Potato virus Y Transmission Efficiency from Potato Infected with Single or Multiple Virus Strains

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ABSTRACT

There has been a recent shift in the prevalence of *Potato virus Y* (PVY) strains affecting potato with the ordinary strain PVY^O declining and the recombinant strains PVY^{NTN} and PVY^{N:O} emerging in the United States. Multiple PVY strains are commonly found in potato fields and even in individual plants. Factors contributing to the emergence of the recombinant strains are not well defined but differential aphid transmission of strains from single and mixed infections may play a role. We found that the transmission efficiencies by *Myzus persicae*, the green peach aphid, of PVY^{NTN}, PVY^{N:O}, and PVY^O varied depending on the potato cultivar serving as the virus source. Overall transmission efficiency

was highest from sources infected with three virus strains, whereas transmission from sources infected with one or two virus strains was not significantly different. Two strains were concomitantly transmitted by individual aphids from many of the mixed-source combinations, especially if PVY^O was present. Triple-strain infections were not transmitted by any single aphid. PVY^O was transmitted most efficiently from mixed-strain infection sources. The data do not support the hypothesis that differential transmission of PVY strains by *M. persicae* is a major contributing factor in the emergence of recombinant PVY strains in the U.S. potato crop.

Potato (*Solanum tuberosum* L.) is an economically important, high-value crop in the United States and is the major noncereal staple food crop worldwide (FAO 2008). *Potato virus Y* (PVY), the type member of the family *Potyviridae*, is distributed in potato-growing areas throughout the world (Gray et al. 2010; Karasev and Gray 2013; Kerlan and Moury 2008) and can severely constrain potato production by affecting yield and tuber quality (Gray et al. 2010; Ragsdale et al. 2001). PVY is the major reason for the rejection of seed lots by U.S. and other national seed certification programs (Frost et al. 2013). An emerging concern regarding PVY is the increasing prevalence of recombinant strains relative to the ordinary strain, PVY^O (Gray et al. 2010; Karasev and Gray 2013). Unlike PVY^O, which generally causes readily observable foliar mosaic, necrosis, and leaf drop symptoms, the recombinant strains (PVY^{NTN}, PVY^{NA-NTN}, PVY^{N:O}, and PVY^{N-Wi}) often induce mild or transient foliar mosaic symptoms. Moreover, the recombinant strains are more likely to cause potato tuber necrotic ringspot disease of varying severity in many important North American potato cultivars, including ‘Yukon Gold’, ‘Red Norland’, ‘Yukon Gem’, ‘Highland Russet’, ‘Alturas’, and ‘Ranger Russet’ (McDonald and Singh 1996; Nolte et al. 2004; van den Heuvel et al. 1994; Whitworth et al. 2006).

Recombinant PVY strains were first reported in North America from Canada in 1991 (Singh 1992) and the United States in 2002

(Crosslin et al. 2002). Subsequent reports and surveys have shown a rapid shift in geographic distribution and relative incidence of PVY strains in potato in the United States and Canada (Ellis et al. 1997; Gray et al. 2010; McDonald and Kristjansson 1993; Piche et al. 2004; Singh et al. 2003). These new strains are a product of recombination in several regions of the genome of two parental strains (PVY^O and PVY^N); most notable are exchanges spanning the P1 and HC-Pro cistrons and spanning the VPg, NIa, NIb, and CP cistrons (Gray et al. 2010; Karasev and Gray 2013). The recombinant PVY^{N:O} and PVY^{NWi} strains now predominate in most U.S. production areas. Although the tuber-necrotic PVY^{NTN} strain is still a relatively minor component of the overall PVY incidence, it is distributed in all U.S. seed production areas (Gray et al. 2010). Meanwhile the two parental strains of most recombinants are either rapidly decreasing in prevalence (PVY^O) or have all but disappeared (PVY^N). PVY^N has only been recovered from potato growing on two farms in one state since national surveys began in 2004. Multiple PVY strains are routinely found within a single seed lot or field, and strain mixtures in single plants are not uncommon (Crosslin et al. 2006; Gray et al. 2010). The reasons for the strain shifts are the subjects of ongoing studies but several factors are likely contributors, including the unintended selection for recombinant strains in seed certification programs and rogueing operations due to their milder foliar symptoms, a sizeable number of potato varieties with partial resistance genes effective against PVY^O but not the recombinant strains (Hane and Hamm 1999; Sturz et al. 1997), and differences in relative transmission efficiency of PVY strains by different aphid vectors (Mello et al. 2011; Mondal et al. 2016a; Verbeek et al. 2010).

