Preferential acquisition and inoculation of \( \text{PVY}^{\text{NTN}} \) over \( \text{PVY}^{\text{O}} \) in potato by the green peach aphid \( \text{Myzus persicae} \) (Sulzer)

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In the past decade, the incidence and distribution of the recombinant, tuber necrotic strain of Potato virus Y (PVYNTN) has been increasing in the US seed potato crop while the ordinary strain (PVYO) has been decreasing. The transmission efficiency of both strains was determined from two potato cultivars when acquired sequentially by the same aphid or when acquired by separate aphids and inoculated to the same plant. PVYNTN was transmitted more efficiently than PVYO and the order of acquisition or inoculation did not affect the preferential transmission of PVYNTN. When a recipient plant became infected with both strains, PVYNTN maintained higher titre than PVYO and would facilitate the acquisition of PVYNTN. Furthermore, the acquisition and transmission of PVYNTN over PVYO was enhanced in the potato cultivar that expressed a strain-specific \( \text{Ny} \)-like resistance gene that confers partial resistance to PVYO.
The green peach aphid, *Myzus persicae* (Sulzer), clone used in transmission tests had been maintained on caged Physalis floridana (Rydb.) plants in a plant growth room at our laboratory for approximately 15 years (Gray, 2008). Aphids, starved for at least 1 h, were placed on detached PVY⁰- or PVY⁰TN-infected source leaves and observed for probing behaviour during a 1–3 min acquisition access period (AAP). Individual aphids were transferred to the youngest fully developed leaf on a healthy Red Maria seedling and the plant caged for an inoculation access period (IAP) of at least 2 h. The sequential acquisition experiments, designated O/NTN and NTN/O, used a single *M. persicae* given an AAP on a leaf infected with one strain and then moved immediately to a leaf infected with the other strain for the second AAP before being placed on the recipient plant (Fig. 1). The sequential inoculation experiments, designated O + NTN and NTN + O, used two aphids per plant each given an AAP on a single strain. There was an approximately 20–30 min lag between placing the first and second aphids on the recipient plant (Fig. 1). Control treatments consisted of uninoculated plants and plants inoculated with single aphids that had been given an AAP on PVYO- or PVY⁰TN-infected leaves. The experiment was repeated six times, twice using Pike source tissue and four times using Superior source tissue. In each experiment 20 to 22 recipient plants were used per treatment. Following the IAP, the plants were treated with a mixture of pymetrozine, Endeavour 50WG, at 1.6 g (93 m²)⁻¹ [0.058 oz (1000 ft²)⁻¹] and bifenthrin, Talstar P, at 15 ml (93 m²)⁻¹ [0.5 fl oz (1000 ft²)⁻¹] to kill all aphids before being moved to the greenhouse.

Inoculated plants were observed for symptom development and tested at 35 to 42 days post-inoculation (days p.i.) by TAS-ELISA (Agdia) using a monoclonal antibody (4C3) that recognizes all strains of PVY or monoclonal antibodies specific for PVYO/C serotypes (Mab2) or for PVYN serotypes (1F5) (Baldauf et al., 2006). Source leaves and a subset of plants infected with PVYO and/or PVY⁰TN were also tested by immunocapture multiplex reverse transcription (RT)-PCR (Lorenzen et al., 2006).

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**Fig. 1.** Schematic of the sequential acquisition treatments O/NTN and NTN/O where one aphid was given an acquisition access period (AAP) sequentially on source tissue infected with each strain (PVYO and PVY⁰TN) before being placed on a recipient plant and of the sequential inoculation treatments O + NTN and NTN + O in which two aphids, each given an AAP on tissue infected with one of the virus strains, were used to sequentially inoculate a single recipient plant.
Statistical analyses of experiments were conducted using effect likelihood ratios and odds ratios determined by fitting data to a nominal logistic model, contingency analysis, distribution probability analysis and chi-square tests. Experiment was not significant and experimental results were combined for analyses shown. All analyses were done with JMP Pro version 10.0.2 (SAS Institute) or QuickCalcs (GraphPad Software).

