DIFFERENTIATING PVY INFECTION FROM NITROGEN DEFICIENCY IN POTATO USING SPECTRAL REFLECTANCE

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Potato Virus Y (PVY) infection and nitrogen (N) deficiency cause similar symptoms (chlorosis and stunting) on potato foliage. While conventional methods, including ELISA and petiole testing, require destructive sampling and a longer time to diagnose, spectral analysis can be non-destructive, rapid and efficient. Spectral reflectance for potato cultivars representing three market types, chip processing, red-skinned fresh, and fresh and processing russets, were assessed in separate greenhouse trials in response to three N rates (90, 200, and 290 kg/ha) and two $PVY^{N:O}$ infection levels (clean and infected) at 4, 6, and 8 weeks after inoculation (WAI). Normalized Difference Vegetation Index (NDVI) was able to differentiate clean and $PVY^{N:O}$ infected samples of red-skinned and chip processing cultivars, at 4 and 8 WAI, respectively. Overall, cultivars differed in their spectral responses, indicating the importance of studying cultivar-specific spectral responses against PVY infection in future.

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DEDICATION

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LIST OF ABBREVIATIONS

PVY.....Potato Virus Y

N.....Nitrogen

WAI.....Weeks after inoculation with $PVY^{N:O}$

ESN.....Environmentally Smart Nitrogen

ELISA.....Enzyme-linked immunosorbent assay

ANOVA.....Analysis of variance

NIR.....Near Infra-red

SWIR.....Shortwave Infra-red

VI.....Vegetation index

NDVI.....Normalized difference vegetation index

SPAD.....Soil plant analysis development

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INTRODUCTION

Potato Virus Y (PVY), an aphid vectored virus, is considered a major pathogen responsible for considerable potato yield loss and tuber quality defects worldwide (Shukla et al. 1994; Piche et al. 2007; Singh et al. 2008). An increase in PVY incidence in potato crops has been observed in North America over the last decade (Piche et al. 2007; Singh et al. 2007; Gray et al. 2010). Symptoms of infection vary by PVY strain, PVY isolate, potato cultivar, plant physiological condition (plant age), environmental factors (temperature and light intensity), and most importantly, whether the infection is primary (current season infection) or secondary (tuber-borne) (Schubert et al. 2007; Whitworth et al. 2007). In general, PVY infection symptoms include chlorosis, mild to severe mosaic or mottling, which in severe cases may result in leaf drop, as well as leaf and stem necrosis; infected plants may also be stunted (Nie et al. 2012). In addition to the range of foliar expression, some PVY strains may cause potato tuber necrotic ring spot disease, resulting in unmarketable tubers for both consumption and processing, or for use as certified seed (Nie et al. 2012). Relatively newer recombinant PVY strains (e.g., $PVY^{N:O}$) to North America induce very mild symptoms in foliage compared to the ordinary strain (PVY^{O}), and often are not distinguishable as infected with PVY during visual inspection in the field or in the greenhouse (Nie et al. 2012). Additionally, due to the chlorosis exhibited in foliage, symptoms can often be similar to other biotic and abiotic stresses (e.g., nitrogen deficiency) (Stafford 2000; Mahlein et al. 2010). Plant tissue analysis by enzyme-linked immunosorbent assay (ELISA) is one of the most reliable means used for detection of PVY, which can be both laborious and time consuming, when done on a large scale (Naidu et al. 2009). Remote sensing technology has been applied to assess plant diseases in a rapid and non-destructive manner, based on the measurement of reflected light energy from the subject of interest, mainly in the

visible (390-770 nm), Near Infra-red (NIR) (770-1300 nm), and mid infrared (mid IR) (1300-2500 nm) wavelengths (Reynolds et al. 2009). Many virus infections are known to cause changes in leaf pigments, bio-chemical components and metabolic alteration in leaves that influence the spectral signature of the vegetation in their own characteristic way (Mahlein et al. 2013). For instance, due to chlorosis or yellowing (decreasing chlorophyll content) in both PVY infected and nitrogen (N) deficient plants, there may be a decline in the amount of reflected light energy, particularly in the VIS and NIR wavelengths, when compared to healthy plants (Sahoo et al. 2015). As hyperspectral (a series of narrow wavelength bands) remote sensing techniques are capable of measuring the different amounts of light reflection (Reynolds et al. 2009), these may be useful tool, not only to determine PVY symptoms effectively, but also in differentiating PVY symptoms from N deficiency symptoms. Prior to that, it is necessary to study the spectral signature (leaf/canopy) of a specific crop (cultivar or genotype) in response to a particular stress (abiotic and biotic). To our knowledge, this is the first study to investigate the potential of leaf spectral signatures of ten potato cultivars, differentiating $PVY^{N:O}$ infection from N deficiency.

Various vegetation indices were used previously to represent different approaches characterizing plant health in response to stresses (Sims and Gamon 2002; Ray et al. 2011; Lowe et al. 2017). Normalized difference vegetation index (NDVI), a simple, most widely used vegetation index, is determined as a ratio of reflectance in the red and NIR wavelengths, and most importantly, reported to have a stable relationship with the fraction of photosynthetically active radiation absorbed by the plant (Chavez et al. 2009). Therefore, NDVI of ten potato cultivars was also analyzed in this study to determine if we can differentiate PVY infected samples from N deficient ones. This rapid, economical, non-destructive, and potentially more

accurate diagnostic tool could aid potato certification personnel and seed potato producers in avoiding the risk of inaccurate or delayed diagnosis.

LITERATURE REVIEW

History of potato production

Potato is the fourth most important food crop in the world, after wheat, rice, and maize (Birch et al. 2012; Zaheer and Akhtar 2016; Stevenson 2017). The origin of potato was believed to be in southern Peru and northwestern Bolivia at altitudes of 1,220 to 1,829 meters (Extension_section PAA 2010), but recently was amended to include Chile (Spooner et al. 2012). Spanish explorers found a large number of species and land-races of potatoes under cultivation in 1524 during their conquest in South America (Stevenson 2017). It is believed that the native Incas may have been cultivating potatoes 2000 years prior to the Spanish conquering them (Spooner et al. 2012). Soon after, potato was introduced to Spain in about 1570; it quickly spread across the other European countries including Italy, Belgium, Germany, and Ireland (Stark et al. 2003). The introduction of potato in North America is not exactly known, but believed to be by a group of Presbyterian Irish in 1719 (Extension_section PAA 2010).

Botany of the potato

Potato (*Solanum tuberosum* L.) is tetraploid, having 48 chromosomes, and belongs to the nightshade family, Solanaceae (Extension_section PAA 2010). There are about 160 tuber bearing species of Solanums differing in ploidy, and only 20 are considered as cultivated (Extension_section PAA 2010). The economically important part of the potato plant is its underground tubers, which are modified stems (Extension_section PAA 2010). Potato is classified as an herbaceous dicot and is primarily propagated asexually (Zaheer and Akhtar 2016). True potato seed (TPS) is occasionally used to grow potatoes by peasant farmers around the world, but primarily by potato breeders in controlled environments for breeding purposes (Dwelle et al. 2003). Some potato cultivars may produce few or no flowers during their life

cycle, but still may yield better than others because tuberization is independent of flowering (Extension_section PAA 2010).

Potato production in the US

The Food and Agriculture Organization Corporate Statistical Database (FAOSTAT, 2018) ranked the United States (US) fifth in terms of potato production in the world. Total potato production in the US was about 23.6 million metric tonnes in 2018; Idaho ranked first among states, with production of 6.86 million metric tonnes, while North Dakota ranked fourth, producing 1.22 million metric tonnes (NASS 2018).

Potato Virus Y

PVY is a member of the *Potyviridae* family, responsible for potato mosaic disease and considered a major economic disease causal agent in both commercial and seed potato production (Gray et al. 2010). PVY affects both potato yield and tuber quality. In cases of severe infection, 50-80 percent yield loss has been observed in commercial potato fields in Wisconsin (Schramm et al. 1999). Seed potato growers are also at potential risk of having their seed lots rejected from certification due to PVY infection. As a part of seed certification, a strict PVY tolerance level (below one percent in North Dakota and below three percent in Minnesota) has to be maintained; not maintaining the tolerance level can result in seed lot rejection from certification (Schramm et al. 1999). In recent years, such incidents have been reported more frequently than in the past, and thus PVY has been brought back to the center of attention (Fuller et al. 2017; Piche et al. 2007).

PVY has been reported worldwide, not only affecting potatoes, but also other Solanaceous crops, including tomato (*Solanum lycopersicum* L.), tobacco (*Nicotiana tabacum* L.), pepper (*Capsicum spp.* L.*)* and eggplant (*Solanum melongena* L.) (Gray et al. 2010). There

are approximately 50 aphid species that can spread PVY; the green peach aphid (*Myzus persicae* S.) is considered the most efficient (Gray et al. 2010). PVY is transmitted in a non-persistent manner, by which aphids acquire the virus on their piercing stylet while feeding from an infected plant, and release the virus into another plant during probing (Karasev and Gray 2013). It takes aphids less than one minute to acquire and inoculate the virus into several healthy plants (Karasev and Gray 2013). This rapid spread is one of the reasons why applying insecticides has not been found effective in controlling PVY transmission (Nolte et al. 2002; Fuller et al. 2017). Another PVY vector, the soybean aphid (*Aphis glycines* M.), was identified in North Dakota and Minnesota in 2000 (MacRae and Koch 2012). Interestingly, the soybean aphid was found to feed solely on soybean leaves, but in order to look for this suitable food source, they probe other plants such as potato, thus transmitting PVY (MacRae and Koch 2012). Transmission by soybean aphid is efficient only when high numbers (or populations) of soybean aphids are present, because individual soybean aphids are not considered efficient (MacRae and Koch 2012).

PVY can also be transmitted mechanically, by which infected plants can transmit PVY to their neighboring healthy plants through wounds caused by mechanical means, farming, human activity, wind and others (Nolte et al. 2002). If plant sap from a wounded, PVY infected plant touches another wounded, but healthy plant, PVY can be transmitted to that plant via the sap (Schramm et al. 1999). Compared to the rapid and efficient aphid transmission, mechanical transmission is considered to be a slow and inefficient means of spread (Nolte et al. 2002). Mechanical seed cutting can be a means of PVY spread from one seed tuber to another, which is why mechanical seed cutters need to be sanitized properly during use and prior to starting each

day and seed lot (Schramm et al. 1999). However, seed cutting was not found to be a major contributor to the spread of PVY in recent studies (Draper et al. 2002; Fageria et al. 2015).

Visual assessment of PVY symptoms has been very successful as a tool for potato seed certification procedures in the past; however, visual assessment has been found to be risky and inaccurate in recent years (Gray et al. 2010), primarily due to the shifts of PVY strains producing visible mosaic symptoms to strains that induce mild or no symptoms (Karasev et al. 2017). Disease symptoms range from no symptoms, to severe yellowing, and even dying of plants, based on different strains of PVY and the potato cultivar (Nolte et al. 2002; Gray et al. 2010; Fuller et al. 2017). For instance, Russet Norkotah and Shepody show no or very mild foliar symptoms (asymptomatic), but stunting and severe mosaic symptoms have been observed in Russet Burbank (Nolte et al. 2002).

Four strains of PVY have been genetically identified to date (PVY^{O}, PVY^{C}, PVY^{Z} and PVY^{N}), among which PVY^{O}, PVY^{C} and PVY^{N} were found to have non-recombinant genomes (Karasev et al. 2017). Further molecular characterization revealed that the genome possessed by PVY^{Z} strain is recombinant, and built of PVY^{O} and PVY^{N} parental sequences (Karasev et al. 2017). Other recombinant strains ($PVY^{N\text{-}Wi}$, $PVY^{N:O}$, PVY^{NE11}) of PVY were classified as well, based solely on their molecular characteristics, but are not yet defined genetically (Karasev et al. 2017). According to a PVY strain survey in 2004-2006, PVY^{O} was found to be the most abundant PVY strain in North America; this has changed to $PVY^{N:O}$ in 2012, and beyond (Karasev et al. 2017). The ordinary PVY strain (PVY^{O}) exhibits leaf mosaic, rugosity, crinkling, severe leaf mottling, and in some cases, stunting of potato plants (Gray et al. 2010). A relatively uncommon PVY strain, PVY^{C} produces stipple streak in some cultivars and causes leaf drop and dwarfing (Gray et al. 2010). Recombinant strains like PVY^{NTN} and $PVY^{N:O}$ may cause severe

necrotic symptoms in tubers, called potato tuber necrotic ringspot disease (PTNRD) (Extension_section PAA 2010). It results in unmarketable tubers for both the fresh and processing markets (Nolte et al. 2002; Gray et al. 2010; Karasev and Gray 2013). Necrotic PVY strains were first reported in 2002 in northern US potato production fields (Crosslin et al. 2002), and since then, many other recombinant strains of PVY have been identified. Surprisingly, necrotic strains like PVY^{NTN} and $PVY^{N:O}$ display mild or no foliar symptoms compared to the common PVY^{O} strain, and therefore, often go unnoticed during visual inspection of potato fields (Nie et al. 2012).

Nitrogen deficiency

Nitrogen (N) is one of the major limiting factors in growth and development of potato plants (Bélanger et al. 2007; Jain et al. 2007; Birch et al. 2012). Overapplication of N can cause excessive vegetative growth in expense of tuber growth, degrade tuber quality, and pose a risk of N losses to the environment, resulting in groundwater contamination (Jaynes et al. 2001; Jain et al. 2007; Goffart et al. 2008). Nitrogen deficiency greatly reduces potato yield and affects tuber quality (Borhan et al. 2004; Bélanger et al. 2007). Nitrogen is a highly mobile nutrient in soils and most potato cultivars require high amounts of N for optimum yield and tuber quality (Cohen et al. 2010). Compared to the other major crops such wheat, maize, or sugar beet, potato has a rather poorly developed root system, contributing to its relatively low nitrogen use efficiency, ranging between 50 to 60% (Tyler et al. 1983; Rourke 1985; Porter and Sisson 1991; Goffart et al. 2008). Therefore, meeting the high N requirement of potatoes depends on how efficiently N is managed under field conditions (Cohen et al. 2010). Proper N management is a combination of many factors, including determination of field N status, precise and timely application, environmental concerns, and cultivar specific N management (Jain et al. 2007; Cohen et al.

2010). Mismanagement of a single factor can result in overcompensation or a nitrogen deficiency problem under field conditions (Jain et al. 2007).

PVY infection symptoms, such as mild to severe chlorosis and stunting are also common symptoms of nitrogen (N) deficiency (Stark and Westermann 2003; Stafford 2000). Therefore, PVY infected plants producing these symptoms may be mistaken as N deficient plants during visual inspection in potato fields. Effective management techniques cannot be implemented in the crop until the stresses are differentiated. Existing methods for predicting crop N status are based on analysis of randomly collected samples from the field, thus spectral reflectance via remote sensing may provide more precision and accuracy in estimating crop N requirements (Gitelson et al. 2003; Bélanger et al. 2007; Sahoo et al. 2015). The use of remote sensing techniques detecting crop N deficiencies is increasing as a part of precision agricultural practices (Jain et al. 2007; Cohen et al. 2010). Distinguishing between two different stresses has recently been reported using spectral techniques (Gazala et al. 2013; Feng et al. 2016).

Basis of spectral analysis

When electromagnetic radiation (ER) from the sun hits objects on earth, the objects absorb a portion of that energy and reflect the rest (Lowe et al. 2017). The incoming ER is called incident energy (IE), and the amount that reflects after absorption is called reflected energy (RE) (Lowe et al. 2017). Generally, the extent of absorption or reflectance varies from object to object, because of differences in surface features (color, texture, structure etc.), physical and chemical state, as well as geometric circumstances (e.g. incident angle of the sunlight) (Xu et al. 2007; Albayrak 2008). Therefore, the ratio of RE to IE, called spectral reflectance (SR) in remote sensing, has great potential in distinguishing a particular surface or material, in terms of physical, chemical and geometric attributes (Haboudane 2004). Each object has its own spectral signature,

which is graphically represented by plotting all the variations in spectral reflectance as a function of different ER wavelengths (Sahoo et al. 2015).

In plants, leaves are the primary surface for energy and gas exchange (Xu et al. 2007). Regardless of species, the general pattern of the spectral reflectance curve or spectral signature for healthy and green vegetation is similar (Sahoo et al. 2015). However, the presence of different light absorbing pigments (chlorophyll a, chlorophyll b, carotenoids, xanthophylls, anthocyanins, polyphenols etc.), their quantity in leaves, as well as the internal cellular structure of leaf tissues, greatly influence the spectral attributes among plant species (Botha et al. 2010; Gazala et al. 2013). Based on these biochemical and biophysical attributes of vegetation, the reflectance curve is found to be highly responsive in three defined spectral domain: visible (400-700 nm), Near Infra-red (700-1300 nm), and mid infrared (1300-2500 nm) (Sahoo et al. 2015). For instance, in the upper pallisade parenchyma of healthy green leaves, the optimum content of chlorophyll a, chlorophyll b, and other light absorbing pigments cause maximum absorption in both the blue and red regions of the visible domain (Sahoo et al. 2015). Therefore, the reflectance is very low in the blue and red regions, but high in the green region. This is the reason we see plants as green in nature (Sahoo et al. 2015). On the other hand, absorption is very low in the NIR spectrum, as it is not affected by any leaf pigments, and directly reaches to the internal spongy parenchyma of leaves (Mahlein et al. 2010). Spongy parenchyma consists of irregularly shaped cells, and therefore, reflection reaches its maximum due to the refraction of NIR energy in various directions (Strachan et al. 2002; Jain et al. 2007). The third domain, mid infrared, also known as the Shortwave Infra-red (1300-2500 nm) spectrum, is mainly affected by water and other foliar constituents (Sahoo et al. 2015). Overall, healthy vegetation shows low

spectral reflectance in the visible (blue and red region) domain, but maximum reflectance in the NIR domain (Lowe et al. 2017).

Spectral analysis in stress detection

In contrast to healthy plants, plants under different stresses (abiotic and biotic) show significant deviation in their spectral signature response when compared to healthy vegetation (Carter and Knapp 2001; Osborne et al. 2002; Strachan et al. 2002; Zhao et al. 2005; Blackburn 2007; Jain et al. 2007; Grisham et al. 2010). Healthy vegetation tends to show very high spectral reflectance in the NIR region (700 nm – 950 nm) compared to the spectral reflectance of stressed vegetation. In contrast, spectral reflectance for stressed vegetation is higher in the visible region (400 nm to 700 nm) compared to the reflectance for healthy vegetation.

