Advanced spectroscopic techniques for plant disease diagnostics. A review

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ABSTRACT

Timely plant pathogen diagnostics can save up to 50% of the total agricultural yield worldwide. Current molecular and imaging methods for detection and identification of plant diseases have many limitations. This sparked interest in the development of minimally invasive and substrate general spectroscopic techniques that can be used directly in the field for confirmatory plant disease diagnostics. This review discusses recent progress in development of reflectance, infrared, Raman and surface-enhanced Raman spectroscopy for detection and identification of plant diseases. It also shows advantages and disadvantages of these optical spectroscopy methods compared to the most common molecular and imaging techniques.

1. Introduction

As the human populations grows from its current size of 7 billion to the projected 9.7 billion in 2050 and 11.2 billion in 2100, we will need to produce 70–100% more food in the near future [1]. One way to address this issue is to increase the amount of agricultural areas. Although many developing countries follow this strategy, it has limited applicability and essentially is destructive to nature [2]. The second strategy is focused on increasing the yield of existing crops. This includes directed plant breeding and timely diagnostics of plant biotic and abiotic stresses [3]. Currently, biotic stress accounts for about 11.5% of crop loss in the United States, up to about 1–2% higher in other places around the world [4]. Total biotic stress caused by various pathogens, pests, and weeds accounts for 20–40% of agricultural productivity loss worldwide [5]. To help minimize these massive losses in food, several molecular and imaging methods are employed to enable detection of diseases at early stages. However, when it comes to disease, time and reliability are of the essence and these requirements are not both met by current methods of analysis. These issues have cultivated interest in alternative methods, such as infrared (IR) and Raman (RS) spectroscopies, for rapid confirmatory detection and identification of plant pathogens in the field.

Various measures to prevent and contain crop diseases including treatment with pesticides, use of genetically modified plants and timely removal of diseased plants have had variable success [6]. Chemical treatment by pesticides and fungicides can be used to prevent development of plant diseases as well as reduce amount of pest that feed on them. One study showed that in 22 crops, application of fungicides improved yield by ~50%, equivalent to about 44 billion kilograms of food or 12.8 billion U.S. dollars’ worth [7]. Using pesticides, however, can potentially harm both humans and the environment. Development of genetically modified organisms (GMOs) is an alternative approach which allows production of transgenic crops with pathogen-specific resistance coded into their DNA. Although using transgenic crops has raised yields by 22%, increasing profits by 68%, according to a review of 147 studies [8], the potential risk of introducing transgenic plans into the ecosystem is not yet fully understood. Lastly, removing infected plants in order to contain the spread of the disease may be costly, and failure to detect a disease outbreak at its onset often makes this containment measure impractical. The limitations of the current disease prevention and containment practices further elevate the demand for reliable, fast and cost-effective methods for early-stage detection of crop diseases.

2. Current methods in plant disease detection

A variety of methods are currently used in the agricultural industry to detect plant pathogens (Table 1). These techniques can be
divided on three groups: molecular, imaging and spectroscopic methods. Sensitivity is the capacity of a method to correctly identify its target (in this case, a particular pathogen), while specificity is the method’s ability to correctly identify when its target is not present [9].

2.1. Molecular methods

Molecular methods used for the detection and identification of plant diseases include polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), fluorescence in situ hybridization (FISH), immunofluorescence, aptamer-based diagnostics and flow cytometry. These methods are based on either direct detection of the pathogen or associated molecules, such as DNA or toxins. The current review will only briefly discuss the first two methods due to their broad application in plant pathology. Detailed discussions of molecular methods can be found in several excellent reviews by Fang and Ramasamy [10], Martinelli and co-authors [11], as well as Donoso and Valenzuela [12].

PCR and, in particular, quantitative (q)PCR, are the work horses of plant pathology [13,14]. PCR has high sensitivity, which enables early stage disease detection, and high specificity, which ensures precise identification of fungal, bacterial and viral pathogens [15–17]. However, PCR is a labor-intensive and destructive technique that requires extensive sample preparation and is generally performed in a laboratory by highly skilled personnel. The method involves sample homogenization, extraction and purification of the DNA material, as well as gel electrophoresis and gel imaging, which add to the cost and duration of analysis of individual samples. Furthermore, PCR requires primer preparation and consequently determination of the DNA sequence of the pathogen of interest. Despite great progress in optimization of PCR-based disease diagnostics over the last few decades, this technique remains expensive for routine detection and identification of plant diseases. Also, PCR is highly sensitive to contamination from environmental DNA, can be inhibited by small quantities of organic solvents [18].

ELISA is based on using specially raised bodies to bind antigens of interest to confirm their presence; it is typically a colorimetric assay [19]. Like PCR, this assay is invasive and destructive, but ELISA generally has lower sensitivity. ELISA is also highly sensitive to photobleaching of chemical dyes used in this technique. Since this method’s inception in the early 1970s [20], many modifications to this method have been made such as voltammetric detection and the use of electrical-impedance spectroscopy (EIS). Others retained the optical approach using Lateral Flow Immunoassays (LFI) or surface plasmon resonances (SPR) [21].