When using infected Pike leaves as the source of virus, the transmission efficiency of PVYNTN was similar across all treatments described in Fig. 1 ($\chi^2 = 1.36, P = 0.8507$). The number of plants infected with PVYNTN was similar whether the AAP was on PVYNTN-infected tissue (18.6 %, $n=43$), on PVYNTN and then PVYO (16.7 %, $n=42$), on PVYO and then on PVYNTN (19.5 %, $n=41$) or when two aphids, each given an AAP on one virus, were placed on the recipient plant in either order (PVYNTN first, 14.0 %, $n=43$; PVYO first, 23.3 %, $n=43$).

In contrast to the mild mosaic symptoms observed on Pike plants infected with PVYNTN, infection with PVYO led to vein necrosis and chlorotic/necrotic spots on leaves. PVYO was not transmitted from Pike source tissue in any of the experiments. The variety ‘Pike’ may possess one or more of the poorly characterized Ny resistance genes that confer incomplete resistance to PVYO, manifested as a hypersensitive response and restriction in virus movement (Rowley et al., 2015). This could have prevented PVYO acquisition and transmission. Ny resistance is often strain specific, temperature sensitive and ineffective against the recombinant strains of PVY, e.g. PVYNTN. Ny-like resistance to PVYO is common in North American cultivars, and reduced spread of PVYO by aphids and through tubers from those cultivars could be contributing to the shift in PVY strain predominance from PVYO to PVYNTN and other recombinant PVY strains.

The Superior plants infected with PVYO or PVYNTN expressed typical mosaic symptoms without vein or leaf necrosis. When leaves from PVY-infected Superior plants were used as the virus source, the number of PVY-infected recipient plants was significantly different across treatments ($\chi^2 = 13.82, P = 0.0168$) (Table 1). PVYNTN transmission efficiency was higher, 28.1 %, than PVYO, 17.5 %. When a single aphid was fed sequentially on PVYO then PVYNTN, or the reverse order, the transmission efficiencies were 19.5 % (O/NTN) and 22.2 % (NTN/O), respectively. The highest transmission efficiencies, 35.4 % and 36.6 %, were in the sequential inoculation treatments, O + NTN and NTN + O, respectively. These higher PVY transmission efficiencies were perhaps expected, since these plants were inoculated by two potentially viruliferous aphids. The transmission efficiencies for PVYO in treatments O + NTN (15.9 %) and NTN + O (19.5 %) were not significantly different ($\chi^2 = 0.38, P = 0.8274$) from the PVYO control (17.5 %) (Table 1). Similarly, PVYNTN transmission efficiency was 24.4 % in both sequential inoculation treatments and not significantly different ($\chi^2 = 0.38, P = 0.8268$) from the PVYNTN control (28.1 %) (Table 1). These results suggest that while multiple viruliferous aphids landing on the plant will increase the probability of infection, the probability of either PVYO or PVYNTN being inoculated does not change, i.e. overall inoculation efficiency by multiple aphids carrying different virus strains is additive relative to single aphids inoculating the plant.

Aphids sequentially acquiring both virus strains in treatments (O/NTN or NTN/O) actually inoculated a successful mixed infection in only one out of 163 plants (0.6 %) (Table 1). Although rare, these results show a single aphid is capable of probing sequentially on two plants, each infected with a different strain, and transmitting both strains to a single plant. Therefore, an aphid need not acquire both strains from one source plant in order to transmit multiple viruses. In fact, acquisition and subsequent transmission of both virus strains from a mixed-infected plant is not generally efficient (Srinivasan et al., 2012; Syller & Grupa, 2014) and mixed-infected plants are not abundant in the field (Gray et al., 2010). Our results suggest that mixed-infected plants would more likely be generated in the field via inoculation by multiple aphids that have fed on plants infected with different strains than by single aphids probing multiple plants singly.

### Table 1. Number of Red Maria seedlings infected with PVY, PVYO, PVYNTN or mixed-infected (Mix) in the four experiments, using Superior as source tissue

Mixed-infected plants are counted also as both O-infected and NTN-infected. Letters next to the numbers indicate significant differences in the odds ratios for uninfected plants across treatments within each Total column.