This deviation is believed to be stress specific, and several studies have been conducted to identify the specific wavelengths that are associated with specific stress conditions (Gazala et al. 2013; Feng et al. 2016). Detection becomes more important when two or more stresses cause similar, mild, or no symptoms at all (Gazala et al. 2013). For example, N deficiency (an abiotic stress) and PVY infection (a biotic stress) in potatoes may produce similar chlorosis in leaves; some strains of PVY even cause mild or no symptoms in potato plants (Schramm et al. 1999; Gray et al. 2010). Therefore, visual screening of PVY symptoms becomes difficult and should not be relied on for seed potato certification (Gray et al. 2010).

Although there is no direct research showing how PVY infection affects photosynthesis in potato, studies have found that viral diseases reduce stomatal conductance in sugar beet (Clover et al. 1999), damage thylakoid membranes in potatoes (Salazar et al. 2000), and infect phloem cells (Bélanger et al. 2007). Significant reduction in photosynthesis has been observed in sugar beet (*Beta vulgaris* L.) plants infected with Beet Yellow Virus (BYV) (Clover et al. 1999).

Early detection of potato yellow vein virus (PYVV) was demonstrated in a greenhouse study, where specific changes were observed in the spectral signature due to disturbance of light reflection in infected leaves caused by alterations in internal leaf structures (Chávez et al. 2009). Chávez et al. (2009) also showed that the detection of PYVV disease was possible much earlier using a combination of spectral and multifractal analysis techniques.

Considerable changes in spectral reflectance have been reported in plants with various diseases, such as the infection of Grapevine Leafroll-associated Virus-3 in wine grape cutivars (Naidu et al. 2009), sugarcane yellow leaf virus (Grisham et al. 2010), and potato yellow vein virus (Chávez et al. 2009). Recently, significant changes in spectral reflectance have been observed when healthy soybean plants were compared to soybean plants with yellow mosaic disease (YMD) (Gazala et al. 2013). In that particular study, R688 (688 nm in the red wavelengths) and R750/R445 (750 nm and 445 nm in the red wavelengths) were identified as the yellow mosaic sensitive bands for the first time. Another study showed consistent results in both field and greenhouse studies detecting powdery mildew sensitive spectral bands between 580 nm and 710 nm (Feng et al. 2016).

The potential of predicting plant nutrient content, especially nitrogen, using spectral analysis has been explored by many researchers (Al-Abbas et al. 1974; Zarco-Tejada et al. 2001; Haboudane et al. 2004; Blackburn 2007; Botha et al. 2010; Cohen et al 2010). Chlorophyll content of leaves is suggested to be proportional to the nitrogen content in plants (Mauromicale et al. 2006). Carter and Knapp (2001) showed that the leaf chlorophyll content can precisely be estimated based on the reflectance response in the far-red (700nm) wavelengths. A number of studies demonstrated the response of different crops in terms of their decrease in chlorophyll content under N deficiency (Cartelat et al. 2005; Zhao et al. 2005; Read et al. 2002; Mercure

2004). Osborn et al. (2002) detected N and P deficiency in corn based on leaf chlorophyll concentration using spectral radiance measurements. The sensitivity of hyperspectral reflectance properties of sorghum under N deficiency was reported by Zhao et al. (2005). Canopy reflectance was reported as a parameter for growth assessment of lint yield based on the estimation of N content in the cotton canopy (Zhao et al. 2007). Nitrogen deficiency was detected with 80% precision in potato plants using the combined parameters of reflectance and fluorescence methods (Bélanger et al. 2007).

In addition to nutrient management, spectral analysis has also been considered as a useful tool in turf grass management and forage quality maintenance. Lamb et al. (2002) showed that leaf N concentration and total N content of ryegrass are highly correlated with the leaf reflectance in the 690-740 nm wavelengths. A strong correlation has been observed between forage quality variables (N, P, K acid detergent fiber, and neutral fiber content) and canopy reflectance (mainly in the blue, green, red and NIR bands) in sainfoin (*Onobrychis sativa* Lam.) pastures (Albayrak 2008). Similar studies conducted by Starks et al. (2004) on Bermuda grass indicated a close and linear correlation for forage quality attributes with canopy reflectance using stepwise regression methods. Therefore, spectral analysis has not only been identified as a useful tool in predicting different nutrient content of plants, but also shows potential in differentiating between quality attributes (Albayrak 2008).

PVY infection causes a wide range of symptoms (from latent, to mild chlorosis, to severe mosaic of potato leaves) in potatoes, depending on PVY strain or isolate, cultivar, the type of infection, light intensity, and plant age (Draper et al. 2002; Schubert et al. 2007; Whitworth et al. 2007). In addition to these factors posing an enormous challenge in detecting PVY symptoms in potato, N deficiency may make it more confusing due to their symptom similarities (for example

stunting and chlorosis of leaves). Detecting and distinguishing specific nutrient deficiencies using spectral techniques is possible in crops (Osborne et al. 2002; Christensen 2004; Zhao et al. 2005), including potato (Jain et al. 2007). Spectral analysis was found to be efficient and more precise in predicting N status, compared to other standard methods, such as petiole sap tests, foliar analyses, and use of a chlorophyll meter (Bélanger et al. 2007). For standard methods, direct contact with plants is required, in addition to longer sampling time and plant tissue analysis in the laboratory (Bélanger et al. 2007); whereas, the spectral reflectance technique is both rapid and non-destructive (Gitelson et al. 2003; Bélanger et al. 2007; Sahoo et al. 2015). However, only a few studies reported the potential of using spectral reflectance as a tool for detecting N deficiency in potato (Bélanger et al. 2007; Jain et al. 2007). Remote sensing techniques have also been successfully used to differentiate healthy plant samples from diseased, in various crops (Zhang et al. 2002; Bravo et al. 2003; Steddom et al. 2007; Delalieux et al. 2009). However, the use of spectral reflectance differentiating between stresses has been investigated by very few (Gazala et al. 2013; Feng et al. 2016), and therefore, remains challenging (Stafford 2000). Additionally, crops have specific spectral responses, depending on the stresses they are exposed to (Gitelson and Merzlyak 1996; Mahlein et al. 2010), as each stress has its own characteristic way of affecting a crop by inducing changes in leaf structural, physical and biochemical properties (Gitelson and Merzlyak 1996; Mahlein et al. 2010). Thus, proper knowledge of variety specific spectral response caused by a specific stress is essential.

Vegetation indices (VI) are widely used to analyze the changes in spectral curves due to stress-induced chemical and physiological responses of vegetation (Sims and Gamon 2002; Ray et al. 2011; Lowe et al. 2017). NDVI is the most popular, and widely used, and is highly correlated with plant properties such as chlorophyll content and N concentration (Westerveld et

al. 2003; Dunn et al. 2015). NDVI has also been used for measuring the general health status of crops (Baker et al. 1980; Lasaponara and Masini 2007), prediction of forage quality (Albayrak 2008), detecting shield bug (*Eurygaster integriceps* P.) and late blight disease infestation in major crops, including wheat and potato, respectively (Genc et al. 2008; Ray et al. 2011). The amount of red (668 nm-683 nm) and NIR (898 nm-913 nm) light reflected by the leaf or canopy is measured to calculate NDVI (Reynolds et al. 2009). The advantages of NDVI over other vegetative indices include mathematical simplicity, low sensitivity of soil reflection, and reduced interference by environmental conditions (Gilabert et al. 1997; Chávez et al. 2009).

This search of the literature found no previous studies reporting spectral signatures of potato cultivars in response to both PVY (strain specific) infection and N deficiency. Therefore, the broad objective of this study was to determine the potential of differentiating PVY infection ($PVY^{N:O}$) from N deficiency in ten potato cultivars (representing three market types), using leaf spectral reflectance. The specific objectives were to:

1. Study spectral signatures for ten potato cultivars, in response to three N rates (90, 200, and 290 kg/ha) and two infection levels (clean and $PVY^{N:O}$ infected), at 4 , 6, and 8 WAI (weeks after inoculation with $PVY^{N:O}$).
2. Determine if spectral reflectance can be used to differentiate PVY infection from N deficiency in those ten potato cultivars.

MATERIALS AND METHODS

Plant material

Research trials were conducted at the Agricultural Experiment Station (AES) greenhouse complex located on the North Dakota State University campus, in Fargo, ND. A total of ten potato cultivars representing three market types, were used in the experiments (Table 1). Cultivars were grouped by market type and two separate greenhouse trials (trial 1 and trial 2) were conducted for each market type, for validation purposes (Table 1).

Table 1. List of trials based on three different potato market types (chip processing, red-skinned fresh market and russet) with the length of each trial and the cultivars used in the current experiment

Market types	Trials per market type	Length of trial	Cultivars
Chip processing	Trial 1	May 14, 2018 – July 16, 2018	Atlantic Dakota Pearl Waneta
	Trial 2	May 28, 2018 – July 29, 2018*	Atlantic Dakota Pearl Waneta
Red-skinned fresh market	Trial 1	May 28, 2018 – July 29, 2018	Chieftain Red LaSoda Red Norland
	Trial 2	August 22, 2018 –October 17, 2018	Chieftain Red LaSoda Red Norland
Russet	Trial 1	May 28, 2018 – July 29, 2018	Dakota Russet Russet Burbank Russet Norkotah Shepody
	Trial 2	August 22, 2018 – October 17, 2018	Dakota Russet Russet Burbank Russet Norkotah Shepody

*Trials with the same planting date were conducted in separate greenhouse rooms

A mixture of nutrient-free peat moss and vermiculite were used as a growing medium at a ratio of 8:1 by volume. Each 10L pot was filled with the media mixture, keeping the ratio consistent throughout the six trials. Each pot was considered as an individual experimental unit and consisted of two plants. Two virus-free and optimally sprouted minitubers of the ten cultivars were planted at a one inch depth in each pot, for corresponding trials.

Fertilizer supply

Granular mono-ammonium phosphate (MAP) (11-52-0), Environmentally Smart Nitrogen (ESN) (44-0-0), granular tri-super phosphate (TSP) (0-45-0) and muriate of potash (MOP) (0-0-60) were used in this experiment, as the sources of three major macronutrients, N, phosphorus (P), and potassium (K).

At 20 metric tons yield potential, the recommended rate of N, P and K for potato are 200, 230 and 370 kg/ha, respectively (Franzen 2018). In all the pots, P and K were applied at the recommended rates; only N varied. Nitrogen treatments included: base N (90 kg/ha), optimum N (200 kg/ha), and high N (290 kg/ha). Liquid solutions of $ZnSO_4$, $CuSO_4$, and $MnSO_4$ were applied as a source of Zn, Cu and Mn at the rate of 10mg (Zn/Cu/Mn) per plot, respectively. Two grams of powdered $CaCO_3$ and $MgCO_3$ were applied in each pot as a source of Ca and Mg, respectively. All fertilizers were well incorporated into the media prior to planting.

Experimental design

Separate trials were conducted for each market type. The three factors for individual trials included - N rate (90 kg/ha, 200 kg/ha, and 290 kg/ha N), PVY infection (clean, infected with $PVY^{N:O}$), and cultivar (four for the dual-purpose russet trial, three each for the chip processing and red/fresh trials). Each trial was blocked by PVY infection. Within the blocks, N rate and cultivar were randomized within each of four replicates. Data was collected at two-week

intervals, starting at four weeks after inoculation (WAI) with $PVY^{N:O}$. All three factors and the time (weeks) were considered as fixed factors.

Mechanical inoculation of PVY

Plants were inoculated according to the inoculation technique described by Karasev et al. (2017). Inoculum plants were grown from PVY infected potato tubers. PVY infection was confirmed in inoculum plants by the Enzyme-Linked Immunosorbent Assay (ELISA) prior to inoculation. ELISA testing was carried out as described in Singh et al. (2007). The strain of PVY was confirmed as $PVY^{N: O}$ from immunocapture results (Mallik et al. 2012).

Fresh leaves from infected inoculum plants were diluted in phosphate buffer (50 mM sodium phosphate, pH 7.0) at a dilution rate of 1:10 with mortar and pestle on ice. Plants were mechanically inoculated at the six to ten leaf stage. A very fine abrasive, carburandum, was used to create wounds on three leaflets per plant. The clean treatments were also wounded, likewise using carburandum, and inoculated with water and buffer.

All plants were subjected to ELISA testing to confirm PVY infection, or lack thereof (clean treatment), prior to data collection.

Petiole collection for measuring petiole nitrate concentration

Plant tissue analysis was conducted according to the technique described by Stark and Westermann (2003). The fourth petiole from the top axis of each plant was collected to determine plant nutrient status, starting from four weeks after inoculation (WAI) through eight weeks, at two-week intervals. If the fourth petiole was not available, the next petiole below was collected. Leaflets were detached from the petiole immediately after sampling and petioles were stored in a cooler at 3.3° C until delivery to Agvise Laboratories (Northwood, ND) for determination of plant nitrate-N concentration.

SPAD data collection

Chlorophyll content of plants was measured according to the technique described by Yadav and Ibaraki (2010). SPAD readings were taken from both plants of a plot, for each evaluation date. Initially, a stem was selected from each plant randomly, and a petiole of each selected stem was then marked with a zip tie, in order to keep track of the leaf. A SPAD (Soil Plant Analysis Development) 502 plus Chlorophyll Meter was used to acquire SPAD readings from the two primary leaflets of those marked petioles. Four individual measurements were taken from each of the two primary leaflets and averaged to obtain a mean SPAD value per plant. The two SPAD values from two plants were averaged to obtain a single mean SPAD reading per plot. SPAD value represent the proportion of two absorbance values at the red (640 nm) and at Near Infra-red (940 nm) wavelengths. SPAD value does not have a unit.

Measurement of leaf spectral reflectance

A handheld spectrometer (Ocean Optics, Dunedin, FL) was used to collect leaf spectral reflectance. The hyperspectral spectrometer consisted of two separate spectrometers, an Oceanoptics USB200 +XR-ES (measures reflectance from 200-950 nm wavelengths) and an Oceanoptics NIR-Quest (measures reflectance in the 1050-2500 nm wavelengths). Spectral wavelengths are comprised of visible (400 nm -700 nm), Near Infra-red (NIR) (700 nm -950 nm), and Shortwave Infra-red (SWIR) (1050 nm -2300 nm) regions. Spectral reflectance measurements were recorded at a sampling interval of 0.4 nm for the 200 nm - 950 nm, and 7 nm for the 1050 nm and 2500 nm, by the instrument. To keep the light interference consistent throughout the experiment, measurements were generally taken between 1100 to 1600 h central standard time. There were some cloudy days when the yellow tangsten light in the greenhouse was constantly on and could not be avoided during data collection. The machine was calibrated

using a white and dark reference panel every 30 minutes, and also between trials during data collection, on the same day. Reflectance data was acquired at four, six and eight weeks after $PVY^{N:O}$ inoculation, from the same two primary leaflets the SPAD readings were taken from, throughout the three evaluation dates. Similar to SPAD readings, spectral reflectance does not have a unit.

Deriving Normalized Difference Vegetation Index (NDVI) from leaf spectral reflectance

NDVI values were calculated for samples using the following equation in Excel (Chavez et.al. 2009; Reynolds et al. 2009; Ray et al. 2011):

$$NDVI = (NIR - RED) / (NIR + RED)$$

where, RED = The average reflectance between 620 nm -690 nm wavelengths

NIR = The average reflectance between 700 nm -950 nm wavelengths

Data analysis

Distributions of two datasets (trial 1 and 2 for each market type) for each trait were tested using the Shapiro-Wilk normality test (Shapiro and Wilk 1965). Two datasets (trial 1 and trial 2 for each market type) for each trait (petiole nitrate-N concentration and SPAD value) were combined to perform statistical analysis, when found homogeneous ($P > 0.05$). Separate Analysis of Variance (ANOVA) was carried out for the combined dataset for each trait, by the week of data collection, to determine if significant factor responses occurred. Statistical analysis was done using GLIMMIX procedure in SAS Statistical Software version 9.4 (SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513). Significant differences were tested at the levels of <0.001, <0.01 and <0.5. Mean separation was done by LS means $\alpha = <0.05$, where appropriate. Pearson product moment correlations between petiole nitrate-N concentration and SPAD measurements were performed for each market type, to see the association between two traits.

Scatterplots (SPAD vs petiole nitrate-N concentration) were created for each market type, combining both trials of the same market type (trial 1 and trial 2), using R studio software version 3.5.1 (Rstudio Inc., 250 Northern Avenue Boston., MA 02210). Co-efficient (R^2) values were determined, indicating the strength of association between the two variables. Significance of co-efficients (*P* value) were also estimated.

The entire spectrum (200 nm – 2500 nm) could not be used due to the near zero and high negative values obtained in some regions (200 nm to 399 nm, and 2300 nm -2500 nm), as a form of noise. Two leaf reflectance measurements assessed from the same plot were averaged per wavelength (400 nm to 950 nm and 1050 nm to 2200 nm) in Excel, to provide one reflectance measurement per plot, for analysis. Reflectance datasets were subjected to pre-processing, prior to creating spectral signatures. The Min-Max normalization technique was used to fit reflectance values throughout the wavelengths, in a defined range (0 to 1). The following equation was used to perform the normalization (Al Shalabi et al. 2006):

$$X' = ((X - min\ value\ of\ R) / (max\ value\ of\ R - min\ value\ of\ R) \times (new\ max - new\ min)) + new\ min$$

Where, R = the range of reflectance values per plot, from visible to SWIR wavelengths (each wavelength has it's corresponding reflectance value)

X'= normalized reflectance value of X

X = one reflectance value from R, subjected to normalization

New min = new minimum set value for the normalized reflectance dataset, 0

New max = new maximum set value for the normalized reflectance dataset, 1

Spectral signatures were generated for each potato cultivar, plotting normalized leaf spectral reflectance against wavelengths (visible-NIR (400 nm-950 nm) and SWIR (1050 nm-2200 nm)),

at 4, 6, and 8 WAI with $PVY^{N:O}$. The combined dataset (trial 1 and trial 2) of NDVI for each market type was subjected to ANOVA for detecting significant main or interaction effects of factors. Significant differences were tested at the levels of <0.001, <0.01 and <0.5. Mean separation was done by LS means at α = <0.05, where appropriate. Statistical analysis was carried out using GLIMMIX procedure in SAS Statistical Software version 9.4 (SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513).

RESULTS AND DISCUSSION

Chip processing trials

ELISA testing

To determine if the non-inoculated block was clean and the inoculated block was infected, ELISA testing was done 21 days after inoculation. ELISA testing was carried out as described by Singh et al. (2007). According to ELISA testing, all inoculated plants were $PVY^{N:O}$ infected and all non-inoculated plants were clean (not infected).

