Research

Greenhouse Comparison of Two Detection Methods for *Potato virus Y*^{N-Wi} at Four Potato Growth Stages

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Abstract

Potato virus Y (PVY) causes significant crop and monetary losses. Owing to the prevalence of newly emerging strains of PVY such as PVY^{N-Wi}, which often cause asymptomatic to mild reactions on certain potato cultivars, accurate tools are required to detect the virus in potato production. This study compared the sensitivity of a rapid field detection method (immunostrips) with a common laboratory detection method (triple antibody sandwich ELISA) on cultivar Chieftain, grown under isolated conditions in a greenhouse and mechanically inoculated with PVY^{N-Wi}, at four potato growth stages (emergence, preflower, postflower, and senescence). Plants inoculated at emergence displayed severe

symptoms of mosaic, veinal necrosis, and leaf drop. Plants inoculated at preflower, postflower, and senescence had veinal necrosis but low or no incidence of mosaic and leaf drop. Overall, few or no tuber symptoms were observed, but a trend of lower tuber yield occurred for emergence-inoculated plants. Low variability in PVY^{N-Wi} detection occurred in both tests for emergence-inoculated plants, whereas those inoculated at preflower and postflower had more variability. Because symptom expression may differ depending on the growth stage when a plant becomes infected, these variations should be heeded with either detection method when collecting samples for PVY testing.

Potato virus Y (PVY) is one of the oldest known plant viruses, and it can cause significant crop and monetary losses (Karasev and Gray 2013). In recent years, PVY has presented significant challenges to the seed potato industry and potato production in the United States, largely owing to the emergence of recombinant strains like PVY^{NTN} and PVY^{N-Wi}, which often cause asymptomatic or only mild reactions on certain potato cultivars (Gray et al. 2010; Karasev and Gray 2013). On foliage, PVY can cause a range of symptoms depending on the cultivar (Gray 2014), and on tubers, PVY can induce potato tuber necrotic ringspot disease (PTNRD) (Gray et al. 2010) and other symptoms such as necrosis and canoe-shaped cracks (Benedict et al. 2015), all capable of impacting both yield and quality.

Reliable and sensitive detection methods are essential for PVY diagnosis and disease management. To obtain immediate confirmation of PVY in field settings, growers have been encouraged to use portable, rapid immunostrip detection kits such as Agdia ImmunoStrips (Elkhart, IN). These kits include a sterile pouch with buffer; a small leaf section is added and macerated within the pouch, and a lateral flow immunostrip then tests for the presence or absence of PVY proteins. To meet seed potato certification standards in some states, many growers send samples to plant diagnostic laboratories and utilize other serological tests such as enzyme-linked immunosorbent assay (ELISA) for PVY confirmation. ELISA is performed in a microtiter plate that uses a series of buffers and washes to detect the protein coat of viruses. ELISA generally is the

most cost-effective method for detection because multiple samples (96 or more) can be processed at once. Some growers utilize polymerase chain reaction (PCR) or quantitative PCR for PVY detection, which are sensitive molecular tests that amplify a specific nucleic acid sequence of interest. However, not all seed potato growers or state Departments of Agriculture have access to these tests, often owing to cost, proximity to a laboratory, or lack of knowledge (Beissinger et al. *in press a*). As a result, many states' seed potato certification standards, such as Washington's, do not require laboratory testing to certify potatoes, and they rely mostly on visual symptoms for detection (Washington State Department of Agriculture 2014).

PVY^{N-Wi} was reported in the Pacific Northwest in 2006 (Crosslin et al. 2006) and in western Washington in 2012 (Benedict et al. 2015) on Chieftain and Yukon Gold cultivars. Now, PVY^{N-Wi} is considered to be the most predominant strain in the region (Dupuis 2017; Mondal et al. 2017). In western Washington, potato growers often depend on immunostrips for PVY detection. But since this shift in PVY strain dominance, some growers have expressed concern about the accuracy of immunostrips that are used during late-season field scouting, and some growers have reported that they have observed different results from immunostrips when they were used to test different leaflets on the same plant at the same or different times. Given the increasingly widespread occurrence of PVY^{N-Wi} in the region and that symptoms caused by PVY^{N-Wi} can be difficult to discern visually (Benedict et al. 2015), the relative reliability of immunostrips for PVY^{N-Wi} has needed verification. Thus, the first objective of this study was to contrast PVY^{N-Wi} detection sensitivity of immunostrips with ELISA, another readily accessible detection method that Washington growers are increasingly interested in utilizing.

