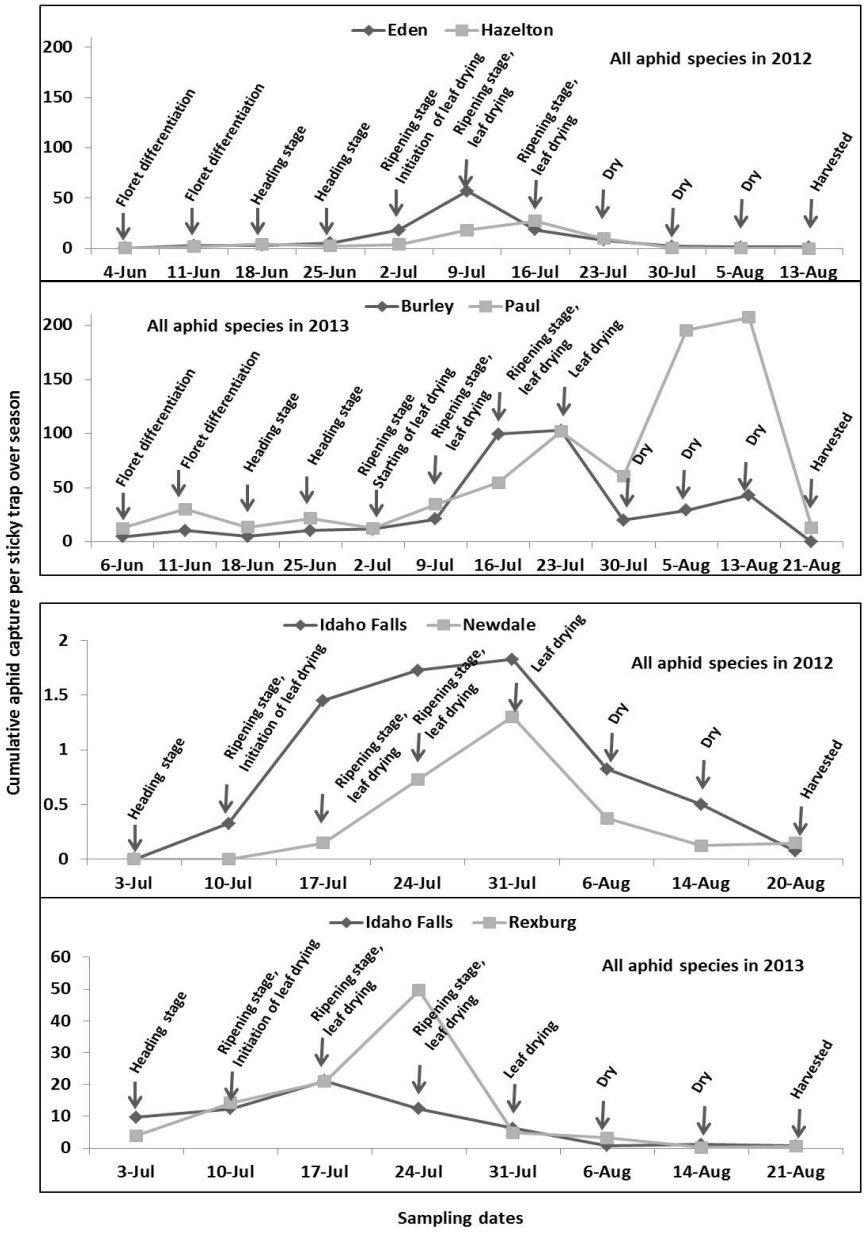

Fig. 3.11: Cumulative aphid captures (all species together) per sticky trap measured over time for both 2012 and 2013. The upper panel represents the fields from south-central Idaho, and lower panel represent the fields from south-eastern Idaho. Arrows note the cereal crop stage on the day of aphid sampling. Note the different y-axis scales.

Chapter 4

Response of aphid vectors of *Potato leaf roll virus* to potato varieties in Southern Idaho

Abstract

Potato leaf roll virus (PLRV) can constrain potato production as a result of impacts on tuber yield and quality. PLRV is transmitted in a persistent, circulative manner by several aphid species, but in the Pacific Northwest (PNW), green peach aphid [GPA; *Myzus persicae* (Sulzer)] and potato aphid [PA; *Macrosiphum euphorbiae* (Thomas)] are the two most important potato-colonizing vectors. The objective of this study was to compare responses of aphid vectors of PLRV to the three predominant potato varieties grown in the PNW. During the 2011-2013 growing seasons, aphids were sampled in field plots at Kimberly, Idaho from 'Russet Burbank' (RB), 'Ranger Russet' (RR), and 'Russet Norkotah' (RN), which represent 63% of the commercial potato cultivation in the PNW. Aphids were sampled weekly from plants in a replicated field trial. In all three years, GPA was more abundant than PA, representing at least 97% of samples. GPA density did not differ among varieties across years. PLRV infection in different varieties also did not differ significantly, although the percent of PLRV-infected plants varied among years (46% in 2013, 29% in 2011, and 13% in 2012). For RR and RN, PLRV infection rate was positively correlated with total aphid abundance and the proportion of total aphids that were viruliferous, whereas in RB only total aphid abundance was positively correlated with PLRV infection. Information generated here regarding variety-specific risk of PLRV

infection can be used to improve monitoring and modelling systems to mitigate this important potato pathogen.

Introduction

Commercial and seed potato (*Solanum tuberosum*) production is constrained by several viral diseases in terms of yield and quality reduction (Ragsdale et al. 2001). Among the 28 viral species that infect potato, *Potato leaf roll virus* (PLRV, genus *Polerovirus*, family Luteoviridae) is one of the most important viruses known to cause potato crop and tuber loss worldwide (Salazar 1996). Typical PLRV symptoms include rolling of leaves, chlorosis, yellowing, stunting of infected plants, and tuber net necrosis in some potato varieties including 'Russet Burbank,' the most widely grown potato variety in the USA (Douglas and Pavek 1972, Nault 1997, Alvarez and Srinivasan 2005). PLRV has a wide host range including plants from the Solanaceae, Amaranthaceae, Crucifereae, and Chenopodiaceae families (Hanafi et al. 1989, Thomas 1993, Hanafi et al. 1995, Srinivasan and Alvarez 2008, Smith et al. 2012).

PLRV is readily transmitted by several potato-colonizing and non-colonizing aphid species in a persistent, circulative, and non-propagative manner (Nault 1997, Syller 1996, Robert and Bourdin 2001, Radcliffe and Ragsdale 2002). Green peach aphid (GPA; *Myzus persicae* Sulzer) and potato aphid (PA; *Macrosiphum euphorbiae* Thomas) are the two main potato-colonizing aphid vectors of PLRV in the Pacific Northwest (PNW) USA (Ragsdale et al. 2001, Radcliffe and Ragsdale 2002). GPA is the most efficient and economically important vector of PLRV (Harrison 1958, Van den Heuvel et al. 1991, Halbert et al. 1995),

whereas PA transmits PLRV less efficiently than GPA (Tamada et al. 1984, Woodford et al. 1995).

Russet Burbank (RB), Russet Norkotah (RN), and Ranger Russet (RR) are the most widely grown potato varieties in Idaho, representing 53%, 21%, and 14%, respectively, of the total Idaho state potato acreage. The predominance of these three varieties is similar for the seven major potato-growing states of the USA, with 39.7%, 13.1%, and 9.9% of the total potato acreage in RB, RN, and RR, respectively (NASS 2013). Potato varieties vary in regard to susceptibility to PLRV (Corsini et al. 1994, DiFonzo et al. 1994). GPA and PA colonization also has been found to differ among potato varieties, although patterns may change from year to year depending on frequency of insecticide use, prevalence of natural enemies, and weather parameters (Flis et al. 2005, Davis et al. 2007). However, the recent patterns of GPA and PA colonization in the three predominant potato varieties are not well documented. In addition, RB, RN, and RR are susceptible to PLRV infection (Corsini and Brown 2001), but the degree of susceptibility is unknown for these varieties (Corsini et al. 1994, R. Novy and J. L. Whitworth. personal communication).