Petiole nitrate-N concentration

Nitrogen plays a key role in the production of chlorophyll, which is critical for photosynthesis in plants (Chen et al. 2007). Determining N status in crops is necessary for farmers to avoid crop loss due to N deficiency, as well as minimizing the overapplication of N fertilizers (Goffart et al. 2008). Plants take up N as a mineral nutrient from the soil in the forms of ammonium and nitrate (Kraiser et al. 2011). Petiole nitrate-N concentration is commonly measured in potatoes, as it is closely correlated to plant N status and is the best method for monitoring N status in-season (Zhang et al. 2002; Wu et al. 2007; Goffart et al. 2008). Petioles of the first fully expanded leaf from a stem are collected, leaflets removed, and petioles analyzed for nitrate concentration (Stark and Westermann 2003). An exception of sampling the fourth petiole may result in interpretation errors, because the nutrient content of leaves and plant parts changes during their maturity (Westermann et al. 1994). To interpret the results of petiole tests, researchers have established sufficiency ranges for nutrients at different growth stages of potato petioles (Franzen et al. 2018; Rosen 2018). Suggested sufficiency ranges for nitrate-N in potato petioles are 17,000-22,000 ppm, 11,000- 15,000 ppm, and 6,000-9,000 ppm at the vegetative, tuber bulking, and maturation stages, respectively (Rosen 2018; Franzen et al. 2018). Potato

plants exhibiting petiole nitrate-N concentrations lower than the suggested sufficiency ranges are considered N deficient. Variety specific potato petiole nitrate-curves are also used by many growers as a guide for in-season N fertilization (MacKerron et al. 1995; Bélanger et al. 2003; Alva 2007).

In this study, petiole samples were collected from three chip processing varieties (Atlantic, Dakota Pearl and Waneta), treated with three rates of N (90, 200 and 290 kg/ha), and two PVY infection levels (clean and infected with $PVY^{N:O}$), at 4, 6, and 8 WAI with $PVY^{N:O}$. All plants emerged within one week of planting. Chip cultivars were inoculated with $PVY^{N:O}$ at the 6-9 leaf stage (7 days after emergence), and petiole testing began at 4 weeks post inoculation, approximately 34-36 days after emergence. Therefore, chip cultivars were reaching the tuber initiation stage during the first date of petiole nitrate-N assessment (4 WAI with $PVY^{N:O}$). The second and third petiole tests were conducted approximately at 48-50 (tuber bulking stage) and 62-65 (tuber bulking stage) days after emergence, respectively. Petiole nitrate-N concentration was analyzed by Agvise Laboratories (Northwood, ND), in parts per million (ppm). Distributions of two petiole nitrate-N datasets (trial 1 and 2 of chip processing cultivars) were tested using the Shapiro-Wilk normality test (Shapiro and Wilk 1965); the distributions were not normal at $P < 0.05$. Therefore, log transformation was performed for the petiole-nitrate datasets reducing the skewness, although it did not make the distribution normal. However, homogeneity ($P > 0.05$) between two chip trials allowed them to be combined and analyzed. Analysis of Variance (ANOVA) was performed using the log transformed, combined petiole nitrate-N dataset (see Appendix Table A1). The combined ANOVA results (Appendix Table A1) indicated that Time, in interactions with all other factors (Variety, N rate and PVY infection), was highly significant. This indicates petiole nitrate-N concentration for chip processing cultivars significantly differed

by assessment date. To better understand how factors (Variety, N rate and PVY infection) affected petiole nitrate-N concentration at 4, 6, and 8 WAI, the combined dataset was analyzed by week of petiole nitrate-N assessment (Table 2).

Table 2. Analysis of variance (ANOVA) of log petiole nitrate-N concentration for chip processing varieties Atlantic, Dakota Pearl, Waneta, in response to three N rates (90, 200 and 290 kg/ha) and two infection levels (clean, $PVY^{N:O}$ infected), and three dates (4, 6 and 8 WAI[*]). Petiole nitrate-N data for two replicated chip trials (trial 1 and trial 2) were combined for ANOVA.

	4 WAI		6 WAI		8 WAI	
SOV[a]	**F Value**	**Pr > F**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Variety	33.553	0.000***	0.242	0.785	5.988	0.003**
N rate	28.062	0.000***	12.111	0.000***	7.732	0.001**
PVY	5.561	0.020*	8.435	0.004**	5.325	0.022*
Variety × N rate	9.497	0.000***	8.472	0.000***	1.933	0.111
Variety × PVY	9.938	0.000***	3.280	0.061	0.195	0.831
N rate × PVY	11.512	0.000***	9.030	0.000**	0.894	0.412
Variety × N rate × PVY	7.715	0.000***	2.233	0.070	1.911	0.110

[*]WAI = weeks after inoculation with $PVY^{N:O}$
[a]Source of variation
Level of significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

ANOVA (Table 2) results indicated that, the three-way interaction of the factors (Variety × N rate × PVY) were only significant ($p < 0.001$) at 4 WAI. All possible two-way interactions were significant ($p < 0.001$) at 4 WAI, but none were significant ($p > 0.05$) at 8 WAI. At 6 WAI, the interaction terms N rate × PVY and Variety × N rate were significant at the $p < 0.001$ level, but the interaction between Variety × PVY was non-significant ($p > 0.05$). The main effect of Variety was significant at both 4 WAI and 8 WAI, but non-significant ($p > 0.05$) at 6 WAI. Petiole nitrate-N concentration for chip processing cultivars significantly differed with N rate, at all three assessment dates. PVY was also a significant main factor for differentiating petiole nitrate-N concentration, at all three assessment dates.

Treatment means for the three-way interaction were compared at 4 WAI, and significant differences in petiole nitrate-N concentrations were determined both within and between

treatments, using least square means (Table 3). Overall, petiole nitrate-N concentrations for the three chip processing cultivars were much lower than the sufficiency ranges suggested by various researchers (Rosen 2018; Franzen et al. 2018) for potato petiole nitrate-N levels at different growth stages. The purpose of providing 90, 200 and 290 kg/ha N rates was to attain deficient, optimum, and excessive levels of petiole nitrate-N, respectively. That purpose was not fulfilled by this study, since the three chip cultivars were N deficient based on their petiole nitrate concentrations, regardless of the N rate provided (Appendix Table A2).

Table 3**.** Mean petiole nitrate-N concentration (ppm) for chip processing varieties Atlantic, Dakota Pearl and Waneta averaged across nitrogen rates (90, 200 and 290 kg/ha) and infection level (clean and $PVY^{N:O}$ infected), at 4 WAI (weeks after inoculation with $PVY^{N:O}$).

Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for chip processing cultivars at 4 WAI		
		Atlantic	Dakota Pearl	Waneta
90	Clean	87.5 de*	70.5 de	142.0 cde
	$PVY^{N:O}$ infected	48.0 e	99.5 de	118.0 de
200	Clean	250.0 cd	82.5 de	347.5 bc
	$PVY^{N:O}$ infected	38.0 e	70.5 de	249.5 cde
290	Clean	71.0 de	106.0 de	1109.5 a
	$PVY^{N:O}$ infected	246.0 cde	512.5 b	550.5 b

*Means within and between columns followed by the same letter are not significantly different at α=0.05, based on least squares (LS) means.

According to the literature search, lack of N management, potential leaching of N, unfavorable environmental conditions, and low N-use efficiency of crops are some of the factors that can induce N deficiency in plants (Choudhury and Kennedy 2005; Zhao et al. 2005; Lemaire et al. 2008; Kraiser et al. 2011). Rosen et al. (2013) found a one-time application of ESN (44-0-0) at emergence (broadcasted and incorporated) increased the yield of Russet Burbank over conventional split urea (46-0-0) applications of N (112 kh/ha N at emergence, 23 kg/ha at hilling and four application of 23 kg/ha N during post hilling period at 2-week intervals) in Minnesota. ESN (44-0-0) has also been found to be effective in reducing N leaching in soil (Wilson et al.

2010). In our study, two nitrogenous fertilizers including MAP (11-52-0), and ESN (44-0-0) were used as the sources of N. MAP (11-52-0) was applied as the source of readily available N to plants, whereas ESN (44-0-0) (slow release) was applied to ensure the supply of N later in the growing period. All fertilizers (including the sources of other essential macro and micronutrients) were well incorporated into the growing media, prior to planting. No split doses of N were applied in-season. Plants were watered daily. An increase in soil temperature and moisture increases N release from ESN (Golden et al. 2011). Daily water application, soil temperature, and N release from ESN were not monitored in our study. Thus, definitive conclusions regarding the extremely low petiole nitrate-N concentrations for chip processing cultivars, at all three N rates, cannot be drawn. The method (broadcasting, incorporating) and timing (pre-plant, post-emergence) of ESN application have also been reported by researchers as significant factors influencing N release, and indirectly influencing petiole nitrate-N concentrations in corn and cotton (Yu et al. 2010; Halvorson and Del Grosso 2012; Wang et al. 2013). Therefore, further investigation is needed to elucidate N-rate effects.

Petiole nitrate-N concentrations varied among cultivars in response to the three N rates and two virus infection levels (Table 3). Overall, Atlantic and Waneta had the lowest and the highest mean petiole nitrate-N concentrations, respectively. Dakota Pearl and Waneta petiole nitrate-N levels peaked with the 290 kg/ha N rate, regardless of virus infection level. An increase in N supply is expected to increase the petiole nitrate-N concentration (Dinh et al. 2017). $PVY^{N:O}$ infected samples for Waneta had significantly lower petiole nitrate-N values than clean samples for the 290 kg/ha N rate (Table 3). $PVY^{N:O}$ infected samples of Atlantic had significantly lower mean petiole nitrate-N concentrations when compared to clean (uninfected) samples, at 200 kg/ha N. In contrast, Dakota Pearl $PVY^{N:O}$ infected samples had significantly higher mean

petiole nitrate-N concentrations than non-infected, clean samples at the high N rate (290 kg/ha N) (Table 3). Overall, no clear pattern was observed for varying petiole nitrate-N concentration in response to N rate and PVY infection. To understand how the interaction between N rate and PVY infection affect petiole nitrate-N concentration for chip processing cultivars, further investigation is warranted.

Soil Plant Analysis Development (SPAD)

Chlorophyll is the most important pigment in leaves, capable of channeling the energy of sunlight into chemical energy, by the process of photosynthesis (Chen et al. 2007). Leaf chlorophyll content is used as an indirect measure of N status in plants (Dunn et al. 2018). Chlorophyll from leaves can be extracted conventionally in the laboratory (Murdock et al. 1997; Dunn et al. 2018); however, it is time consuming, destructive, laborious and costly (Loh et al. 2002). The use of a SPAD chlorophyll meter is much simpler, quicker, and non-destructive, and therefore, has been widely used in agricultural research (Loh et al. 2002). The SPAD meter measures the ratio of light transmittance at the red wavelength (640 nm), which is absorbed by chlorophyll, and at infrared (940 nm wavelength), where no chlorophyll absorption occurs (Perry and Davenport 2007). SPAD readings are often correlated with N content to indirectly measure N status (Dunn et al. 2015; Dunn et al. 2018).

In chip processing cultivar trials (trial 1 and trial 2), one young, fully expanded leaf was chosen per plant for measuring SPAD values, beginning at 4 WAI, and at two week intervals through 8 WAI. Each experimental unit (plot) contained two potato plants; therefore, two SPAD measurements were obtained per plot, from the two terminal leaflets of chosen petioles (each from one stem per plant). Measurements were averaged as a single SPAD data point per plot. SPAD datasets for both chip processing trials were homogeneous ($P > 0.05$), and thus, combined

for ANOVA (Appendix Table A3). In the combined ANOVA, time was found to be a significant factor differentiating SPAD readings of chip processing cultivars, in all possible interactions (four-way, three-way, and two-way) with other factors (Appendix Table A3). Weekly assessments (4, 6, and 8 WAI) were subjected to ANOVA separately, to determine how N and PVY treatments affected SPAD values of chip cultivars at each assessment date (Table 4). ANOVA was performed using SAS Statistical Software version 9.4 (SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513). Treatment means were compared when appropriate, using LS means ($p<0.001$) in SAS. ANOVA for 4, 6 and 8 WAI (Table 4) indicated that SPAD values for chip processing cultivars significantly differed ($P<0.001$) for nearly all possible interactions (except for the interaction of variety × PVY at 6 WAI) and the main effects of the factors (except for variety at 8 WAI).

Table 4**.** Analysis of variance (ANOVA) of SPAD values for chip processing varieties Atlantic, Dakota Pearl, and Waneta in response to three N rates (90, 200 and 290 kg/ha), and two virus levels (clean, $PVY^{N:O}$ infected), at three times (4, 6 and 8 WAI[*]). SPAD data for the two replicated chip trials (trial 1 and trial 2) were combined for ANOVA.

	4 WAI		6 WAI		8 WAI	
SOV[a]	**F Value**	**Pr > F**	**F Value**	**Pr > F**	**F Value**	**Pr > F**
Variety	397.232	0.000***	9.121	0.000 ***	2.913	0.058
N rate	301.012	0.000***	43.921	0.000***	17.161	0.000***
PVY	336.294	0.000***	191.560	0.000***	144.021	0.000***
Variety × N rate	220.220	0.000***	38.241	0.000***	30.898	0.000***
Variety × PVY	10.071	0.000***	0.409	0.671	4.863	0.009**
N rate × PVY	15.425	0.000***	16.384	0.000***	15.012	0.000***
Variety × N rate × PVY	20.783	0.000***	9.188	0.000***	5.413	0.001***

[*]WAI= weeks after inoculation with $PVY^{N:O}$
[a]Source of variation
Level of significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

SPAD values have been reported to be positively correlated with leaf chlorophyll content by many researchers (Carter and Knapp 2001; Gitelson and Merzlyk 1996, Gitelson et. al. 2003). Chlorophyll tends to decline compared to other pigments, such as carotenoids and anthocyanins,

as plants are under stress or approaching senescence (Gitelson and Merzlyk 1996, Gitelson et.al. 2003). Figure 1 indicates a considerable decline in SPAD values for all three chip cultivars with increasing time (4, 6, and 8 WAI) post inoculation with $PVY^{N:O}$. As N deficiency results in chlorosis of leaves due to a decline in chlorophyll content, this decline in SPAD values was expected (Zhao et al. 2005). In our study, N deficiency symptoms (chlorosis) were not visually present in leaves at 4 WAI; but by 5 WAI, plants began yellowing, which became more prominent in the following weeks. By 8 WAI, the three chip processing cultivars were senescing. As expected, a decline in SPAD values increased with N deficiency and senescence. SPAD values of chip processing cultivars were the lowest at 8 WAI (Figure 1iii, 1vi, 1ix), compared to the other two SPAD assessment dates (Figure 1i, 1iv, 1vii, 1ii, 1v, 1viii).

At 4 WAI, SPAD values for chip processing cultivars were significantly reduced by $PVY^{N:O}$ infection for all N rates (Figure 1i, 1iv, 1vii). At 6 and 8 WAI, PVY infection significantly affected SPAD values for Atlantic and Waneta, for the low and high N rates (Figure 1ii, 1viii, 1iii, 1ix). However, for Dakota Pearl, significant reductions in SPAD values were observed due to PVY infection, at both 6 and 8 WAI, regardless of N rate (Figure 1v, 1vi). These results suggest that PVY infection can negatively affect SPAD values. Rosyara et al. (2007) reported that spot blotch disease resulted in significantly lower SPAD values for susceptible wheat cultivars compared to tolerant wheat varieties; this was attributed to lower leaf chlorophyll content in leaves of susceptible wheat cultivars.

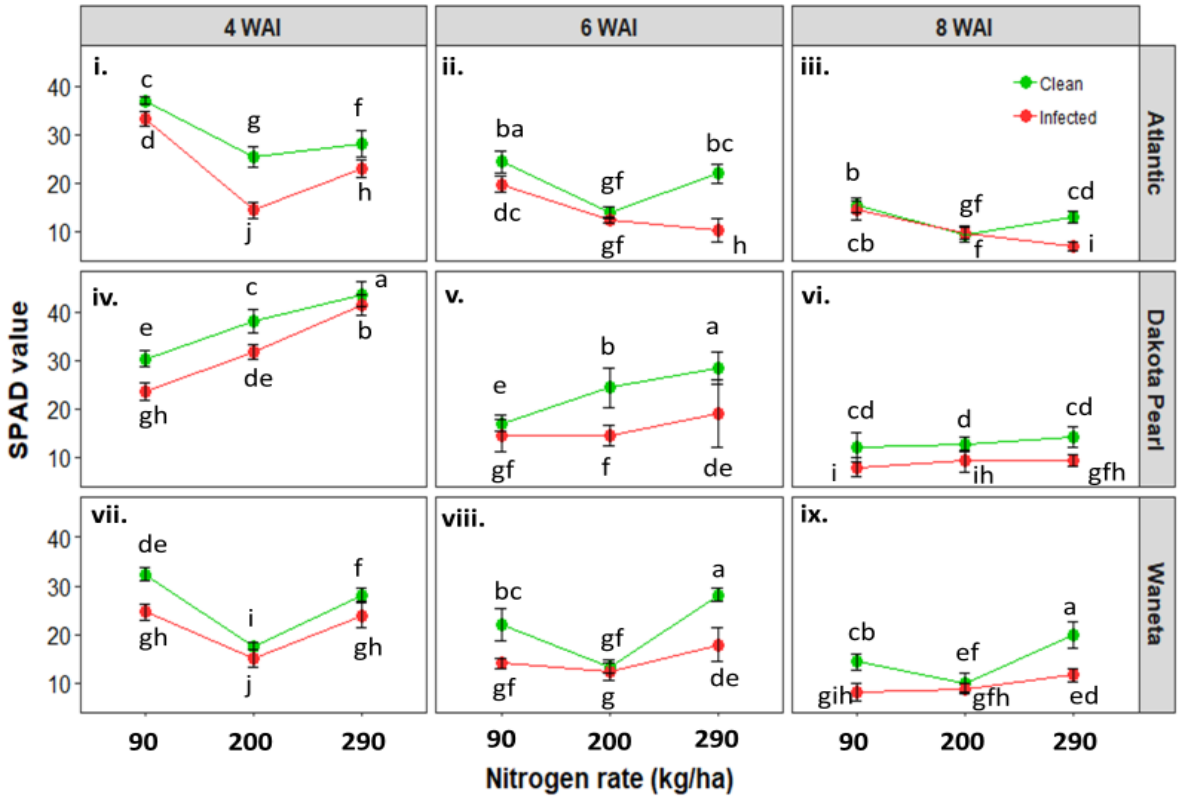

Figure 1**.** SPAD values for chip processing cultivars (Atlantic, Dakota Pearl, Waneta) in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean, $PVY^{N:O}$ infected), at 4, 6, and 8 WAI. WAI = weeks after inoculation with $PVY^{N:O}$. The X axis denotes N rate (90, 200, and 290 kg/ha) and the Y axis represents the SPAD values. Each of the three horizontal plots (from left to right, on the same row) represent SPAD values for the same chip processing cultivar, at 4, 6, and 8 WAI, respectively. Each of the three vertical plots (from top to bottom, on the same column) represent SPAD values assessed on the same date for Atlantic, Dakota Pearl, and Waneta, accordingly. Means were compared within and between each of the three vertical plots, using LS means followed by the same letter are not significantly different at $\alpha = 0.05$. Green and red dots connected with green and red lines denote SPAD values of clean and infected samples, respectively.