Potato cultivars are known to be differentially affected by different strains of PVY (Beissinger et al. *in press b*; Nolte et al. 2004;

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Nie et al. 2012; Shrestha et al. 2014). Much of the existing research, however, focuses on potato cultivars used in processing markets and only describes the obvious foliar symptoms that PVY can induce. Effects of plant growth stage at the time of plant inoculation (Shrestha et al. 2014) are not fully understood. Thus, the second objective of this study was to determine whether inoculation with PVY^{N-Wi} at four potato growth stages (emergence, preflower, postflower, and senescence) under controlled conditions in a greenhouse affects foliar symptom progression, tuber yield, and tuber quality on fresh market cultivar Chieftain.

Virus Preparation

White Burley tobacco seeds (*Nicotiana tabacum*) were sown in Redi-Earth potting mix (Sun Gro Horticulture, Agawam, MA) and misted with tap water in growth chambers lined with fine mesh fabric to prevent insect entry. The chambers were kept at 19°C with fluorescent lighting (Phillips F17T8/TL741, 17 W) adjusted to maintain a 16-h daylight and 8-h dark period. After 8 weeks, the seedlings were transplanted into 2 liter pots with Sunshine potting mix (Sun Gro Horticulture) and watered daily using 20-20-20 fertilizer (Scotts, Marysville, OH) at a 1:100 ratio. Three weeks later, the tobacco plants were mechanically inoculated with PVY^{N-Wi} (isolate N1; serotype O; GenBank genome sequence HQ912863) kindly provided by Alexander Karasev (University of Idaho, Moscow). When the tobacco plants became symptomatic, young infected leaves were collected from two tobacco plants and frozen at -80°C in separate bags. To confirm that the integrity of the PVY^{N-Wi} strain had been maintained, selected tobacco leaves were tested by triple antibody sandwich ELISA (TAS-ELISA) and with reverse transcription PCR at the Karasev Lab (Moscow, ID).

Potato Plant Inoculations

Two trials of the experiment (trials I and II) were conducted in a controlled greenhouse to eliminate the possibility of unwanted aphid transmissions of PVY. Certified virus-free cultivar Chieftain nuclear seed tubers (mini-tubers) were obtained from Valley Tissue Culture (Halstad, MN) and stored in a potato storage cooler maintained at 3 to 5°C and near 100% humidity until planting. On August 4 and 12, 2015, 52 mini-tubers were removed from storage, placed in a paper bag, and set to warm on a bench at approximately 25°C. On August 5 and 13, the nuclear seed tubers were planted in 3.8 liter pots with Sunshine potting mix and were arranged on a bench in a greenhouse maintained at 20°C.

The experiment consisted of four inoculation time points (emergence, preflower, postflower, and senescence) with PVY^{N-Wi} on the plants grown from the nuclear seed tubers. In each trial, three potato plants in four replicates were inoculated at each time point, making a total of 12 plants per time point or 48 test plants. Reference controls

in each trial were included by mechanically inoculating one additional plant per time point with inoculation buffer only. The experiment had a randomized complete block design with a one-way treatment structure, with replicate assigned as block and time point as treatment factor. Care was taken to keep plants from touching one another as they grew.

