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Review

A review of advanced techniques for detecting plant diseases

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ABSTRACT

Diseases in plants cause major production and economic losses in agricultural industry worldwide. Monitoring of health and detection of diseases in plants and trees is critical for sustainable agriculture. To the best of our knowledge, there is no sensor commercially available for real-time assessment of health conditions in trees. Currently, scouting is most widely used mechanism for monitoring stress in trees, which is an expensive, labor-intensive, and time-consuming process. Molecular techniques such as polymerase chain reaction are used for the identification of plant diseases that require detailed sampling and processing procedure. Early information on crop health and disease detection can facilitate the control of diseases through proper management strategies such as vector control through pesticide applications, fungicide applications, and disease-specific chemical applications; and can improve productivity.

The present review recognizes the need for developing a rapid, cost-effective, and reliable health-monitoring sensor that would facilitate advancements in agriculture. It describes the currently used technologies that can be used for developing a ground-based sensor system to assist in monitoring health and diseases in plants under field conditions. These technologies include spectroscopic and imaging-based, and volatile profiling-based plant disease detection methods. The paper compares the benefits and limitations of these potential methods.

Contents

1. Introduction

Plant diseases cause major production and economic losses in agriculture and forestry. For example, soybean rust (a fungal disease in soybeans) has caused a significant economic loss and just by removing 20% of the infection, the farmers may benefit with an approximately 11 million-dollar profit (Roberts et al., 2006). It is estimated that the crop losses due to plant pathogens in United Stated result in about 33 billion dollars every year. Of this, about 65% (21 billion dollars) could be attributed to non-native plant pathogens (Pimentel et al., 2005). Some of the diseases caused by introduced pathogenic species are chestnut blight fungus, Dutch elm disease, and huanglongbing citrus disease (Pimentel et al., 2005; Li et al., 2006).

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The bacterial, fungal, and viral infections, along with infestations by insects result in plant diseases and damage. There are about 50,000 parasitic and non-parasitic plant diseases of plants in United States (Pimentel et al., 2005). Upon infection, a plant develops symptoms that appear on different parts of the plants causing a significant agronomic impact (López et al., 2003). Many such microbial diseases with time spread over a larger area in groves and plantations through accidental introduction of vectors or through infected plant materials. Another route for the spread of pathogens is through ornamental plants that act as hosts. These plants are frequently sold through mass distribution before the infections are known. An early disease detection system can aid in decreasing such losses caused by plant diseases and can further prevent the spread of diseases.

After the onset of plant disease symptoms, the presence of disease in plants is verified using disease detection techniques. Presently, the plant disease detection techniques available are enzyme-linked immunosorbent assay (ELISA), based on proteins produced by the pathogen, and polymerase chain reaction (PCR), based on specific deoxyribose nucleic acid (DNA) sequences of the pathogen (Prithiviraj et al., 2004; Das, 2004; Li et al., 2006; Saponari et al., 2008; Ruiz-Ruiz et al., 2009; Yvon et al., 2009). In spite of availability of these techniques, there is a demand for a fast, sensitive, and selective method for the rapid detection of plant diseases. Disease detection techniques can be broadly classified into direct and indirect methods. Fig. 1 summarizes some of these methods of disease detection. An advanced plant disease detection technique can provide rapid, accurate, and reliable detection of plant diseases in early stages for economic, production, and agricultural benefits.

In the present paper, advanced techniques of ground-based disease detection that could be possibly integrated with an automated agricultural vehicle are reviewed. In ground-based disease detection studies, both field-based and laboratory-based experiments are discussed in this paper. The field-based studies refer to studies that involve spectral data collection under field conditions, whereas laboratory-based studies refer to data collection under laboratory conditions. The laboratory-based experiments provide strong background knowledge (such as the experimental protocol and statistical algorithm for classification) for the field-based applications.

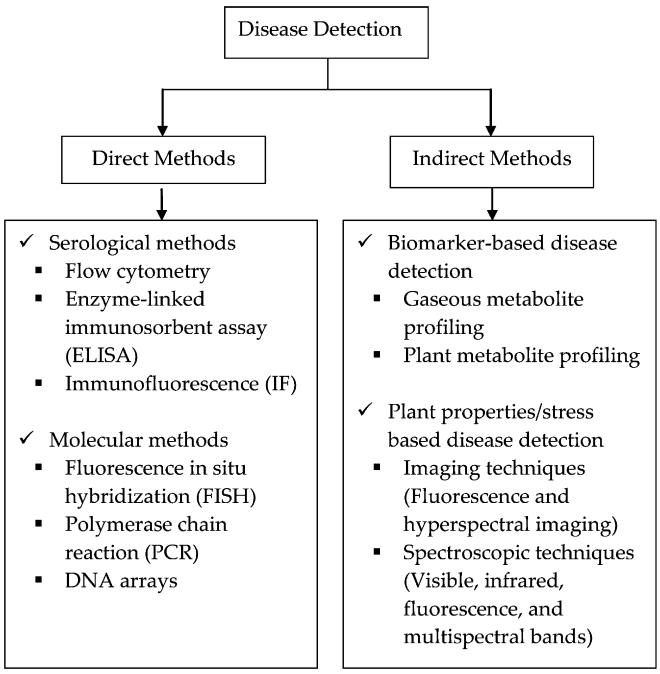

Fig. 1. Methods of plant disease detection.

This paper describes two approaches taken to detect plant diseases. The first approach involves the application of spectroscopic and imaging techniques for disease detection, whereas the second approach describes the application of volatile organic metabolites as possible biomarkers for disease detection. These two approaches were selected as they could be easily integrated with an agricultural vehicle for a fast, reliable, and real-time plant disease monitoring for disease control and management. The early detection of plant diseases (before the onset of disease symptoms) could be a valuable source of information for executing proper pest management strategies and disease control measures to prevent the development and the spread of diseases.

2. Molecular techniques of plant disease detection

In recent years, molecular techniques of plant disease detection have been well established. The sensitivity of the molecular techniques refers to the minimum amount of microorganism that can be detected in the sample. López et al. (2003) reported that the sensitivity of the molecular techniques for detecting bacteria ranged from 10 to 10^6 colony forming units/mL. The commonly used molecular techniques for disease detection are ELISA and PCR (PCR and real-time PCR). Other molecular techniques include immunoflourescence (IF), flow cytometry, fluorescence in situ hydridization (FISH), and DNA microarrays.

In the ELISA-based disease detection, the microbial protein (antigen) associated with a plant disease is injected into an animal that produces antibodies against the antigen. These antibodies are extracted from the animal's body and used for antigen detection with a fluorescence dye and enzymes. In presence of the disease-causing microorganism (antigen), the sample would fluoresce, thus confirming the presence of a particular plant disease. In PCR-based disease detection, the genetic material (DNA) of the disease-causing microorganism is extracted, purified, and amplified before performing the gel electrophoresis. The presence of a specific band in gel electrophoresis confirms the presence of the plant-disease causing organism. Different types of immunological and PCR techniques are described by López et al. (2003). There are number of studies on disease detection using the molecular techniques. Efforts are ongoing to improve the efficiency of these techniques. Table 1 summarizes few studies on plant disease detection using molecular techniques. López et al. (2003) reviewed the various molecular techniques used for detecting pathogenic viruses and bacteria in plants. Their review paper elaborates the molecular methods of plant disease detection, including different types of PCR and ELISA-based techniques. Schaad and Frederick (2002), and Henson and French (1993) described the applications of PCR technique for the diagnosis of plant diseases. Alvarez (2004) reported that there are about 97 commercially available immunodiagnostic test kits for the detection of bacterial pathogens in plants.