During the late 1900s and early 2000s, PLRV incidence increased due to dispersal of aphid vectors in the major potato growing areas of the USA, including the Columbia Basin in the state of Washington and the Northern Great Plains (Ragsdale et al. 2001). Many environmental factors including low-level jet streams and high and fluctuating temperatures contributed to this aphid vector dispersal, particularly to the spring migration that influenced PLRV incidence (Thomas et al. 1997, Davis et al. 2006, Zhu et al. 2006). Furthermore, increasing prevalence of alternative weed hosts and invasive aphid species can also enhance

the field spread of PLRV (Thomas 1993, Davis and Radcliffe 2008, Srinivasan et al. 2013, Hutchinson 2014).

Management of aphid vectors of PLRV is challenging in part because of their reproductive potential, diverse host range, and tendency to develop resistance to insecticides. One of the most common management approaches is prophylactic use of systemic and contact insecticides; however in many cases, this approach has been proven not to be adequate to manage PLRV despite providing adequate aphid control (DiFonzo et al. 1995, Mowry 2001, 2005, Ragsdale et al. 2001). This is in part because aphids remain viruliferous throughout their lives after PLRV acquisition (Radcliffe and Ragsdale 2002). Furthermore, potential drawbacks of this approach include resistance to a wide group of insecticides, negative impacts on natural enemies and other beneficial organisms, and high costs associated with insecticide application (Foster et al. 2008, van Toor et al. 2008). Although neonicotinoid insecticides significantly suppressed aphid and PLRV spread in potato for many years, recent development of resistance by GPA against this insecticide group underscores the need for improved integrated pest management strategies for the aphid complex responsible for PLRV transmission (Foster et al. 2000, Nauen and Denholm 2005, Puinean et al. 2010, Bass et al. 2011, 2014, Fray et al. 2014).

Although PLRV inoculum has been reduced due to use of virus-free seed tubers, virus inoculum level in regional aphid populations and alternative weed hosts is unpredictable from year to year. A better understanding of the phenology and population dynamics of aphid vectors in relation to various varieties will aid in predicting variety-specific risk to aphid infestation and PLRV infection. Aphid responses and PLRV

susceptibility among the predominant potato varieties of the PNW are not well documented. The primary aim of the present study was to test the hypothesis that GPA and PA densities and PLRV incidences differ among different potato varieties. In addition, the study was conducted to clarify the relationships between aphid abundance (total and viruliferous) and PLRV incidence in plants in order to determine whether variety-specific differences exist.

Material and methods

Planting, cultivation, and plot layout

Field plots were located at the University of Idaho Kimberly Research and Extension Center during 2011, 2012, and 2013. In all three years, research plots were located adjacent to other studies that featured PLRV inoculum and release of non-viruliferous green peach aphids, although aphid phenology in this study appeared to reflect natural infestation rather than spread of the released aphids. Seed tubers of each variety, namely Russet Burbank (RB), Russet Norkotah (RN), and Ranger Russet (RR) were hand cut to seed pieces of ca. 70 g. Prior to planting, tubers were treated with P.S.T Plus Bark® (J.R. Simplot Company, Boise, ID), which is comprised of the fungicide Mancozeb (ethylene bisdithiocarbamate) and the micronutrients Mn and Zn at a rate of 10 g product per kg potato tuber seed. The tubers were planted with a 30.5 cm spacing within rows and a 91.5 cm row spacing at a rate of 2.3 t/ha (ca. 33,300 seed pieces/ha) during the last week of April each year. Standard agronomic practices were followed for fertilizer, irrigation, and weed management. Plots were 3 rows wide by 7.6 m long and replicated eight times in a randomized complete block design. The study area was flanked on each side by at least two rows of RB, which served as a buffer. In order to protect the plots from defoliation by Colorado potato beetle (*Leptinotarsa decemlineata*), the first generation of beetles was treated by chemigation with

spinosad (Blackhawk Naturalyte®; Dow Agro Science, LLC) at a rate of 69.9 g a.i. per ha. Spinosad was used for this study because of its low aphid toxicity.

Aphid sampling

Green peach aphid and potato aphid are the only two potato-colonizing aphid species known to occur in the study area. In 2011, 2012, and 2013, sampling for these aphids were conducted once a week throughout the growing season, from plant emergence to vine kill. Aphids were collected from the abaxial surface of six lower leaves from each of five plants in the center row of each plot using a No. 2 sable hair paint brush. Aphids were placed in 95% ethyl alcohol and stored at −20°C for later species-level identification and diagnostic testing. Aphids were assayed with reverse transcription polymerase chain reaction (RT-PCR) to test for presence of PLRV (see below).

Virus incidence in aphids

A subsample (50% of the total aphids collected each week of 2011 and 2012, and 20% of total aphids collected each week of 2013) was tested with RT-PCR to estimate the proportion of viruliferous aphids. Aphids from each subsample were tested either singly or by grouping (for larger aphid sample). Each aphid sample was placed in a 1.5 ml micro-centrifuge tube containing 70% ethyl alcohol. The ethyl alcohol then was removed and the sample allowed to dry for a short period before being homogenized using a pellet pestle motor. Then 100 µl of DNase extraction buffer [100 µl buffer containing 10 µl 0.1 M Tris-HCL, 10 µl of 2.5 mM $MgCl_2$, 20 units/ml DNase (RNase free), and 80 µl of ultrapure nuclease-free sterile water] was added to the aphid sample and homogenized again. For small aphid samples (fewer than 5 per plant) individual aphids were assayed separately. For

larger samples (5-20 aphids per plant), aphids were grouped, (2, 5, or 10 aphids together) as necessary. For aphid samples in which the number was more than 20 per plant), 2, 5, or up to 20 aphids from the same plant were assayed together. For larger samples of aphids, the volume of RNA extraction buffer was increased at a rate of ca. 100 µl per 5 aphids (Singh et al. 1995). In the following steps of RNA extraction, reagent quantities were adjusted similarly. After homogenization, each sample was incubated at 37°C for 10 minutes; then 10 µl of 10% SDS and 5 µl of proteinase K (10 mg/ml) was added to the sample and incubated again at 65°C for 10 minutes. Then 100 µl of water-saturated phenol was added to the sample, mixed vigorously by vortex, and then spun down at 12,000 rpm (4°C) for 10 minutes. The lower-phase phenol was discarded and the supernatant was transferred into a new 1.5 ml micro-centrifuge tube, into which 100 µl of chloroform-isoamyl alcohol solution (24:1) was added. The sample then was mixed vigorously by vortex and centrifuged at 12,000 rpm (4°C) for 10 minutes. The resulting supernatant was removed and transferred to a new 1.5 ml micro-centrifuge tube containing 250 µl chilled ethyl alcohol (95%), 10 µl 3M sodium acetate (pH 5.5), and 1 µl glycogen (20 mg/ml; RNA grade, Invitrogen). The solution was mixed by vortex and allowed to precipitate overnight at −20°C. The RNA was spun down at 13,000 rpm (4°C) for 30 minutes; the resulting pellet was washed with 70% ethyl alcohol, and dried in a 37°C incubator for 10 minutes. The dried RNA pellet was then suspended in 10 µl of nuclease-free sterile water and stored at −20°C before performing the PCR reaction.