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PVY is transmitted to potato by more than 50 colonizing and noncolonizing aphid species in a nonpersistent, stylet-borne manner (Bradley 1954; Sigvald 1984). Aphids acquire virus during very short (<1 min) feeding probes in epidermal leaf cells and subsequently transmit the virus to healthy plants by short-duration probes with no latent period (Perez et al. 1996); therefore, both potato-colonizing and transient noncolonizing aphids are important vector species (Difonzo et al. 1997; Mondal et al. 2016b). *Myzus persicae* (Sulzer), the green peach aphid (GPA), is the most efficient aphid vector (Boquel et al. 2011; Kaliciak and Syller 2009; Mondal et al. 2016a; Verbeek et al. 2010), whereas most other species of aphids tested in controlled transmission studies are inefficient vectors (Davis et al. 2005; Halbert et al. 2003; Katis and Gibson 1985; Mello et al. 2011; Mondal et al. 2016a; Sigvald 1984). Therefore, the vast majority of studies on PVY transmission efficiency and specificity have been conducted using GPA. Although it is difficult to directly compare many of the transmission efficiency studies, most GPA clones and populations appear to be efficient vectors. In previous studies, variation in transmission efficiency could be linked more to PVY strains, isolates within a strain, or source or recipient plant species than to GPA clone (Basky and Almási 2005; Harrington and Gibson 1989; Mello et al. 2011; Mondal et al. 2016a; Shrestha et al. 2014; Sigvald 1984; Verbeek et al. 2010).

The experiments described in this study took place in New York and in Idaho and were designed to examine the effects of mixtures of PVY strains in single plants of different potato cultivars on the ability of GPA to efficiently acquire and transmit those strains to other potato plants. This will provide valuable information on whether mixed infections and potato cultivar influence PVY transmission, thereby contributing to the shift in PVY strains over the past decade.

MATERIALS AND METHODS

Aphids, virus isolates, and plant hosts. The New York GPA clone has been reared on PVY-free *Physalis floridana* Rydb. in a growth room maintained at 21 to 23°C with constant light for over 15 years. The Idaho GPA colony, initially collected from potato by Guy Bishop, Parma Research & Extension Center, University of Idaho, was reared on Chinese cabbage (*Brassica pekinensis* Ruprecht) in a growth chamber maintained at 21 to 26°C, 90% relative humidity, and a photoperiod of 14 h of light and 10 h of darkness.

Several virus isolates used in the study were collected during national surveys of seed potato from 2004 to the present and included PVY^O-NY09-31, PVY^{N:O}-NY09-4, PVY^O-CA14, PVY^{N:O}-WI62, and PVY^{NTN}-NY09-29. Isolate PVY^{NA-NTN}-RRA1 (Lorenzen et al. 2006a) was obtained from Dr. Jonathan L. Whitworth. Additional isolates of PVY^O, PVY^{NWi}, and a PVY^{O/NWi} mixture were obtained from infected ‘Lamoka’ potato tubers harvested from 2010 field experiments conducted in Ithaca, NY. All isolates except those obtained from the Lamoka tubers were maintained long term in lyophilized tobacco (*Nicotiana tabacum* L. ‘Samsun-NN’) leaves stored at -20°C. Strain types of the infecting isolates was determined using a multiplex reverse-transcription polymerase chain reaction (RT-PCR) assay developed by Lorenzen et al. (2006b) or by a novel multiplex RT-PCR (see below).

Virus source plants for the experiments conducted in New York were derived from either PVY-infected Lamoka tubers, described above, or PVY-infected ‘Goldrush’ or ‘Red Maria’ plants that were initially infected by mechanical inoculation. Virus source plants were subsequently propagated through cuttings treated with rooting hormone (Bontone; 0.1% indole-3-butyric acid). Plants were grown in Cornell Mix (Boodley and Sheldrake, 1982) potting soil in plastic pots (10 by 10 by 15 cm) and maintained in a greenhouse screened to exclude aphids.

Virus source plants for the Idaho experiments were virus-free tissue-culture plantlets of ‘Russet Burbank’ potato. The plantlets

were potted in plastic pots (10 by 10 by 15 cm) in a 2:2:1 sand/peat/vermiculite mix with encapsulated fertilizer of N-P-K at 14:14:14 (Osmocote; Scotts Miracle Gro, Marysville, OH). Plants were infected by mechanical inoculation and maintained in a greenhouse at 19 to 27°C, 70% relative humidity, and a photoperiod of 16 h or light and 8 h of darkness.

