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Preliminary Studies of Age-Related Resistance to *Potato virus Y* in Potato

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Potato virus Y (PVY) management has proven to be a challenge due to complex strain composition, non-persistent transmissibility by many aphid species, vegetative propagation and extensive trade in seed potato tubers, and difficulties associated with breeding for PVY resistant cultivars. Currently, PVY is a major cause of the degeneration of seed that requires a regular flushing-out of seed potatoes after limited field production cycles.

Management of PVY in seed potato production is a multi-faceted affair, each strategy only capable of suppressing a fraction of PVY infection. It is important, therefore, to investigate all potential avenues for reducing the increase in PVY infection rate from generation to generation in seed potato lots. Much effort has recently been placed on late-season PVY management but perhaps not enough on the early weeks of the season. We have, therefore, focused some of our recent efforts on the role and importance of early-season PVY transmission in the overall PVY problem.

In this article we report on preliminary investigations of a potentially important aspect of PVY management called age-related resistance (ARR). Generally, ARR can be defined by changes in resistance of whole plants or plant tissues to plant pathogens in correlation with plant development (Panter and Jones, 2002). In potato, ARR, referred to as the mature plant resistance, was described and studied for many European cultivars against PVY strains, demonstrating development of the ARR through reduced and blocked systemic movement of the virus and translocation into the progeny tubers, starting at 3-4 weeks after emergence (Beemster 1976; Sigvald 1985; Gibson 1991). In North America, PVY^{NTN} comprises 10 to 30% of PVY strains in the field, is a major cause of potato tuber necrotic ringspot disease (PTNRD), and a key focus of our research program. Yukon Gold is highly susceptible to PTNRD caused by PVY^{NTN} making it an ideal cultivar for preliminary studies of the importance of ARR in North American potato production.

The major objective of the current research was to investigate the relationships between the age of Yukon Gold plants at the time of PVY^{NTN} infection and: 1) rates of primary systemic infection, 2) incidence and severity of PTNRD, 3) tuber yield, 4) virus translocation rates into progeny tubers, and 5) rates of secondary infection from primarily infected tubers. The data

obtained suggest that ARR may be important in controlling PVY infection at later stages of potato plant development.

Procedures

Experiments were conducted in the greenhouse of the University of Idaho, Moscow campus during May to August of 2017 and 2018. The PVY^{NTN} isolate HR1 and the PTNRD-susceptible potato cv. Yukon Gold were used. HR1 (GenBank accession number FJ204166) was originally isolated from a potato tuber with PTNRD collected from southern Idaho in 2007. Virus-free tissue culture-propagated plantlets of Yukon Gold were planted in gallon pots of sunshine potting soil mix with slow release fertilizer (Ozmocote™) and grown in a greenhouse. Growing conditions were set at temperature range of 22-24/14-18°C day/night, with 16/8 hrs day/night and natural light conditions during May-August of 2017 and 2018.

A randomized block design (RBD) was used with three blocks and nine treatments. Three replicates of 5 plants each were mechanically inoculated weekly starting from W1 to W8 after transplanting. On each plant, 3 leaflets (typically the leaves 3-5 from the top) were marked and inoculated. When multiple stems on the same plant were encountered, at least 1 leaflet per stem was inoculated. To test for systemic infection, inoculated (1 per plant) and upper non-inoculated leaflets (3 per plant) on each inoculated plant were sampled and tested for PVY infection using TAS-ELISA and IC-RT-PCR (Nikolaeva et al., 2012; Chikh Ali et al., 2013).

Foliage of each plant was pulled out at 12 weeks post-transplanting, and a week later, the tubers were harvested from each plant. For each plant, total tuber number, number of PTNRD-positive tubers, number of necrotic rings/arcs per tuber, and tuber weight were recorded. Tubers from each plant were kept separately in a cloth bag and stored in a dark place at room temperature. At one month after harvest the tubers were again scored for PTNRD expression.

Three months after harvest, two sprouting tubers harvested from the same plant were planted in 4-inch pots, with a total of 30 tubers per treatment (W1-W8 plus the healthy controls), and allowed to grow under greenhouse conditions with 16/8 hrs day/night and temperature of 22°C. Six to seven weeks after planting, emerging plants were scored for systemic foliar symptoms and tested for systemic infection using PVY-specific TAS-ELISA and IC-RT-PCR, to determine possible tuber-borne, systemic PVY infection.