Single step RT-PCR was performed using a multiplex primer as described by Singh (1998). Reaction master mix was prepared using the BIO-RAD iTaq Universal Probes one step kit (Bio-Rad, Hercules, CA) and Rediload gel loading dye (Invitrogen). Each 20 µl

reaction mix contained 10 µl of BIO-RAD PCR reaction mix (0.5 mM of each dNTP [dATP, dCTP, dGTP, dTTP], Mg^{++}, antibody-mediated hot start Taq DNA polymerase, stabilizers), 4.5 µl of nuclease-free (DNase/RNase) water, 1 µl each of both the forward and reverse primer (PLRV-Singh F- 5'-CGCGCTAACAGAGTTCAGCC-3' and PLRV Singh R- 5'GCAATGGGGGTCCAACTCAT-3'), 2 µl of Rediload gel loading dye (Invitrogen), 0.5 µl of iScript RT (50X formulation of iScript RNase H+ MMLV reverse transcriptase), and 1 µl of sample RNA extract. The thermo-cycles for the PCR reaction consisted of 15 min at 50°C for cDNA synthesis, 5 min at 94°C for iScript reverse transcriptase inactivation, 30 cycles of 15 sec at 94°C, 1 min at 58°C, and 30 sec at 72°C followed by a final extension at 72°C for 5 minutes and final storage of PCR product at 4°C. After the PCR reaction, 15 µl of the final amplified PCR product was analyzed by gel electrophoresis (2% agarose gel; Ultrapure Agarose®, Invitrogen) by staining the product with ethidium bromide (0.5 mg/ml), and observing the gel under UV light (302 nm) in Alpha Manager MINI (Cell Biosciences).

The resulting PCR product of the sample aphid RNA produced a diagnostic 336 base pair band in the agarose gel indicating presence of PLRV. All the samples showing this band were scored as viruliferous. For samples containing multiple aphids from the same plant, all the aphids were considered positive if the sample showed the identifying band.

Virus incidence in potato plants

PLRV incidence in plants in each plot was determined three times throughout the growing season. The sampling dates were 30 June, 30 July, and 29 Aug in 2011; 17 June, 17 July, and 16 Aug in 2012; 5 June, 30 July, and 23 Aug for 2013. The final sample data

occurred shortly before vine kill. On each sample date, a composite leaflet sample was taken from the top, middle, and bottom portion of each of ten plants sampled from all three rows of each plot. Composite leaf samples were ground using an electric leaf press. Sap was collected and mixed with 0.5 ml sample buffer (1× phosphate-buffered saline [pH 7.4] containing 3.2 mM Na_2HPO_4, 0.5 mM KH_2PO_4, 1.3 mM KCl, 135 mM NaCl, 0.05% Tween 20, and 2 g / liter of powdered non-fat dry milk). The samples were tested with double-antibody sandwich enzyme linked immuno sorbent assay (DAS-ELISA; Clark and Adams, 1977) using polyclonal coating IgG and monoclonal conjugate (Anti PLRV polyclonal and Anti PLRV monoclonal conjugated with AP; BIOREBA, Reinach, Switzerland) to determine infection status in the field plants.

Statistical analysis

Analysis of variance was used to compare the mean aphid density among different varieties across different sampling dates in 2011, 2012, and 2013. All analyses were conducted using SAS 9.2 (SAS Institute, Cary, NC, USA). Analyses were conducted separately for the two aphid species collected. Cumulative degree days (accumulated product of time and temperature between the developmental thresholds of green peach aphid for each day) were calculated for each sampling date for each year, and statistical comparisons among treatments within each year were made for three sample dates over the season with similar degree days across all three years. Three sample dates were chosen rather than comparing all dates to facilitate the analysis, by testing three uniform data points across years. The biological reason associated with this was to find data points across years where environmental conditions were similar for aphid population development. Poisson

distribution was assumed for aphid counts. Comparisons of mean aphid density among varieties across years were made using PROC-GENMOD with logit-link transformation. In addition, comparisons of mean aphid density among the three varieties within each year were made using the same procedure and transformation. Comparisons of mean PLRV infection rates among different potato varieties across years were made using PROC-GENMOD with logit-link transformation assuming a binomial distribution. Correlations among aphid density, proportion of viruliferous aphids within samples, and PLRV infection in plants were evaluated using PROC-CORR (Spearman method).

Results

Aphid densities

GPA represented at least 96.8% of total aphids collected each year. Mean GPA density across the varieties differed significantly among sampling dates within years, although overall densities did not differ significantly among varieties for any year (Table 4.01, Fig. 4.01). When aphid densities were compared in the same variety with nearly similar sampling cumulative degree- days (Table 4.02) across years, there was no significant difference among the three years (Table 4.01, Fig. 4.01).

Pairwise comparison among the three potato varieties revealed no significant differences in GPA densities across years (Table 4.03). However, GPA densities did differ among the varieties within each year, displaying slightly different patterns each year (Table 4.04). During 2011, mean GPA densities over the season were higher on RR than on the other two varieties, which did not differ between each other (Table 4.04, Fig. 4.03). During 2012, densities were higher on RR and RB (which did not differ between each other) than

on RN (Table 4.04, Fig. 4.03). During 2013, GPA densities were higher on RR than on RB, which had higher densities of GPA than did RN (Table 4.04, Fig. 4.03). Overall mean GPA density was considerably higher during 2013 than during the previous two years. In 2011 and 2012, aphid density was numerically higher in RR than RN late in the season, but not at earlier sampling times (Fig. 4.01).

Due to the low numbers of PA sampled (ca. 3% of aphid samples each year), statistical analyses were possible only for a few cumulative degree-days. However, absence of PA in the same degree-days and less statistical power associated with those low numbers precluded an analysis of PA density statistically across years. Numerically, overall PA density peaked around 550-950 cumulative degree-days, but this pattern was not the same for the GPA density pattern except in 2012 (Fig. 4.03). For all years, no significant differences were observed among varieties for PA densities (Table 4.04, Fig. 4.03).

Relationships among aphids, virus incidence, and disease incidence

During 2011 and 2012, the ratio of GPA to PA was 1:0.03 and 1:0.02, respectively; however, in 2013 the ratio was greater at 1:0.0005.

In 2011, 2012 and 2013, 10.3%, 5.2%, and 56.8%, respectively, of aphids sampled were viruliferous. Although PA abundance was low, 5.7%, 9.3%, and 9.7% of PA were viruliferous in 2011, 2012, and 2013, respectively.

Across all years and varieties, the percent PLRV infection was ca. 25% (Fig. 4.04). The total number of aphids (including viruliferous and non-viruliferous aphids) and the total number of viruliferous aphids, were positively correlated with the percent PLRV incidence

in RR and RN across years, whereas PLRV incidence in RB was not correlated with the total number of viruliferous aphids but was correlated with total number of aphids (including viruliferous and non-viruliferous aphids) (Table 4.05).

PLRV infection in the three potato varieties

PLRV incidence in potato did not differ significantly among RB, RR, and RN pooled across years (Table 4.06, Fig. 4.04). However, PLRV incidence did differ significantly among years, with 2013 showing the highest infection (44.7%) while incidence did not differ significantly between 2011 (17.5%) and 2012 (13.1%) (Fig. 4.04). PLRV incidence in potato plants differed significantly among sampling dates within each year and in all years for all varieties. The third sampling date showed the highest infection followed by the second and first dates (Table 4.07).

Discussion

Despite considerable differences in overall aphid abundance among years, all three potato varieties in this study showed similar patterns of aphid colonization across years and similar relationships between PLRV incidence in plants and aphid abundance or proportion of viruliferous aphids.