Virus source plants were mechanically inoculated with one, two, or three PVY strains. Briefly, PVY-infected leaf tissue was ground in 0.1 M phosphate buffer (1:5), pH 7.4. Carborundum was applied to leaves of 3-week-old potato plants and sap was rubbed on the adaxial surface of all fully opened leaves of each plant. To obtain double- and triple-strain mixtures in source plants, sap extracted from plants infected with single strains was mixed in equal ratios and used as inoculum. All virus source plants were tested with multiplex RT-PCR to check for the presence of the desired strains. Young, fully expanded, systemically infected leaves were detached from the plant and used as the virus source for the aphid transmission experiments.

Recipient plants for the experiments conducted in New York were Red Maria seedlings generated from true seed. This provided a high-throughput method to generate many seedlings of the same size for transmission experiments. We had previously tested a large population of these seedlings ($n = 340$) to ensure that there was no variation in their susceptibility to PVY (Mello et al. 2011). True potato seed were treated with gibberellic acid-3 (750 ppm) for 5 days and seeded in Cornell Mix potting soil in 98-cell plug trays. Seedlings were transplanted at the three-leaf stage individually into pots (10 by 10 by 15 cm) and used as recipient plants for aphid inoculation at the four- to six-leaf stage. Recipient plants for the Idaho experiments were virus-free tissue-culture plantlets of Russet Burbank, grown as described above. Recipient plants were at the four- to six-leaf stage.

Transmission assays. Experiments in New York comparing the transmission of two PVY strains and the mixture of both viruses were done using PVY^O and PVY^{NWi} sourced from field-infected Lamoka plants and using PVY^O and PVY^{NTN} (isolates NY09-31 and NY09-29, respectively) sourced from mechanically inoculated Goldrush or Red Maria plants. Additional experiments, one in Idaho using Russet Burbank as source and another in New York using Goldrush as source, tested the transmission efficiency by GPA of single, double, and triple infections of PVY^O, PVY^{N:O}, and either PVY^{NA-NTN} or PVY^{NTN}. The PVY^O, PVY^{NWi}, and mixture experiment using Lamoka source tissue was repeated eight times with 20 to 40 recipient plants in each of the three treatments. All other experiments were repeated five times using 8 to 24 recipient plants for each treatment. Detached leaves from noninoculated plants were used as controls in each experiment.

Adult apterous GPA, previously starved for 1 to 2 h in covered plastic dishes, were allowed a 2- to 5-min acquisition access period (AAP) on a young, fully expanded source leaf systemically infected with the appropriate virus strain or combination of virus strains. Single aphids were transferred to and caged on healthy recipient potato plants for a 24- to 48-h inoculation access period. Plants were subsequently fogged with an insecticide mixture of pymetrozine (Endeavor 50WG) at 1.6 g (93 m²)⁻¹ and bifenthrin (Talstar P) at 15 ml (93 m²)⁻¹ to kill all aphids, moved to a greenhouse, and observed for symptom development for 2 to 3 weeks. At 3 to 5 weeks postinoculation, plants were tested for PVY infection using triple-antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA) (Ellis et al. 1996). Infected plants were subsequently tested by immunocapture multiplex RT-PCR to determine the infecting strains.

Detection and differentiation of PVY strains and strain mixtures. All PVY strains used in these experiments were initially detected and differentiated using TAS-ELISA (Ellis et al. 1996) using the following monoclonal antibodies (Agdia Inc., Elkhart, IN): 4C3 detects all PVY strains, MA62 is specific for the PVY^O and PVY^{N:O/NWi} isolates, and 1F5 is specific for the PVY^{NTN} isolates.

Subsequently, strain and strain mixtures were identified by multiplex RT-PCR (Lorenzen et al. 2006a). Although this multiplex RT-PCR can distinguish mixtures of most of the strains, it cannot differentiate a mixture of PVY^O, PVY^{N:O}, and PVY^{NTN} from a mixture of PVY^O and PVY^{NTN} (Fig. 1A, lanes 7 and 11). This was the reason for using PVY^{NA-NTN} rather than PVY^{NTN} in initial experiments, until a new assay was developed that could resolve a triple mixture of PVY^O, PVY^{N:O}, and PVY^{NTN} (Fig. 1B).