Results and Discussion

For both 2017 and 2018 experiments, the non-inoculated controls showed no symptoms in foliage and were found negative for systemic PVY infection. PVY^{NTN} was detected in both inoculated and non-inoculated leaves of plants inoculated at W1 to W3 and inoculated and most upper non-inoculated leaves of plants inoculated at W4 (Fig. 1), which demonstrates a susceptibility to PVY infection in young plants.

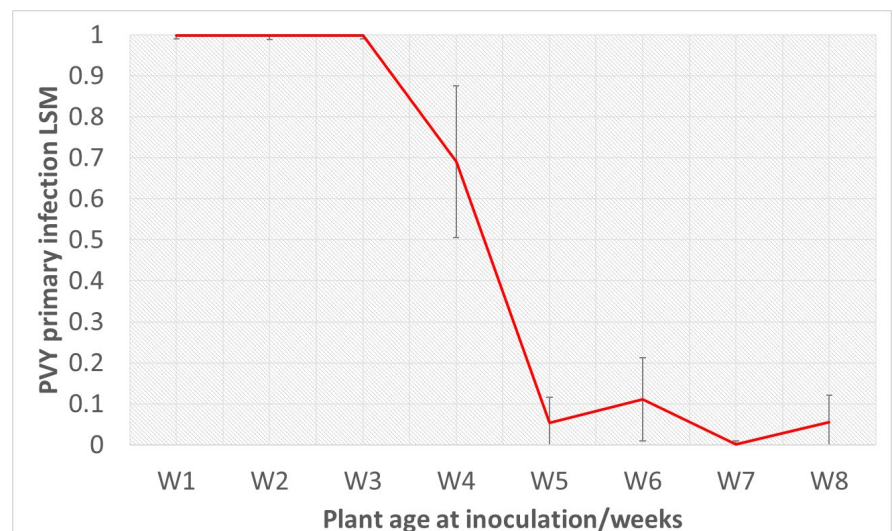


Figure 1. The relationship between the mean rate of systemic primary infection of Yukon Gold (Y axis) following the inoculation with PVY^{NTN} at eight consecutive weeks (W1-8; X axis) after transplanting in a greenhouse experiment in 2018. LSM, Least Square Mean.

Foliage for most of these plants was dead about two months after inoculation while a few plants lasted longer but looked stunted, particularly those of W1 to W3. Plants inoculated at W5 to W8 developed local necrotic reactions on inoculated leaves, typical of a hypersensitive response (HR), followed by the death of the inoculated leaflets similar to earlier weeks. PVY^{NTN} was easily detected in inoculated leaves until their death, but rarely in the upper, non-inoculated leaves for these W5 to W8 plants (**Fig. 1**). In the 2018 experiment, for instance, PVY was detected in inoculated leaves of nine out of fifteen plants of W8, while only a single plant exhibited systemic PVY infection when the upper leaves were tested, and in this single case ELISA signal (OD₄₀₅) exceeded the negative control only by two-fold. It is worth mentioning that even this plant (W8-plant with upper, non-inoculated leaves positive for PVY) produced tubers without PTNRD, and two of these tubers, tested in a subsequent grow-out, produced plants negative for systemic PVY infection. This indicates a restriction of virus systemic movement and increased resistance to PVY in older plants.

Secondary (or tuber-borne) infection showed a similar trend. In 2017 and 2018, all tubers from W1 and W2 plants produced PVY-positive plants (systemic secondary infection; **Table 1**). The number of PVY-positive plants emerging from tubers harvested from plants inoculated at different time points declined starting from W4 (**Table 1**). There were rare cases where late PVY infection did not lead to systemic spread of the virus in the foliage yet resulted in production of PVY-infected tubers. Conversely, some upper, non-inoculated leaves of plants inoculated later in the season (W4 to W8-inoculations) were found positive for PVY (systemically infected with PVY) but produced PVY-negative tubers (impairment of virus translocation to tubers). That was noted in 3 plants in W4 and W8 of 2017 (4% of plants W4 to W8), and 2 plants of W8 of 2018 (3% of plants W4 to W8) where the upper, non-inoculated leaves tested positive, but the two progeny tubers sampled were negative. On several occasions, specifically 9 PVY-positive or PVY-negative plants out of 150 plants at W4 to W8 inoculation time-point, for both 2017 and 2018, produced a mixture of infected and healthy tubers.