Potato varieties vary in their susceptibility to PLRV and aphid colonization (Corsini et al. 1994, DiFonzo et al. 1995, Corsini and Brown 2001). Although the predominant potato varieties in the PNW, RB, RN, and RR are susceptible to PLRV infection and exhibit varying levels of virus incidence (83%, 67%, and 69% mean infection percentage in RB, RN, and RR, respectively, in a replicated field evaluation from 2000-2012) (Corsini et al. 1994, R. Novy and J. L. Whitworth, personal communication), aphid colonization patterns

in these varieties are not well documented. In the present study, aphid colonization by GPA and PA did not differ among RB, RR, and RN. This result was in accordance with the findings from Davis et al. (2007). Aphid arrestment and colonization is influenced, often increased, by elevated headspace volatile organic compounds (VOC) as a result of PLRV infection in different potato varieties (Eigenbrode et al. 2002, Ngumbi et al. 2007, Werner et al. 2009, Rajabaskar et al. 2013a, 2013b). Our results suggest that all the varieties in this study may have similar headspace VOC profiles given the similar aphid colonization and PLRV incidence among varieties, however additional studies are required to clarify this. RR exhibited numerically higher aphid density than RN late in the season, but this may be due to dispersal of aphids from the early-maturing RN plants to the later maturing RR plants. Although there were slight differences in aphid numbers later in season, it did not appear to have been enough to change PLRV infection levels among varieties.

Several studies have documented a host-vector manipulation phenomena by plant viruses in diverse pathosystems (Mauck et al. 2010, Bosque-Pérez and Eigenbrode 2011, Ingwell et al. 2012, Shrestha et al. 2012, Moreno-Delafuente et al. 2013) as well as in the PLRV-aphid pathosystem (Eigenbrode et al. 2002, Werner et al. 2009, Rajabaskar, et al. 2013, Rajabaskar et al. 2014). Behavioral manipulations of aphid vectors may be one way to describe the disease progression pattern observed in the present study. Small numbers of viruliferous aphids were found to colonize plants at the beginning of the season. These viruliferous aphids might have preferentially landed on healthy plants and created a primary source of inoculum by infecting them. Secondary spread in a plant-to-plant mode from that primary inoculum might be possible due to later colonization by non-viruliferous summer migrants and their conditional preference for the PLRV-infected plants followed by the

preference for the healthy plants as soon as they turn viruliferous. The disease progression model developed by Roosien et al. (2013) showed that conditional vector preference aids in the spread of plant pathogens.

PLRV is transmitted by aphids in a persistent and circulative manner which makes disease dynamics highly complex due to the vector's ability to transmit the virus throughout its life, the plethora of natural dissemination means, and alternative hosts (Thomas et al. 1997, Gray and Banerjee 1999, Gray and Gildow 2003, Zhu et al. 2006, Srinivasan and Alvarez 2008, Srinivasan et al. 2013). In the present study, a positive correlation between viruliferous aphid abundance and PLRV infection was found in RN and RR, which may suggest that viruliferous aphids preferred to colonize these varieties. However, aphids might have preferred to land on RB and RN early in the season as more viruliferous aphids were detected early in the season on RB and RN (data not shown). Molecular monitoring for PLRV incidence on field-collected aphids (Singh et al. 1996, 1997, Singh 1999) early in the season may be useful in detecting primary PLRV inoculum levels. Furthermore, the present study showed that aphid abundance was positively correlated with PLRV incidence in plants which is similar to the findings from Flanders et al. (1991). Thus, it might not be necessary to test every aphid to predict inoculum level; rather, monitoring total aphid abundance may be useful to assess risk of virus infection. This also suggests that the three major varieties in the PNW are at a similar risk in the event of PLRV infection. PLRV susceptibility also depends on the plant age at inoculation, inoculum pressure, and seed tuber infection (DiFonzo et al. 1994). In the present study, a variation of PLRV incidence within year was found, which may be correlated to different inoculum pressure in different years.

Although potato growers incidentally manage non-viruliferous aphid populations to manage PLRV by frequent foliar spray of aphidicides, in-furrow treatment, or seed treatment targeted against colonizing aphids, they may be able to reduce management costs by applying management tools only when they find a viruliferous population of aphids. In PNW potato production, information on the historical trends of PLRV inoculum level in regional populations of aphids is unknown. Also, the PLRV inoculum threshold that can result in economic loss for different potato varieties has not been determined. A high PLRV inoculum with low aphid numbers may be more destructive than vice versa. Therefore, a monitoring system (aphid trapping and diagnostic tests on those aphids) should be developed to determine the proportion of viruliferous aphids among the spring migrants. Furthermore, a subsample of the colonized aphids should also be tested on a weekly or biweekly basis to predict the risk of secondary spread. Future research is also needed to determine the PLRV inoculum level in seed and commercial crops sufficient to cause economic impact. A combination of management practices may help reduce PLRV spread in commercial and seed growing areas (Robert et al. 2000, Davis and Radcliffe 2008, Vučetić et al. 2013).

Widespread use of certified virus-free seed tubers and application of neonicotinoid insecticides by potato growers have dramatically reduced PLRV in the PNW (Halterman et al. 2012). However, PLRV has potential to further constrain potato production due to resistance development by aphids to neonicotinoid insecticides (Puinean et al. 2010, Bass et al. 2011, 2014, Beckingham et al. 2013, Fray et al. 2014). The present study featured nearby PLRV field inoculum, inoculative release of aphids, and no aphidicide applied to plants;

therefore, aphid and virus pressure were relatively high, which may represent the potential for disease pressure where insecticide resistance occurs.

Jones et al. (2010) and Jones (2014) suggested new detection technology, improved information systems, and disease modelling to predict viral outbreaks. The present study has offered some potential disease monitoring approaches and risk mitigation strategies that can be incorporated in potato production systems. Molecular detection technology can enhance earlier detection of virus in the aphids; thus, the primary inoculum from spring migrants can be detected and potentially minimized. Weekly trapping and counting of aphids and testing a proportion of them for the presence of virus can be considered for developing a disease progression model. Furthermore, as spring aphids migrate from the main host to potato through different alternative hosts, the capture and testing of spring migrants with PCR might give an idea of the regional potential inoculum in aphid populations within a region. The three widely grown varieties tested in this study are predisposed to risk of future PLRV outbreaks; thus, the ongoing efforts of potato breeding programs to develop PLRV resistant varieties will help reduce the problem.

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Table 4.01: Comparison of green peach aphid densities among three potato varieties on three different sampling dates (degree days) within and across three years.

Variable[a]	df	$\chi 2$	Pr> $\chi 2$
Year	2	18.0	<0.0001
Variety	2	0.5	0.769
Date	2	29.7	<0.0001
Date*Variety	4	0.3	0.990
Year*Date	4	33.7	<0.0001
Year*Variety	4	0.6	0.961
Year*Date*Variety	8	1.5	0.992

[a]GPA densities were compared among three potato varieties Russet Burbank (RB), Russet Norkotah (RN), and Ranger Russet (RR) in 2011, 2012, and 2013. Cumulative degree days were calculated for each sampling date for each year, and statistical comparisons among treatments within each year were made for three sample dates over the season with similar degree days across all three years. Differences in least square means of aphid density were compared using PROC GENMOD (assuming Poisson distribution).

Table 4.02: Degree days used for statistical analyses for each sample point during each year.

Years[a]	1st sampling dates	2nd sampling dates	3rd sampling dates
2011	683	1,030	1,266
2012	561	1,099	1,232
2013	611	982	1,288

Table 4.03: Pairwise comparisons of green peach aphid densities among three potato varieties across three years.