To differentiate triple-strain mixtures from mixtures of PVY^O and PVY^{NTN}, a novel immunocapture RT-PCR was developed using three PVY strain-specific primer pairs. Immunocapture was conducted by coating wells of a 96-well PCR plate (Laboratory Products Sales, Inc., Buffalo, NY) with 100 μ l of anti-PVY immunoglobulin G (0.1 μ g/ml) in 20 mM sodium carbonate coating buffer (pH 9.6) and incubating at 37°C for 2 h. The plate was then washed three times with phosphate-buffered saline containing 0.1% Tween 20 (PBST). Leaf samples homogenized in PBS containing 2% polyvinylpyrrolidone at 1:10 (wt/vol) were added to each well and incubated at 4°C overnight. The plate was washed with PBST four times, rinsed with deionized water, and air dried. To release viral RNA, 7.5 μ l of 1% Triton X100 was added to each well and the plate heated to 65°C for 5 min, then placed on ice for 5 min. An RT master mix was prepared using ImProm-II Reverse Transcription System (Promega Corp., Madison, WI), with some minor modifications (3.3 mM MgCl₂, 0.5 mM each dNTP, and 0.375 μ g of random hexamer primer) and 7.5 μ l was added to each sample well. The plate was incubated at 45°C for 30 min, followed by 1 h at 42°C, and then increased to 65°C for 15 min for inactivation of the transcriptase. PCR was carried out using GoTaq Green Master Mix (Promega Corp.) following the manufacturer's instructions with six strain-specific primers (0.8 μ M each). The primer pairs O1962F (5'-TCAACATTCTATCCACCAAC-3')/O2296R (5'-ACGTTT GAGTGTCTATGGT-3') and N:O1008F (5'-GCACGTTCCAAG GTTACC-3')/N:O1703R (5'-TCGCTTAGCATGATATTCCCT-3')

were designed to amplify 335- and 695-bp fragments from the HC-Pro cistron for PVY^O and PVY^{N:O}, respectively. PVY^{NTN} was identified by primer pair NTN7350F (5'-ACATCACCAGATGAG CAGG-3') and NTN8266R (5'-GTACATACCCTCGATTAGCA-3') producing a 918-bp amplicon from the NIb cistron (Fig. 1B). cDNA (3 μ l) was added to the PCR mixture and adjusted to a final volume of 20 μ l using nuclease-free water. PCR was carried out in an ABI 2720 Thermal Cycler (Applied Biosystems, Foster City, CA) with thermal cycles of 94°C for 3 min; 35 cycles of 94°C for 30 s, 62°C for 30 s, and 72°C for 1 min; final extension at 72°C for 7 min; and final hold at 4°C. PCR products were analyzed by electrophoresis in 1.5% agarose gel stained with GelRed Nucleic Acid Gel Stain (Biotium Inc., Hayward, CA) or ethidium bromide and visualized on a UV transilluminator.

Statistical analysis. Statistical analyses of experiments using source plants derived from infected Lamoka tubers were conducted using effect likelihood ratios and odds ratios determined by fitting data to a nominal logistic model, contingency analysis, distribution probability analysis, and χ^2 tests. Analyses were done with JMP Pro (version 10.0.2; SAS Institute Inc., Cary, NC).

A generalized linear model was fitted to compare means among the treatments for both the varietal comparison of the overall infection percentage using Goldrush and Red Maria source plants for the PVY^O and PVY^{NTN} single- and double-strain treatments, as

Fig. 1. A, Detection of *Potato virus Y* (PVY) strains PVY^O, PVY^{N:O}, and PVY^{NA-NTN} and their mixtures by reverse-transcription polymerase chain reaction (RT-PCR) (Lorenzen et al. 2006b). Lane 1, healthy Russet Burbank; 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; and L lanes, GeneRuler 100-bp DNA Ladder (Thermo Fisher Scientific). **B**, Detection of PVY^O, PVY^{N:O} and PVY^{NTN} by multiplex immunocapture RT-PCR in ethidium-bromide-stained agarose gel (2%). Lane H, healthy Goldrush; lane 1, O (CA14 isolate); lane 2, N:O (WI62 isolate); lane 3, NTN; lane 4, O+N:O; lane 5, N:O+NTN; lane 6, O+NTN; lane 7, O+N:O+NTN; and L lanes, GeneRuler 100-bp DNA Ladder (Thermo Fisher Scientific).