Some of the limitations of the molecular techniques are that they are time-consuming and labor-intensive, and require an elaborate procedure, especially during sample preparation (collection and extraction) to obtain reliable and accurate results on plant disease detection. In addition, these techniques require consumable reagents that must be tailored to detect each specific pathogen (e.g. sequence-specific primers for PCR). The molecular techniques could be used as robust tool to ensure the presence of plant diseases, but cannot be used as a preliminary screening tool for processing large number of plant samples due to the time involved in the process. Thus, spectroscopic techniques can be potential method for rapid detection of plant diseases. In the present review paper, molecular methods of plant disease detection are not dis-

Table 1
Examples of some studies on plant disease detection using molecular techniques.

Plant/Trees	Pathogen	Type	Molecular method	Reference
Grapevine	*Xylella fastidiosa*	Bacteria	PCR, ELISA	Minsavage et al. (1994)
Onion	*Sclerotium cepivorum*	Fungi	PCR	Anwar Haq et al. (2003)
Olive	*Pseudomonas savastanoi* pv. *savastanoi*.	Bacteria	PCR, Hybridization	Bertolini et al. (2003)
Sweet orange	*Candidatus* Liberibacter asiaticus.	Bacteria	PCR	Das (2004)
Sweet orange	*Candidatus Liberibacter asiaticus*, *Ca. L. americanus*, *Ca. L. africanus*	Bacteria	PCR	Teixeira et al. (2005)
Citrus	*Candidatus Liberibacter*	Bacteria	PCR	Li et al. (2006)
Citrus	*Xylella fastidiosa*, *Methylobacterium mesophilicum*	Bacteria	PCR	Lacava et al. (2006)
Citrus	*Citrus tristeza virus*	Virus	PCR, ELISA	Saponari et al. (2008)
Sweet orange	*Candidatus Liberibacter asiaticus*	Bacteria	Isothermal, chimeric primer-initiated amplification of nucleic acids + cycling probe technology	Urasaki et al. (2008)
Rice	*Burkholderia glumae*	Bacteria	Fluorescence PCR	Fang et al. (2009)
Potato	*Candidatus Liberibacter solanacearum*	Bacteria	PCR	Li et al. (2009a)
Citrus	*Citrus leaf blotch virus*	Virus	PCR	Ruiz-Ruiz et al. (2009)
Tomato	*Pepino mosaic virus*	Virus	PCR, ELISA	Gutiérrez-Aguirre et al. (2009)
Almond	*Candidatus Phytoplasma prunorum*	Bacteria	PCR	Yvon et al. (2009)

cussed in detail as a number of review papers are available in the literature.

3. Spectroscopic and imaging techniques for disease detection

Recent developments in agricultural technology have lead to a demand for a new era of automated non-destructive methods of plant disease detection. It is desirable that the plant disease detection tool should be rapid, specific to a particular disease, and sensitive for detection at the early onset of the symptoms (López et al., 2003). The spectroscopic and imaging techniques are unique disease monitoring methods that have been used to detect diseases and stress due to various factors, in plants and trees. Current research activities are towards the development of such technologies to create a practical tool for a large-scale real-time disease monitoring under field conditions.

Various spectroscopic and imaging techniques have been studied for the detection of symptomatic and asymptomatic plant diseases. Some the methods are: fluorescence imaging (Bravo et al., 2004; Moshou et al., 2005; Chaerle et al., 2007), multispectral or hyperspectral imaging (Moshou et al., 2004; Shafri and Hamdan, 2009; Qin et al., 2009), infrared spectroscopy (Spinelli et al., 2006; Purcell et al., 2009), fluorescence spectroscopy (Marcassa et al., 2006; Belasque et al., 2008; Lins et al., 2009), visible/multiband spectroscopy (Yang et al., 2007; Delalieux et al., 2007; Chen et al., 2008), and nuclear magnetic resonance (NMR) spectroscopy (Choi et al., 2004). Hahn (2009) reviewed multiple methods (sensors and algorithms) for pathogen detection, with special emphasis on postharvest diseases.

The spectroscopic and imaging techniques could be integrated with an autonomous agricultural vehicle that can provide information on disease detection at early stages to control the spread of plant diseases. This technology can also be applied to identify stress levels and nutrient deficiencies in plants. In regard to plant disease detection, significant research is ongoing on the prospective of this technology from last few decades. Spectroscopic technology has been successfully applied for plant stress detection such as water-stress detection and nutrient-stress detection. In addition, there have also been significant applications for monitoring the quality of postharvest fruits and vegetables. Some of the commonly used spectroscopic and imaging techniques are described in the following sections. Tables 2 and 3 summarize few studies on plant disease detection using spectroscopic and imaging techniques. Some postharvest applications have also been discussed in this paper, as the knowledge on statistical models and methodology for data collection for postharvest applications is transferrable for real-time plant disease detection.

3.1. Fluorescence spectroscopy

Fluorescence spectroscopy refers to a type of spectroscopic method, where the fluorescence from the object of interest is measured after excitation with a beam of light (usually ultraviolet spectra).

For the last twenty years, the laser-induced fluorescence has been used for vegetative studies, such as to monitor stress levels and physiological states in plants (Belasque et al., 2008). Two types of fluorescence: (i) blue-green fluorescence in about 400–600 nm range, and (ii) chlorophyll fluorescence in about 650–800 nm range, are produced by green leaves. The fluorescence spectroscopy can be utilized to monitor nutrient deficiencies, environmental conditions based stress levels, and diseases in plants (Cerovic et al., 1999; Belasque et al., 2008).

Belasque et al. (2008) employed fluorescence spectroscopy to detect stress caused by citrus canker (bacterial disease caused by *Xanthomonas citri–X. axonopodis* pv. *citri*) and mechanical injury. A portable fluorescence spectroscopy system was taken to the greenhouse and the measurement probe was placed 2 mm above the leaf (attached to greenhouse plants) for collecting data from different samples during the period of study (60 days). The spectral data were further processed and analyzed in the laboratory. A 532 nm 10 mW excitation laser was used for excitation and ratios between fluorescence at different wavelengths were employed to monitor the stress caused by bacterial infection. The samples of leaves collected from the field (detached leaves) as well as leaves from greenhouse plants (attached leaves) were analyzed using the system. The three ratios used were: (i) ratio between fluorescence intensity at 452 and 685 nm, (ii) ratio between fluorescence intensity at 452 nm and 735 nm, and (iii) ratio between fluorescence intensity at 685 nm and 735 nm. Fluorescence of citrus leaves was monitored for 60 days under four different conditions: leaves with no stress, leaves with mechanical stress, leaves with disease, and leaves with disease and mechanical stress. The studies reported the potential of fluorescence spectroscopy for disease detection and discrimination between the mechanical and diseased stress. A similar approach was taken to detect water stress and differentiate citrus canker leaves from variegated chlorosis leaves (Marcassa et al., 2006). The above studies could classify healthy from citrus canker-affected leaves, but were unable to identify water stress and distinguish between variegated chlorosis and citrus canker-infected leaves. The authors did not yet present any statistical analysis to evaluate the

Table 2
Examples of studies on plant disease detection using spectroscopic techniques.