Variety[a]	Variety compared with	df	$\chi 2$	Pr> $\chi 2$
Russet Burbank	Russet Norkotah	1	0.3	0.591
Russet Burbank	Ranger Russet	1	0.02	0.893
Russet Norkotah	Ranger Russet	1	0.5	0.504

[a]GPA densities were compared pairwise among varieties across 2011, 2012, and 2013. Cumulative degree days were calculated for each sampling date for each year, and statistical comparisons among treatments within each year were made for three sample dates over the season with similar degree days across all three years. Differences in least square means of aphid density were compared using PROC GENMOD (assuming Poisson distribution).

Table 4.04: Pairwise comparisons of green peach aphid and potato aphid densities among three potato varieties within each sampling year.

Aphid species	Sampling year	Varieties[a]	Varieties compared with	df	$\chi 2$	Pr> $\chi 2$
GPA	2011	Russet Burbank	Russet Norkotah	1	0.7	0.403
		Russet Burbank	Ranger Russet	1	27.7	<0.0001
		Russet Norkotah	Ranger Russet	1	9.8	<0.0001
	2012	Russet Burbank	Russet Norkotah	1	129.9	<0.0001
		Russet Burbank	Ranger Russet	1	1.4	0.245
		Russet Norkotah	Ranger Russet	1	154.9	<0.0001
	2013	Russet Burbank	Russet Norkotah	1	288.3	<0.0001
		Russet Burbank	Ranger Russet	1	3,498.5	<0.0001
		Russet Norkotah	Ranger Russet	1	5,675.4	<0.0001
PA	2011	Russet Burbank	Russet Norkotah	1	0.97	0.324
		Russet Burbank	Ranger Russet	1	0.04	0.845
		Russet Norkotah	Ranger Russet	1	1.39	0.239
	2012	Russet Burbank	Russet Norkotah	1	1.02	0.313
		Russet Burbank	Ranger Russet	1	0.8	0.381
		Russet Norkotah	Ranger Russet	1	3.4	0.065
	2013	Russet Burbank	Russet Norkotah	1	0.13	0.721
		Russet Burbank	Ranger Russet	1	0.43	0.510
		Russet Norkotah	Ranger Russet	1	0.09	0.762

[a]GPA densities was compared pairwise among varieties separately for 2011, 2012, and 2013. Cumulative degree days were calculated for each sampling date for each year, and statistical comparisons among treatments within each year were made for three sample dates over the season with similar degree days across all three years. Differences in least square means of aphid density were compared using PROC GENMOD (assuming Poisson distribution).

Table 4.05: Correlation between PLRV infection percent and either total aphid number or number of viruliferous aphids in different potato varieties.

Variety[a]	Spearman correlation coefficient of total aphid number and infection percent	Spearman correlation coefficient of viruliferous aphid number and infection percent
Russet Burbank	0.766 (0.027)	0.596 (0.119)
Russet Norkotah	0.764 (0.027)	0.820 (0.013)
Ranger Russet	0.766 (0.026)	0.873 (0.005)

[a]Because PA abundance was very low relative to GPA, both species were counted together for correlation analyses. Viruliferous aphids were estimated by RT-PCR from the field-collected aphids. PLRV infection status in the plants was determined by DAS-ELISA. The values in parenthesis are the P-values associated with the respective correlation coefficient.

Table 4.06: Comparison of PLRV incidence in three different potato varieties across years 2011, 2012, and 2013.

Variety[a]	Varieties compared with	df	$\chi 2$	Pr> $\chi 2$
Russet Burbank	Russet Norkotah	1	0.5	0.497
Russet Burbank	Ranger Russet	1	0.8	0.388
Russet Norkotah	Ranger Russet	1	0.04	0.838

[a]Composite leaf samples were collected from field plots and assayed with DAS-ELISA. Differences in least square means of percent PLRV infection in different potato varieties were compared pairwise using PROC GENMOD (assuming binomial distribution).

Table 4.07: PLRV incidence in three different potato varieties in the years 2011, 2012, and 2013 over three sample dates throughout the potato growing season.

Years	Variety	Infection percent[a]		
		1st Sampling	2nd Sampling	3rd Sampling
2011	Russet Burbank	0 a	10.0 b	22.5 c
	Russet Norkotah	0 a	5.0 b	15.0 c
	Ranger Russet	0 a	7.5 b	15.0 c
2012	Russet Burbank	0 a	5.0 b	15.3 c
	Russet Norkotah	0 a	7.5 b	12.5 c
	Ranger Russet	0 a	2.5 b	11.1 c
2013	Russet Burbank	0 a	13.8 b	42.5 c
	Russet Norkotah	0 a	14.2 b	41.3 c
	Ranger Russet	0 a	15.4 b	50.4 c

[a]Composite leaf samples were collected from field plots on three sampling dates in each year—30 June, 30 July, and 29 Aug in 2011; 17 June, 17 July, and 16 Aug in 2012; 5 June, 30 July, and 23 Aug for 2013—and assayed with DAS-ELISA. Statistical analysis was carried out separately for each year. Mean infection percentages within each horizontal panel that share the same letter are not significantly different.

