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Research Paper

Nondestructive detection of zebra chip disease in potatoes using near-infrared spectroscopy

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Near-Infrared (NIR) spectroscopy (900–2600 nm) was evaluated as a rapid, non-destructive method for detection of zebra chip disease (ZC) in potatoes. Two models were tested; one that directly correlated spectra with ZC and one that measured sugar concentrations which in turn are known to be correlated with ZC. Applying stepwise regression in conjunction with canonical discriminant analysis to raw spectra, total classification accuracy of 98.35% was achieved in discriminating infected potatoes from control, with 2% false negative and 1% false positive error rates. The same analysis applied to 2nd derivative spectra yielded 97.25% accuracy with equal false negative and false positive error rates. Canonical discriminant analysis applied to sucrose, glucose, and fructose concentrations previously determined by high-performance liquid chromatography yielded 96.7% classification accuracy, with 4.3% false positive and 2.3% false negative rates. Accuracy did not significantly differ when fructose was excluded from the model. Partial least squares regression models built to predict sugar concentrations from the 2nd derivative NIR spectra resulted in R^2 for actual vs. predicted concentrations of 0.7 and 0.72 respectively for sucrose and glucose, 0.63 for fructose, and 0.81 for total sugars. Given the relatively low R^2 values in measuring sugar concentrations directly from the spectra it was concluded that classification accuracy is highest for models that directly correlate spectral features to ZC without considering sugar concentrations. Furthermore, this indicates that although NIR can detect infection, it may not be effective for evaluating severity of ZC in fresh potatoes.

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1. Introduction

Zebra chip (ZC), a disease of potato (*Solanum tuberosum* L.), was first documented in Mexico in 1994 and has since spread across much of the United States, Central America, and New Zealand (Munyaneza, 2012; Secor et al., 2009). In the United States, it has been reported in Texas, Nebraska, Colorado, Kansas, Wyoming, Utah, New Mexico, Arizona, Nevada, California, Idaho, Oregon, and Washington (Crosslin et al., 2012; Munyaneza, 2015; Munyaneza, Crosslin, & Upton, 2007). In addition to decreased yield due to arrested growth and/or

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rapid plant death, above-ground symptoms of ZC are very similar to those of psyllid yellows and purple top diseases, including upward rolling leaves with yellow and purple discolouration, shortened internodes, and the formation of aerial tubers, which are commonly seen on plants that are injured or damaged (Buchman, Fisher, Sengoda, & Munyaneza, 2012; Ewing & Wareing, 1978; Munyaneza et al., 2007; Secor et al., 2009). Below ground symptoms include collapsed stolons, necrotic flecking of internal tissues and streaking of the medullary ray tissues in tubers (Buchman et al., 2012; Munyaneza et al., 2007). These symptoms become more conspicuous with processing and frying, with the appearance of dark blotches and streaks which presumably inspired the name "zebra chip". Although consuming diseased potatoes is not known to have an effect on human health, the visual and sensory effects on the tubers render the diseased potatoes unacceptable for the consumer market and result in significant economic losses (Crosslin, Munyaneza, Brown, & Liefting, 2010; Munyaneza, 2012).

The causal agent of ZC has only recently been identified as "*Candidatus* Liberibacter solanacearum" (Lso) (Liefting, Weir, Pennycook, & Clover, 2009). The bacterium is closely related to the liberibacters associated with Huanglongbing (i.e. citrus greening disease). The liberibacters are gram-negative, unculturable bacteria and are primarily spread by psyllid vectors (Liefting et al., 2009; Wallis et al., 2014). Lso primarily infects solanaceous plants, such as potato, tomato, pepper, and eggplants, but was also recently discovered in non-solanaceous plants such as carrot and celery (Munyaneza et al., 2010; Munyaneza, Lemmetty, Nissinen, Sengoda, & Fisher, 2011). Transmission of Lso has been observed in several psyllid species, and is expected to have more host plants and insect vectors than currently identified (Munyaneza, 2012).