2.2. Imaging methods

Imaging methods include thermography, hyperspectral, as well as RGB and fluorescence imaging. Their coupling to unmanned aerial vehicle (UAV) allows for monitoring of large agricultural areas. These techniques enable indirect disease diagnostics via detected changes in color, texture or temperature of the plant. Thermography detects the heat emitted by objects and survey large areas in a single image. Because pathogens induce metabolic and structural changes in the host, both associated with temperature, thermography can be used to detect plant diseases and other stresses [22]. For instance, infection of tobacco by tobacco mosaic virus causes closing of the plant stomata. This affects transpiration rates and results in overheating of the plant [22,23]. The change in plant temperature, relative to either the temperature of adjacent plant parts or the temperature of a reference healthy plant, can be detected by thermal imaging cameras. This technique has also been shown to be able to detect stress-induced thermal changes in plants [24]. Thermography, while capable at surveying large areas at a time, is non-specific and only sensitive to some types of pathogens, is entirely non-invasive. It requires

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Limitations</th>
<th>Advantages</th>
<th>Time of analysis</th>
<th>Portability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymerase chain reaction (PCR) and quantitative polymerase chain reaction (qPCR)</td>
<td>High</td>
<td>Yes</td>
<td>Requires DNA sequencing and design of primers. Sensitive to organic contaminants. Labor intensive. Photobleaching</td>
<td>Requires small sample amount. Provides high specificity and accuracy</td>
<td>Long</td>
<td>No</td>
</tr>
<tr>
<td>Enzyme-linked immunosorbent assay (ELISA)</td>
<td>Medium</td>
<td>Yes</td>
<td></td>
<td>Easy to operate. Low cost</td>
<td>Long</td>
<td>No</td>
</tr>
<tr>
<td>Fluorescence in-situ hybridization (FISH), immunofluorescence imaging (IFI), flow cytometry (FCM)</td>
<td>Medium</td>
<td>Yes</td>
<td>Autofluorescence and photobleaching</td>
<td>High specificity and accuracy of detection</td>
<td>Long</td>
<td>No</td>
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### Imaging Methods

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Thermography</td>
<td>Low</td>
<td>No</td>
<td>High cost. Poor specificity. Requires calibration.</td>
<td>Probes large agricultural areas. Can be used to detect “danger” areas.</td>
<td>Long</td>
<td>Yes</td>
</tr>
<tr>
<td>RGB and fluorescence imaging</td>
<td>Low</td>
<td>No</td>
<td>Poor specificity. Requires calibration.</td>
<td>Probes large agricultural areas. Can be used to detect “danger” areas.</td>
<td>Long</td>
<td>Yes</td>
</tr>
<tr>
<td>Hyperspectral Imaging</td>
<td>Low</td>
<td>No</td>
<td>High cost, complexity, and long data acquisition. Sophisticated and time-consuming data analysis.</td>
<td>Probes large agricultural areas.</td>
<td>Long</td>
<td>Yes</td>
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### Spectroscopic Methods

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<tr>
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<th>Portability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visible (VIS) and Near-Infrared (NIR) Transmittance and Reflectance spectroscopy</td>
<td>Low</td>
<td>No</td>
<td>Poor specificity</td>
<td>Fast and inexpensive.</td>
<td>Fast</td>
<td>Yes</td>
</tr>
<tr>
<td>Infrared Spectroscopy (IR)</td>
<td>High</td>
<td>Yes</td>
<td>Presence of water</td>
<td>Fast and accurate.</td>
<td>Fast</td>
<td>Yes</td>
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only a thermal scan of the plant surface, which requires no contact.

**RGB and fluorescence imaging** detect pathogens by probing changes in transmittance or fluorescence of various plant parts caused by pigmentation. In RGB imaging, a series of photographs is consecutively taken is through red, green and blue filters. Like thermography, images taken of a plant at multiple points in time can be compared to each other in order to see potential differences in the levels of these colors. These differences can be caused by a change in intensity in the transmittance of these color by the various pigments in a plant [25] and therefore can be used to identify certain pathogens.

Chlorophyll fluorescence imaging measures the difference in fluorescence intensity across plant area or relative to the signal from surrounding plants. Plant fluorescence typically decreases under stressful conditions [26]; this method is therefore non-invasive and non-destructive; however, it is also non-conclusive as many very similar changes in plant color and texture can be induced by abiotic and biotic stresses.

A more advanced imaging technique known as **hyperspectral imaging** utilizes reflectance data collected over a wide spectral range, typically 350–2500 nm, to reconstruct a spatial image of the plant leaf analyzed with special image processing methods [22]. Being an information rich method, it enables detection of a wider range of diseases compared to RGB imaging. Among disadvantages of hyperspectral imaging are high cost, complexity, long data acquisition times and complex data analysis, which all together limit its practical application especially in cases where quick response times or screening of large areas are desired [27].