<table>
<thead>
<tr>
<th>Sequential treatment</th>
<th>Plants (n)</th>
<th>Total PVY</th>
<th>% PVY</th>
<th>Total PVYO</th>
<th>% PVYO</th>
<th>Total PVYNTN</th>
<th>% PVYNTN</th>
<th>Total Mix</th>
<th>% Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>O control</td>
<td>80</td>
<td>14 a</td>
<td>17.5</td>
<td>14 b</td>
<td>17.5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>O/NTN</td>
<td>82</td>
<td>16 a</td>
<td>19.5</td>
<td>3 a</td>
<td>3.7</td>
<td>14 a</td>
<td>17.1</td>
<td>1 ab</td>
<td>1.2</td>
</tr>
<tr>
<td>NTN/O</td>
<td>81</td>
<td>18 ab</td>
<td>22.2</td>
<td>4 a</td>
<td>4.9</td>
<td>14 a</td>
<td>17.3</td>
<td>0 a</td>
<td>0.0</td>
</tr>
<tr>
<td>NTN control</td>
<td>82</td>
<td>23 abc</td>
<td>28.1</td>
<td>–</td>
<td>–</td>
<td>23 a</td>
<td>28.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>O + NTN</td>
<td>82</td>
<td>29 bc</td>
<td>35.4</td>
<td>13 b</td>
<td>15.9</td>
<td>20 a</td>
<td>24.4</td>
<td>4 bc</td>
<td>4.9</td>
</tr>
<tr>
<td>NTN + O</td>
<td>82</td>
<td>30 c</td>
<td>36.6</td>
<td>16 b</td>
<td>19.5</td>
<td>20 a</td>
<td>24.4</td>
<td>6 c</td>
<td>7.3</td>
</tr>
</tbody>
</table>

http://jgv.microbiologyresearch.org
infected with different strains. In the sequential inoculation treatments, four of 29 and six of 30 plants became infected with both viruses in the O+NTN and NTN+O treatments, respectively (Table 1).

When single aphids were given sequential AAPs on both strains of the virus there were significant differences in the numbers of PVYO, PVYNTN- or mixed-infected plants in each of the two treatments (O/NTN, \(\chi^2=15.89, P=0.0004\); NTN/O, \(\chi^2=17.33, P=0.0002\)) (Fig. 2a). PVYNTN was transmitted 3–4 times more efficiently than PVYO, PVY NTN or both strains (Mixture) within the sequential transmission treatments using Superior as source tissue. Letters above the bars indicate significant differences in the distribution of infected plants. (a) Sequential acquisition in which one aphid given an AAP sequentially on each strain, the first listed strain first, was placed on a recipient plant. The order in which the strain was acquired by the same aphid had no significant effect on the numbers of plants infected with either PVYN and PVYNO infected more recipient plants in the sequential inoculation treatments (O+NTN, NTN+O) (Table 1, Fig. 2b), although differences in the number of plants infected with each strain or strain mixture were only significant in the O+NTN treatment (\(\chi^2=7.71, P=0.0055\)). The mechanism for this outcome is unknown, but it is unlikely both aphids would inoculate the same or closely associated cells. Therefore, any synergistic or competitive effects of both strains would occur after many rounds of replication and cell-to-cell or systemic movement. The order of inoculation and perhaps the time interval between inoculations likely influence the outcome. Perhaps PVYN and PVYNO has a stronger silencing suppressor (Kasschau & Carrington, 1998) than PVYO, which could facilitate superinfection of PVYO in a PVYN-infected plant with suppressed plant defences, although PVYN appears to win over PVYO more of the time. Interestingly, in the 11 mixed-infected plants from all treatments (Table 1), PVYN always outcompeted PVYNO. At 35 to 42 days p.i., the average ELISA absorbance values for PVYN were similar in mixed-infected (1.952, \(n=11\)) and singly infected (2.304, \(n=28\)) plants, while the average ELISA absorbance values for PVYNO were 0.230 (\(n=11\)) and 2.469 (\(n=17\)) in mixed-infected and singly infected plants, respectively. At 98 to 140 days p.i., nine surviving mixed-infected plants were retested with TAS-ELISA, multiplex RT-PCR and by two bioassays: back-inoculation to a systemic host, *Nicotiana tabacum*, and to a local lesion host, *Chenopodium amaranticolor*, Coste & A. Reyn. Seven of the nine plants failed to test positive for PVYNO, the other two plants gave slightly above background levels for PVYNO with TAS-ELISA and RT-PCR, but PVYNO was not detected by the plant bioassays. Aphids were allowed an AAP on leaves from these two plants and 20 aphids from each source leaf were transferred to a single recipient plant. PVYNO was transmitted from one of the two mixed infected plants. Finally, tubers collected from the mixed-infected plants were sprouted and the sprouts tested for PVY by TAS-ELISA and RT-PCR; only PVYN was detected in the developing sprouts. Syller & Grupa (2014) also reported that in their experiments PVYN and PVYNO were transmitted with similar efficiencies, suggesting fasting is not a likely factor.