Plant	Disease/Damage	Statistical Methods	Optimum spectral range	Reference
Fluorescence Spectroscopy				
Citrus	Citrus canker	–	452, 685 and 735 nm	Belasque et al. (2008)
Visible and Infrared Spectroscopy				
Rice	Infested with brown planthopper (*Nilaparvata luge*)	–	737–925 nm	Yang and Cheng (2001)
Wheat	Powdery mildew and take-all disease	Analysis of variance, correlation and regression analysis	490_{780}, 510_{780}, 516_{1300} and 540_{1300} nm	Graeff et al. (2006)
Rice	Brown planthopper and leaffolder infestation	Linear regression models	426 nm	Yang et al. (2007)
Kiwifruit	Gray mold, Sclerotinia rot	Principal component analysis	–	Costa et al. (2007)
Wheat	Yellow rust	Regression	–	Huang et al. (2007)
Tomato	Leaf miner damage	–	800 to 1100 nm, 1450 and 1900 nm	Xu et al. (2007)
Grapevine	Grapevine leafroll disease	Discriminant analysis	752, 684 and 970 nm	Naidu et al. (2009)
Nuclear Magnetic Resonance (NMR) Spectroscopy				
Catharanthus roseus (ornamental plant)	Phytoplasmas causing yellowing disease	Principal component analysis	–	Choi et al. (2004)

ability of the technique to discriminate or classify different plant conditions.

Lins et al. (2009) conducted field experiments to discriminate citrus canker-stressed leaves from chlorosis-infected (caused by *Xylella fastidiosa* bacteria) and healthy leaves. In addition, they conducted leaf detachment experiments to monitor effect of time (up to 12 h) on fluorescence of detached leaves using fluorescence spectroscopy. In their study, two indices/figures of merit were used to assess the difference between healthy and citrus canker-infected leaves. In spite of having a clear discrimination between the healthy and citrus canker-infected leaves, the citrus canker-affected leaves showed similar fluorescence output as that of chlorosis-affected leaves under field condition. The paper reported that one of the indices (figure of merit-1) was less influenced by the leaf detachment time (1–12 h) than the other (figure of merit-2). The above report recommended the application of a range of spectral data rather than fluorescence alone for a reliable discrimination results, and further work needs to be done to classify different plant conditions using statistical tools.

Methods such as principal component analysis (PCA), discriminant analysis, and neural network-based classification algorithms can be applied to analyze the results obtained from fluorescence spectroscopy. Methods such as PCA, parallel factor analysis, cluster analysis, partial least square (PLS) regression, and Fischer's linear discriminant analysis (LDA) can be applied for classifying fluorescent spectrometric data having two or more classes (Guimet, 2005).

3.2. Visible and infrared spectroscopy

Similar to fluorescence spectroscopy, visible and infrared spectroscopy have been used as a rapid, non-destructive, and cost-effective method for the detection of plant diseases. It is a fast-developing technology used for varied applications (Ramon et al., 2002; Delwiche and Graybosch, 2002; Pontius et al., 2005; Gomez et al., 2006; Zhang et al., 2008a,b; Guo et al., 2009; Sundaram et al., 2009). Studies have also been conducted on the detection of stress, injury, and diseases in plants using this technology (Polischuk et al., 1997; Spinelli et al., 2006; Naidu et al., 2009). The visible and infrared regions of the electromagnetic spectra are known to provide the maximum information on the physiological stress levels in the plants (Muhammed, 2002, 2005; Xu et al., 2007) and thus, some of these wavebands specific to a disease can be used to detect plant diseases (West et al., 2003), even before the symptoms are visible. In general, visible spectroscopy is used for disease detection in plants in combination with infrared spectroscopy (Malthus and Madeira, 1993; Bravo et al., 2003; Huang et al., 2004; Larsolle and Muhammed, 2007).

Spinelli et al. (2006) assessed the near infrared (NIR)-based technique for detecting fire blight disease in the asymptomatic pear plants under greenhouse conditions. The NIR technique did not exhibit potential for classifying infected plants from that of healthy ones, while electronic nose system showed a better potential to classify diseased plants. The authors reported that the possible

Table 3
Examples of studies on plant disease detection using imaging techniques.

Plant	Disease/Damage	Statistical Methods	Optimum spectral range	Reference
Wheat	Scab (Fusarium head blight)	Step discrimination and discriminant analysis	568, 715 nm (550, 605, 623, 660, 697 and 733 nm)	Delwiche and Kim (2000)
Tomato	Late blight disease	Minimum noise fraction transformation and spectral angle mapping-based classification	700–750 nm, 750–930 nm, 950–1,030 nm, and 1,040–1,130 nm	Zhang et al. (2003, 2005)
Wheat	Yellow rust, nutrient deficiency	Self-organizing map neural network, quadratic discriminant analysis	680, 725 and 750 nm	Moshou et al. (2005, 2006)
Wheat	Yellow rust	Regression analysis	–	Huang et al. (2007)
Grapefruit (fruit)	Citrus canker	Principal component analysis	553, 677, 718 and 858 nm	Qin et al. (2008)
Vidalia sweet onions	Sour skin disease	Image analysis	1,150–1,280 nm	Wang et al. (2009)
Sweet orange	Blue mold, Browning rot	Difference in reflectance	540 and 680 nm	Sighicelli et al. (2009)

reason for the inability of the NIR based technique to distinguish diseased from healthy plants could be due to a very small leaf scan area (2 mm^2 in this study). The authors recommended that a multidimensional image analysis could provide more information on the diseased plants rather than a smaller field of view. This signifies that the spectroscopy-based imaging techniques could be more robust in disease identification at early stages in plant diseases than spectroscopic methods alone.

Purcell et al. (2009) investigated the application of NIR spectroscopy for the determination and rating of sugarcane resistance against Australian sugarcane disease, Fiji leaf gall. The leaf samples from the cane stalks were analyzed with a Fourier transform (FT)-NIR instrument in 2–4 days after the sugarcane stalks were removed. The signal in the spectral range of 11,000–4000 cm^{-1} was procured. Principal component analysis and PLS-based statistical methods were used to analyze the data. The second derivative of the signal in the spectral range was also determined to verify if the signal is better represented the disease for analysis. The authors reported that the PLS-based method was effective in predicting the disease rating in sugarcane.

The data analysis procedure used for the classification of postharvest food products can be applicable for plant disease detection. Thus, the knowledge from one application (on statistical algorithm, data processing, experimental protocol, etc.) can be transferred to other possible applications. For example, Sirisomboon et al. (2009) used visible spectra along with NIR spectra (600–1100 nm) to identify defective pods during soybean processing. In addition to PCA, soft independent modeling of class analogy (SIMCA) and PLS-discriminant analysis were used to classify the groups. The authors reported that the SIMCA-based model was able to discriminate different groups of soybean better than the PLS-based model.