Fig. 2. A, Number of recipient Red Maria plants infected by single *Myzus persicae* (green peach aphid) adults given an acquisition access period on Lamoka potato plants infected with *Potato virus Y* (PVY) strains PVY^O, PVY^{NWi}, or a mixture of both strains. Numbers of infected plants were calculated from eight experiments using a total of 252 recipient plants per source tissue. Letters above the bars indicate significant difference in odds ratios ($P = 0.0118$) determined with a nominal logistic model. **B**, Identification of the PVY strains infecting the 29 plants (A, mixture) inoculated by aphids fed on the source tissue infected with both PVY^O and PVY^{NWi}. Virus strain was determined by immunocapture multiplex reverse-transcription polymerase chain reaction (Lorenzen et al. 2006b). Letters above the bars indicate significant difference in distribution probability analysis ($\chi^2 = 8.08$, $P = 0.0176$, $df = 2$).

well as the experiment where mixed-strain treatments (single, double, and triple) were compared separately for Russet Burbank and Goldrush source cultivars. Differences and interactions within the treatments were evaluated using logistic regression by PROC GENMOD with a logit-link transformation in SAS version 9.4 (SAS Institute). For the varietal comparison, pairwise contrasts were used to compare the infection status of the strain treatments of the two varieties. In addition, maximum-likelihood of percent infection from the two-way strain mixtures in the experiment with Goldrush and Red Maria cultivars was estimated in a proportional odds model with random effects, assuming a multinomial distribution using PROC-GLIMMIX with generalized logit link transformation in SAS version 9.4. For the mixed-strain treatments, differences in the numbers of single-strain, double-strain, and triple-strain infections in plants inoculated from a double-strain source and triple-strain source were compared by χ^2 pairwise contrasts. There were significant interactions in regard to percent infection by specific strains or strain combinations among mixtures; therefore, data were sorted by mixture and analyzed separately within each mixture. Within the double mixtures, there were significant interactions between combinations; therefore, data were analyzed within each combination separately.

RESULTS

PVY^O-PVY^{NW} mixture experiments. The transmission efficiency of PVY^{NW} (15.5%) was significantly higher than PVY^O (8.3%) when aphids were allowed an AAP on Lamoka source plants infected with the single strains (odds ratio $P = 0.0118$ for PVY^O and PVY^{NW} source strains). Transmission efficiency from source plants infected with a mixture of both strains was intermediate (11.5%) but not significantly different from either single source alone (Fig. 2A). When the 29 recipient plants infected with PVY by the aphids given an AAP on the mixed-infection source plant were analyzed, PVY^O infected significantly more plants (58.6%) than

either PVY^{NW} alone (24.1%) or both strains together (17.2%) (Fig. 2B).

PVY^O-PVY^{NTN} mixture experiments. Although neither cultivar effect nor strain effect alone was significant (cultivar: $P = 0.432$, $\chi^2 = 0.62$, df = 1; strain: $P = 0.107$, $\chi^2 = 4.46$, df = 2), the cultivar-strain interaction ($P = 0.025$, $\chi^2 = 7.37$, df = 2) had a significant effect on transmission efficiency (Fig. 3). The transmission efficiency of PVY^O was less than PVY^{NTN} when aphids were given an AAP on either Goldrush or Red Maria plants infected with either strain alone, although the differences were not significant. Transmission efficiency from plants infected with both strains was lower from Red Maria (22%) than from Goldrush (48%) (Fig. 3) and the composition of strains transmitted differed between the cultivars. Single aphids allowed an AAP on Goldrush infected with both PVY^O and PVY^{NTN} transmitted each of the strains and the mixture in nearly equal proportions (Fig. 3A, inset). In contrast, aphids allowed an AAP on Red Maria infected with both PVY^O and PVY^{NTN} transmitted PVY^O more than twice as often than either PVY^{NTN} alone or both strains together (Inset Figure 3B).

Three-strain mixture experiments. Overall transmission efficiency, as measured by percentage of recipient plants infected, regardless of strain transmitted, varied significantly among the virus strain treatments using both Russet Burbank and Goldrush as virus source plants (Fig. 4). Although transmission efficiency was highest from plants infected with three virus strains for both cultivars, transmission efficiency from plants infected with one or two strains of PVY was variable, indicating that mixed infections are not always better sources of virus that could contribute more toward secondary virus spread than singly infected plants.