Significant differences in SPAD values were observed in response to N rate, among cultivars, for most SPAD assessment dates (Figure 1). Atlantic and Waneta responded similarly (decreasing SPAD value with N rate 200 kg/ha N, followed by an increase in SPAD with higher N rate 290 kg/ha N) for SPAD value, throughout the course of study, for each N rate. SPAD values for Dakota Pearl increased with increasing N rate, for all assessment dates (Figure 1iv, 1v, 1vi). Several studies reported an increase in SPAD value with an increase in N rate, mainly due

to high chlorophyll content in leaves (Blackmer and Schepers 2013; Fox et al. 2001; Jia et al. 2007; Dinh et al. 2017). According to some studies, SPAD value did not show a consistent trend with N rate (Dunn et al. 2015; Loh et al. 2002; Basyouni et al. 2015).

Correlation between petiole nitrate-N concentration and SPAD value

In this study, two independent variables, petiole nitrate-N concentration and SPAD value were subjected to correlation analysis. Datasets for each variable were comprised of both chip trials (trial 1 and trial 2). Treatment means were averaged across replicates to determine the association of SPAD value and petiole nitrate-N concentration by Pearson product moment correlation. The non- transformed dataset for SPAD value and petiole nitrate-N concentration was used in the correlation analysis. Figure 2 summarizes the results of correlation analysis. Nonsignificant correlation ($p > 0.05$) was found between the SPAD value and petiole nitrate-N concentration for most of the combinations of chip processing cultivars (Atlantic, Dakota Pearl and Waneta) and infection levels (clean and $PVY^{N:O}$ infected), except for Atlantic ($PVY^{N:O}$ infected) and Waneta (clean). SPAD and petiole nitrate-N concentrations were significantly correlated for Atlantic ($PVY^{N:O}$ infected) and Waneta (clean) combinations at the significance levels of 0.001 and 0.01, respectively. SPAD readings have been reported to be highly and positively correlated with leaf N content for various field and ornamental crops, such as wheat (*Triticum aestivum* L.) (Jia et al. 2007), corn (*Zea mays* L.) (Blackmer and Schepers 2013; Fox et al. 2001), and geranium (*Pelargonium* spp.) (Dunn et al. 2015). Nonsignificant correlations observed between SPAD value and petiole nitrate-N concentration in this experiment are contradictory to those studies. Previous studies focused primarily on determining the effects of N rate or chlorophyll content on petiole nitrate-N and SPAD readings in various crops (Vos and Bom 1993; Zhang et al. 2002; Wu et al. 2007; Cohen et al. 2010; Dunn et al. 2015; Dunn et al.

2018). The effects of disease severity on these two traits were also studied by a few researchers (Rosyara et al. 2007; Rashid 2018); however, there are no studies where the responses of SPAD and petiole-nitrate concentration were assessed for both N rate and PVY infection in potatoes.

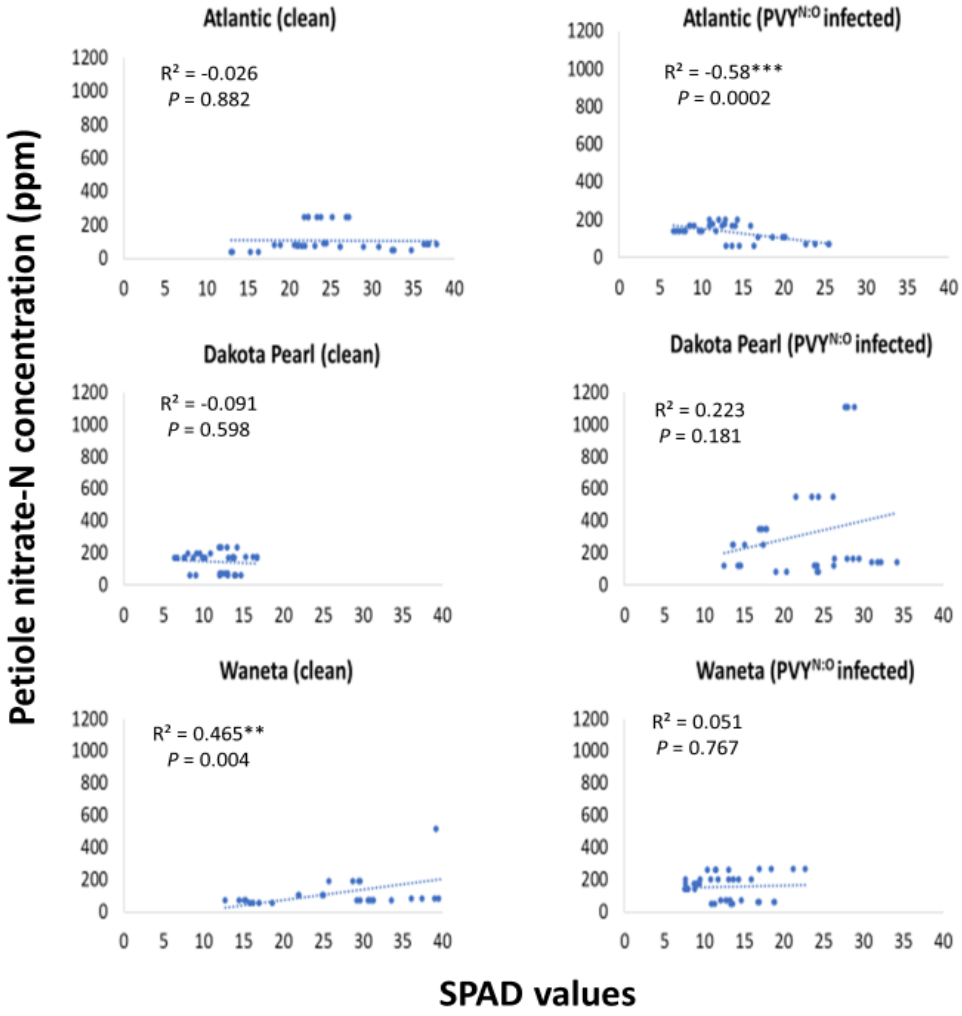

Figure 2. The Pearson product moment correlation between SPAD and petiole nitrate-N concentration, for three chip processing cultivars (Atlantic, Dakota Pearl, and Waneta), in response to three N rates (90, 200, and 290 kg/ha) and two virus infection levels (clean and $PVY^{N:O}$ infected), at 4, 6, and 8 WAI. R represents the correlation coefficient and *p* value denotes the significance of the correlation. Level of significance *** $p < 0.001$; ** $p < 0.01$. SPAD and petiole-N concentration datasets for trial 1 and trial 2 were combined for the correlation analysis.

Therefore, it is not clear if the correlation between SPAD and petiole nitrate-N concentration was affected by other factors that were not considered in this experiment. For

instance, SPAD readings were recorded from the same primary leaflets of marked petioles for three assessment dates; however, petiole samples were collected from more than two stems per plot, but not exclusively from the stem holding the marked petiole used for SPAD reading. Additionally, when the fourth petiole was absent due to previous petiole collection, the next available petiole below the fourth was collected for the next date of petiole nitrate-N assessment. These might have induced sampling inconsistencies and consequently contributed to the lack of correlation between the two parameters. Correlation may also be affected by time of sampling (Dunn et al. 2015), leaf age (Loh et al. 2002), leaf thickness (Peng et al. 1992), and water stress (Basyouni et al. 2015). Therefore, future studies predicting N status from SPAD value and petiole nitrate-N concentration, should consider possible factors that may induce variability in SPAD values and petiole nitrate-N concentrations.

Spectral response

Spectral curves allow researchers to see how different wavelengths in the spectra respond to the treatments provided (Naidu et al. 2009). Leaf spectral reflectance was measured for three chip processing cultivars (Atlantic, Dakota Pearl and Waneta), in response to three N rates (base 90 kg/ha N, optimum 200 kg/ha N and high 290 kg/ha) and two virus infection levels (clean, and infected with $PVY^{N:O}$). Measurements were recorded three times (4, 6, and 8 WAI) following inoculation with $PVY^{N:O}$, using a hand-held spectrometer. All reflectance responses were grouped into two major wavelength sections (a.Visible and NIR (400 nm to 950 nm) and b. Shortwave Infra-red (SWIR) region (1050 nm to 2250 nm)). The spectrometer was not able to record reflectance measurements in between 950 nm and 1050 nm wavelengths. Two leaf reflectance measurements were recorded from the same primary leaflets SPAD readings were captured for, and averaged to obtain one mean spectral response per plot.

Prior to creating spectral signatures, spectral reflectance datasets were normalized, using a Min-Max normalization technique (Al Shalabi et al. 2006). Normalized reflectance measurements for replicates were averaged per treatment (clean + 90 kg/ha, infected + 90 kg/ha, clean + 200 kg/ha, infected + 200 kg/ha, clean + 290 kg/ha and infected + 290 kg/ha) to produce six spectral curves, plotted against their corresponding wavelengths, to create a spectral signature for each chip processing cultivar, at 4, 6 and 8 weeks after inoculation with PVY $^{N:O}$ (Figure 3, 4, and 5). Spectral curves were generated using Excel. For chip trial 1, all reflectance data recorded at 4, 6, and 8 WAI were usable (Figure 3, 4, and 5); however, for chip trial 2, only reflectance data assessed at 4 WAI could be used (Figure 6). The 6 and 8 WAI reflectance measurements for trial 2 could not be used due to extreme noise in the spectra.

The SWIR region provided more distinguished spectra for individual treatments when compared to the visible and NIR regions for Atlantic (Figure 3). For all three assessment dates, spectral reflectance difference increased between clean versus infected samples, with increasing time after inoculation, becoming most prominent at 8 WAI (Figure 3). N treatments were also clearly differentiated at 8 WAI, both in the visible-NIR (except 850-950 nm) and SWIR regions (Figure 3).

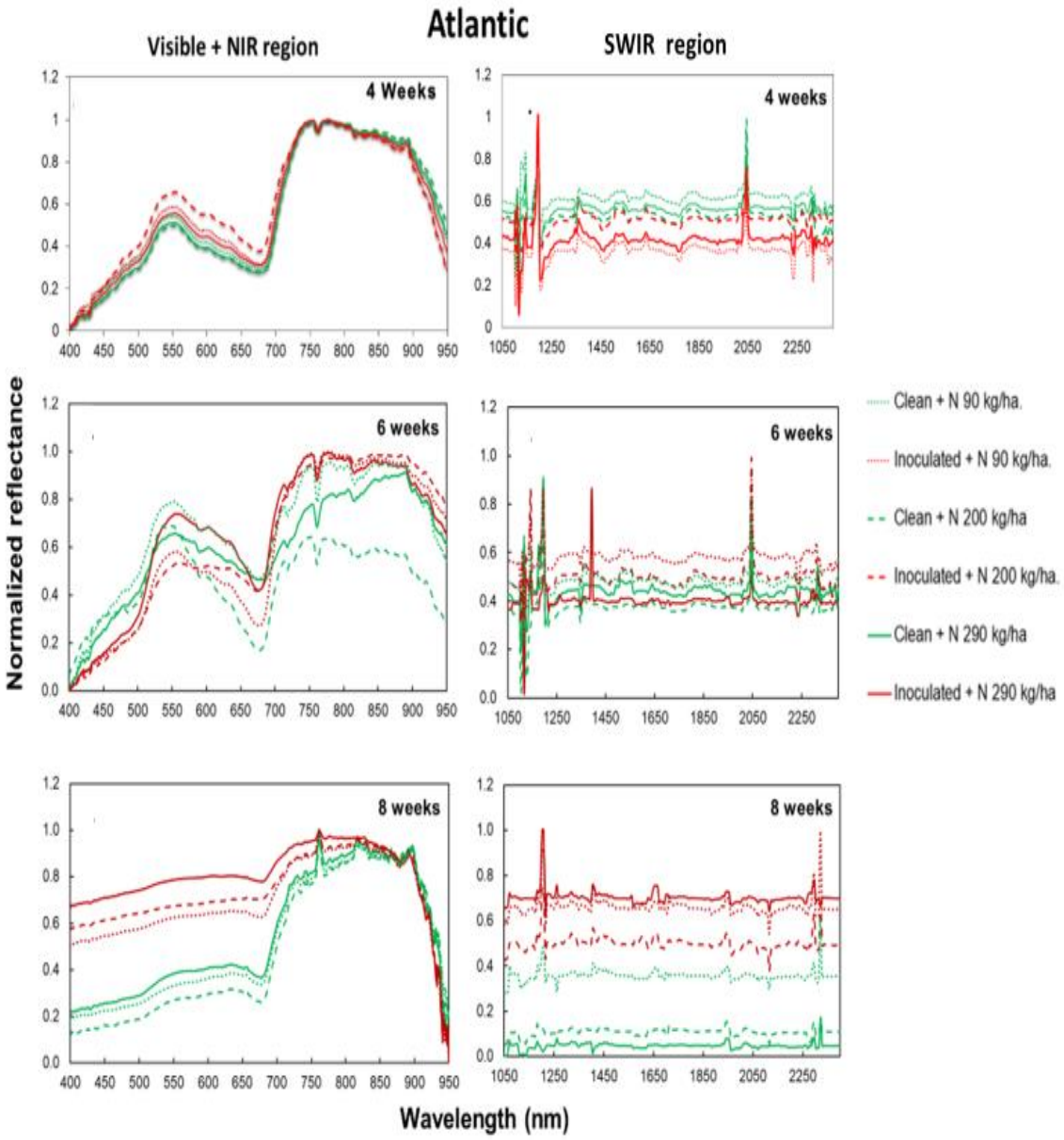

Figure 3**.** Normalized spectral reflectance curves for Atlantic (chip trial 1) in the visible, Near Infra-red (NIR), and Shortwave Infra-red (SWIR) regions recorded 4, 6, and 8 weeks after inoculation with $PVY^{N:O}$. Three nitrogen rates (90, 200, and 290 kg/ha) in combination with two virus infection levels (clean and infected with $PVY^{N:O}$) were tested.

Spectral curves distinguished clean and $PVY^{N:O}$ infected samples for Dakota Pearl at 4, 6 and 8 WAI, most noticeably at 4 and 8 WAI (Figure 4), specifically in the range of 400-600 nm wavelengths (visible range). N rate was also clearly differentiated at 8 WAI, especially in the SWIR region (Figure 4), although not as clearly as for Atlantic (Figure 3).

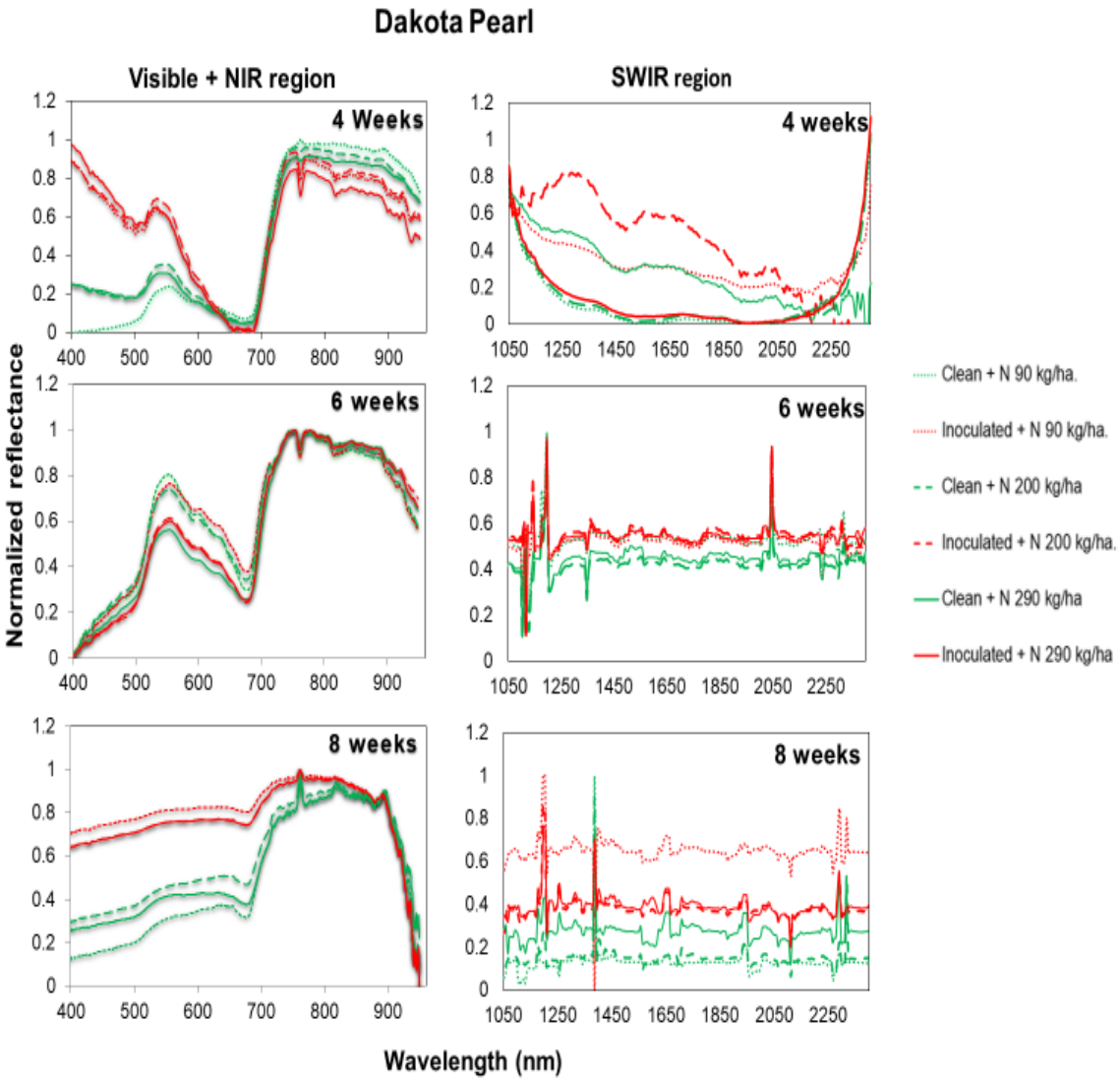

Figure 4**.** Normalized spectral reflectance curves for Dakota Pearl (chip trial 1) in the visible, Near Infra-red (NIR), and Shortwave Infra-red (SWIR) regions recorded 4, 6, and 8 weeks after inoculation with $PVY^{N:O}$. Three nitrogen rates (90, 200, and 290 kg/ha) in combination with two virus infection levels (clean and infected with $PVY^{N:O}$) were tested.

