Computers and Electronics in Agriculture 74 (2010) 329–335

Contents lists available at ScienceDirect

Computers and Electronics in Agriculture

journal homepage: www.elsevier.com/locate/compag

Original paper

Diagnosis of bacterial spot of tomato using spectral signatures

C.D. Jones[a,*], J.B. Jones[b], W.S. Lee[a]

[a] Department of Agricultural and Biological Engineering, University of Florida, Gainesville, FL 32611, USA
[b] Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA

ARTICLE INFO

Article history:
Received 27 August 2009
Received in revised form 1 August 2010
Accepted 14 September 2010

Keywords:
Bacterial leaf spot of tomato
NIRS
Significant wavelength
Reflectance
Plant disease
PLS

ABSTRACT

Ultraviolet, visible, and near-infrared reflectance spectroscopy was used to determine the disease severity of tomato (*Lycopersicon esculentum*) leaves infected with *Xanthomonas perforans*, the causal agent of bacterial leaf spot of tomato. Chemometric methods were used to identify significant wavelengths and create spectral-based prediction models. Significant wavelengths were identified through analysis of the *B*-matrix from partial least squares (PLS) regression, analysis of a correlation coefficient spectrum, and through the use of a stepwise multiple linear regression (SMLR) procedure. These analysis methods revealed several significant regions wavelengths and produced predictive models of disease severity based on absorbance spectra. The best model predicted the disease severity of the validation data set with a root mean square difference (RMSD) of 4.9% and a coefficient of determination (R^2) of 0.82. The results of this initial study indicate the potential for the use of spectral technology to detect bacterial leaf spot of tomato in the field.

1. Significance of problem

Tomato (*Lycopersicon esculentum*) is an important commodity in Florida, with this State accounting for over 40% of the fresh-market tomato production in the United States between 1997 and 2006 with production in 2006 valuing $551 million (United States Department of Agriculture, 2006). Despite the high productivity of the State in this market, the high humidity, warm temperatures, and high rainfall rates typical for Florida make crops particularly susceptible to bacterial spot disease (Pohronezny and Volin, 1983). The disease affects all aboveground plant parts, but is most noticeable as spots on leaves and fruit (Kucharek, 2000). It has been shown to be endemic (Jones et al., 1986) and has been demonstrated to cause severe reduction in marketable fruit yield (Dougherty, 1978; Pernezny et al., 1996).

Currently, treatments for bacterial spot do exist. Copper bactericide (Jones et al., 1991) and bacteriophage (Obradovic et al., 2004) applications have been shown to reduce incidence of bacterial spot effectively, and these treatments have been shown to be economically beneficial to growers (Pernezny et al., 1996; McAvoy, 2006). Despite these available proven applications, many fields remain significantly affected. This is because current methods of disease detection in many cases are inadequate.

Assessment of crop disease damage is typically determined using field scouting, which has been shown to be expensive, time-consuming, and difficult for large farms (Lucas, 1998). Field scouting is usually conducted on a weekly interval, and treatments are applied if disease is detected. For large-scale operations, however, conventional ground scouting has been shown to be incapable of providing efficient disease monitoring in an economical manner (Zhang et al., 2003). Remote sensing has the potential to detect crop diseases for large scale operations in a rapid and spatially specific manner (Zhang et al., 2003). Early and accurate diagnosis of bacterial spot in the field could enhance the ability of farmers to implement beneficial disease treatments in an economical manner, reducing the incidence of disease, the amount of chemical applications, the cost of these chemical applications, and the monitoring cost. This approach could also be useful to researchers for determining the effectiveness of chemical control strategies by allowing more accurate and quantitative disease assessment.

Numerous studies have demonstrated the ability to use imaging techniques to detect plant diseases. Polischuk et al. (1997) were able to diagnose tomato mosaic tobamovirus infection using spectral measurements. Graeff et al. (2006) were able to identify and discriminate between powdery mildew and take-all disease in wheat using leaf reflectance measurements. Moshou et al. (2004) were able to distinguish between healthy wheat and wheat infected with yellow rust using reflectance measurements and neural networks. Naidu et al. (2009) took reflectance measurements and used stepwise selection and discrimination procedures to detect Grapevine leafroll-associated virus-3 in two cultivars of grapevine. Wu et al. (2008) took reflectance measurements of eggplant leaves

* Corresponding author at: Frazier Rogers Hall, P.O. Box 110570, Gainesville, FL 32611, USA. Tel.: +1 352 392 1864x238; fax: +1 352 392 4092.
E-mail address: cujo@ufl.edu (C.D. Jones).

0168-1699/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.compag.2010.09.008

infected with gray mold and used principal component analysis (PCA), PLS, and neural networks to detect infection with 85% accuracy. Huang and Apan (2006) were able to determine *Sclerotinia* rot disease in celery using reflectance measurements and PLS regression with RMSD of 11.1%. Liu et al. (2007) were able to determine the percentage of leaf diseased with rice brown spot disease with RMSD as low as 2.0% using leaf reflectance measurements and SMLR, principal component regression (PCR), and PLS. The subsequent spectral signatures of these various plant diseases can be utilized in remote sensing applications to allow for rapid, inexpensive, large-scale disease assessments.