Naidu et al. (2009) used leaf spectral reflectance to identify viral infection (under field conditions) in grapevines (*Vitis vinifera* L.) that cause grapevine leafroll disease. A portable spectrometer was used to collect reflectance data from each leaf of the plant using a plant-probe attachment device having a leaf clip. In addition to the green, near infrared, and mid infrared region of the spectra, vegetative indices were used to assess the applicability of spectral reflectance in identifying the disease. Discriminant analysis was performed to classify the infected leaves with and without symptoms with that of non-infected leaves. The different categories of leaves could be clearly differentiated with improved accuracies when both the vegetative indices and individual reflectance bands were used. A maximum of 75% accuracy was achieved in the study. Huang and Apan (2006) collected hyperspectral data using portable spectrometer under field conditions to detect *Sclerotinia* rot disease in celery. PLS regression analysis was performed to analyze the spectral reflectance data. The first and second derivatives were estimated to test their effectiveness in reducing the root mean square error during the validation of the developed model. It was reported that the raw data-based model produced lower root mean square errors than the first and second derivatives. The authors also stated that the reflectance in the visible and infrared range from 400 to 1300 nm were sufficient in acquiring similar results as that of entire spectra (400–2500 nm). The cross-validation results using raw, first derivative, and second derivative data provided a prediction error of 11–13%.

Chen et al. (2008) investigated the application of hyperspectral reflectance to identify cotton canopy infected with Verticillium wilt. The data were collected using a portable spectroradiometer under field conditions and it was analyzed in the laboratory. The authors reported that among the visible and infrared spectra, the first derivative of the infrared spectra in the wavelength range between 731 and 1317 nm were most effective in predicting the Verticillium wilt in cotton canopy accurately based on the developed models. Other sensitive regions for prediction of infection severity levels were found to be from 780 to 1300 nm and the first derivative of the spectra from 680 to 760 nm. Yang et al. (2007) studied brown planthoppers and leaf-folder infestations in rice plants. The infested conditions of the plants were ranked and efforts were made to identify the extent of infestations using spectroscopic reflectance (350–2400 nm) data collected under field conditions. The results indicated that the spectral range from 426 to 1450 nm showed the maximum correlation intensity. The changes in spectral properties were low in visible and ultraviolet (UV) range, whereas the infrared region (740–2400 nm) yielded the maximum change in spectral signature.

Delalieux et al. (2007) used hyperspectral reflectance data (350–2500 nm) to detect apple scab caused by *Venturia inaequalis*. The study involved the identification of infected trees and selection of wavelengths best suited for classifying the infected leaves from those of the healthy leaves. The spectral data were analyzed using methods as LDA, logistic regression analysis (for each wavelength), partial least squares logistic discriminant analysis (PLS-LDA), and tree-based modeling for classifying the infected and healthy leaves. The paper reported that the spectral features from 1350 to 1750 nm and 2200 to 2500 nm were effective for the classification of the infected leaves from healthy leaves at early stages, whereas 580–660 nm and 688–715 nm were effective in identifying infected leaves at their developed stages of infection. Among the statistical methods, logistic regression analysis, PLS-LDA, and tree-based modeling were preferred for classification. The authors recommended PLS-LDA and tree-based modeling methods as they are simpler, and less computationally- and time-intensive. Kobayashi et al. (2001) utilized multispectral radiometer and airborne multispectral scanner for the identification of panicle blast in rice. The spectral range for airborne multispectral scan was selected based on ground experiments. The four spectral bands of 400–460 nm, 490–530 nm or 530–570 nm, 650–700 nm, and 950–1100 nm were utilized for scanning (instantaneous field of view = 2.5 mrad, ground resolution = 0.94 m at 300 m height). The magnitude of reflectance ratios (R_{470}/R_{570}, R_{520}/R_{675}, and R_{570}/R_{675}) decreased with an increase in frequency of panicle blast occurrence. Wang et al. (2002) used PLS and artificial neural network (ANN) models on the visible-IR reflectance data for classifying damaged soybean seeds. The authors reported that the ANN yielded higher overall as well as individual-class classification accuracies than PLS models.

Various studies have used different methods/models for the classification of diseases/conditions of plants based on spectral data. For an instance, Roggo et al. (2003) utilized eight classification models (linear discriminant analysis, *k*-nearest neighbors, soft independent modeling of class analogy, discriminant partial least squares (DPLS), procrustes discriminant analysis (PDA), classification and regression tree, probabilistic neural network, and learning vector quantization-based neural network) for qualitative determination of sugarbeet. They found that SIMCA, DPLS, and PDA yielded the highest classification accuracies that those of other models. Wu et al. (2008) used PCA-based back-propagation neural network (BPNN) model and PLS wavelength-based BPNN for detection of *Botrytis cinerea*-affected eggplant leaves prior to the visibility of symptoms under laboratory conditions. The BPNN model yielded a maximum of 85% classification accuracy for predicting fungal infections.

In addition to the statistical models for classification, many spectroscopy-based studies use different vegetative indices for evaluating the change in spectral reflectance at different plant conditions (diseased or healthy plant). Some of the vegetative indices are summarized in Table 4.

Table 4
Vegetative indices used in spectroscopic studies for disease detection.

Vegetative Index	Estimation	Reference
Disease index (f_D) (specific for individual study)	$f_D = \frac{I_{550\,nm}}{I_{550\,nm}+I_{690\,nm}}$	Moshou et al. (2005)
Normalized difference vegetation index (NDVI)	$NDVI = \frac{R_{NIR}-R_{RED}}{R_{NIR}+R_{RED}}$	Yang and Cheng (2001), Bravo et al. (2004), Yang et al. (2007), Naidu et al. (2009)
Green normalized difference vegetation index (Green NDVI)	$Green\ NDVI = \frac{R_{GREEN}-R_{RED}}{R_{GREEN}+R_{RED}}$	Yang et al. (2007)
Water Band Index (I_{WB})	$I_{WB} = \frac{R_{950\,nm}}{R_{900\,nm}}$	Xu et al. (2007)
Soil-adjusted vegetation index (SAVI)	$SAVI = \frac{(R_{NIR}-R_{RED})(1+L)}{R_{NIR}+R_{RED}+L}$, $L = 0.5$	Yang et al. (2007)
Other indices	$(R_{NIR} - R_{RED})$, $\frac{R_{RED}}{R_{NIR}}$, $\frac{R_{GREEN}}{R_{RED}}$, $\frac{R_{NIR}}{R_{RED}}$	Yang et al. (2007)
Photochemical reflectance index (PRI)	$PRI = \frac{R_{531\,nm}-R_{570\,nm}}{R_{531\,nm}+R_{570\,nm}}$	Huang et al. (2007), Naidu et al. (2009)
Red-edge vegetation stress index (RVSI)	$RVSI = \frac{R_{714\,nm}+R_{752\,nm}}{2}-R_{733\,nm}$	Naidu et al. (2009)
Modified chlorophyll (a and b) absorption in reflectance index (MCARI)	$MCARI = [(R_{700\,nm} - R_{670\,nm}) - 0.2(R_{700\,nm} - R_{550\,nm})] \times \frac{R_{700\,nm}}{R_{670\,nm}}$	Naidu et al. (2009)
Visible atmospherically resistance index (VARI)	$VARI = \frac{R_{GREEN}-R_{RED}}{R_{GREEN}+R_{RED}-R_{BLUE}}$	Naidu et al. (2009)
Water Index (WI)	$WI = \frac{R_{900\,nm}}{R_{970\,nm}}$	Naidu et al. (2009)

I: Fluorescence intensity; R: Reflectance.

3.3. Fluorescence imaging

The change in blue-green fluorescence and chlorophyll florescence of the plants upon ultraviolet excitation could provide the status of physiological condition of the plant (Belasque et al., 2008). Fluorescence imaging is an advancement of fluorescence spectroscopy, where fluorescence images (rather than single spectra) are obtained using a camera. A xenon or halogen lamp is used as a UV light source for fluorescence excitation, and the fluorescence at specific wavelengths are recorded using the charge-coupled device (CCD)-based camera system (Bravo et al., 2004; Lenk and Buschmann, 2006; Chaerle et al., 2007; Lenk et al., 2007).