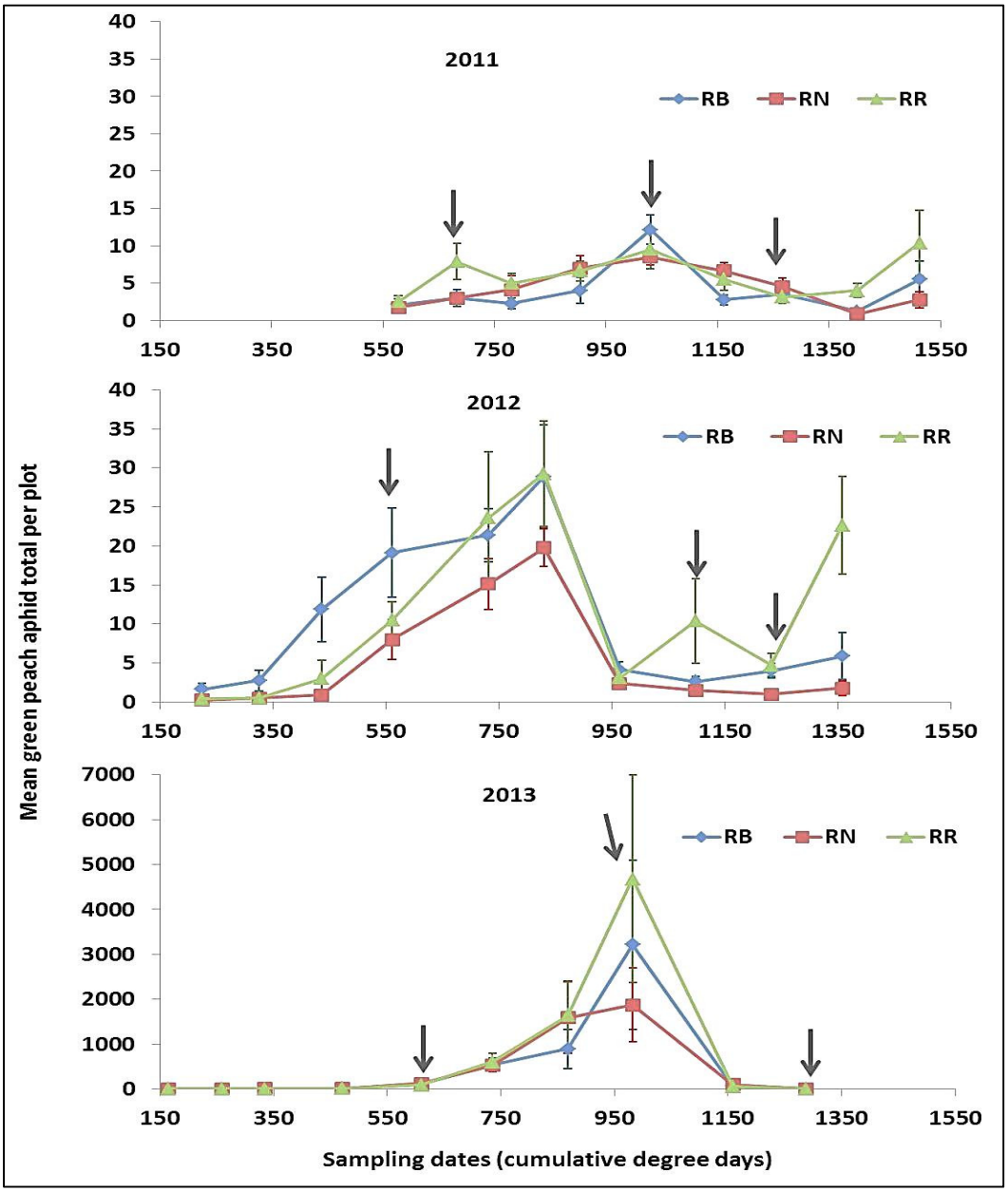

Fig. 4.01: Mean GPA densities were compared among Russet Burbank (RB), Russet Norkotah (RN), and Ranger Russet (RR) across different sampling dates in 2011, 2012, and 2013. Cumulative degree days were calculated for each sampling date for each year, and statistical comparisons among treatments within each year were made for three sample dates over the season with similar degree days across all three years (indicated by arrows). Error bars represent SEM.

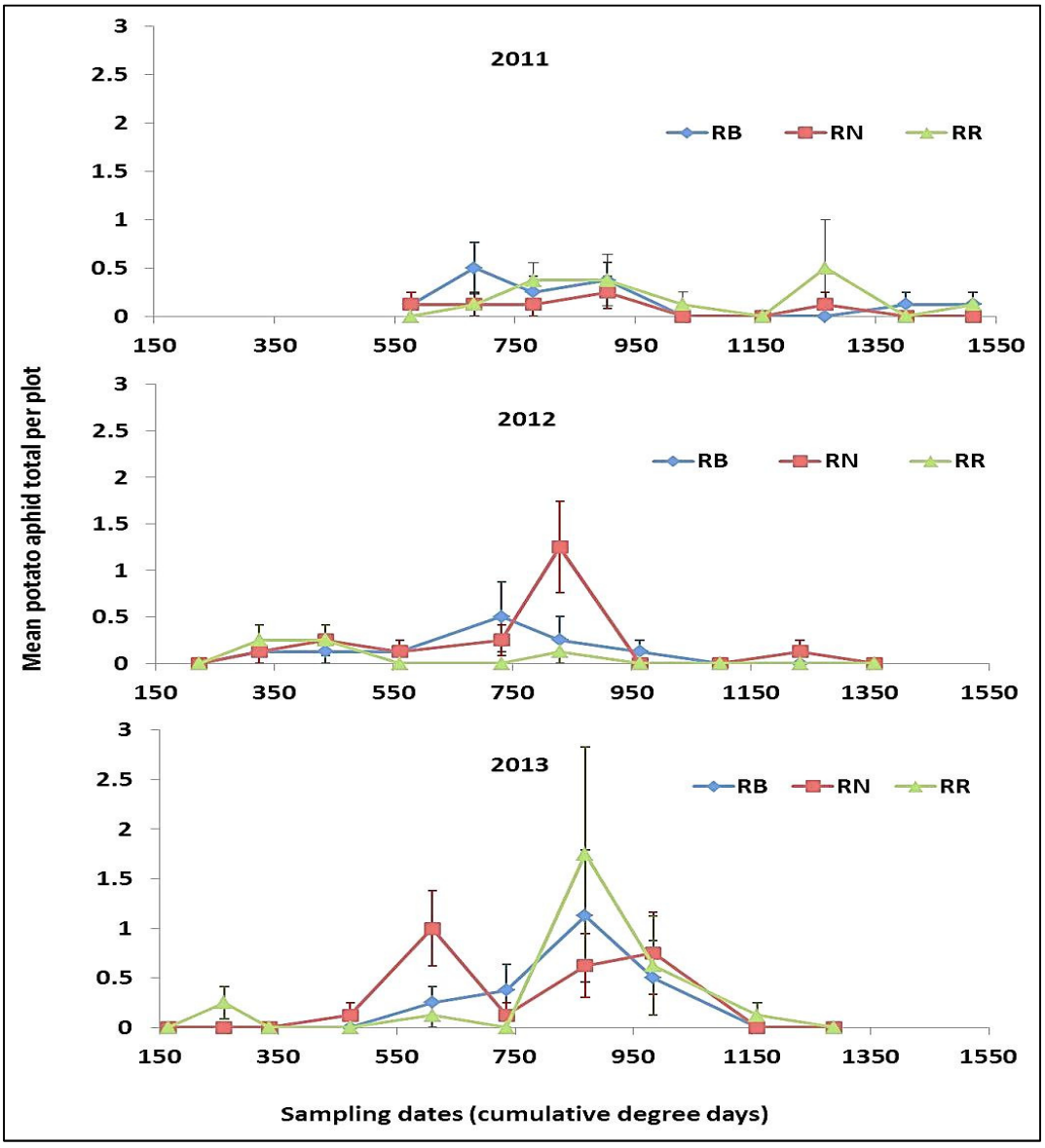

Fig. 4.02: Mean PA densities were compared among Russet Burbank (RB), Russet Norkotah (RN), and Ranger Russet (RR) across different sampling dates in 2011, 2012, and 2013. Cumulative degree days were calculated for each sampling date for each year, and statistical comparisons among treatments within each year were made for three sample dates over the season with similar degree days across all three years. Statistical comparisons were not possible due to low number of PA captured. Error bars represent SEM.