The frozen infected tobacco tissue was used to mechanically inoculate the test plants in both trials. Frozen leaves first were ground in cold buffer (500 ml of deionized water, 6.7 g of Na₂HPO₄, and 3.3 g of KH₂PO₄) at a ratio of 1:10 (w/v) in a sterile, cold mortar and pestle, and then gently rubbed onto the potato leaves following a light dusting with carborundum (silicon carbide). The inoculated potato leaves were rinsed with a stream of deionized water to remove excess homogenate. Inoculations took place at approximately 21, 40, 56, and 77 days after planting (emergence, preflower, postflower, and senescence, respectively). These dates corresponded to August 25, September 15, October 7, and October 23 for trial I and September 3, September 23, October 16, and October 29 for trial II. PVY presence was confirmed for each batch of prepared inoculum by testing with an Agdia ImmunoStrip prior to inoculation.

During the course of the trials, fine mesh nylon fabric was hung on all doors leading into the greenhouse bay to help prevent aphid entry. The insecticide Entrust (spinosad, Dow AgroSciences, Indianapolis, IN) was applied preventatively at a rate of 0.066 ml/liter to potatoes on September 14 and October 5 to mitigate against a thrips infestation. Plants were examined at each watering to make sure that no aphids were present. Lighting conditions in the greenhouse were maintained with incandescent lamps so that potatoes also were exposed to a 16-h daylight and 8-h dark period. Plants were fertigated daily as described for tobacco above. Plants were observed weekly for foliar symptom development and were rated for presence or absence of typical PVY symptoms such as mosaic, veinal necrosis, and leaf drop (early senescing and necrotic leaves that remain attached to the main stem). The presence of a localized infection (symptoms appearing in an isolated occurrence) or systemic infection (symptoms affecting the entire plant) also was noted.

Twelve weeks after planting, all vines were clipped at the soil line using sterile metal shears. The potato pots then were covered with brown paper and kept in the greenhouse at 20 to 22°C for 2 weeks to allow for the periderm of progeny tubers to mature. During this period, any stem regrowth was cut with sterile metal shears. On November 17 (trial I) and November 23 (trial II), tubers were harvested and evaluated for symptoms. Pots were emptied and tubers gently rinsed with tap water and dried with paper towels. Individual tubers from each plant were visually inspected, counted, weighed, and categorized using a scale of either healthy or 50% or greater of the surface with tuber cracking.

TABLE 1
Number of positive Chieftain plants inoculated with *Potato virus Y*^{N-Wi} at four different potato growth stages and detected by using Agdia ImmunoStrips and triple antibody sandwich ELISA^a

Time of inoculation	Time of testing							
	Emergence		Preflower		Postflower		Senescence	
	Strip	ELISA	Strip	ELISA	Strip	ELISA	Strip	ELISA
Emergence	0	1	20	21	24	24	24	24
Preflower			0	0	10	6	13	14
Postflower					0	1	9	12
Senescence							0	0

^a Trials I and II combined; *n* = 24 plants per time point.

PVY Detection

One week after each time point inoculation, one leaflet section per plant from the second or third trifoliate leaf was removed using sterile metal scissors and was placed into a labeled plastic bag. Plants were immediately tested for PVY using Agdia ImmunoStrips reactive to all known strains of PVY per manufacturer instructions. A positive or negative result was recorded, and all individual strips were preserved. Afterward, the sample trifoliate leaf sections were stored at -80°C . The detection testing occurred in succession such that inoculated plants continued to be tested throughout the trial (i.e., plants inoculated at emergence stage were retested for PVY at the preflower, postflower, and senescence growth stages) to observe if detection sensitivity changed during the experiment. At the end of each trial, approximately 1 week following the final immunostrip testing, all frozen samples were tested for PVY with TAS-ELISA (Ellis et al. 1996) (Agdia PathoScreen kits, PSA 20001/0096) specific to all strains of PVY. Of 240 inoculated samples tested in total with the immunostrips, 100 were positive (41.67%) for PVY. Of 240 inoculated leaflet samples tested in total by ELISA, 103 were positive (42.92%) for PVY.

All statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC) with a statistical significance level of $\alpha = 0.05$. Total tuber yield per plant (in grams) was analyzed using the SAS GLM procedure. Pairwise comparisons were carried out between each inoculation time point. There was no interaction between the two trials (see below), so the data (detection method sensitivity, symptom expression, and tuber yield) were pooled together for all analyses.