The regions of electromagnetic spectra that are commonly used for fluorescence imaging are blue (440 nm), green (520–550 nm), red (690 nm), far red (740 nm), and near infrared (800 nm) (Lenk and Buschmann, 2006; Chaerle et al., 2007). Lenk et al. (2007) described the multispectral fluorescence and its possible application in monitoring fruit quality, photosynthetic activities, tissue structures, and disease symptoms in plants; and the basic instrumentation required.

The chlorophyll fluorescence imaging can be an effective tool in monitoring leaf diseases (Chaerle et al., 2004; Scharte et al., 2005; Lenk et al., 2007). Chaerle et al. (2007) used blue-green fluorescence to evaluate the effectiveness of this technique in observing the development of tobacco mosaic virus (TMV) infection in tobacco plants. A temporal effect of TMV infection on the fluorescence (blue-green and chlorophyll fluorescence) of infected plants was observed. The reflectance image at 550 and 800 nm were acquired and considered as the reference images. The authors reported an increase in blue, green, and chlorophyll fluorescence after about 40–55 h upon inoculation of TMV. The fluorescence imaging demonstrated a visible difference between the infected and non-infected leaves in short period of time (50 h) in comparison to the reference images (14 days for visible symptoms of infection).

Bravo et al. (2004) used fluorescence imaging for detecting yellow rust in winter wheat. They acquired two fluorescence images: a background image without the xenon lamp source and a fluorescence image with the xenon lamp source during the experiments. The fluorescence image utilized for the analysis was obtained by subtracting fluorescence image from the background image. The fluorescence was measured at 450, 550, 690, and 740 nm. The authors stated that the difference between the fluorescence at 550 and 690 nm were higher in the diseased portion of the leaves, while it was very low for healthy regions of the leaves. Quadratic discriminant analysis (QDA) was utilized to differentiate the healthy from the infected plants. Results indicated that though the methods was not effective in differentiating the healthy from mildly infected plants, QDA could classify healthy and diseased plants with an accuracy of 71% and 96%, respectively.

Moshou et al. (2005) investigated the applicability of hyperspectral reflectance imaging in combination with multispectral fluorescence imaging through sensor fusion to detect yellow rust (*Puccinia striiformis*) disease of winter wheat. The hyperspectral imaging was performed under ambient condition in winter wheat plots, whereas fluorescence images were procured upon UV-excitation. The authors reported that when the sensor information from the fluorescence and multispectral imaging were combined, QDA-based classification accuracy of the healthy plants improved from 71–90% to 97%. The classification accuracy of the diseased plants and healthy plants further improved to 98.7% and 99.4%, respectively, when the self-organizing map (SOM)-based neural network was used for the plant classification. The above studies indicate the possibility for using imaging techniques for disease identification.

The imaging techniques are an improvement over spectroscopic techniques as these methods acquire spectral information over a larger area and provide three-dimensional spectral information in the form of images.

3.4. Hyperspectral imaging

In recent years, hyperspectral imaging is gaining considerable interest for its application in precision agriculture (Okamoto et al., 2009). In the hyperspectral imaging, the spectral reflectance of each pixel is acquired for a range of wavelengths in the electromagnetic spectra. The wavelengths may include the visible and infrared regions of the electromagnetic spectra. The hyperspectral imaging

is similar to multispectral imaging, the difference being a broader range of wavelengths (more number of spectral bands) being scanned for each pixel in the hyperspectral imaging. The resulting information is a set of pixel values (intensity of the reflectance) at each wavelength of the spectra in the form of an image. Hyperspectral imaging is often used for monitoring the quality of food products (Kim et al., 2001, 2002; Mehl et al., 2004; Yao et al., 2005; Lee et al., 2005; Tallada et al., 2006; Gowen et al., 2007; Mahesh et al., 2008; Sighicelli et al., 2009). Aleixos et al. (2002) used multispectral imaging of citrus fruits to assess the quality of the fruits for developing a machine vision system. Gowen et al. (2007) reviewed the application of hyperspectral imaging for food quality control and food safety applications. The authors discussed the components of hyperspectral imaging system, different image processing techniques, and various applications in food quality and safety.

Some of the major challenges in hyperspectral imaging-based plant disease detection are the selection of disease-specific spectral band and selection of statistical classification algorithm for a particular application, which depends on the data acquisition setup under field conditions. For an example, Lu (2003), Xing and Baerdemaeker (2005), Xing et al. (2005), Nicolaï et al. (2006) and ElMasry et al. (2008) used hyperspectral imaging for the detection of bruises in apples and acquired different results. Lu (2003) reported that 1000 nm to 1340 nm were best for bruise detection, whereas Xing et al. (2005) and ElMasry et al. (2008) reported bands within range 558–960 nm were suitable for the identification of bruises in apple.

Blasco et al. (2007) applied multi-spectral computer vision using non-visible (ultraviolet, IR, and fluorescence) and visible multiple spectra for citrus sorting. The anthracnose was classified better with NIR images (86%), whereas green mold was more accurately classified with fluorescence imaging (94%). The stem-end injury was classified up to 100% using the ultraviolet spectra in this study. This study showed the utilization of hyperspectral bands for detecting different aspects of a single problem. Similarly, the hyperspectral imaging could be used for detecting different features within a plant to identify diseases.

Each spectral region provides unique information about the plant. For instance, the reflectance at visible wavelength provides the information on the leaf pigmentations while, reflectance at infrared wavelength provides the physiological condition of the plant (Huang et al., 2007). Much attention has been drawn towards utilizing this technology for the plant disease detection for precision agriculture-based applications.

Bravo et al. (2003) investigated the application of visible-NIR hyperspectral imaging for the early detection of yellow rust disease (*Puccinia striiformis*) in winter wheat. A discrimination model was developed using quadratic discriminant analysis for the classification of diseases from the healthy plants. The classification model yielded about 92–98% classification accuracy while classifying diseased plants. Similarly, Moshou et al. (2004) utilized a spectrograph to acquire spectral images from 460 to 900 nm to detect yellow rust in wheat. Statistical techniques such as QDA, SOM-, and multilayer perceptrons (MLP)-based artificial neural networks were used to classify the diseased from healthy wheat plants. Information from four wavebands, namely 543, 630, 750, and 861 nm were used for the classification models. The classification accuracies based on QDA and neural network (MLP) for the discriminating individual healthy plants were 92.0% and 98.9%, and diseased plants were 97.8% and 99.4%, respectively. The study indicated a possibility of using this technology for early detection of diseases such as yellow rust in field conditions.

Shafri and Hamdan (2009) used air-borne hyperspectral imaging for the detection of ganoderma basal stem rot disease in oil palm plantations. The authors used various vegetative indices and red edge techniques to classify the diseased from healthy plantations. The reported classification accuracies using different methods ranged from 73 to 84%. The results indicated that an aerial hyperspectral imaging could be used for disease detection and management of plantations in large scale. Qin et al. (2009) obtained hyperspectral images in the wavelength range 450–930 nm to detect citrus canker and other damages to Ruby red grapefruit. A spectral information divergence (SID)-based classification method yielded about 96% classification accuracy for discriminating the diseased, damaged, and healthy fruits. Similarly, Lee et al. (2008) investigated the applicability of aerial hyperspectral imaging for the detection of greening in citrus plantation. Efforts were made to identify citrus greening in tree canopies using hyperspectral image, by classifying the image with spectral angle mapping (SAM) and spectral feature fitting (SFF) classification techniques (ENVI program). The authors were not able to identify the diseased canopies with high classification accuracies due to a large variability within the data.