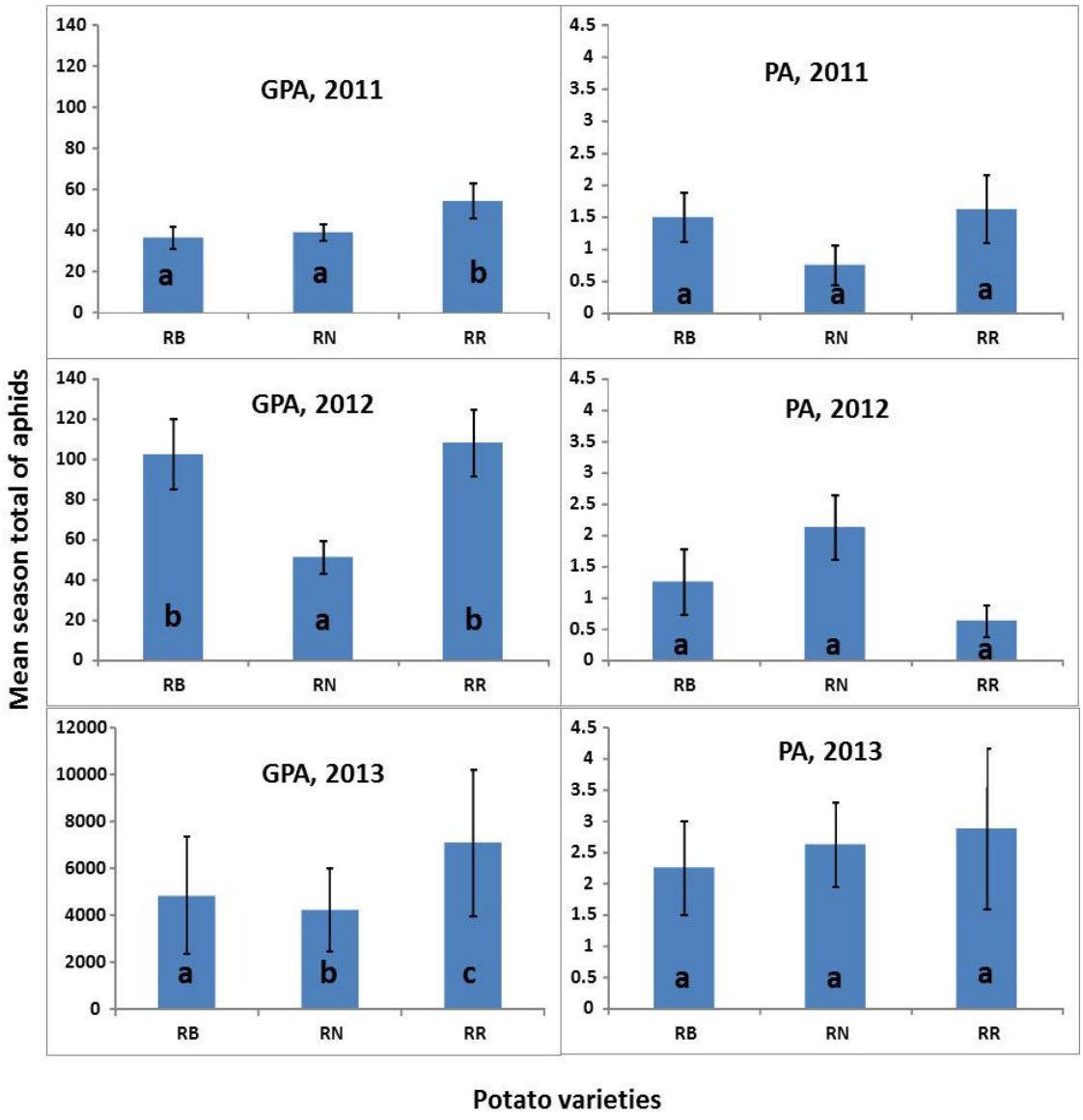

Fig. 4.03: Mean green peach aphid (GPA) and potato aphid (PA) densities were compared pairwise among potato varieties on different sampling dates separately for 2011, 2012, and 2013. Means within each group that share the same letter are not significantly different. Error bars represent SEM. Note the different y-axis scales.

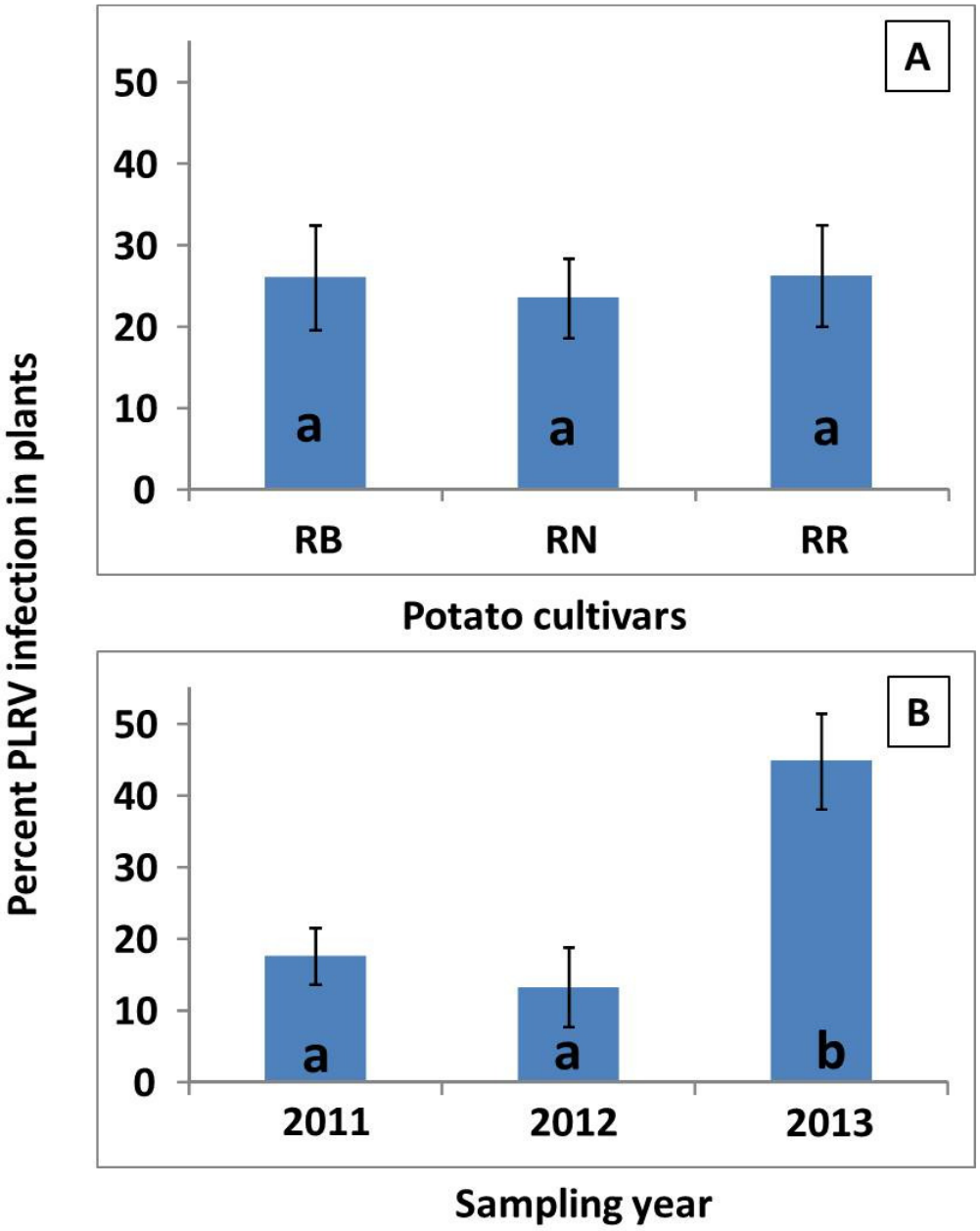

Fig. 4.04: PLRV incidence in potato (percent infection) compared among Russet Burbank (RB), Russet Norkotah (RN), and Ranger Russet (RR) (A) and overall among 2011, 2012, and 2013 (B). Means within each group that share the same letter are not significantly different. Error bars represent SEM.

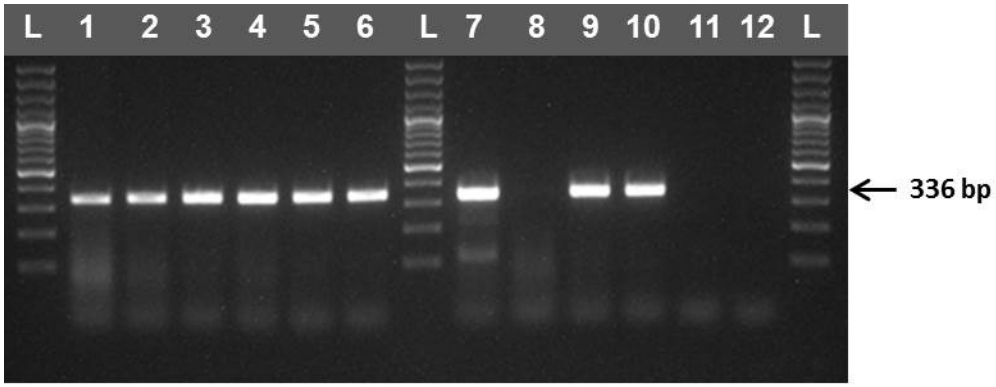

Fig. 4.05: Detection of PLRV in aphids by RT-PCR in ethidium bromide stained agarose gel (2%). RT-PCR product of RNAs of Lane 1 –Single aphid; Lane 2 –Two aphids; Lane 3 – Five aphids; Lane 4 – Ten aphids; Lane 5 – Fifteen aphids; Lane 6 – Twenty aphids; Lane 7 – PLRV infected potato plant from field plot; Lane 8 –Non-viruliferous aphid; Lane 9 – Positive check (plant); Lane 10 – Positive check (aphid); Lane 11 – Negative check (plant); Lane 12 – Negative check (aphid); Lane L –100bp ladder.