As airplane and satellite technologies continue to advance, interest in using spectral information with remote sensing to monitor crop diseases has grown (Bryant and Moran, 1999; Deguise et al., 1998). Many studies have demonstrated the ability to identify plant diseases using remotely sensed data. Apan et al. (2004) were able to discriminate between sugar cane with severe orange rust disease from non-diseased areas using hyperspectral imagery. Qin and Zhang (2005) used multispectral images and created disease indices that predicted rice sheath blight severity with correlation coefficients higher than 0.62. Chen et al. (2007) were able to detect wheat infested with take-all disease using Landsat Thematic Mapper imagery processed with masking and PCA techniques. Jacobi and Kuhbauch (2005) demonstrated that Quickbird satellite images could be used to discriminate between wheat with fungal infection and nitrogen deficient wheat. Zhang et al. (2003) used hyperspectral remote sensing data obtained via low-altitude aerial photography of tomato fields and were able to distinguish healthy and lightly diseased plants from moderate to severely diseased plants with late blight disease. Wang et al. (2008) applied a back-propagation neural network to Advanced Visible Infrared Imaging Spectrometer (AVIRIS) hyperspectral images taken from low altitude flights and were able to determine late blight tomato disease infection with an R^2 of 0.67.

2. Objectives

Applications for sensing disease remotely depend on initial data linking spectral responses to disease severity. In fact, the identification of spectral signatures for bacterial spot of tomato is the first step in applying these technologies towards improved disease monitoring and control. Considering the economic impact of tomato leaf spot, this research could prove beneficial for regional tomato growers by improving disease assessment compared to current techniques. Previous studies have shown the ability to use remote sensing to determine the presence or severity of various crop diseases. However, no studies have been conducted to apply this approach to diagnosing bacterial spot of tomato. Therefore, the objectives of this study were to determine the spectral signature of tomato leaves infected with bacterial spot, to analyze the diffuse reflectance spectra to find the most significant electromagnetic wavelengths and components for predicting disease severity, and to create a model capable of determining the severity of tomato leaf spot infection based on measured spectral characteristics.

3. Materials and methods

3.1. Plant growth, leaf sampling, and reflectance measurement

Bonnie Best tomato variety seedlings were transplanted 10 days after sowing into 10-cm diameter pots containing Scott's Metro Mix 350 soilless medium (Denver, CO) and grown in Gainesville, Florida (29.67°N, 82.33°W) in a glass greenhouse with temperatures ranging from 25 to 35 °C (night:day), ambient humidity, and 30% shading. Twelve plants were planted in the first trial and 24 plants were planted two weeks later for a second trial. The plants were inoculated 4 weeks after germination using the dip inoculation method described by Whalen et al. (1991). In order to do this, *Xanthomonas perforans* strain 91–118 was grown for 24 h at 28 °C. The bacterium was suspended in sterile tap water and the concentration was adjusted by adding sterile tap water until a spectrophotometer measured the absorbance at 600 nm to be 0.3, which corresponds to 10^8 colony forming units per ml (CFU/ml). A 1000-fold and a 100-fold dilution were then made with tap water to create suspensions of 10^5 and 10^6 CFU/ml to be used for the two treatments. Silwet L77 surfactant was added to the solutions at 0.025% so the solutions would better wet the leaf surface and better facilitate disease incidence. Each plant was submerged in the solution for 15 s. One third of the plants were inoculated with the 10^5 CFU/ml solution, one third with the 10^6 CFU/ml solution, and one third with no bacterium.

Samples were collected 8 and 11 days after inoculation for the first trial and after 9, 14, and 20 days for the second trial, with 30 and 32 samples collected on the respective days for the first trial and 25, 22, and 47 samples collected on the respective days for the second trial. Leaf samples were not collected randomly, but rather were selectively chosen in an attempt to sample a range of disease severities, which is more useful for the creation of a prediction model. Samples were collected after various time intervals to allow sampling of early and late developed disease and to obtain a range of disease severity. The third sampling date was conducted during the second trial with a larger number of samples being taken to obtain more samples with greater disease severity because earlier sampling had a greater number of samples with lesser disease severities.

The disease severity of each sample was determined based on visual estimation of the percentage of the infected leaf surface area, as is often done to obtain leaf-level estimates of disease severity for spectral disease identification (Graeff et al., 2006; Liu et al., 2007; Luedeling et al., 2009). Fig. 1 shows leaves with varying disease severities. Damage estimates were conducted by the same person for all samples to ensure consistency. Damage percentages were determined in increments of 5%, except for samples with less than 3% damage, which were determined in increments of 1%.

The spectral diffuse reflectance of the leaf samples was measured from 200 nm to 2500 nm in 1 nm increments using a spectrophotometer (Cary 500, Varian Inc., Palo Alto, CA, USA) with mercury and UV lamp light sources. The system was allowed at least 30 minutes to warm-up before any measurements were taken. An integrating sphere (DRA-CA-5500, Labsphere Inc., North Sutton, NH, USA) with an interior coating of white polytetrafluoroethylene (PTFE) was attached to the spectrophotometer. The full width at half maximum of the spectrophotometer was 2 nm. The sample measurement port had a diameter of 38 mm. The size and shape of tomato leaves were not sufficient to cover the port, so a Teflon sheet with a 25 mm diameter hole and 3.175 mm thickness was used. In order to obtain an optical reference standard, each day before any sample measurements were taken, the hole on the Teflon sheet was covered with a 50 mm PTFE disc and the diffuse reflectance was recorded. A total of 156 spectra were obtained from the measurement of 156 leaf samples.

3.2. Identification of significant wavelengths

Pre-treatment. According to the Beer–Lambert law, the concentration of an absorber is directly proportional to the sample absorbance (Williams and Norris, 2001). So in order to better relate the spectral data to disease severity, the diffuse reflectance data were converted to absorbance data using the relationship $A = \log(1/R)$, where A is the absorbance and R is the reflectance. All analyses were conducted using absorbance data.