3.5. Other imaging techniques

The other imaging techniques that can used for detecting plant diseases are infrared thermography, terahetz spectroscopy, NMR spectroscopy, and X-ray imaging. As these techniques are not cost-effective, the present paper provides an overview of these methods and does not discuss them in detail.

Infrared thermography refers to an imaging technique that utilizes the thermal energy of the infrared band and transforms the procured information into a visible image. Infrared thermography similar to other imaging techniques can be used for non-destructive monitoring of physiological status of plants (Chaerle et al., 1999, 2001). Lenthe et al. (2007) used infrared thermography to examine the possible relationship between the leaf microclimate and fungal diseases in wheat fields. Although microclimate can be determined from infrared thermography, direct diseased leaf area identification could not be established. Chaerle et al. (2003) and Chaerle and Van Der Straeten (2000) reviewed the application of imaging techniques in agronomy and stress detection. They reported that the stomatal changes in the leaves of the plants upon pathogen infection could be monitored by thermography (e.g. hydrogen peroxide produced by *Pseudomonas syringae* induces stomata in the leaves to close). Similarly, local temperature changes due to plant defense mechanisms against diseases can also help in monitoring plant diseases. Tobacco leaves produces salicylic acid (promoting thermal and stomatal change) that can be monitored using thermography. Other studies include presymptomatic detection of cucumber downy mildew- *Pseudoperonospora cubensis* (Lindenthal et al., 2005; Oerke et al., 2005, 2006), fungal infection by *Cercospora beticola* in sugarbeet (Chaerle et al., 2004), and *Brassica napus* infection with *Phoma lingam* (Lamkadmi et al., 1996) among others.

In recent years, terahertz (THz) frequencies (0.1–10 THz) are being utilized for measuring the water content in leaves. The water stress can be observed by utilizing this frequency, as terahertz frequency is absorbed greatly by water molecules (Hadjiloucas et al., 2009). Nuclear magnetic resonance and X-ray-based imaging techniques can also be used for detecting infections, different types of stress, and other health conditions in trees/fruits (Goodman et al., 1992; Williamson et al., 1992; Karunakaran et al., 2004; Pearson and Wicklow, 2006). Goodman et al. (1992) used NMR microscopic imaging for identification of the fungal pathogen *Botrytis cinerea* in red raspberry. Narvankar et al. (2009) applied X-ray imaging to identify fungal infections in wheat. The authors employed statistical discriminant models and ANN to classify the images of the wheat kernels. Statistical classifiers, especially the Mahalanobis discriminant classifier performed better (92–99% accuracy) than the ANN-based classification.

4. Profiling of plant volatile organic compounds for disease detection

The volatile organic compounds (VOC) released by plants and trees contribute about two-thirds of the total VOC emissions present in the atmosphere (Guenther, 1997). There are number of factors that affect the volatile metabolic profile of a plant or tree. The VOCs released by the plants depend on various physico-chemical factors such as humidity, temperature, light, soil condition, and fertilization, as well as biological factors such as growth and developmental stage of the plant, insects, and presence of other herbs (Vallat et al., 2005; Vuorinen et al., 2007). The physico-chemical factors either directly or indirectly affect the physiological condition of the plant, thereby influencing the VOC profile of the plant. These plant volatiles in turn influence their relationship between the plants and other organisms including pathogens (Vuorinen et al., 2007). For example, acetaldehyde is released by the leaves of young poplar trees are controlled by the transfer of ethanol to leaves through transpiration (Kreuzwieser et al., 2001).

Dudareva et al. (2006) reviewed a range of plant volatiles released by the plants due to biotic and abiotic interactions. Some of the commonly found secondary plant volatiles are terpeniods, volatile fatty acids (such as *trans*-2-hexenal, *cis*-3-hexenol and methyl jasmonate), phenylpropanoids and benzenoids, and amino acid volatiles (such as aldehydes, alcohols, esters, acids, and nitrogen- and sulfur-containing volatiles derived from amino acids). The abiotic and biotic stresses can result in a change in the volatile profile of the plants that can be utilized for the plant disease detection. Cevallos-Cevallos et al. (2009) reported that the compounds (extracted from leaves) such as hesperidin, naringenin, and quercetin present in the leaves could be used as a biomarker to identify huanglongbing diseases in citrus trees. The volatiles of these compounds could be tested in the atmosphere near the citrus plantations to detect the presence of huanglongbing disease. Tholl et al. (2006) reviewed practical methods to study the plant volatiles suitable for various applications. The authors described the methods for VOC sampling and analysis for in-situ experiments as well as some for field experiments.

The focus of the present paper is towards the application of plant VOC profile monitoring for detecting diseases in plants. The VOCs released by the plants change when the plant is infected with a disease due to change in its physiology. These emissions are expected to vary from the VOCs released under normal plant health conditions. This technology could facilitate the detection of plant diseases in real-time, thereby preventing the spread of plant diseases. Such techniques will provide agricultural and financial benefits for the growth and development of our economy.

The volatile metabolic gas profile analysis has been evaluated by few researchers (Vuorinen et al., 2007; Laothawornkitkul et al., 2008; Li et al., 2009b) to identify plant diseases. Studies have been performed in natural/field conditions (Vuorinen et al., 2007; Staudt and Lhoutellier, 2007; Laothawornkitkul et al., 2008) and controlled environments/in situ (Li et al., 2009b). A handful of studies have been performed to detect diseases or infections in postharvest fruits/vegetables (Prithiviraj et al., 2004; Li et al., 2009b). The two common methods used for assessing the profile of volatile metabolites released by plants are using gas chromatography (GC)-based and electronic nose system-based techniques. The following sections describe the studies performed on plant disease analysis using these two techniques.

4.1. Electronic nose system

An electronic nose system consists of a series of gas sensors that are sensitive to a range of organic compounds. As each sensor has specific sensitivities, the sensitivities of a series of sensors could be used to discriminate different compounds present in the atmosphere. Electronic nose systems have been used for multiple applications. They have been used to determine food quality (Evans et al., 2000; Di Natale et al., 2001; Zhang et al., 2008a,b), identify diseases in humans (Gardner et al., 2000; Lin et al., 2001; Dragonieri et al., 2007), and detect microorganisms in food products (Falasconi et al., 2005; Rajamäki et al., 2006; Balasubramanian et al., 2008; Concina et al., 2009) among others. The application of electronic nose systems for identifying plant diseases is relatively new domain for its application.