In all instances where PVY^O was present in the mixed-infected source tissue (PVY^O and PVY^{NTN}, PVY^O and PVY^{NTN/NA-NTN}, or all three strains), it was found to infect the highest number of recipient plants regardless of the source cultivar (Fig. 5). However, the difference was only significant for the PVY^O and PVY^{NA-NTN} mixture and the three-way mixture acquired from Russet Burbank source tissue. This is similar to the significant difference observed

Fig. 3. Mean transmission (percentage of Red Maria recipient potato plants infected) by single *Myzus persicae* (green peach aphid) adults given an acquisition access period on **A**, Goldrush or **B**, Red Maria potato plants infected with *Potato virus Y* (PVY) strains PVY^O, PVY^{NTN}, or a mixture of both strains. Experiments were repeated five times using 8 to 24 recipient plants for each treatment. Means within each group that share the same letters are not significantly different. Although a cultivar-strain interaction was found and pairwise comparison was conducted accordingly, significance is shown separately for two cultivars. Error bars denote standard error. Statistical inference was based on logit-link transformed data; untransformed means are shown. Pie chart insets indicate the percentage of the recipient plants inoculated by aphids fed on the mixed-infected source plants that were infected by each or both of the PVY strains.

with Lamoka PVY^O and PVY^{NW_i} mix-infected source tissue (Fig. 2B) and the nonsignificant trend observed with Red Maria PVY^O and PVY^{NTN} mix-infected source tissue (Fig. 3B, inset). In the absence of PVY^O, both strains PVY^{N_iO} and PVY^{NTN} alone and together were transmitted at similar efficiencies from Russet Burbank source plants, whereas PVY^{NTN} was transmitted at significantly higher efficiencies from the Goldrush source plants (Fig. 5). All single strains and all two-strain mixtures were transmitted from Goldrush source plants infected with all three strains. The relative transmission efficiencies of single and two-strain mixtures were different from Russet Burbank source plants infected with all three strains; the mixture of PVY^{N_iO} and PVY^{NA-NTN} was not transmitted. No single aphid transmitted all three strains from triple-infected source plants of either cultivar (Fig. 5).

DISCUSSION

The ordinary strain of PVY (PVY^O) is declining as an important virus strain affecting seed potato production in the United States, while the recombinant strains, especially PVY^{NW_i}, are becoming more prevalent (Gray et al. 2010; Karasev and Gray 2013). Whether differential aphid transmission efficiency of PVY strains is contributing to this strain shift has been the subject of many studies. Several studies have investigated the transmission efficiency of PVY strains and strain mixtures (Basky and Almási 2005; Harrington and Gibson 1989; Mello et al. 2011; Mondal et al. 2016a; Srinivasan et al. 2012; Sigvald 1984; Syller and Grupa 2014; Verbeek et al. 2010) and results have been generally inconclusive that recombinant strains are transmitted more efficiently. One consistency among all the studies is the use of *M. persicae* (GPA) as the vector, simply because it is the most efficient. Most other aphid species transmit at relatively low efficiencies, which does not allow accurate measurements, especially if single aphids need to be transferred to recipient plants to tease apart the differential transmission from mixed infections under controlled conditions. There are some differences among GPA clones with respect to PVY transmission efficiency (Verbeek et al. 2010) but all are still efficient vectors. The inconsistencies in results of this and previous

studies on differential transmission of PVY strains by GPA under laboratory conditions are more likely attributed to virus isolates, host plants (both source and recipient), and host cultivars. Our studies focused on potato-to-potato transmission and used multiple isolates within strains as well as multiple potato cultivars in an attempt to better understand the most influential factors in PVY strain transmission efficiencies from single and mixed infections.

Our experiment with PVY^O and PVY^{NW_i} provided some evidence that PVY^{NW_i} was transmitted more efficiently than PVY^O from single infections; however, PVY^O was found to be preferentially transmitted from mixed infections of the two strains. Further experiments failed to support more efficient transmission of recombinant strains over the ordinary strain using other PVY^O and PVY^{N_iO} isolates and potato cultivars as virus source. Whether these results are a function of virus isolate, potato cultivar, or both is unknown. Two previous studies (Srinivasan et al. 2012; Syller and Grupa 2014) found that recombinant strains were preferentially transmitted over ordinary strains from a mixture but these contrast with reports that PVY^{N_iO/NW_i} does not have a competitive transmission advantage over PVY^O (Mello et al. 2011; Mondal et al. 2016a; Verbeek et al. 2010).