Reflectance patterns for Waneta at 4 and 8 weeks post $PVY^{N:O}$ inoculation indicated distinct differentiation between clean and $PVY^{N:O}$ infected samples for all N rates, compared to the assessment at 6 WAI (Figure 5). Clear differentiation between clean and $PVY^{N:O}$ infected samples were observed in the visible-NIR region, mainly from the 400 nm to 700nm wavelengths for the 4 and 8 week assessment times (Figure 5). At 8 WAI, spectral curves for

Waneta were more similar to 8WAI spectral curves for Atlantic and Dakota Pearl. Differentiation between clean and infected samples were also observed throughout the SWIR region, for all three dates of assessment (Figure 5). But no regions were identified as consistently differentiating N treatments for any clone or time.

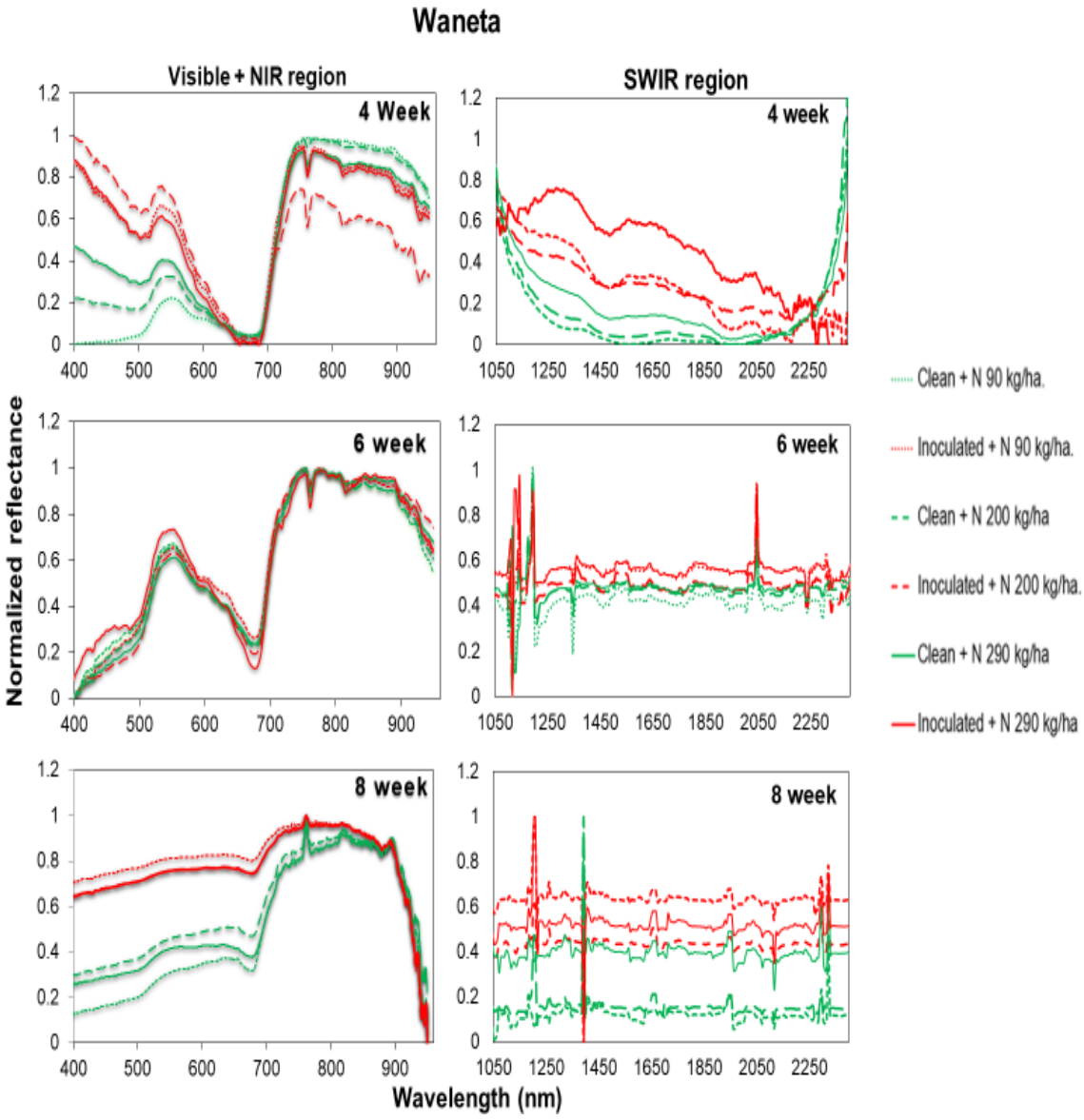

Figure 5**.** Normalized spectral reflectance curves for Waneta (chip trial 1) in the visible, Near Infra-red (NIR), and Shortwave Infra-red (SWIR) regions, recorded 4, 6, and 8 weeks after inoculation with $PVY^{N:O}$. Three nitrogen rates (90, 200, and 290 kg/ha) in combination with two virus infection levels (clean and infected with $PVY^{N:O}$) were tested.

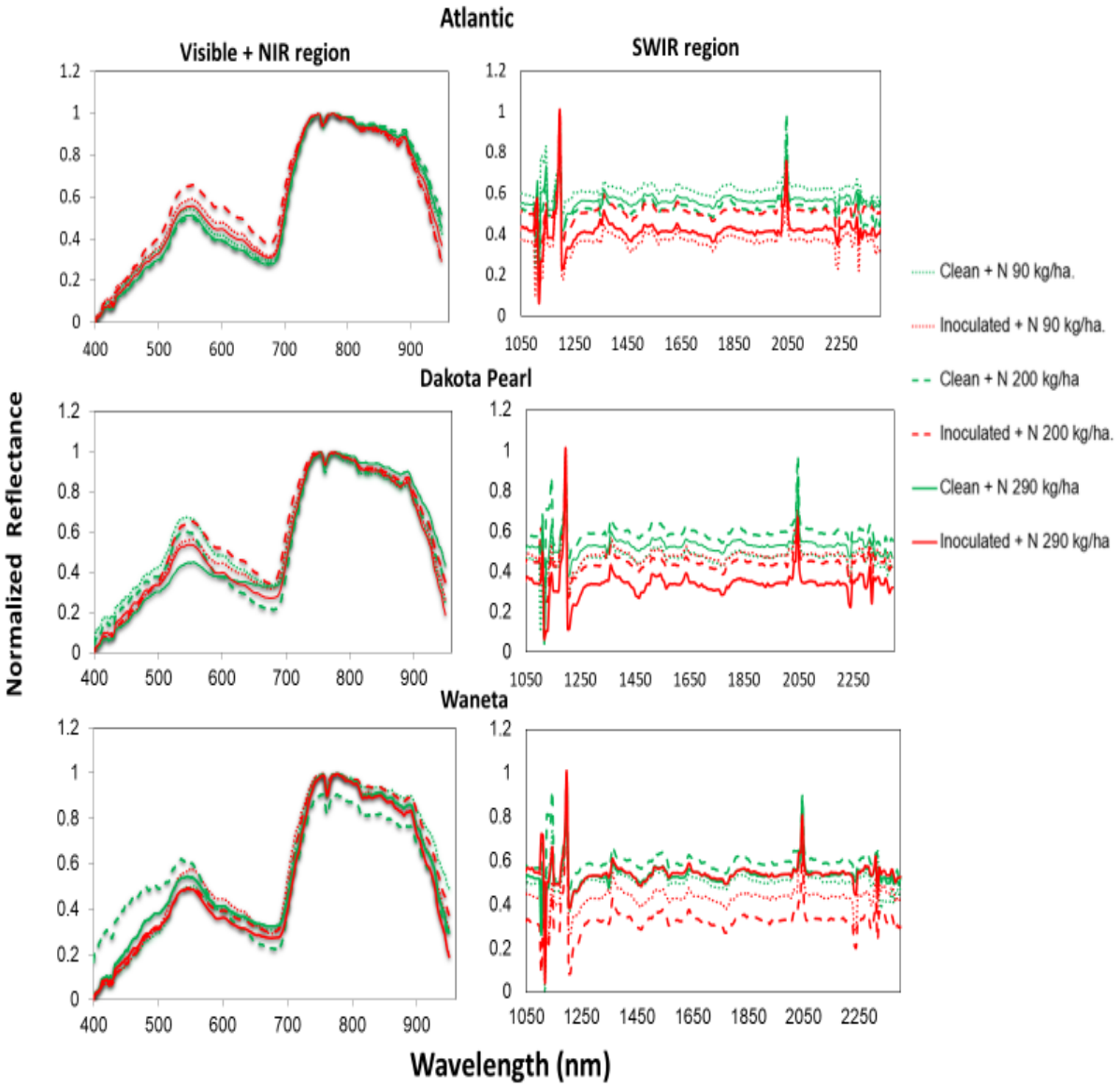

Figure 6**.** Normalized spectral reflectance curves for three chip processing cultivars Atlantic, Dakota Pearl and Waneta, (chip trial 2) in the visible, Near Infra-red (NIR), and Shortwave Infra-red (SWIR) regions recorded 4 weeks after inoculation with $PVY^{N:O}$. Three nitrogen rates (90, 200, and 290 kg/ha) in combination with two virus infection levels (clean and infected with $PVY^{N:O}$) were tested.

Reflectance data collected 4 weeks after inoculation with $PVY^{N:O}$ for chip trial 2 are presented as spectral curves in Figure 6. Distinguishing spectra were not evident in the visible and NIR region for cultivars or treatments (Figure 6). However, clear differentiation among treatments was evident throughout the SWIR region for all cultivars (Figure 6). Reflectance flip-

flopped for Dakota Pearl and Waneta versus trial 1. Interestingly, in both chip trials (trial 1 and trial 2), the spectral curves of Atlantic (at 4 weeks after inoculation) followed a similar pattern (Figures 5 and 8). Whereas, patterns for Dakota Pearl and Waneta were not similar (Figures 6, 7 and 8) at 4 WAI.

For both chip trials, deviation observed in the visible wavelengths (400-680 nm) may be due to the reduction of chlorophyll content in leaves (Reynolds et al. 2009; Naidu et al. 2009; Sahoo et al. 2015). Similarly, the much lower reflectance values for clean samples, compared to the $PVY^{N:O}$ infected samples, are indicative of a reduction of chlorophyll content for $PVY^{N:O}$ infected plants across N rates. For trial 1, the three chip processing cultivars had clear differentiation between clean and PVY infected samples, but mainly at 4 and 8 weeks after inoculation. At 4 WAI, PVY infection symptoms (chlorosis) were not visible in any of the chip cultivars, thus, such clear differentiation in spectra indicates a potential use of spectral reflectance differentiating clean and PVY infected samples, even before any visual symptoms appear. Although practically, at 4 WAI (34-36 days after emergence), this differentiation may be of little use as plants in the field would be approaching to row closure. At 8 WAI, plants had already reached senescence and therefore, differentiation between clean and PVY infected samples using spectral reflectance would not help in seed certification. For future studies, the measurement of leaf chlorophyll content (Sims and Gamon 2002) and the integration of an imaging spectroradiometer (Mahlein et al. 2013) could be helpful.

In contrast to the visible region (400-680 nm), the spectral response in the NIR region (750 to 950 nm) varied from variety to variety for both chip processing trials. NIR is sensitive to the intercellular structure of tissue (Sahoo et al. 2015). In addition to the reduction or degradation of chlorophyll by viral infection, intercellular changes are often reported as a form of thickening

outer cell walls, degeneration of chloroplasts, increase of osmophilic globuli and loosening of thylakoid structures in leaves (ultrastructure) (Alḿsi et al. 1996; Pompe-Novak et al. 2001). In stress-free, healthy leaves, the total internal reflection from the NIR region is generally higher due to the compactness of these cellular structures (Adams et al. 1999; Carter and Knapp 2001; Gazala et al. 2013). Therefore, the spectral variability of different varieties in the NIR regions is probably due to the changes in their leaf ultrastructure, mediated by stress factors.

For chip trial 1 and 2, reflectance in the SWIR region (1250 - 2200 nm) showed very similar distinguished spectral responses for clean versus infected samples. The SWIR region is known to be affected by water stress and foliar constituents (Sahoo et al. 2015). As PVY infection can cause intercellular structural damage and biochemical alterations in potato plants (Alḿsi et al. 1996), which can indirectly affect the foliar constituents; distinguished spectral responses observed in the SWIR region should be studied further for additional insights.

Normalized Difference Vegetation Index (NDVI)

Vegetation indices are used to characterize plant health in response to stresses (Sims and Gamon 2002; Ray et al. 2011; Lowe et al. 2017). NDVI is the most common, widely used vegetation index, and is the basis for several commercially available, non-destructive sensors (Naidu et al. 2009). NDVI is calculated based on the difference between the maximum absorption of radiation in red wavelengths (620 nm to 690 nm) and the maximum reflectance in the NIR (700 nm - 950 nm) spectral region (Mahlein et al. 2010). Absorption in red (620 nm to 690 nm) and NIR (700 nm - 950 nm) wavelengths are dependent on chlorophyll pigments and leaf cellular structure, respectively (Mahlein et al. 2010). Previous studies have found NDVI to be highly representative of plant photosynthetic capacity (Benedetti and Rossini 1993), as well as having the ability to differentiate disease damage in the field (Calderón et al. 2013). Patil et al.

(2007) reported that N deficient wheat plants had very low NDVI values compared to optimally fertilized plants. However, variability in NDVI may occur due to soil in the background, brightness, and canopy shade (Xue and Su 2017). The increasing brightness of the background can increase NDVI systematically (Xue and Su 2017).

Derived NDVI values for both chip trials (trial 1 and trial 2) were combined and subjected to ANOVA. Trials were homogeneous ($P > 0.05$) and thus, could be combined for ANOVA. Significant differences between treatments were tested at the levels of <0.001, <0.01 and <0.5. Treatment means were compared, when appropriate, using LS means ($p<0.001$) in SAS statistical software version 9.4 (SAS Institute Inc., 100 SAS Campus Dr., Cary, NC 27513).

ANOVA results (Table 5) indicated that the four-way and three-way interactions of the factors were not statistically significant ($p > 0.05$). NDVI for chip processing cultivars significantly differed for the two-way interaction Variety × Time, and PVY × Time, at $p < 0.01$ and $p < 0.001$, respectively. The main effects of PVY and Time were also significant at the levels of $p < 0.01$ and $p < 0.00$, respectively.

Table 5**.** Analysis of variance (ANOVA) of NDVI for chip processing varieties Atlantic, Dakota Pearl, and Waneta, in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean, $PVY^{N:O}$ infected), at three dates (4, 6 and 8 WAI[*]). NDVI datasets for two replicated chip trials (trial 1, trial 2) were combined for ANOVA.

SOV[a]	Df[b]	Sum Sq	Mean Sq	F value	Pr(>F)
Rep	1	0.088	0.088	3.493	0.063
Variety	2	0.098	0.049	1.943	0.147
N rate	2	0.017	0.009	0.338	0.714
PVY	1	0.209	0.209	8.293	0.005 **
Time	2	16.434	8.217	326.421	0.000 ***
Variety×N rate	4	0.078	0.019	0.775	0.543
Variety×PVY	2	0.004	0.002	0.074	0.929
Variety×Time	4	0.428	0.107	4.249	0.003 **
N rate×PVY	2	0.058	0.029	1.158	0.317
N rate×Time	4	0.068	0.017	0.678	0.608
PVY×Time	2	1.480	0.740	29.393	0.000***
Variety×N rate×PVY	4	0.215	0.054	2.138	0.078
Variety× N rate×PVY×Time	24	0.661	0.028	1.095	0.356
Residuals	377	4.028	0.025		

[*]WAI= weeks after inoculation with $PVY^{N:O}$
[a]Source of variation
[b]Df =Degree of freedom
Level of significance *** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$

Mean separation of the interaction PVY × Time was done to determine how NDVI changes with the time after $PVY^{N:O}$ inoculation (Table 6). Results indicated that mean NDVI values were significantly reduced with time post inoculation (Table 6). Similar reduction in NDVI with increasing time was reported as the effect of senescence in sugarbeet, when leaves became more chlorotic due to declining leaf chlorophyll content (Mahlein et al. 2010). Our results also indicated that NDVI significantly differentiated clean and infected samples, but only late, at the 8 WAI assessment date (Table 6). Similarly, detection of take-all disease in wheat was possible using NDVI, relatively later in the growing season (Chen et al. 2007). In another study, Phytophthora root rot in cranberry was also reported to be most consistently detected in late-season assessments (Pozdnyakova et al. 2002), using NDVI.

Table 6. Mean NDVI averaged across three N rates (90, 200 and 290 kg/ha) and three chip processing cultivars Atlantic, Dakota Pearl, and Waneta, representing the interaction of two infection levels (clean and $PVY^{N:O}$ infected) and three dates of assessments (4, 6 and 8 WAI[*]).

PVY×Time	N	NDVI
Clean×4 WAI	36	0.891a[§]
Infected×4 WAI	36	0.951a
Clean×6 WAI	36	0.473bc
Infected×6 WAI	36	0.514b
Clean×8 WAI	36	0.401c
Infected×8 WAI	36	0.101d

N=observation
[*]WAI = weeks after inoculation with $PVY^{N:O}$.
[§]Mean grouped with the same letter are not significantly different

When it may be useful for other crops to detect a disease at any growth stage, detecting PVY infection in certified seed potato production is of little use, unless accomplished at a very young stage. At 8 WAI, the three chip cultivars were senescing, therefore, this result of NDVI detecting PVY infection at this stage will not be helpful.

Red-skinned fresh market trials

ELISA testing

As for the red-skinned fresh market trials, the PVY inoculated block and the non-infected (control) blocks were evaluated using ELISA (Singh et al. 2007). All plants in the PVY inoculated block were positive and plants in the untreated block were negative for PVY infection.