Li et al. (2009b) used a Cyranose® 320 (an array of 32 conducting polymer-based sensors) to detect postharvest fungal disease in blueberries in a controlled environment. Blueberries were disinfected with ethanol to eliminate any naturally present fungal spores and bacteria. Once the blueberries were rinsed with distilled water to remove residual ethanol, they were inoculated with spore suspensions of three fungal species: *Botrytis cinerea*, *Colletotrichum gloeosporioides*, and *Alternaria* spp. that cause gray mold, anthracnose, and Alternaria fruit rot in postharvest blueberries, respectively. The berries were placed in a 500 mL bottle and headspace gases were tested using Cyranose® 320. GC–MS (Gas chromatography–mass spectroscopy) analysis was also performed to identify specific compounds that could be related to fungal diseases. Principal component analysis plots indicated a clear delineation between the control (fresh berries) and berries with fungal infections. The berries with *C. gloeosporioides* could be distinctively differentiated from the other groups, though there was some overlap in the VOC profiles of the berries infected with *B. cinerea* and *Alternaria* spp. The authors suggested that as *B. cinerea* and *Alternaria* spp. mainly infect the same region (stem scar region) of blueberry fruit that might have resulted in a similar VOC profile. When linear Bayesian classifier was used, the study found that the type I error for classifying infections caused by *B. cinerea*, *C. gloeosporioides*, and *Alternaria* spp. were 18%, 11%, and 13%, respectively. In addition, PCA results on GC–MS data (relative concentration of VOCs with respect to the class) indicated that styrene, 1-methyl-2-(1-methylethyl) benzene, eucalyptol, undecane, 5-methyl-2-(1-methylethyl)-2-cyclohexen-1-one, and thujopsene contributed to the classification of the four groups (three diseases and one healthy). The research work described above demonstrates the potential for applying VOC profiling-based technique for non-destructive detection of plant diseases.

Laothawornkitkul et al. (2008) evaluated the potential of plant volatile signature for pest and disease monitoring in cucumber, pepper, and tomato plants. Similar to Li et al. (2009b), an electronic nose system and GC–MS were used to identify and distinguish the volatiles released by the plants under different conditions. The authors used Bloodhound® ST214 electronic nose (an array of 14 conducting polymer-based sensors with one sensor as a reference) for determining sensors' responses to VOCs released by the plant leaves in experiments conducted in a greenhouse. The healthy pepper plant VOC profile was compared to that of wounded pepper plant; the healthy cucumber plant VOC profile was compared to that of wounded and spider mite-infested cucumber plant; and healthy tomato plant VOC profile was compared to that of wounded, mildew-infected, and hornworm infested tomato plant. Statistical methods such as PCA, discriminant function analysis (DFA), and cluster analysis were utilized to analyze the data to classify healthy plants from the unhealthy ones.

The profile of VOCs released by three different plants (as determined using electronic nose) was distinctly different when the data were analyzed using DFA model. Similarly, the VOC profile of wounded and healthy plants could be clearly discriminated

using DFA and cluster analysis techniques. When the VOC profile of healthy, wounded, and spider-mite infested cucumber plants as determined by electronic nose and GC–MS were compared, the different groups were distinctively separable. The gases that might have contributed to the variation in the electronic nose sensor responses were identified using GC–MS analysis. Similar results were obtained for VOC profile analysis of tomato plants. The authors stated that the electronic nose results were comparable to the GC–MS data, facilitating a portable means of detecting VOC profile for plant disease monitoring under field conditions.

Zhang and Wang (2007) applied PEN2 electronic nose system (Win Muster Airsense Analytics Inc., Germany) consisting of an array of 10 metal oxide-based sensors for measuring the profile of VOCs released from wheat damaged with age and insects. The authors were able to classify the different categories of wheat grains using PCA and LDA techniques. Spinelli et al. (2006) evaluated the near infrared and electronic nose system-based techniques to detect fire blight (asymptomatic stage) in pear plants. It was reported that the electronic nose system was able to provide a distinct olfactory signature required to identify the disease. The disease could be detected as early as 6 days after the infection. The study indicated that electronic nose system could be used as effective tool for the early diagnosis of the plant disease under natural conditions. Markom et al. (2009) used an electronic nose system to detect basal stem rot disease in oil palm plantation during field experiments. The authors reported that two principal components based on PCA were able to account for 99.32% variability in the data, and MLP-based artificial neural network could classify infected from healthy trees with high accuracy.

The above studies revealed the prospective of the electronic nose-based VOC monitoring systems in conjunction with advanced statistical techniques for detecting different stress and health conditions in plants. This indicates that the profile of volatile metabolites released by plants could be used as a disease-monitoring tool for early and rapid detection of plant diseases.

4.2. GC–MS

The GC–MS is commonly used technique for a qualitative as well as quantitative analysis of volatile metabolites released by plants/trees in different environmental and physiological conditions. The GC–MS studies have been performed to evaluate the change in volatiles caused by bacterial or fungal infection in various food products (Table 5).

Prithiviraj et al. (2004) assessed the variability in the volatiles released from onion bulbs infected with bacterial (*Erwinia carotovora* causing soft rot) and fungal species (*Fusarium oxysporum* and *Botrytis allii* causing basal and neck rots) using HAPSITE, commercial portable GC–MS instrument. The study indicated that 25 volatile compounds (among the 59 consistently detected compounds) released from onion can be used to identify the disease based on VOC profiling. Although no statistical analyses were performed to determine the discriminatory ability of an algorithm in classifying the VOC profiles for disease detection, model development and software development were recommended for the purpose.

Similar studies on potato tubers inoculated with *Erwinia carotovora* subsp. *carotovora*, *E. carotovora* subsp. *atroseptica*, *Pythium ultimum*, *Phytophthora infestans*, or *Fusarium sambucinum* using solid phase microextraction (SPME) fiber along with GC-flame ionization detector (FID) indicated the potential of the VOC profiling for disease detection (Kushalappa et al., 2002). The amount of volatiles increased with an increase in disease severity. A BPNN model was applied to classify the volatile metabolite profiles with respect to the diseases. The gas retention time of the volatile compounds (GC feature) was used as the input data and two hidden layers were used for cross-validation. The cross-validation probabilities (using BPNN) were >67% (67–75%) for all groups except potato tubers infected with *Phytophthora infestans*. Unlike other studies, this study did not determine the specific compounds that resulted in VOC peaks in the FID.

Lui et al. (2005) inoculated potato tubers with *Phytophthora infestans*, *Pythium ultimum*, or *Botrytis cinerea* and analyzed the VOC profile using GC–MS. The compounds in the headspace of the potatoes were identified and their abundance in terms of peak area was determined. Stepwise discriminant analysis was performed using the 32 compounds that were consistently present in the headspace and mass ions from the MS data as the input. The developed discriminant analysis models categorized the diseases with a classification accuracy of 13–100%. Comparing studies conducted by Lui et al. (2005) and Kushalappa et al. (2002), BPNN-based classification provided higher classification accuracy than discriminant analysis based models. However, the results may be highly sensitive to experimental conditions, diseases, and type of fruit or vegetable.

Vuorinen et al. (2007) utilized VOC emission pattern of silver birch to determine whether the plants were damaged by larvae (herbivore arthropod *Epirrita autumnata*), infected with pathogenic leaf spot (*Marssonina betulae*) or if they were healthy. The VOCs were collected from pathogen-inoculated leaves, herbivore-damaged leaves, and undamaged leaves from the top of the branches as well as undamaged detached twigs. Different plant conditions produced specific patterns of VOCs. The herbivore damaged leaves released VOCs such as methylsalicylate, linalool, etc., especially after 72 h of feeding. It was also reported that higher quantities of (Z)-ocimene and (E)-β-ocimene were released from the pathogen-infected twigs than from the control twigs. Though simple statistical methods as *t*-test and ANOVA were performed in the study, a detailed discussion on the statistical results was

Table 5
Examples of studies on disease detection in vegetables and fruits using GC–MS.