The competitive advantage in transmission efficiency of PVY^{NTN} over PVY^O has been reported by several studies (Basky and Almási 2005; Carroll et al. 2016; Mondal et al. 2016a; Srinivasan et al. 2012; Syller and Grupa 2014). Although the results were not statistically significant, our data also indicate that PVY^{NTN} isolates were transmitted with greater or equal efficiency than PVY^O when acquired from the various potato cultivars infected with one virus strain (Figs. 3 and 4). PVY^{NTN} is increasing in incidence and geographic distribution within the U.S. seed potato crop but it has remained a relatively minor component of overall PVY incidence. Similar to results for PVY^O + PVY^{NW_i} mixed infections, transmission from PVY^O + PVY^{NTN} mixed infections favored PVY^O transmission (Figs. 3B and 5). These results are in contrast to those reported by Syller and Grupa (2014), where transmission from PVY^O and PVY^{NTN} mixed infections favored PVY^{NTN}. Although no general conclusions can be drawn from the multitude of transmission studies on PVY strains, it is apparent that transmission

Fig. 4. Mean transmission (percentage of Red Maria recipient potato plants testing positive for *Potato virus Y* [PVY]) by single *Myzus persicae* (green peach aphid) adults given an acquisition access period on **A**, Russet Burbank or **B**, Goldrush potato plants infected with PVY^O, PVY^{N_iO}, PVY^{NTN/NA-NTN}, or all possible mixtures of the strains. Experiments were repeated five times using 8 to 24 recipient plants for each treatment. Means within each panel that share the same letters are not significantly different. Error bars denote standard error of mean. Statistical inference was based on logit-link transformed data; untransformed means are shown.

efficiency as measured by the number of plants that become infected following inoculation by viruliferous aphids is far too simplistic an approach to tease out factors that may have profound effects on PVY strain-specific dispersal within and between potato fields.

Virus acquisition by aphids and subsequent transmission efficiency may be affected by vector and host specificity, viral fitness, distribution and concentration of strains and isolates within the plant host, and synergistic and antagonistic interactions among coinfecting viruses, strains, and isolates (Carroll et al. 2016; Cervantes and Alvarez 2011; Domingo 2010; Mondal 2014; Mondal et al. 2016a; Robert and Bourdin 2001; Syller and Grupa 2014; Verbeek et al. 2010; Wargo and Kurath 2012). Host specificity is affected by various factors, including the age of the plant at infection, distribution of virus particles within the plant, the physiological responses to virus infection, and plant resistance genes (Chikh-Ali et al. 2014; Cockerham 1970; Jones 1990). A faster-replicating virus in a coinfection often has a competitive advantage (Zwart et al. 2009). The difference in virus titer and distribution of specific strains and isolates within potato cultivars might partially explain the altered transmission efficiency of the same isolates of PVY^O and PVY^{NTN} from Goldrush, Red Maria, and Russet Burbank. Differences in systemic virus movement and

distribution were reported by Basky and Almási (2005), in which PVY^N was found to increase more rapidly and infect more segments and progeny tubers than PVY^O. A similar phenomenon of higher virus titer was also found in PVY^{NTN}-infected hairy nightshade and Russet Burbank compared with PVY^O and PVY^{N:O} (Cervantes and Alvarez 2011) as well as in PVY^O and PVY^{NTN} mix-infected potato plants derived from true seed of Red Maria (Carroll et al. 2016).

Additionally, host responses to viral infection and plant-resistant genes should be taken into consideration for their effects on transmission efficiencies. PVY^O infection in Goldrush was often accompanied by a systemic vein necrosis and necrotic areas in the systemically infected leaves. This is indicative that Goldrush is carrying one or more of the poorly characterized *Ny* resistance genes (Cockerham 1970) that tend to be strain specific, most notably against PVY^O, and convey a type of resistance manifested as a systemic hypersensitive response restricting local virus movement but allowing systemic movement. Extreme hypersensitive reactions related to *Ny* genes are apparent in some cultivars (e.g., 'Pike') that ultimately result in plant death but also limit virus acquisition by aphids while the plant is growing (Carroll et al. 2016). Interestingly, no visible hypersensitive reactions were observed in Goldrush plants infected with mixtures of PVY^O with PVY^{NTN} or PVY^{NW} (data not shown). The presence of the second

Fig. 5. Identification of the *Potato virus Y* (PVY) strains infecting the **A**, Russet Burbank and **B**, Goldrush plants inoculated by single aphids allowed an acquisition access period on virus source plants infected with two or three PVY strains. Bar graphs represent the breakdown of the total percentage of infected plants into percentages of plants that were infected by each strain or mixture of strains. Means within each inoculation group that share the same letters are not significantly different. Error bars denote standard error of mean. Statistical inference was based on logit-link transformed data; untransformed means are shown. The pie chart in each panel is a graphical representation of the bar graph data based on 100%.