PVY^{N-Wi} Infection and Detection Results

PVY infection. PVY^{N-Wi} infection differed by potato growth stage. Infections were more numerous among plants inoculated at emergence, and they occurred in a higher proportion compared with those inoculated at preflower, postflower, or senescence (Table 1). Plants inoculated at senescence had the fewest number of PVY infections.

The types of symptoms differed by potato growth stage. On foliage, mosaic, veinal necrosis, and leaf drop were consistently observed on plants. Those inoculated at emergence had the highest percentage of severe and systemic symptoms, relative to the other growth stages. Plants inoculated at emergence showed mosaic symptoms 2 weeks after inoculation (week 5 after planting) and veinal necrosis and leaf drop symptoms approximately 3 weeks after inoculation (week 6 after planting) (Fig. 1). At 4 weeks after inoculation (week 7 after planting), mosaic symptoms coalesced on plants inoculated at emergence, and the classic light green and dark green patches became difficult to distinguish. Plants inoculated at preflower had low occurrence of mosaic and the next highest percentage of veinal necrosis; both of these symptoms presented in a local, more isolated fashion and began to appear 2 weeks after inoculation (week 8 after planting). Plants inoculated at postflower did not develop mosaic or leaf drop but did have veinal necrosis, which initiated 1 week after inoculation (week 10 after planting). Only a few plants inoculated at senescence expressed symptoms, and these were mostly as veinal necrosis beginning 1 week after inoculation (week 11 after planting). In terms of tuber symptoms, incidence of tuber cracking either was low or absent.

FIGURE 1

Number of weeks after inoculation that Chieftain plants inoculated at four different potato growth stages with *Potato virus Y*^{N-Wi} expressed mosaic (A), veinal necrosis (B), and leaf drop (C) symptoms. Trials 1 and 2 combined; $n = 24$ plants per time point.

There was no significant interaction between trial and time of inoculation ($P = 0.5924$), so tuber yield data from both trials were statistically analyzed collectively. Tuber weight did not differ significantly ($P = 0.1012$) by inoculation time (Fig. 2), although there was a trend toward lower tuber yield for plants inoculated at emergence.

PVY detection. There was little difference in PVY detections between the immunostrip and the TAS-ELISA methods (Table 1). The plants that were inoculated at emergence revealed low to no variability in PVY detection sensitivity between the two methods, especially when tested later in the season at postflower and senescence. Plants inoculated at preflower and tested at postflower showed slight variability between the two methods, and plants inoculated at postflower and tested at senescence exhibited some variability between the two detection methods. Neither method detected PVY in plants that were inoculated at senescence. Finally, no control plants tested positive for PVY by either detection method.

Study Implications

Many studies have shown that plant growth stage can have an effect on susceptibility of plants to virus infection (Beemster 1976; Sigvald 1985; Venekamp and Beemster 1980). With the relative shift in distribution of PVY toward recombinant strains in potato growing regions of the Pacific Northwest and United States (Karasev and Gray 2013; Mondal et al. 2017; Piche et al. 2004), it is important to understand how potato plants react to the time at which PVY^{N-Wi} infections occur. Although ideally, it would have been preferable to set up this study in the field, it was important to eliminate the possibility of current season PVY infections by aphid vectors, which commonly occur during any potato growing season (Johnson 2008). Similar to other studies that have examined PVY and potato yield (Whitworth et al. 2006), this study showed that cultivar Chieftain exhibited severe foliar symptom expression and a trend toward lower tuber yield when inoculated with PVY^{N-Wi} at an early plant growth stage. Although a high incidence of tuber symptoms (such as PTNRD or tuber cracking) was not noted in this experiment, others have reported such findings (Benedict et al. 2015) with PVY^{N-Wi} infections in commercial field settings. Reduction in tuber quality owing to PVY should remain a concern for potato growers and a focus of their PVY management.