Plant/Fruit/Vegetable	Cause of disease	No. of VOCs	Statistical Methods	Classification accuracy (%)	Reference
Timber	*Serpula lacrymans* (dry rot fungus) and *Coniophora puteana* (cellar fungus)	12–15	–	–	Ewen et al. (2004)
Onion bulbs	*Erwinia carotovora* ssp. *carotovora*, *Fusarium oxysporum* and *Botrytis allii*	25	–	–	Prithiviraj et al. (2004)
Potato tubers	*Phytophthora infestans, Pythium ultimum* and *Botrytis cinerea*	32	Discriminant analysis	13–100	Lui et al. (2005)
Carrots	*Botrytis cinerea, Erwinia carotovora* subsp. *carotovora*, *Aspergillus niger* and *Fusarium avenaceum*	39	Discriminant analysis	30–90	Vikram et al. (2006)
Mango	*Lasiodiplodia theobromae* (stem-end rot) and *Colletotrichum gloeosporioides* (anthracnose),	35	Discriminant analysis	33–88	Moalemiyan et al. (2007)

Table 6
Comparison of various types of plant disease detection.

Characteristics	Molecular techniques	Imaging and spectroscopic techniques	VOCs profiling-based techniques
Accuracy of the method	- Molecular techniques are presently the most accurate method for plant disease detection. - Efforts are ongoing to make molecular methods more reliable and simpler as well as develop molecular detection kits for field applications. However, it is difficult to develop kits for all diseases. They are usually focused towards commonly found and harmful diseases.	- The accuracy of imaging and spectroscopic techniques is plant and disease specific. - Higher the visible symptoms, better is the accuracy of the technique. Nevertheless, the non-visible regions of spectra can be utilized for improving the accuracy of the method.	- Accuracy is currently unknown, as this method is in the developmental stages and has been utilized in recent years. - Identification of disease-specific biomarker volatiles (most challenging step) can improve the accuracy significantly.
Cost	- Moderately expensive. Quite often: is labor intensive and requires specific instrumentation. - Trained personnel are also required for careful handing of samples and results.	- Expensive, especially if techniques as hyperspectral imaging are used. Fewer the wave bands used, cheaper will be the instrument. - Require computers/laptops for data analysis.	- The cost of technique depends on the desired accuracy for VOC profiling. - The cost can range depending on the detector required for biomarker identification.
Applicability for rapid detection	- Speed depends on the samples required to be analyzed, number of personnel, and equipments and materials. - The technique is not fast for a huge amount of samples.	- The focus on the development of this technique is due to its ability for rapid detection.	- The technique shows the potential for rapid plant disease detection.
Applicability for field work/Ruggedness	- Field kits are being developed. However, it is difficult to develop kits for all diseases. - The field kits are rugged, but require good accuracy for reliable results.	- Moderately ruggedness. The ruggedness of the spectrometer or the scanner depends on the base on which the sensor is mounted.	- Moderately rugged, depending on the detector used for sensing VOCs.
Speed of detection	- May require 24 - 48 h for reliable results. -Molecular kits are faster.	- Once this technique is established, may require minutes for disease detection. -The speed depends on the computational speed of the computer as well as speed of the scanner.	- This method may require significantly less time, if proven as an effective method for a particular disease. - Speed would depend on the detector speed and computational speed.
Others aspects	- One of the limitations of this technique is that it is difficult to automate the process for rapid detection. - Requires personnel for monitoring plant disease by this method.	-This method can be automated or disease detection can be performed through remote operation easily. -This method can be used as preliminary screening of diseases, so that fewer samples can be confirmed by molecular technique. - The methods in combination with other methods as molecular detection can be effective in rapid detection of plant diseases.	- Selection of biomarker is a critical step for determining the applicability of this technique. -Once established this method can be automated with a robotic vehicle for plant disease detection.

absent from the paper. Staudt and Lhoutellier (2007) evaluated the VOC release profile of the damaged and undamaged leaves of holm oak tree infested by gypsy moth larvae. The researchers stated that the leaves released linalool, homoterpene (E)-4, 8-dimethyl-1,3,7-nonatriene, germacrene D, â-caryophyllene, and several other sesquiterpenes upon days of caterpillar growth on the leaves. These gases were not present in the VOCs released by the control plants.

Moalemiyan et al. (2006, 2007) employed VOC profiling to detect fungal diseases (*Lasiodiplodia theobromae* causing stem-end rot and *Colletotrichum gloeosporioides* causing anthracnose) in mangoes. Discriminant analysis models were used to classify the groups using the gaseous metabolite profile and mass ions. Though the methods showed potential for detecting fungal diseases, the classification accuracy of the models needs to be further increased to make it a feasible method for postharvest disease detection.

5. Future directions

Recent reports in the literature support the notion that both volatile profiling and changes in spectral reflectance can be used for non-invasive field monitoring of plant diseases. Plants and trees release volatile organic compounds (VOCs) as a result of the metabolic activities taking place within its shoots, leaves, flowers, or fruits. The VOC profile of each plant differs significantly based on its physiological condition and the species. Various factors influence the profile of VOCs from a particular plant or tree, which may include changes in plant metabolism as a result of environmental changes, the age of plant, developmental stage of a plant, effect of stress on plants, and the presence of disease/herbivore in a plant.

One of the biggest challenges in the utilization of plant gaseous metabolites as an indicator for the presence of plant diseases is the natural variation in the VOC profile within plant species. The variation in VOCs released by plants may mask the changes due

to the stress and the presence of diseases. Therefore, there is a need to identify distinct volatile biomarker specific for a particular plant and disease that would be different from the VOCs produced due to an environmental or nutrient stress. For practical applications, development of a robust and reliable system for real-time monitoring of plant diseases is required.

Similar to VOC profile of the plants, the environmental conditions affect the spectral reflectance from the object (Griffin and Burke, 2003). Therefore, there is a need to identify suitable approach to overcome this problem. The possible way to overcome this problem could be identification of wavelength range or index that is not only sensitive to a specific plant disease but also is least affected by the changes in the environmental condition.

It is feasible to incorporate the imaging and VOC profiling techniques into an autonomous robot as these techniques are well established for other industrial applications. Once these techniques are well established for a specific disease detection application, these methods can be integrated with an autonomous agricultural vehicle for real-time monitoring for plant diseases. The overall comparisons of three major techniques are summarized in Table 6.

6. Summary and conclusions

The present paper reviews and summarizes some of the non-invasive techniques that have been used for plant disease detection. The two major categories for non-invasive monitoring of plant diseases are: (i) spectroscopic and imaging techniques, and (ii) volatile organic compounds profiling-based technique for recognizing plant diseases. The spectroscopic and imaging techniques include fluorescence spectroscopy, visible-IR spectroscopy, fluorescence imaging, and hyperspectral imaging. The VOC profile-based disease detection involves using electronic nose or GC–MS based volatile metabolite analysis released by healthy and diseased plants as a tool for identifying diseases. Some of the challenges in these techniques are: (i) the effect of background data in the resulting profile or data, (ii) optimization of the technique for a specific plant/tree and disease, and (iii) automation of the technique for continuous automated monitoring of plant diseases under real world field conditions. The review suggests that these methods of disease detection show a good potential with an ability to detect plant diseases accurately. The spectroscopic and imaging technology could be integrated with an autonomous agricultural vehicle for reliable and real-time plant disease detection to achieve superior plant disease control and management.

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