strain may suppress the PVY^O-specific host defense response or may facilitate the local movement of both viruses. These may explain the increased transmission efficiency of PVY^O from Goldrush plants infected with strain mixtures compared with plants infected with PVY^O alone. There is no evidence that Red Maria or Russet Burbank are carrying any of the *Ny* genes. More studies will be needed to better understand how various strains interact with different cultivars and how these interactions may change with the introduction of mixtures of strains in the same plant. Virus movement and distribution in different cultivars will affect not only the importance of these plants as sources of virus for aphids but also the vertical transmission of viruses to progeny tubers.

Differences in PVY strain transmission from single and mixed infections measured as percentage of recipient plants inoculated also does not take into account the potential virus interactions with the aphid. Clearly, from our results and previous studies, a single aphid is capable of concomitant transmission of two strains of PVY, albeit at a lower frequency than transmission of any one PVY strain. Transmission efficiency is influenced by the accessibility of virus to the feeding aphid (i.e., both viruses must be present in the same epidermal cells or in closely associated cells). Although we determined that different virus strains were present in the source tissue on which the aphids were feeding, we have no information on the relative distribution or titers of the different strains in the mixed infections. Facilitative and antagonistic interactions between plant viruses in mixed infections are possible (DaPalma et al. 2010; Dietrich and Maiss 2003; García-Cano et al. 2006; Rochow and Ross 1955; Syller 2012; Takeshita et al. 2004; Vance 1991). Syller and Grupa (2014) reported antagonism between PVY^O and PVY^N and also PVY^O and PVY^{NTN} that resulted in a significant reduction in PVY^O titer. Similar observations were reported by Cervantes and Alvarez (2011) and, in that study, the transmission efficiency of PVY^O from the mixed-infected plants in both studies was much lower than PVY^{NTN}. Data from our unpublished work indicates that relative virus titers of two strains will vary considerably depending on time after infection and the potato cultivar, as well as the strains and isolates within a strain.

Transmission efficiency also is a function of the virus binding to and releasing from virus receptors on the aphid stylets (Blanc et al. 2011; Uzest et al. 2007, 2010). Compounding the stylet binding is a question of whether the helper component (HC-Pro), the virus encoded protein that acts as a bridge to bind virus to the stylet, is strain or isolate specific or generic in its ability to bind multiple strains. The order of HC-Pro-virus acquisition may also affect which strains and their relative proportions are able to bind to the stylets. We have no direct information on which strains may have been bound to or released from the aphid stylets. However, our data suggest that virus availability to the probing aphid in the plant, and not the virus-aphid interaction, is the more important criterion in determining which strain or strains are transmitted. The data in Figure 5 indicate that every two-way combination of virus strains, with the exception of PVY^O + PVY^{NA-NTN}, was able to be acquired and released by individual aphids, but not every combination was easily accessible to feeding aphids. For example, PVY^O + PVY^{N:O} was not transmitted from the double infection in Russet Burbank but, when PVY^{NA-NTN} was added to the mix, PVY^O + PVY^{N:O} were efficiently transmitted together. Similarly, PVY^{N:O} + PVY^{NTN} was not transmitted from the double infection in Goldrush but they were transmitted together from the triple infection. How the addition of the third strain in the infection alters the titer or distribution of the other virus strains to make them more accessible to the aphid is unknown. Less likely, but a possibility, is that the third virus provides a factor that enhances binding or release dynamics in the stylet.

Clearly, the dynamics of PVY strain transmission are complex and influenced by many factors and interactions, and there is much work to be done to better understand all the mechanisms at work. Results from the present study do not support the hypothesis that differential aphid transmission of PVY strains contribute

significantly to the observed recent shift toward recombinant strains in the field; however, controlled laboratory studies cannot mimic all conditions or variables present in the field. The differential transmission of PVY strains observed under field conditions may be different than what has been observed under controlled conditions, especially if other aphid species are the primary vectors. Although difficult, these experiments would best be conducted in the field, where movement of known sources of virus can be attributed to flights of known aphid species moving through the crop. A recent report indicates that spread of PVY^{NTN} is more likely than that of PVY^O, given the experimental conditions in the United Kingdom (Lacomme et al. 2016), and has likely contributed to the increased prevalence of PVY^{NTN} in recent years.

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