Table 1. Percentage of systemically infected potato plants that emerged from tubers originating from plants inoculated with *Potato virus Y* (PVY^{NTN}) at eight different consecutive weeks after transplanting (W1 to W8); two seasons of greenhouse experiments in 2017 and 2018.

Plant age at inoculation ¹	Experiment of 2017 (%) ²	Experiment of 2018 (%) ²
W1	100 (n=20)	100 (n=25)
W2	100 (n=28)	100 (n=21)
W3	83 (n=29)	96 (n=26)
W4	26 (n=30)	57 (n=30)
W5	20 (n=30)	7 (n=30)
W6	7 (n=30)	10 (n=30)
W7	0 (n=26)	0 (n=30)
W8	3 (n=30)	0 (n=30)
Healthy	0 (n=30)	0 (n=30)

¹W, weeks after transplanting at which the inoculation was carried out.

²Total number of tubers that produced PVY-positive plants/total tubers planted n, total tubers tested

A 100% infection rate of Yukon Gold with PVY^{NTN} reduced the average tuber counts by about 50% and the average yield by about 70% as seen in W1- and W2-inoculations. The per plant averages of tuber counts and yield were not affected by the late inoculation (W5-W8) (**Figs. 2, 3, 4**).

More specifically, the dramatic decline in systemic movement of PVY^{NTN} in potato inoculated at W4-W8 (**Fig. 1**) was accompanied by a significant increase in the per plant average of tuber counts and yield compared to W1- and W2- inoculations (**Figs. 2, 3, 4**). Indeed, tuber counts almost doubled in plants inoculated at W4-W8 and equaled the healthy control. Systemic PVY^{NTN} infection in plants of the W3 inoculation did not reduce the tuber counts significantly compared to the healthy control but resulted in significantly smaller tubers and lower average yield (**Figs. 2, 3, 4**).

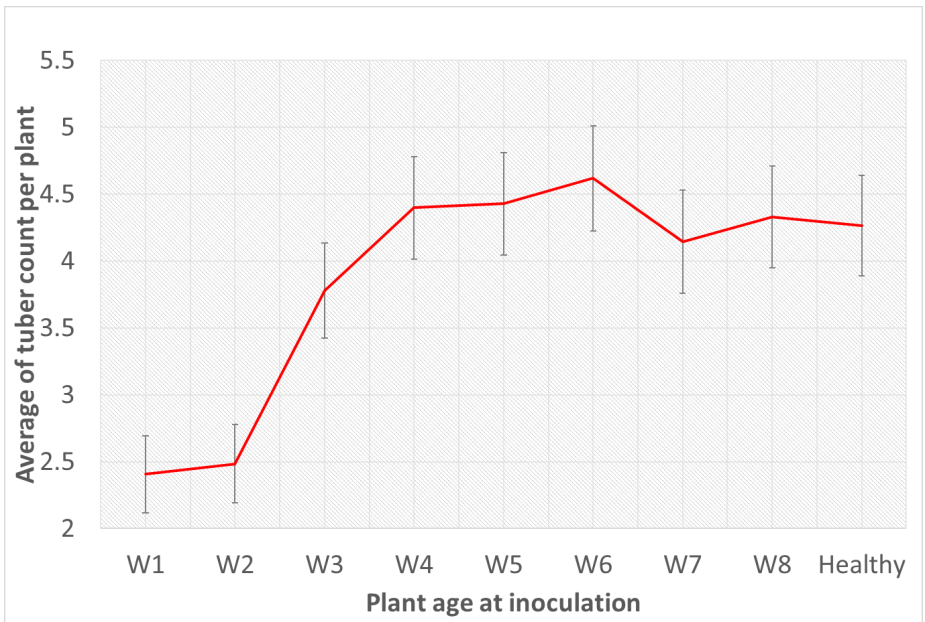


Figure 2. The effects of inoculation with PVY^{NTN} at eight consecutive weeks after transplanting (W1-8; X axis) on the average of tuber counts per a Yukon Gold plant (Y axis) in greenhouse experiments in 2017 and 2018.