FIGURE 2

Mean tuber yield of Chieftain plants inoculated with *Potato virus Y*^{N-Wi} at four different potato growth stages relative to the noninoculated reference control. Trials I and II combined; $n = 24$ inoculated plants per time point; 2 plants per reference control (hence no reference control error bars).

Age-based resistance is a likely factor contributing to lower infection incidence of older potato plants in this study. The results presented here are consistent with other studies that suggest that potato plants are most susceptible to PVY infection within a few weeks of emergence, compared with susceptibility at later physiological growth stages (Beemster 1976; Gibson 1991; Shrestha et al. 2014; Sigvald 1985; Venekamp and Beemster 1980). Further, translocation of PVY has been shown to occur faster in young plants compared with mature ones, owing to a restriction in cell-to-cell movement in leaves (Dupuis 2017). The trend toward lower tuber yields when plants were inoculated with PVY at emergence is consistent with other findings about early plant susceptibility as well (Beemster 1976; Gibson 1991; Shrestha et al. 2014; Sigvald 1985; Venekamp and Beemster 1980).

Reliable and sensitive detection methods are important for disease diagnosis and management. Although there were slight differences in diagnostic test sensitivity in plants that were inoculated at emergence, both tests detected PVY with the same sensitivity when the plants were inoculated at emergence and tested at senescence. However, immunostrip and TAS-ELISA detection results differed somewhat when plants were inoculated at the preflower and postflower stages. Immunostrips detected PVY in 10 plants compared with 6 plants by ELISA when inoculated preflowering and tested postflowering. However, ELISA detected PVY in 12 plants compared with 9 plants by immunostrips when inoculated postflowering and tested at senescence. Possible reasons to explain this difference include low virus titer accumulation in the tested leaves (Liebenberg et al. 2009; Shrestha et al. 2014), uneven distribution of PVY particles throughout the plant (Kogovšek et al. 2011), and rates of virus translocation in the plant (Dupuis 2017). Another reason may be related to the protocol used to sample potato plants in this study. One leaflet per plant from the second or third trifoliate leaf was sampled and was used for the two detection methods. A small section was immediately tested using PVY-specific immunostrips, and all of the remaining leaflet tissue was frozen at -80°C and tested by TAS-ELISA once the trial was harvested. As mentioned earlier, plants that were inoculated with PVY^{N-Wi} at preflower and postflower had higher incidence of veinal necrosis and low to no incidence of mosaic or leaf drop. In this case, if all available symptomatic tissue had been removed for the immunostrip test, the remaining tissue could have had a lower PVY titer, which may have contributed to a difference in detection method sensitivity when compared with TAS-ELISA.

Although this experiment was conducted in a greenhouse, the findings presented in this study can be informative for potato growers and especially seed potato growers, for whom accurate detection of PVY is often imperative. Either method used in this study could suffice when detecting PVY^{N-Wi} in Chieftain prior to plant senescence. To avoid potential discrepancies, it would be best to use a combination of both detection methods if possible. Growers could rely on immunostrips earlier in the season and then utilize ELISA later in the season when a laboratory test might be more sensitive.

From a disease management standpoint, plants that are infected with PVY^{N-Wi} early in the growing season are the most likely to express foliar symptoms and, consequently, be confirmed as positive by a rapid field detection method and/or rogued out of fields. Early detection is important in preventing the spread of PVY^{N-Wi} later in the growing season. Plants infected later in the season are less likely to be rogued out of fields, because they may not exhibit obvious symptoms or provide sufficient living tissue for a positive field test reaction. As a result, such plants may go undetected, posing a risk to other potato plants or even certain weedy hosts of PVY that often surround potato fields. PVY^{N-Wi} that survives in

weeds could serve as an important reservoir and bridge the virus between subsequent growing seasons. Plants subjected to late-season PVY^{N-Wi} infection also may produce asymptomatic infected tubers that are difficult to detect during winter grow-out evaluations (Gray et al. 2010). Detection near the end of the season and at the time of plant senescence is not likely reliable. As such, late-season PVY^{N-Wi} infected plants represent an important source of inoculum that growers should be aware of, especially when timing a PVY detection method.

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