Petiole nitrate-N concentration

Petiole nitrate-N concentration for three fresh market cultivars (Chieftain, Red LaSoda and Red Norland) was determined in response to 90, 200, and 290 kg/ha N rates and two virus infection levels (clean and $PVY^{N:O}$ infection) at 4, 6, and 8 WAI with $PVY^{N:O}$. Mean petiole nitrate-N concentrations for all three cultivars were below the sufficiency range suggested for potatoes (Appendix Table A4), in previous studies (Rosen 2018; Franzen et al. 2018). Therefore,

all plants were N deficient in this experiment, regardless of the N rate. Despite that, to determine how N rate and virus infection level affected petiole nitrate-N concentration, ANOVA was performed separately for each date of assessment, combining petiole nitrate-N concentration datasets from two replicated trials (trial 1 and trial 2) for fresh market cultivars (Table 7). Distribution of the combined dataset was not normal ($P < 0.05$) and therefore, was log-transformed prior to ANOVA.

Table 7**.** Analysis of variance (ANOVA) of log petiole nitrate-N concentrations for the three red-skinned fresh market potato varieties Chieftain, Red LaSoda, and Red Norland, in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean, $PVY^{N:O}$ infected), for three periods (4, 6 and 8 WAI[*]). Petiole nitrate-N data for two replicated red-skinned fresh market potato trials (trial 1 and trial 2) were combined for ANOVA.

	4 WAI		6 WAI		8 WAI	
SOV[a]	F Value	Pr > F	F Value	Pr > F	F Value	Pr>F
Variety	12.024	0.000***	1.883	0.160	0.511	0.613
N rate	0.702	0.501	1.711	0.181	0.184	0.844
PVY	5.459	0.052	0.032	0.863	5.762	0.857
Variety×N rate	1.050	0.391	8.294	0.402	0.465	0.762
Variety×PVY	1.031	0.364	12.713	0.367	1.230	0.305
N rate×PVY	0.716	0.490	9.212	0.378	1.613	0.212
Variety×N rate×PVY	0.832	0.512	5.450	0.415	0.908	0.470

[*]WAI = weeks after inoculation
[a]Source of variation
Level of significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

At 4 WAI (Table 7), only the varietal effect was significant ($p < 0.001$) for petiole nitrate-N. Other main effects nor interactions did not significantly affect ($p > 0.05$) petiole nitrate-N concentration for the three red potato cultivars (Table 7).

SPAD values

SPAD readings were recorded on the same assessment dates petioles were sampled and spectral reflectance determined (4, 6, and 8 WAI). SPAD datasets from both red trials (trial 1 and trial 2) were homogeneous ($P > 0.05$) and thus, subjected to combined ANOVA, per date of assessment.

ANOVA (Table 8) results indicated that none of the factor interactions were significant (p>0.05) for SPAD values for the three fresh market potato varieties, on any assessment date. At 4 and 6 WAI, SPAD values for varieties differed significantly at the p<0.05 level (Table 8). SPAD values for chip processing cultivars (Atlantic, Dakota Pearl, and Waneta) were significantly affected by PVY infection at all three assessment dates, whereas, PVY infection had no significant effect on SPAD values for the fresh market varieties. This result indicates that SPAD readings may differ across different market types of potatoes, in response to similar treatments. Further study of cultivar specific SPAD response, to the interaction of varying N rate and level of PVY infection would be beneficial.

Table 8. Analysis of variance (ANOVA) of SPAD values for the three red-skinned fresh market potato varieties Chieftain, Red LaSoda, and Red Norland, in response to three N rates (90, 200 and 290 kg/ha) and two infection levels (clean, $PVY^{N:O}$ infected), three assessment dates (4, 6 and 8 WAI[*]). SPAD data for the two replicated red-skinned fresh market potato trials were combined for ANOVA.

	4 WAI		6 WAI		8 WAI	
SOV[a]	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Variety	3.969	0.021*	4.660	0.010*	1.137	0.328
N rate	1.239	0.293	0.431	0.652	1.030	0.364
PVY	1.446	0.231	2.433	0.121	1.215	0.275
Variety×N rate	0.430	0.786	0.740	0.561	1.027	0.402
Variety×PVY	0.496	0.610	0.981	0.380	1.042	0.360
N rate×PVY	0.150	0.861	0.222	0.813	1.025	0.366
Variety×N rate×PVY	0.374	0.827	0.631	0.642	1.007	0.412

[*]WAI= weeks after inoculation
[a]Source of variation
Level of significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Correlation between petiole nitrate-N concentration and SPAD value

Combined petiole nitrate-N concentrations and SPAD values for Chieftain, Red LaSoda, and Red Norland (trial 1 and trial 2) in response to PVY infection level (clean and $PVY^{N:O}$ infected) were subjected to Pearson product moment correlation analysis. Homogeneity (P > 0.05) of the trials allowed for a combined analysis. Correlation between petiole nitrate-N

concentration and SPAD value varied depending on the cultivar-infection level combinations. Significant correlation between petiole nitrate-N concentration and SPAD value was found for Red LaSoda (clean), Red Norland (clean) and Red Norland ($PVY^{N:O}$ infected) at the level of 0.001, 0.01, and 0.05, respectively (Figure 7). Non-significant correlations were observed for other three combinations.

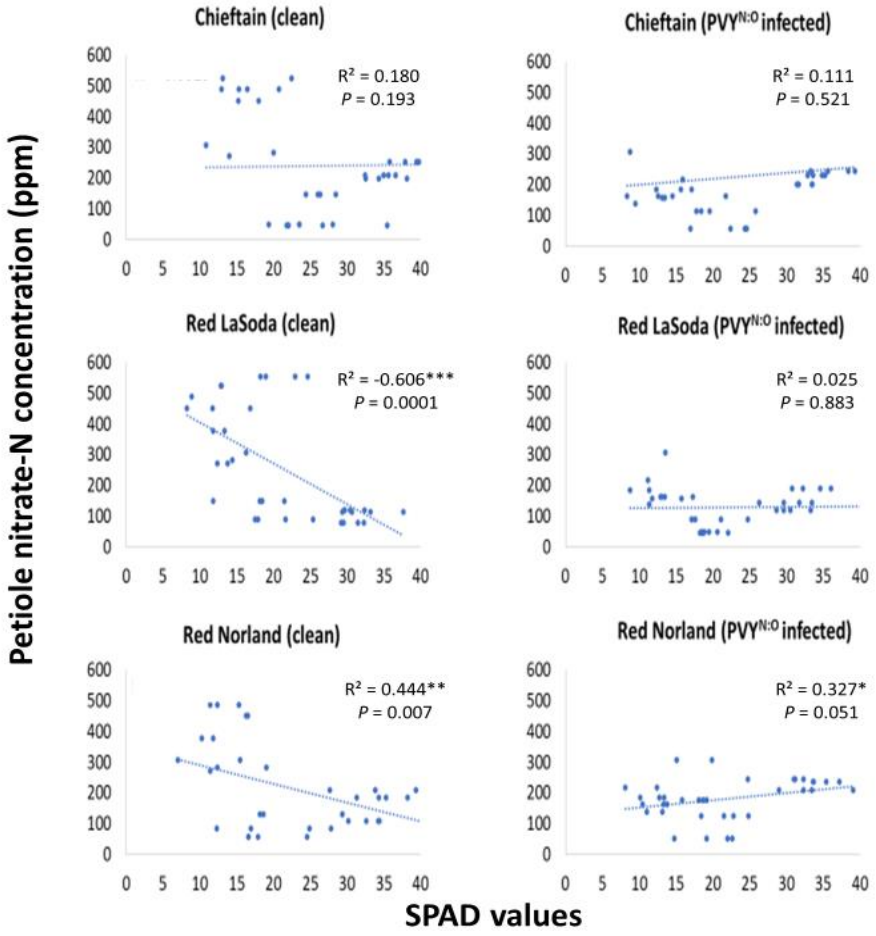

Figure 7. The Pearson product moment correlation between SPAD and petiole nitrate-N concentration (trial 1 and trial 2), for three fresh market potato varieties (Chieftain, Red LaSoda, and Red Norland), in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean and $PVY^{N:O}$ infected), at three assessment dates (4, 6 and 8 weeks after inoculation with $PVY^{N:O}$). R represents the correlation coefficient and *p* value denotes the significance of the correlation. Level of significance *** $p < 0.001$; ** $p < 0.01$; *$p < 0.05$. SPAD and petiole-N concentration datasets for trial 1 and trial 2 were combined for the correlation analysis.

Similar results (i.e. non-significant correlation) between petiole nitrate-N and SPAD values were also observed for most of combinations of the chip processing cultivars and infection levels (Figure 2). These results contradict some previous studies indicating strong, positive correlation between petiole nitrate concentration and SPAD values (Blackmer and Schepers 2013; Dunn et al. 2015; Fox et al. 2001; Jia et al. 2007). Following the extensive review of the literature, a definitive reason for this lack of correlation is not clear. For future investigation, proper fertilizer management, monitored irrigation, and controlled lighting should be ensured.

Spectral response

Spectral reflectance for three red-skinned fresh market cultivars (Chieftain, Red LaSoda, and Red Norland) was recorded separately from the two replicated trials (trial 1 and trial 2). In each trial, leaf spectral reflectance was measured in response to three N rates (90, 200, and 290 kg/ha) and two virus infection levels (clean and $PVY^{N:O}$ infected), at three times after inoculation with $PVY^{N:O}$ (4, 6 and 8 WAI). Spectral curves for the six paired treatments were created in Excel by plotting pre-processed, normalized reflectance values against their corresponding wavelengths. Spectral curves for each cultivar were created for each assessment date. Spectral reflectance measured at 6 and 8 WAI were not useable for trial 1, due to noise in the spectra. For the same reason, reflectance data recorded at 4 and 8 were not useable for trial 2.

In the visible and NIR region (400 -700 nm), spectral curves overlapped in response to the treatments for Chieftain and Red LaSoda (Figure 8a, 8c). Whereas, in the SWIR region (1050 – 2250 nm), the spectral response for the combination of clean and the 200 kg/ha N rate for Chieftain and Red LaSoda was distinguished from other treatments (Figure 8b, 8d). Distinguished spectral response was observed for Red Norland, in between 550 nm to 700 nm

wavelengths (Figure 8e), in response to all the treatments. Overall, the 4 week assessments were not well differentiated, as was the case in most instances for the chip cultivars.

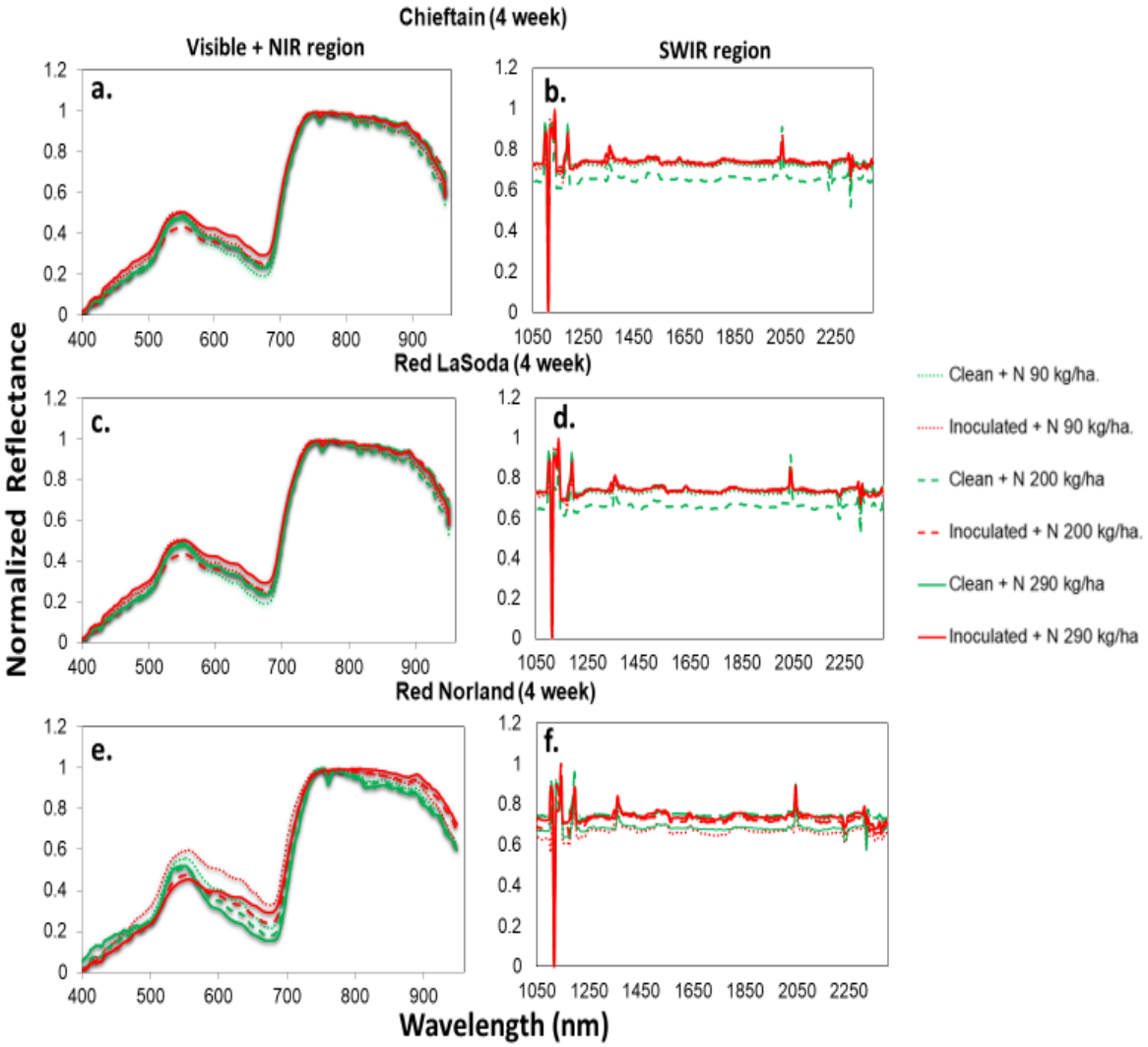

Figure 8**.** Normalized spectral reflectance curves for three red-skinned fresh market potato cultivars (Chieftain, Red LaSoda, and Red Norland) (trial 1) in the visible, Near Infra-red (NIR), and Shortwave Infra-red (SWIR) regions, recorded 4 weeks after inoculation with $PVY^{N:O}$. Three nitrogen rates (90, 200, and 290 kg/ha) in combination with two virus infection levels (clean and infected with $PVY^{N:O}$) were tested.

In the NIR region (750 nm -950 nm), clear spectral differentiation was observed in response to all treatments, for all three cultivars (Figure 9a, 9c, 9e). However, in the SWIR

region, Red Norland had most distinguished spectral curves for all treatments, compared to the other two red cultivars (Figure 9f, 9d, 9b).

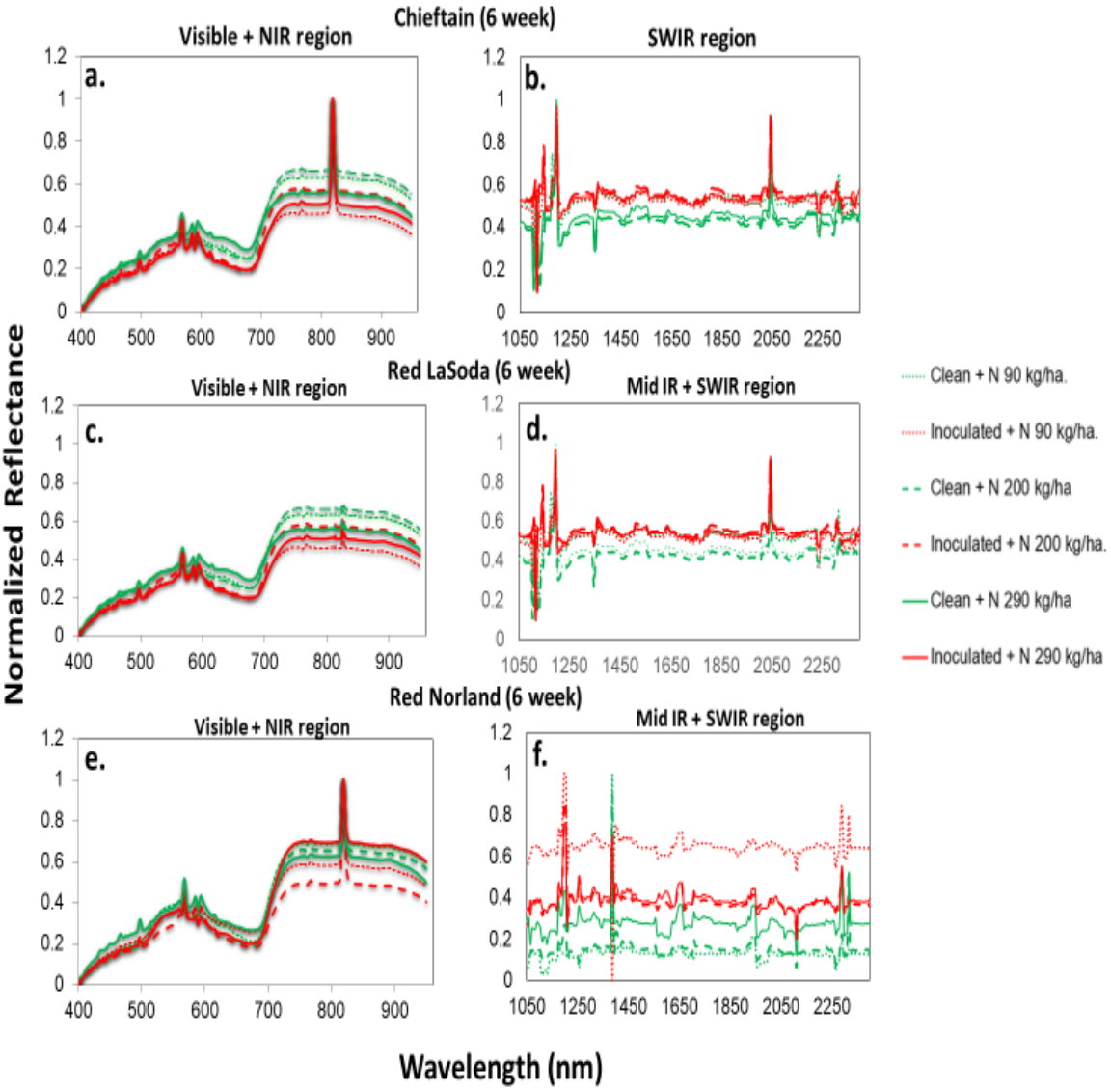

Figure 9**.** Normalized spectral reflectance curves for three red-skinned fresh market potato cultivars (Chieftain, Red LaSoda, and Red Norland) (trial 2) in the visible, Near Infra-red (NIR), and Shortwave Infra-red (SWIR) regions, recorded 6 weeks after inoculation with $PVY^{N:O}$. Three nitrogen rates (90, 200, and 290 kg/ha) in combination with two virus infection levels (clean and infected with $PVY^{N:O}$) were tested.