It is known that symptoms of PTNRD progress during storage (Le Romancer and Nedellec, 1997). Tubers were therefore kept at room temperature (~20°C) and scored for incidence and severity of PTNRD at 1 month postharvest. High correlation was observed between the incidence of PTNRD and the primary and secondary systemic PVY^{NTN} infection in Yukon Gold. Therefore, PVY^{NTN} infection not only reduced yield, but also dramatically affected quality of tubers (**Figs. 4, 5**), which increased the importance of the PVY control early in the growing season. At one-month post-harvest, about 100% of progeny tubers from plants inoculated at W1 and W2 showed necrotic ringspots in both years (**Table 2; Fig. 4**). Tubers of W3-inoculation exhibited necrotic arcs and ringspots in 75% and 97% of progeny tubers in 2017 and 2018, respectively. The incidence of tuber necrosis dropped to about 30% and 50% of total progeny tubers for W4-inoculation for the two years, respectively. Only about 4% of tubers showed necrotic arcs in plants inoculated at W5, while at W6 about 5% of the tubers showed necrotic arcs and dark halos around tuber stolon ends in 2018 with none in 2017. No PTNRD was observed in tubers from plants inoculated at W7 and W8 as well as the healthy control tubers in either year (**Table 2**). The number of rings/arcs was counted on each tuber as an indicator of the severity of PTNRD. Tubers from

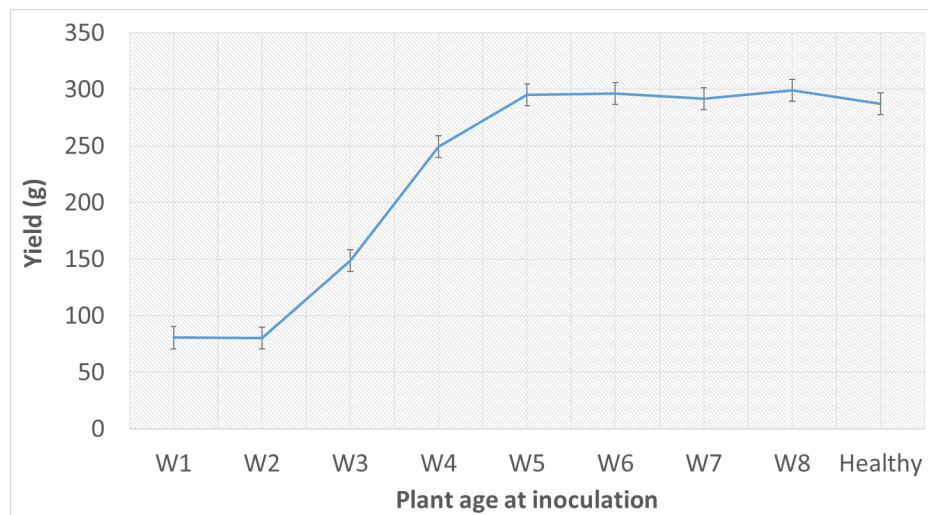


Figure 3. Effects of the inoculation with PVY^{NTN} at eight consecutive weeks after transplanting (W1-8; X axis) on the average of tuber yield per Yukon Gold plant (g; Y axis) in greenhouse experiments during 2017 and 2018 experiments.

Tubers from W7 and W8 as well as the healthy control tubers in either year (**Table 2**). The number of rings/arcs was counted on each tuber as an indicator of the severity of PTNRD. Tubers from



Figure 4. Symptoms of potato tuber necrotic ringspot disease on harvested tubers from plants inoculated at eight consecutive weeks after transplanting in greenhouse experiment 2018.

plants inoculated at W1 and W2 showed the most severe symptoms with more than 6 necrotic rings per tuber, the number decreasing significantly for plants inoculated at W3 to about 2.3 necrotic rings and further in W4 with an average of about 0.5 necrotic ring/arc per tuber. Tubers from plants inoculated at W5 and W6 had a very low average number of necrotic arcs or dark halos around the stolon end, which was not significantly different from W7, W8 and the healthy control.

From a practical standpoint, ARR may be relevant to PVY management in both seed and commercial potato production. The observed ARR in Yukon Gold was established within the first three weeks post-transplantation, and maintained a robust, almost complete control of PVY^{NTN} infection starting from W5-inoculation onward. Consequently, if applicable to other cultivar-strain combinations, and in the absence of a massive early season infestation with PVY, ARR provides sufficient protection to the crop, and commercial potato producers may be assured that the damage to yields and tuber quality caused by late season PVY infection should be negligible as long as they use high-quality certified seed. For seed potato producers, on the other hand, this study points at the early season as a critical time for the efficient protection of potato crops from PVY. If such early season control is provided, it would greatly reduce the number of infection sources in the field available for aphid transmission later in the season and reduce the overall PVY translocation into progeny tubers in otherwise asymptomatic plants missed during visual summer inspections. To assess the practical value of ARR to control PVY, further work on other PVY strains such as PVY^{N-Wi} and main potato cultivars in North America is needed.