**Fig. 2.** The distribution of Red Maria seedlings infected with PVYO, PVYN or both strains (Mixture) within the sequential transmission treatments using Superior as source tissue. Letters above the bars indicate significant differences in the distribution of infected plants. (a) Sequential acquisition in which one aphid given an AAP sequentially on each strain, the first listed strain first, was placed on a recipient plant. (b) Sequential inoculation in which two aphids each given an AAP on a different strain, were placed on a recipient plant, the first strain listed first.
and that PVYO was often undetected by ELISA by 35 days p.i., but detected by back inoculation to tobacco and by aphid transmission. Again, PVYNTN may have a stronger silencing suppressor giving it a competitive edge or perhaps PVYO is more efficiently targeted by the silencing machinery. Alternatively, the RNA of PVYO might be transcapsidated by the PVYNTN coat protein, directly affecting the ELISA, immunocapture multiplex RT-PCR results and the transmission by aphids, or the PVYNTN RNA may outcompete the PVYO RNA for both coat proteins leaving the PVYO RNA more vulnerable to cellular degradation. Clearly, PVYO survives in some plants at low levels, but PVYNTN has a distinct advantage of being acquired and transmitted from these mixed-infected plants.

PVYNTN transmission efficiency by M. persicae as measured by counting infected plants 35 to 42 days p.i. is higher than PVYO transmission from mixed infections (Srinivasan et al., 2012; Syller & Grupa, 2014) despite the use of different clones of M. persicae, different isolates of the virus strains and different host plant species (both as source and recipient). Also, PVYNTN titre in mixed-infected recipient plants is usually higher than PVYO titre (Srinivasan et al., 2012; Syller & Grupa, 2014 and this study). Our results here suggest PVYNTN will also have a competitive advantage over PVYO as a result of sequential acquisition or inoculation. It is likely that these are consistent phenomena across most isolates within these strains. Whether this is true for other aphid vector species remains to be determined. However, what is unknown is the contribution of each step in this process, i.e. do both strains differ in acquisition and inoculation efficiency or is the differential transmission primarily a result of virus–plant interactions that allow PVYNTN to outcompete PVYO in a greater number of instances? Perhaps aphids actually play a minor or no role in differential transmission, since we do not know if both strains are equally acquired (bound) on the aphid styllet or equally inoculated (released) into the recipient plant with equal efficiency. Our results suggest that aphids do play a role and that PVYNTN is either acquired or released more efficiently.

Nonetheless, because transmission efficiency was measured as the number of plants that become infected 35 to 42 days p.i., it may be that both viruses are equally inoculated into plants by the aphid and the resulting final number of plants infected at 35 to 42 days p.i. is strictly a function of plant–virus interactions and competitions. A careful dissection of mechanisms will require additional carefully planned experiments. Regardless of the mechanisms, it is apparent that the end result is a higher likelihood of PVYNTN-infected plants over PVYO-infected plants via aphid transmission, and perhaps tuber transmission. This may, in part, help explain the observed shift in PVY strain distribution and incidence in the US seed potato crop over the past decade.

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References