Overall, in contrast to the results for the chip processing trials, red potato cultivars had no or very little spectral differentiation among treatments in the visual region (400 nm-700 nm). Leaf reflectance in the visual wavelengths depends on the leaf pigment composition (Reynolds et al. 2009; Naidu et al. 2009; Sahoo et al. 2015); thus, varying spectral response between the two market types may be induced due to alteration in leaf pigment composition under stress. The effect of foliar pigmentation on spectral response needs further investigation. On the other hand, at 6 WAI (trial 2), the NIR region had more distinguished spectral response for all six treatments, regardless of the cultivars (Figure 9). Under stressed conditions, deviation in leaf spectral reflectance was previously reported in the NIR region, due to leaf ultrastructure changes (Sahoo et al. 2015).

Normalized Difference Vegetation Index (NDVI)

NDVI for the three red-skinned cultivars were derived from their corresponding spectral signatures following the same procedure as for the chip processing cultivar trials. NDVI was determined for the three red cultivars for the 4 WAI and 6 WAI assessment dates, respectively, for trial 1 and trial 2. Trials were not homogeneous ($P < 0.05$), thus separate ANOVA was performed for NDVI values for each assessment date.

ANOVA results indicated that no interaction effects were significant on NDVI for the three red cultivars (Chieftain, Red LaSoda, and Red Norland) in trial 1, at 4 WAI (Table 9). Only the main effect of PVY infection was significant at the $p < 0.01$ level, indicating that clean and $PVY^{N:O}$ infected samples can be differentiated using NDVI at 4 WAI. On the other hand, no interactions or main effects of factors were found to have significant effects on NDVI for trial 2, at the 6 WAI assessment date (Appendix Table A6). In the chip processing trials, it was possible to differentiate between clean and infected samples using NDVI, but only at the late season

assessment (8WAI). Whereas, for the fresh market trials, NDVI could be used to differentiate between clean and $PVY^{N:O}$ infected samples at relatively earlier stage of assessment (4WAI); which makes it more adoptable for the certification agencies as well as certified seed growers.

Table 9. Analysis of variance of NDVI for three fresh market varieties Chieftain, Red LaSoda, and Red Norland (trial 1), in response to three N rates (90, 200, and 290 kg/ha) and two virus infection levels (clean and $PVY^{N:O}$ infected), at 4 WAI*.

SOV[a]	Df[b]	Sum Sq	Mean Sq	F value	Pr(>F)
Variety	2	0.007	0.003	0.352	0.705
N rate	2	0.011	0.005	0.547	0.582
PVY	1	0.114	0.114	11.839	0.001**
Variety×N rate	4	0.079	0.019	2.054	0.100
Variety×PVY	2	0.026	0.013	1.320	0.276
N rate×PVY	2	0.024	0.012	1.228	0.301
Variety×N rate×PVY	4	0.009	0.002	0.229	0.921

*WAI= weeks after inoculation
[a]Source of variation
[b]Df=Degree of freedom
Level of significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Russet cultivar trials

ELISA testing

All inoculated and non-inoculated plants of russet trials (trial 1 and trial 2) were subjected to ELISA testing (Singh et al. 2007). ELISA test results indicated that all inoculated plants were infected by $PVY^{N:O}$, and all non-inoculated plants were virus-free.

Petiole nitrate-N concentration

Petiole nitrate-N concentrations, for the four russet cultivars (Dakota Russet, Russet Burbank, Russet Norkotah, and Shepody) were determined, in response to three N rates (90, 200, and 290 kg/ha) and two virus infection levels (clean and $PVY^{N:O}$ infected), at 4, 6, and 8 WAI. The three N rates (90, 200, and 290 kg/ha N) were supplied with an aim of attaining N deficiency, N sufficiency, and N excessiveness, respectively. However, similar to the previous trials (chip processing and red-skinned fresh market), petiole nitrate-N concentrations for the

four russet cultivars indicated that all plants were N deficient (Appendix Table A5), regardless of N rate (90, 200, and 290 kg/ha N). ANOVA was performed for petiole nitrate-N concentrations, per date of assessment combining the two replicated russet trials (trial 1 and trail 2), as the trials were homogenous ($P > 0.05$). The distribution of the combined dataset was not normal ($P < 0.05$), and therefore, the dataset was log-transformed.

Table 10**.** Analysis of variance (ANOVA) of log petiole nitrate-N concentration for russet varieties Dakota Russet, Russet Burbank, Russet Norkotah, and Shepody, in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean, $PVY^{N:O}$ infected), at three assessment date (4, 6 and 8 WAI[*]). Petiole nitrate-N data for two replicated russet trials (trial 1 and trial 2) were combined for the same date of assessment.

	4 WAI		6 WAI		8 WAI	
SOV[a]	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Variety	15.311	0.000***	2.314	0.082	9.381	0.000***
N rate	0.583	0.558	13.032	0.000***	1.338	0.273
PVY	7.990	0.012*	2.772	0.101	0.797	0.581
Variety×N rate	2.271	0.041*	1.921	0.080	3.834	0.050
Variety×PVY	12.345	0.000***	6.260	0.001**	3.032	0.032*
N rate×PVY	4.509	0.011*	1.182	0.311	1.500	0.231
Variety×N rate×PVY	2.092	0.066	8.943	0.000***	1.111	0.366

[*]WAI= weeks after inoculation
[a]Source of variation
Level of significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

ANOVA results (Table 10) indicated that at 4 WAI, petiole nitrate-N concentration for dual-purpose cultivars were significantly affected by all possible two-way interactions of the factors, at different levels of significance. The interaction between Variety and PVY infection was highly significant ($p < 0.001$), whereas other two-way interactions were significant at the $p < 0.05$ level. At 6 WAI, the three-way interactions of Variety × N rate × PVY was highly significant at $p < 0.001$ level. The two-way interactions of Variety × PVY was significant at the $p < 0.01$ level. The main effect of N rate was highly significant ($p < 0.001$) for petiole nitrate

concentration, at 6 WAI. At 8 WAI, the only interaction that had a significant effect ($p < 0.05$) on petiole nitrate-N concentrations of the three russet cultivars was between variety and PVY.

Figure 10 demonstrated how mean petiole nitrate-N concentration varied among the varieties for the three assessment dates, when treated with N rates of 90, 200, and 290 kg/ha, and two virus infection levels (clean and $PVY^{N:O}$ infected). Petiole nitrate concentrations for all four russet varieties tended to decrease at 6 WAI, and then increased again at 8 WAI, compared to 4 WAI. At 8 WAI, due to the lack of a fourth petiole, the next available petiole below the fourth (5^{th} or 6^{th}) was collected. The position of petiole sampling was found to have significant effects on petiole nitrate concentrations of potatoes by Westermann in 1994. Overall, unlike the chip processing and red-skinned fresh market cultivars, petiole nitrate-N concentration for the russet cultivars varied among cultivars without following any pattern, in response to three N rates and two infection levels, at all three assessment dates post inoculation with $PVY^{N:O}$.

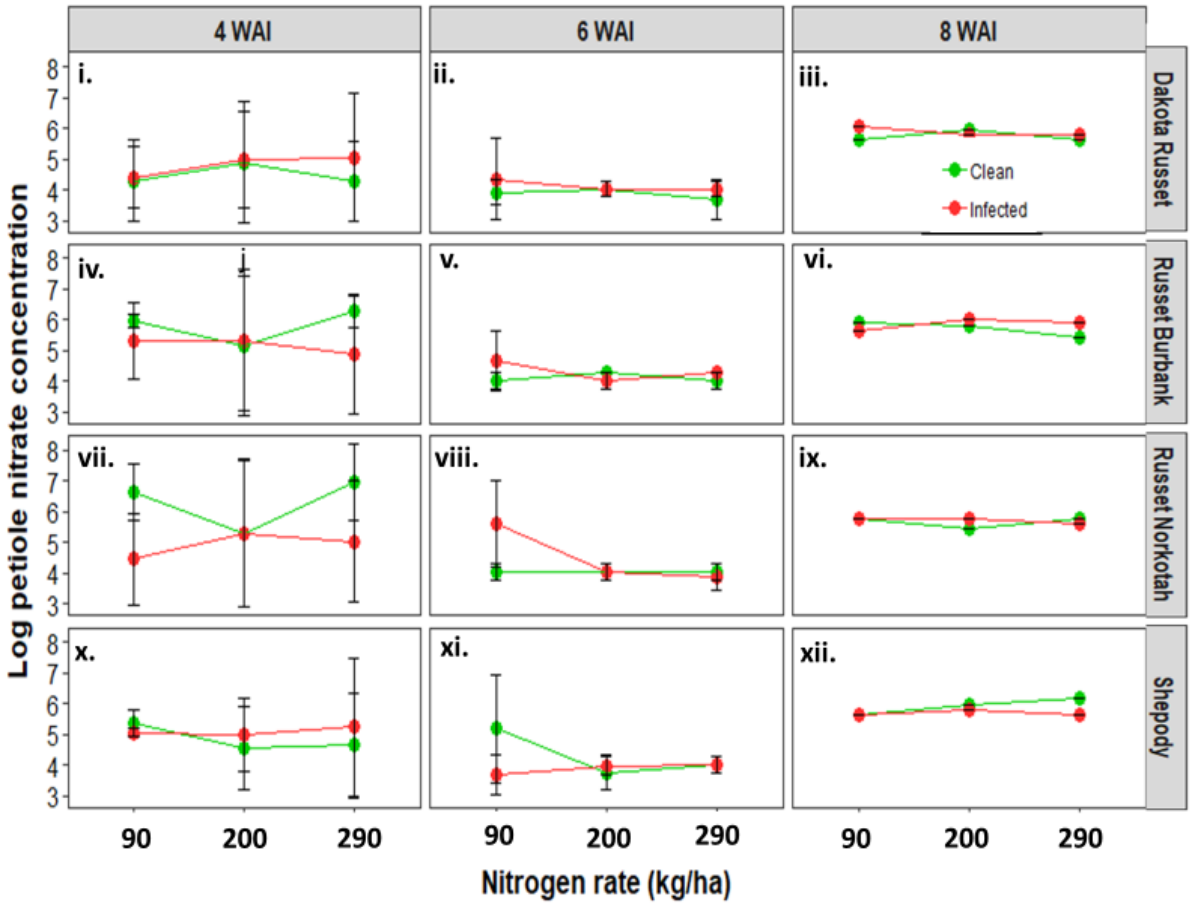

Figure 10**.** Log petiole nitrate-N concentrations for russet cultivars (Dakota Russet, Russet Burbank, Russet Norkotah, and Shepody) in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean, $PVY^{N:O}$ infected), at 4, 6, and 8 WAI. WAI= weeks after inoculation with $PVY^{N:O}$. The X axis denotes N rate (90, 200, and 290 kg/ha) and the Y axis represents the petiole nitrate-N concentration. Each of the three horizontal plots (from left to right, on the same row) represents petiole nitrate-N concentrations for the same dual-purpose cultivar, at 4, 6, and 8 WAI, respectively. Each of the three vertical plots (from top to bottom, on the same column) represents petiole nitrate-N concentrations assessed on the same date for Dakota Russet, Russet Burbank, Russet Norkotah, and Shepody, accordingly. Green and red dots connected with green and red lines denote petiole nitrate-N concentrations of clean and $PVY^{N:O}$ infected samples, respectively.

SPAD values

SPAD values for the four russet cultivars were recorded on the same assessment dates as petioles were collected. SPAD datasets for the two replicated russet trials (trial 1 and trial 2) were homogeneous ($P > 0.05$) and therefore were combined for the same assessment date and subjected to ANOVA. ANOVA results (Table 11) indicated that at 4 WAI, the three-way

interaction of variety, N rate and PVY significantly affected SPAD values at the p <0.05 level. The interaction between N rate and PVY was significant (p < 0.05) for SPAD values only at 4 WAI. The interaction between variety and PVY was significant for petiole nitrate-N at 6 (p <0.05) and 8 (p < 0.01) WAI. For all assessment dates, N rate significantly affected SPAD values for the russet cultivars. Unlike the chip processing trials, no significant effect was observed for virus infection for the russet trials, at any date of assessment post inoculation with $PVY^{N:O}$.

Table 11**.** Analysis of variance of SPAD for russet varieties Dakota Russet, Russet Burbank, Russet Norkotah, and Shepody, in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean, $PVY^{N:O}$ infected), at three assessment dates (4, 6 and 8 *WAI). SPAD datasets for two the replicated russet trials were combined for the same date of assessment.

	4 WAI		6 WAI		8 WAI	
SOV[a]	F Value	Pr > F	F Value	Pr > F	F Value	Pr > F
Variety	7.910	0.000***	6.029	0.015*	9.373	0.000***
N rate	39.191	0.000***	7.656	0.015*	5.851	0.004**
PVY	2.043	0.060	1.552	0.172	1.330	0.251
Variety×N rate	1.003	0.325	1.350	0.251	2.630	0.119
Variety×PVY	4.076	0.012*	3.156	0.034*	5.030	0.002**
N rate×PVY	5.233	0.011*	0.453	0.640	0.771	0.460
Variety×N rate×PVY	2.944	0.014*	0.854	0.530	0.820	0.567

*WAI = weeks after inoculation
[a]Source of variation
Level of significance *** p < 0.001; ** p < 0.01; * p < 0.05

Correlation between petiole nitrate-N concentration and SPAD value

Correlation between petiole nitrate-N concentration and SPAD value for russet cultivars was established by Pearson product moment correlation analysis. Homogeneity (P > 0.05) of two datasets from each of the russet trials (trial 1 and trial 2) allowed those to be combined for the correlation analysis. Correlation analysis (Figure 11) indicates that the two traits are significantly correlated for most of the russet cultivar and infection level combinations, except Dakota Russet (clean) and Shepody (clean). Higher correlation between SPAD and petiole nitrate-N

concentration was observed for russet cultivars, compared to the chip processing and red-skinned fresh market cultivars.

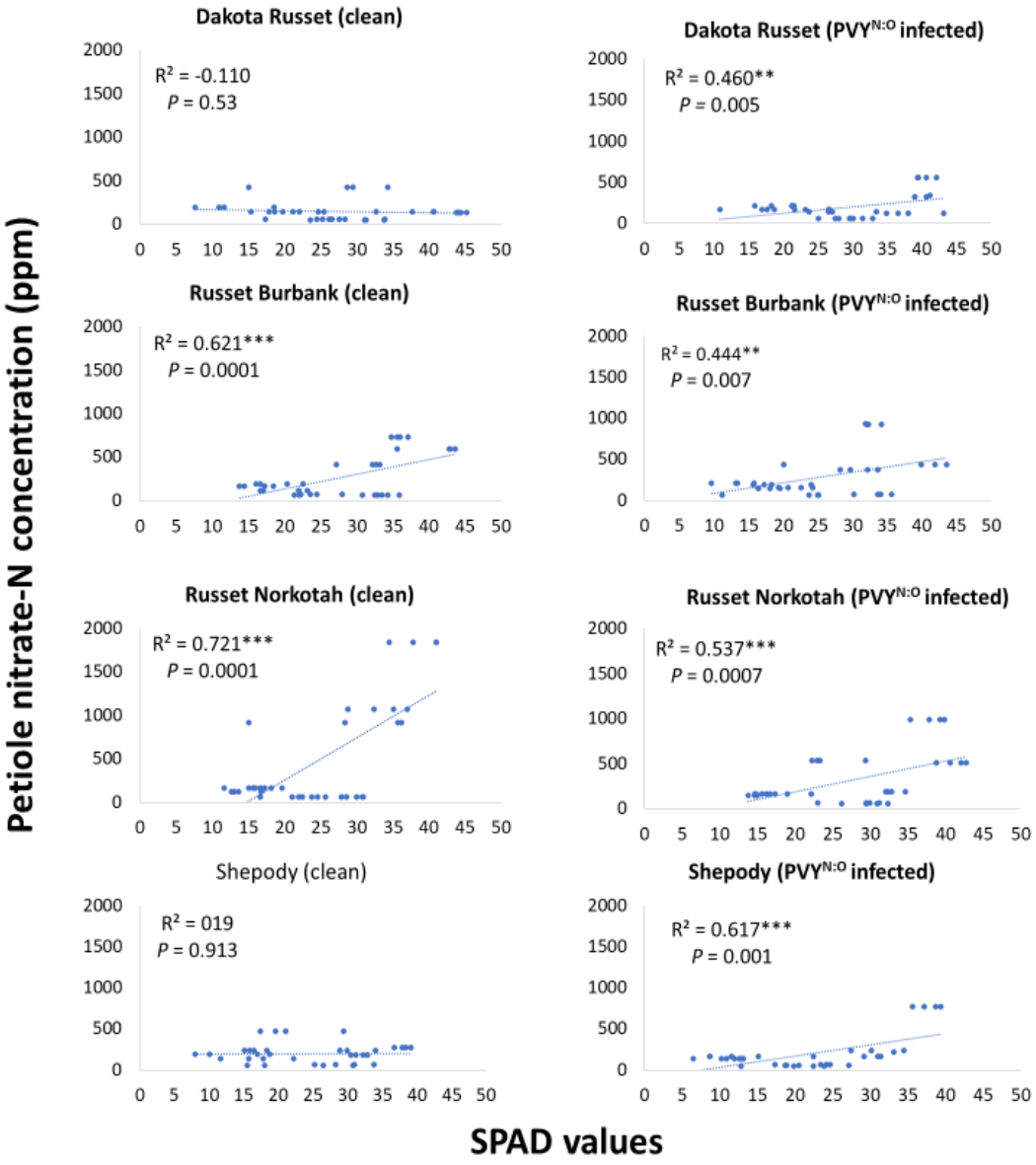

Figure 11. Correlation between SPAD and petiole nitrate-N concentration for four russet potato varieties (Dakota Russet, Russet Burbank, Russet Norkotah, and Shepody) in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean and PVY$^{N:O}$ infected), at three assessment dates (4, 6 and 8 WAI). R represents the correlation coefficient and *p* value denotes the significance of the correlation. Level of significance *** $p < 0.001$; ** $p < 0.01$. SPAD and petiole-N concentration datasets for trial 1 and trial 2 were combined for the correlation analysis.