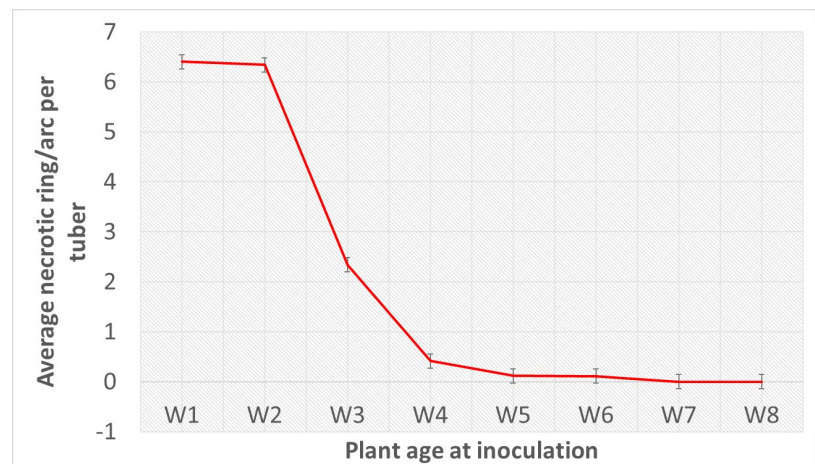


Figure 5. The effects of inoculation with PVY^{NTN} at eight consecutive weeks after transplanting (W1-8; X axis) on severity of potato tuber ringspot disease (PTNRD) on tubers of Yukon Gold plants in greenhouse experiments, in 2017 and 2018 combined.

Table 2. Percentage of tubers of Yukon Gold displaying tuber necrotic ringspots at one month post-harvest from plants inoculated with PVY^{NTN} at eight different consecutive weeks after transplanting; two seasons of greenhouse experiments in 2017 and 2018.

Plant age at inoculation	Average of tubers with PTNRD (%)	
	2017	2018
W1	99	97
W2	100	99
W3	75	97
W4	29	51
W5	5	4
W6	0	6
W7	0	0
W8	0	0
Healthy	0	0

Summary

The recombinant strain of PVY, PVY^{NTN}, is the main cause of potato tuber ringspot disease (PTNRD) in susceptible potato cultivars; it reduces tuber quality and yield. Management of PVY has been a key challenge in most seed potato producing areas. Here, we report the effects of age-related resistance (ARR) in transplants of Yukon Gold under greenhouse conditions. Within the first three weeks after transplanting into soil (W1-3), Yukon Gold plants developed ARR that impaired the systemic movement of PVY^{NTN} into upper, non-inoculated leaves and concomitant translocation into progeny tubers starting from week four (W4) after transplanting. The yield and quality of tubers from PVY-infected plants with the established ARR (W5-W8) were comparable to healthy controls, suggesting late PVY infection would not significantly affect commercial potato production. Potato plants inoculated early (W1-W2), prior to the establishment of the ARR, exhibited 100% primary systemic infection with PVY^{NTN} and produced fewer tubers of smaller sizes, exhibiting PTNRD, which resulted in up to 70% yield reduction compared to plants inoculated later in the season, W5-W8. This ARR greatly restricted the systemic movement of PVY^{NTN} in potato foliage and resulted in very limited translocation rates of the virus into tested progeny tubers at 7.8% and 4.1% in 2017 and 2018, respectively, of all plants inoculated later in the season, W5-W8. This study suggests that, at least in the case of Yukon Gold, PVY management programs should focus more on the early stages of plant development, prior to the onset of the ARR. We hope to build on this preliminary information by conducting related studies on other cultivars and over longer periods of plant growth and development.

For more information, readers are referred to the following research paper, from which the above article was summarized: **Chikh Ali, M., Tran, L., Price, W. and A. V. Karasev (2020) Effects of the age-related resistance to Potato virus Y in potato on the systemic spread of the virus, incidence of the potato tuber necrotic ringspot disease, tuber yield, and translocation rates into progeny tubers. *Plant Disease* 104:269-275.**

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