Chapter 5

Conclusion and remarks

The research presented in this dissertation focused on the virus / aphid pathosystems of two major viruses of potato, *Potato virus Y* (PVY) and *Potato leaf roll virus* (PLRV), both of which impact potato production in terms of yield and quality reduction. The overall objectives of the PVY / aphid pathosystem study were to ascertain some of the factors influencing the recent shift in prevalence of necrotic strains of PVY over ordinary strains and to clarify the biology of this pathosystem to aid in development of PVY management strategies. The objective of the PLRV / aphid pathosystem study was to understand aphid colonization patterns and phenology as well as PLRV incidence in the most widely grown potato varieties of the Pacific Northwest, USA in order to understand the relative risk of PLRV infection among these varieties.

A nation-wide survey in the USA and Canada revealed that the ordinary PVY strains are being replaced by the necrotic and recombinant PVY strains (Gray et al. 2010; Karasev et al. 2014); however, the reason behind this strain shift has remained unclear. Srinivasan et al. (2012) speculated that strain specificity and simultaneous transmission of closely related strains from a strain mixture by aphids could explain the observed shift in the prevalence of PVY strains. The first study presented in this dissertation was conducted to clarify transmission of virus strains by the green peach aphid (GPA) when multiple virus strains were present in the same source tissue with the hypothesis that aphids transmit some strains with higher efficiency than others from a strain mixture. The apparent primacy of O strain transmission from strain mixtures suggests that differences in transmission efficiency for

GPA may not be a contributing factor to the recent necrotic strain emergence. Several other possibilities also may contribute to the strain-shift phenomenon, including antagonistic and/or synergistic reactions within strain mixtures, differences in virus titer level among strains, and varying aphid probing behavior. Some of these questions might be clarified if artificial source tissue could be used with different virus strains of identical titer. Measuring altered aphid feeding behavior using different strains and strain mixtures through electro-penetration graph (EPG) assays would be useful as well. Strain specificity of the aphid acrostyle could be determined using a proteomics approach. All of these possibilities warrant further investigation.

The apparent primacy of the O strain in the differential transmission study suggested that differential transmission of necrotic strains from a strain mixture may not be an important factor in the recent prevalence of necrotic strains. Rather, the many other aphid species found in the field might contribute to the strain shift by transmitting newer necrotic and recombinant strains with better efficiency than ordinary strains. Thus, the second study was aimed at understanding how the newer necrotic PVY isolates are transmitted over ordinary isolates by various colonizing (GPA and potato aphid, PA) and non-colonizing aphid (bird cherry-oat aphid, BCOA) species. Although GPA transmitted PVY isolates most efficiently, the greater transmission efficiency of PVY^{NTN} strain by BCOA observed in this study, as compared with previous reports, suggests BCOA is a more important PVY vector than previously considered. As wheat and barley, hosts of BCOA, are two important cereal crops grown and rotated along with potato in Idaho, studies on the characterization of the aphid complex and their dispersal patterns are required to clarify the contributions of cereal aphids to PVY incidence during their dispersal from drying cereal fields. Despite lower

overall transmission efficiency of cereal aphids relative to GPA, the combination of the sheer numbers of cereal aphids and higher transmission efficiency of necrotic strains by BCOA could facilitate secondary spread of the virus and contribute to PVY incidence in potato.

A field study was conducted during 2012-2013 to examine if cereal aphids disperse to nearby potato fields as cereal crops dry down before harvest. The objective of this study was to characterize the aphid species complex over time in potato fields adjacent to cereal fields and clarify how their transient flight may contribute to PVY incidence. Many aphid species were captured, and a preponderance of them were cereal aphids; however, many other non-colonizing aphids were found that likely came from other hosts, including sugar beet, alfalfa, and/or various weeds. Their importance should not be underestimated considering the diverse landscape of different crops in southern Idaho. PVY incidence in potato increased following peak aphid flights and appeared to be related to aphid abundance. Further research on a landscape scale might be helpful in understanding the contribution of the crop landscape and weed distribution and abundance on that of non-colonizing aphids and PVY. The present study did not determine the origin of the cereal aphids, and indeed many likely came from fields other than the one directly adjacent to the potato field. An experiment could be designed using immunolabeling technology to clarify the proportion of aphids moving into potato from adjacent fields.

The above mentioned studies were aimed at advancing our understanding of the PVY / aphid pathosystem in order to develop improved management strategies for PVY. Despite the availability of numerous aphidicides, PVY management remains challenging due to the

non-persistent transmission of the virus; hence, a better understanding of the aphid transmission mechanism may help in developing PVY management tools. The studies presented here showed that many non-colonizing aphid species might contribute to PVY spread via transient visits to the potato crop from various hosts. Resistant cultivars could be developed that consider aphid probing behavior or their preferences towards particular genotypes. The modality of antixenosis/non-preference of host plant resistance might be applied to develop resistant potato varieties that could mitigate the impact of transient aphid flights from other fields. Small RNA interference (RNAi) could be a future research focus for this purpose. In addition, a landscape ecology approach to understanding the influence of various crop interfaces on aphid distribution and abundance and PVY epidemiology would be essential for developing an area-wide PVY management program.

Although PLRV caused huge economic losses during the end of 1990s and the beginning of 2000, widespread use of certified virus-free seed tubers and application of neonicotinoid insecticides by potato growers have dramatically reduced PLRV in the Pacific Northwest, USA. Despite limited incidence, there are risks of future PLRV outbreaks as the management of aphid vectors of PLRV is challenging in many ways. Challenges to aphid management include high aphid reproductive potential, diverse host ranges, tendency to develop resistance to insecticides, as well as the potential loss of neonicotinoid insecticides due to regulations. However, colonization patterns of aphids in the predominant potato cultivars are not well understood. The objective of the final study presented in this dissertation was to examine the phenology of aphid vectors and PLRV among three predominant potato varieties in the region: Russet Burbank, Russet Norkotah, and Ranger Russet. These potato varieties were sampled over the growing season to clarify the

relationships between aphid numbers (total and viruliferous) and PLRV incidence in plants in order to determine whether variety-specific differences exist. All varieties were found to be at similar risk of PLRV incidence and aphid vector colonization.

Potato growers manage non-viruliferous aphid populations to control PLRV by frequent foliar spray of aphidicides, in-furrow treatment, or seed treatment. Growers might be able to reduce management costs by employing management tools only when viruliferous aphids are found in a field; however, the PLRV inoculum threshold that can result in economic losses for potato—let alone for different varieties of potato—has not been determined. A high PLRV inoculum with low aphid numbers might be more destructive than vice versa. Future research could focus on developing a monitoring system (aphid trapping and diagnostic tests on those aphids) that could determine local aphid densities and virus load among spring migrants. An early detection of viruliferous aphids should ensure timely implementation of control tactics and improve management while reducing overall costs.