Much research has been dedicated to understanding the vector–pathogen–plant interactions, transmission, time between Lso infection and tuber symptom development, and the biochemistry of infected tubers, with the goal of developing control and management strategies for ZC (Rashed, Wallis, Paetzold, Workneh, & Rush, 2013; Wallis, Chen, & Civerolo, 2012). Currently, polymerase chain reaction (PCR) based methods are used for detection of Lso, and diagnosis of subsequent ZC infection is generally based on visual observation of tuber interiors, since other symptoms as described earlier can be difficult to differentiate from other diseases. Rapid, non-destructive detection of infested tubers would benefit potato growers and processors by enabling improved management strategies and quality control of the processing stream, as well as researchers by increasing the speed of conducting relevant studies.

The biochemistry of diseased tubers is well studied and is believed to be linked to the pathogen induction of host defence responses. Concentrations of reducing sugars, aromatic amino acids, phenolic compounds, and other plant-defence-mechanism related enzymes are significantly altered by ZC infection. While some of these changes correlate with the severity of the symptoms, others depend on levels of Lso detected (Buchman et al., 2012; Wallis et al., 2012).

Near-infrared (NIR) spectroscopy is a well-established method for the analysis of the chemical composition of samples. It has also been widely used to determine internal qualities of fresh potato tubers, such as specific gravity, dry matter, water content, starch, proteins, and especially sugars with good results (Chen, Miao, Zhang, & Matsunaga, 2004; Haase, 2006; Hartmann & Büning-Pfaue, 1998; Rady & Guyer, 2015a; Yaptenco et al., 2000). It has also been used commercially to detect defects in tubers, such as hollow heart, greening, rot, spots, and cuts, etc. (Rady & Guyer, 2015b). It is particularly suited for rapid screening of food products due to its non-destructive nature and short processing time. Thus, the objective of this study was to demonstrate the efficacy of NIR spectroscopy coupled with advanced statistical analysis for rapid, non-destructive detection of potato tubers infected with ZC. This could be accomplished directly by correlating spectral features to ZC infection, or indirectly by correlating spectral features to sugar concentration (an already established procedure) which are in turn correlated with ZC. The latter would have the advantage of allowing quantification of the disease in affected tubers.

2. Material and methods

2.1. *Sample preparation*

Certified disease-free seed potatoes of a variety with high susceptibility to ZC infection (Munyaneza, Buchman, Sengoda, Fisher, & Pearson, 2011) (var. Atlantic) were obtained from Thaemert Farms (Quincy, WA) and planted during the last week of May, 2015 at the USDA-ARS Farm at Moxee, WA. The Atlantic potato is primarily used for chipping and grades consistently 64–100 mm in diameter. Whole potato seed tubers were planted 130 mm deep along eight 20 m rows, with a spacing of 2 m between rows and 230 mm between tubers. Along each row, the ground was hilled into a mound approximately 30 cm high. The sandy loam soil was treated with pre-plant herbicides S-ethyl dipropylthiocarbamate (Eptam; Gowan Co., Yuma, AZ) and trifluralin (Treflan; Dow Agrosciences, LLC, Calgary, AB, Canada) at the manufacturer's recommended rates for weed control. Additional weeding was performed manually as needed throughout the season. Fertiliser (Simplot Grower Solutions 16-16-16; Simplot Grower Solutions, Halsey, OR) was side-dressed at planting at a rate of 37.4 g plot^{-1}. Drip tape irrigation (T-Tape; Deere & Co., Moline, IL, USA) was employed throughout the season. Each row was covered with a cage formed from fibreglass poles and insect proof screen as previously reported (Buchman, Sengoda, & Munyaneza, 2011; Munyaneza et al., 2008).

An Lso-infected colony of potato psyllids was established at the USDA-ARS facility in Wapato, WA, with insects originally collected from a ZC-infected potato field in Dalhart, Texas. The insects were maintained at 25 ± 1 °C, 40 ± 5% RH, with a photoperiod of 16:8 (L: D) h. At tuber initiation stage, the plants in half of the rows were exposed to potato psyllids from the infected colony, with the remaining four rows serving as controls. Ideally a randomisation along plot rows or between rows should be implemented, however, such randomisation greatly complicates the generation of samples. It was assumed that potential soil and climate effects between adjacent rows in a single field that would impact NIR spectral