Spectral response

Spectral responses for the four russet cultivars were recorded for six paired treatments (clean × 90 kg/ha N, infected × 90 kg/ha N, clean × 200 kg/ha N, infected × 200 kg/ha N, clean × 290 kg/ha N, and infected × 290 kg/ha N) at 4, 6 and 8 WAI with $PVY^{N:O}$. Due to extreme noise in the spectra, reflectance datasets collected for the 6 and 8 WAI assessment dates were not useable. Therefore, the spectral curves for russet cultivars were created using reflectance data recorded at 4 WAI. Reflectance datasets for the two russet cultivar trials (trial 1 and trial 2) were combined for the 4 WAI assessment date based on homogeneity ($P > 0.05$). None of the russet cultivars had any distinct spectral response distinguishing clean and PVY infected samples, for the three N rates in the visible and NIR regions (Figure 12). Spectral responses for the treatments greatly varied from variety to variety in the SWIR region (Figure 12) in an inconsistent pattern.

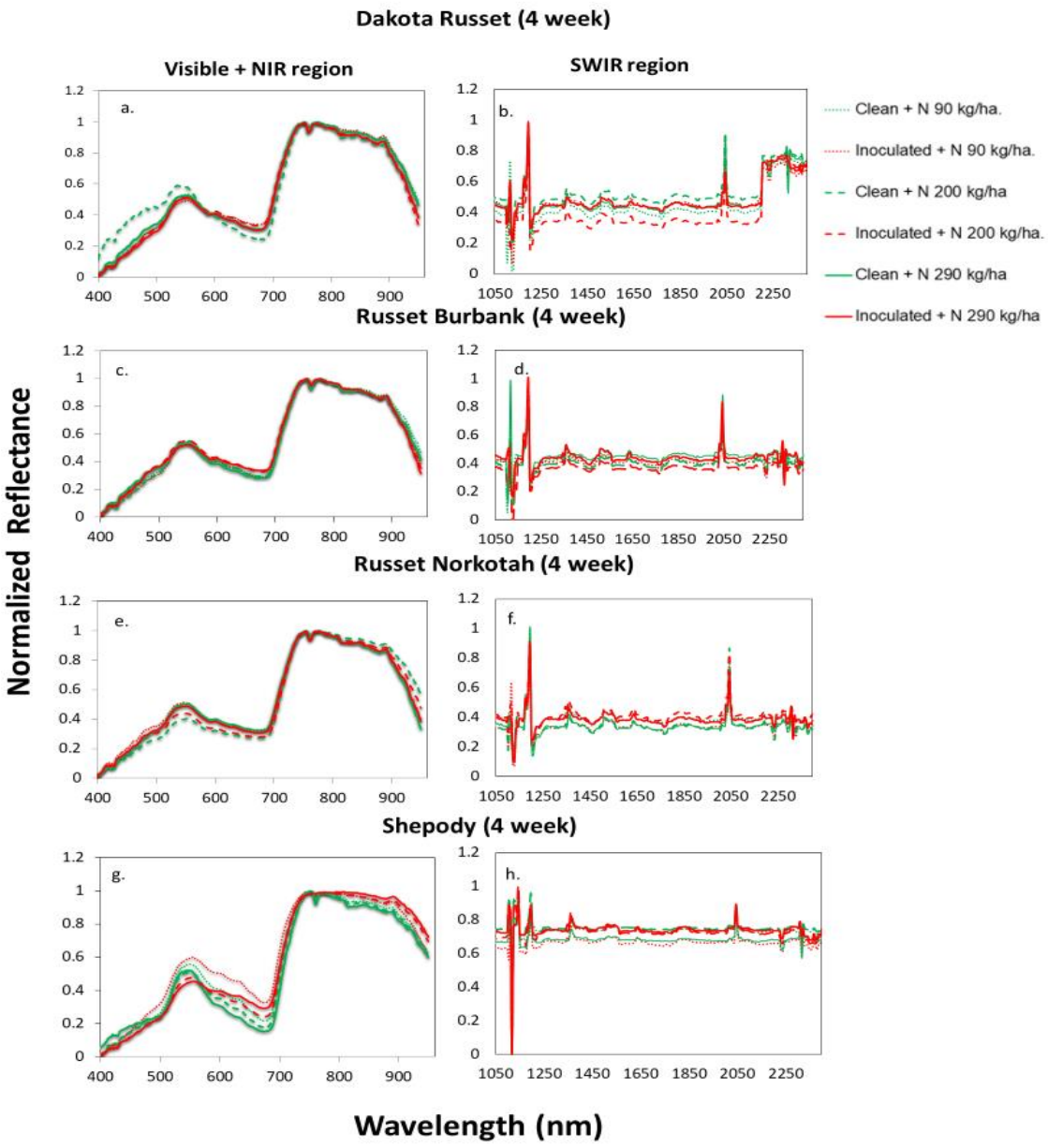

Figure 12. Normalized spectral reflectance curves for four dual-purpose potato cultivars (Dakota Russet, Russet Burbank, Russet Norkotath, and Shepody) (trial 1 and trial 2) in the visible, Near Infra-red (NIR), and Shortwave Infra-red (SWIR) regions recorded 4 weeks after inoculation with $PVY^{N:O}$. Three nitrogen rates (90, 200, and 290 kg/ha) in combination with two virus infection levels (clean and infected with $PVY^{N:O}$) were tested.

Normalized Difference Vegetation Index (NDVI)

NDVI for russet cultivars trials (trial 1 and trial 2) could not be calculated for 6 and 8 WAI since spectral reflectance datasets for those two assessment dates were not useable due to the noise in the spectra. NDVI for russet trials could only be calculated for 4 WAI. Trials were homogeneous ($P > 0.05$) and thus, combined ANOVA was run.

Table 12. Analysis of variance (ANOVA) of NDVI for russet cultivars Dakota Russet, Russet Burbank, Russet Norkotah, and Shepody in response to three N rates (90, 200 and 290 kg/ha) and two virus infection levels (clean, $PVY^{N:O}$ infected), at 4 WAI*. NDVI for two replicated dual-purpose russet trials (trial 1 and trial 2) were combined for ANOVA.

SOV[a]	Df[b]	Sum Sq	Mean Sq	F value	Pr(>F)
Variety	3	0.339	0.113	15.463	0.000***
N rate	2	0.030	0.015	2.049	0.136
PVY	6	0.072	0.012	1.634	0.150
Variety×N rate	1	0.088	0.088	12.054	0.000***
Variety×PVY	3	0.060	0.020	2.745	0.050
N rate×PVY	2	0.004	0.002	0.304	0.739
Variety×N rate×PVY	6	0.043	0.007	0.980	0.445

*WAI = weeks after inoculation
[a]Source of variation
[b]Df=Degree of freedom
Level of significance *** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$

ANOVA result (Table 12) indicated that NDVI for russet cultivars significantly differed ($p < 0.001$) for the effect of two-way interaction, Variety × N rate (Table 12). The main effect of Variety was also highly significant ($p < 0.001$) for NDVI (Table 12). Unlike red-skinned fresh market potato trials, NDVI for dual-purpose russet trials was not significantly affected by PVY infection, at 4 WAI.

SUMMARY

The main objective of this study was to determine if N deficiency and PVY infection can be differentiated in potato cultivars using spectral reflectance. Petiole nitrate-N concentrations and SPAD values for cultivars were studied in this experiment to verify the response to three N rates. Nitrogen sufficiency was not achieved for any of the cultivars in this experiment. As a nutrient-free media was used in this experiment, micro-organisms may not have enough N for maintaining an appropriate C:N ratio, and therefore, may have used the supplied nitrogen (nitrate and ammonia), resulting in a lack of available N for the potato plants. For future studies, the regular greenhouse media (with nutrients) should be used. Split applications of N during the growing season may ensure consistent nutrient availability during growth and development. Since, all the plants were N deficient according to their petiole nitrate-N concentrations, the main objective of this study was not fulfilled. Instead, the study was then focused on distinguishing spectral responses for clean and $PVY^{N:O}$ infected samples, and determining how the three N rates (90, 200, and 290 kg/ha) affect the spectral reflectance at three assessment dates (4, 6, and 8 WAI).

Significant variation in spectral response was observed depending on cultivar and the date of assessment. Spectral responses for the three chip processing cultivars were able to distinguish clean and $PVY^{N:O}$ infected samples in the visible and SWIR region at 8 WAI. NDVI for chip processing cultivars also significantly differed due to PVY infection at 8 WAI. At that stage, it is too late to rogue PVY infected plants or to recover any potential yield or quality damage cause by PVY infection. In this study, all plants were mechanically inoculated with $PVY^{N:O}$ and data collection could not be started until successful inoculation with $PVY^{N:O}$ (4 WAI) was confirmed by ELISA testing. For future research, $PVY^{N:O}$ infected tubers should be

used to ensure systemic infection of $PVY^{N:O}$ in the potato plants, thus allowing the collection of data at a very early stage of plant growth. NDVI for fresh market potato cultivars was also significantly affected by PVY infection, but at 4 WAI. NDVI for russet cultivars did not distinguish clean and infected samples at any date of assessment post inoculation with $PVY^{N:O}$. These cultivar differences in spectral response indicate that spectral response for one cultivar may not be used as a reference for another potato cultivar. Future studies should focus more on studying cultivar-specific spectral response.

To date, this is the first study aimed at characterizing the spectral responses to differentiate PVY infected potato plants at varying N levels. This study may serve as the basis of future in-depth research in developing cultivar-specific spectral bands and vegetation indices to identify PVY infection. Future studies may also focus on how PVY infection affects intercellular structures of leaves, thus impacting cultivar-specific spectral bands.

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APPENDIX

Table A1. Analysis of variance (ANOVA) of Log transformed petiole nitrate-N concentrations for chip processing cultivars Atlantic, Dakota Pearl, and Waneta in response to three N rates (90, 200 and 290 kg/ha) and two infection levels (clean, $PVY^{N:O}$ infected), 4, 6, and 8 weeks after inoculation with $PVY^{N:O}$. Two replicated chip trials (trial 1 and trial 2) were combined for ANOVA.

SOV[a]	Df[b]	Sum Sq	Mean Sq	F value	Pr(>F)
Variety	2	12.631	6.316	20.248	0.000***
N rate	2	18.808	9.404	30.149	0.000***
PVY	1	3.943	3.943	12.640	0.000***
Time	2	38.575	19.287	61.834	0.000***
Variety×N rate	4	6.693	1.673	5.364	0.000***
Variety×PVY	2	4.751	2.375	7.615	0.001***
Variety×Time	4	17.700	4.425	14.186	0.000***
N rate×PVY	2	3.210	1.605	5.145	0.006***
N rate×Time	4	10.722	2.681	8.594	0.000***
PVY×Time	2	0.305	0.153	0.489	0.614
Variety×N rate×PVY	4	5.053	1.263	4.050	0.003***
Variety×N rate×PVY×Time	24	40.430	1.685	5.401	0.000***

[a]Source of variation
[b]Df=Degree of freedom
Level of significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table A2. Mean petiole nitrate-N concentration (ppm) for chip processing varieties Atlantic, Dakota Pearl and Waneta averaged across nitrogen rates (90, 200 and 290 kg/ha) and infection levels (clean and $PVY^{N:O}$ infected), at 4, 6, and 8 WAI*.

Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for chip processing cultivars at 4 WAI		
		Atlantic	Dakota Pearl	Waneta
90	Clean	87.5	70.5	142.0
	$PVY^{N:O}$ infected	48.0	99.5	118.0
200	Clean	250.0	82.5	347.5
	$PVY^{N:O}$ infected	38.0	70.5	249.5
290	Clean	71.0	106.0	1109.5
	$PVY^{N:O}$ infected	246.0	512.5	550.5
Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for chip processing cultivars at 6 WAI		
		Atlantic	Dakota Pearl	Waneta
90	Clean	59.0	59.0	59.0
	$PVY^{N:O}$ infected	38.0	249.5	512.5
200	Clean	71.0	47.5	106.0
	$PVY^{N:O}$ infected	169.0	171.0	169.0
290	Clean	139.0	171.0	258.0
	$PVY^{N:O}$ infected	70.5	246.0	550.5
Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for chip processing cultivars at 8 WAI		
		Atlantic	Dakota Pearl	Waneta
90	Clean	51.0	78.0	163.5
	$PVY^{N:O}$ infected	87.5	142.0	106.0
200	Clean	94.0	82.5	187.0
	$PVY^{N:O}$ infected	175.0	199.0	169.0
290	Clean	175.0	234.0	266.0
	$PVY^{N:O}$ infected	70.5	71.0	1109.5

*weeks after inoculation with $PVY^{N:O}$

Table A3**.** Analysis of variance (ANOVA) of SPAD values for chip processing cultivars Atlantic, Dakota Pearl, and Waneta, in response to three N rates (90, 200 and 290 kg/ha) and two infection levels (clean, $PVY^{N:O}$ infected), at three dates 4, 6 and 8 weeks after inoculation. SPAD data for the two replicated chip trials (trial 1 and trial 2) were combined for ANOVA.

SOV[a]	Df[b]	Sum Sq	Mean Sq	F value	Pr(>F)
Variety	2	1247.291	623.641	132.341	0.000***
N rate	2	2226.864	1113.434	236.274	0.000***
PVY	1	3037.381	3037.381	644.521	0.000***
Time	2	20898.323	10449.163	2217.282	0.000***
Variety×N rate	4	3912.251	978.062	207.542	0.000***
Variety×PVY	2	8.472	4.241	0.900	0.408
Variety×Time	4	2107.304	526.823	111.70	0.000***
N rate×PVY	2	148.142	74.072	15.723	0.000***
N rate×Time	4	411.004	102.750	21.804	0.000***
PVY×Time	2	134.381	67.192	14.262	0.000***
Variety×N rate×PVY	4	283.642	70.910	15.051	0.000***
Variety×N rate×PVY×Time	24	1899.030	79.130	16.792	0.000***
Residuals	377	1776.650	4.710		

a.Source of variation

b. Df=Degree of freedom

Level of significance *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table A4. Mean petiole nitrate-N concentration (ppm) for red-skinned fresh market cultivars Chieftain, Red LaSoda and Red Norland averaged across nitrogen rates (90, 200 and 290 kg/ha) and infection levels (clean and $PVY^{N:O}$ infected), at 4, 6, and 8 WAI*.

Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for red-skinned fresh market cultivars at 4 WAI		
		Chieftain	Red LaSoda	Red Norland
90	Clean	48.0	555.5	58.0
	$PVY^{N:O}$ infected	58.0	90.0	177.5
200	Clean	47.0	90.0	86.0
	$PVY^{N:O}$ infected	651.0	49.0	124.5
290	Clean	146.0	149.0	132.0
	$PVY^{N:O}$ infected	114.0	47.0	52.5
Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for red-skinned fresh market cultivars at 6 WAI		
		Chieftain	Red LaSoda	Red Norland
90	Clean	209.0	115.5	108.5
	$PVY^{N:O}$ infected	201.5	144.0	244.0
200	Clean	197.5	120.5	185.5
	$PVY^{N:O}$ infected	232.0	190.0	238
290	Clean	252.0	78.5	209
	$PVY^{N:O}$ infected	244.0	119.0	208.5
Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for red-skinned fresh market cultivars at 8 WAI		
		Chieftain	Red LaSoda	Red Norland
90	Clean	994.0	746.0	800.0
	$PVY^{N:O}$ infected	360.5	385.8	352.5
200	Clean	782.0	794.5	686.5
	$PVY^{N:O}$ infected	412.8	346.75	469.5
290	Clean	830.5	884.5	800.0
	$PVY^{N:O}$ infected	321.5	350.75	208.5

*weeks after inoculation with $PVY^{N:O}$

Table A5. Mean petiole nitrate-N concentration (ppm) for russet cultivars Dakota Russet, Russet Burbank, Russet Norkotah, and Shepody averaged across nitrogen rates (90, 200 and 290 kg/ha) and infection levels (clean and $PVY^{N:O}$ infected), at 4, 6, and 8 WAI*.

Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for russet cultivars at 4 WAI			
		Dakota Russet	Russet Burbank	Russet Norkotah	Shepody
90	Clean	139.5	407.5	1073.0	235.5
	$PVY^{N:O}$ infected	120.5	365.5	186.0	158.5
200	Clean	426.5	724.0	911.0	181.0
	$PVY^{N:O}$ infected	327.0	925.5	986.0	232.3
290	Clean	133.0	594.0	1836.5	268.5
	$PVY^{N:O}$ infected	558.1	428.6	509.0	769.6
Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for russet cultivars at 6 WAI			
		Dakota Russet	Russet Burbank	Russet Norkotah	Shepody
90	Clean	53.5	58.0	58.0	466.5
	$PVY^{N:O}$ infected	142.0	153.0	58.0	47.0
200	Clean	58.0	72.0	58.0	49.0
	$PVY^{N:O}$ infected	58.0	58.0	535.0	56.0
290	Clean	58.0	58.0	52.5	58.0
	$PVY^{N:O}$ infected	47.0	72.0	58.0	58.0
Nitrogen rate (kg/ha)	Infection levels	Petiole nitrate-N concentration (ppm) for russet cultivars at 8 WAI			
		Dakota Russet	Russet Burbank	Russet Norkotah	Shepody
90	Clean	278.0	372.0	326.0	278.0
	$PVY^{N:O}$ infected	420.0	278.0	326.0	278.0
200	Clean	378.0	326.0	230.0	378.0
	$PVY^{N:O}$ infected	326.0	420.0	326.0	326.0
290	Clean	278.0	230.0	326.0	468.0
	$PVY^{N:O}$ infected	326.0	372.0	278.0	278.0

*weeks after inoculation with $PVY^{N:O}$

Table A6. Analysis of variance (ANOVA) of NDVI for three red-skinned fresh market cultivars Chieftain, Red LaSoda, and Red Norland (trial 2), in response to three N rates (90, 200, and 290 kg/ha) and two infection levels (clean and PVYN:O infected), at 6 WAI[*] with $PVY^{N:O}$.

SOV[a]	Df[b]	Sum Sq	Mean Sq	F value	Pr(>F)
Variety	2	0.054	0.027	1.228	0.301
N rate	2	0.005	0.002	0.113	0.893
PVY	1	0.015	0.015	0.669	0.417
Variety×N rate	4	0.109	0.027	1.234	0.308
Variety×PVY	2	0.031	0.016	0.705	0.499
N rate×PVY	2	0.219	0.109	4.969	0.105
Variety×N rate×PVY	4	0.109	0.027	1.232	0.308

[*]WAI= weeks after inoculation
[a]Source of variation
[b]Df = Degree of freedom
Level of significance *** $p < 0.001$ ** $p < 0.01$ * $p < 0.05$