3. Spectroscopic methods

**Visible and Near-Infrared (Vis-NIR) Transmittance and Reflectance spectroscopy** requires easily accessible and inexpensive experimental setup which is essentially based on a lamp and the photodetector. In reflectance mode (RM), the detector and light source are located on the same side of the sample, whereas in transmittance mode (TM) the detector is located on the opposite side of the illumination. Depending on broadness of the range of electromagnetic radiation of the lamp, spectral acquisition can be achieved in visible and near-infrared regions. Vis-NIR spectroscopy is based on determination of the wavelengths of light a sample best absorbs, reflects or transmits in the Vis and NIR ranges, which are associated with the color of the sample. This simplicity and small size of currently available commercial VIS spectrometers attracted researchers to explore suitability of this technique for detection and identification of plant diseases. Sankaran and co-workers used VIS-NIR RM spectroscopy for detection of Huanglongbing (HLB), or citrus greening, on citrus trees [28]; HLB is a devastating disease of citrus trees caused by the gram-negative *Candidatus Liberibacter asiaticus*, a phloem-limited species vectored by the Asian citrus psyllid, *Diaphorina citri*, and the African citrus psyllid, *Triozia erytreae*. Infected trees exhibit yellowing of leaves, premature leaf and fruit drop, and ultimately, death of the entire plant. The researchers showed that in combination with multivariate statistical analysis, this approach was able to provide high accuracy of HLB diagnostics.

Additionally, several claims have been made that RM VIS-NIR spectroscopy could detect bacterial contamination of spinach leaves, spot disease on wheat and olive leaf spot on olives [29–32]. These works showed the potential of VIS-NIR spectroscopy as a non-invasive and non-destructive approach for plant disease diagnostics. However, several very important questions remain unanswered: 1) can VIS-NIR spectroscopy detect diseases before their visual appearance on plants; 2) can this technique disentangle plant disease and results of abiotic stresses, such as drought, if they have similar visual appearance on the plant; and 3) can several diseases be simultaneously detected on the plant.

Based on the careful analysis of the reported results, one can speculate that it is impossible to answer these questions with TM and RM VIS-NIR spectroscopy. This is primarily because VIS-NIR does not probe structural changes in the plant but rather detects changes in colors which are not necessarily disease specific. Secondly, TM and RM VIS-NIR spectra have broad bands with fluctuating intensities (Fig. 1). This drastically decreases the accuracy of the disease diagnostics.

**Vibrational spectroscopy** is a class of analytical techniques that includes IR and RS. Both provide information about the chemical structure of analyzed samples. They are also, in general, label-free, non-invasive and non-destructive, which makes them ideal for various applications in food science, biochemistry, electrochemistry and medicine. These methods offer an unparalleled capacity to probe structural changes in plants associated with stressors such as disease. Application of vibrational spectroscopy for plant science in general has been previously described in a review by Skolik and co-workers [33].

**Infrared spectroscopy**, including near- and mid-IR, is broadly used for analysis food, grains, animal feeds, minerals and soils. It is based on absorption of infrared radiation corresponding in energy to molecular vibrations. Consequently, the IR absorption spectrum will reflect chemical and structural organization of the sample [34–36]. For instance, Sankaran and co-workers recently showed that mid-IR could be used to detect HLB and nutritional deficiency on citrus trees [37]. The researchers achieved nearly 90% accuracy of prediction based on the collected spectra. Similar results have been independently reported by Hawkins and co-workers [38]. Liqhat and co-workers utilized mid-IR to detect basal stem rot (BSR) disease caused by *Canolderma boninense* [39]. Principal component analysis of the first and second derivatives of the IR spectra enabled overall classification accuracy of 92% in prediction of mild, moderate and severe stages of BSR disease. Kos and co-workers proposed to use diffuse reflection and attenuated total reflection (ATR) IR to detect *Fusarium graminearum* in maize kernels [40]. The researchers observed significant spectral difference between healthy and Fusarium-infected maize and 100% prediction accuracy in ATR-IR approach. One of the major shortcomings of IR-based techniques is the need for extensive sample preparation. Water is highly IR active, so removal of water is a typical step in these experiments. However, in some cases, intact samples can be used: Liqhat and co-workers demonstrated detection of a bacterial infection of potato tubers known as zebra chip disease on intact tubers with NIR [41].

Fig. 1. Reflectance Vis-near IR spectra of healthy and HLB infected citrus leaves. Reproduced with permission from Ref. [29].
Skolik and co-workers showed that ATR mid-IR could be used for non-invasive and highly accurate diagnostics of sour rot infection caused by *Geotrichum candidum* on tomato fruits, Fig. 2 [42]. The researchers showed that mid-IR could detect structural changes in a cuticle, which could be assigned to lignin or cutin, as well as pectin and other polysaccharides. These spectral changes could be evidence of the sour rot disease at early and late stages of its progression on the fruit. The reported results indicate that direct application of mid-IR can be challenging because spectral changes were observed not only for sour rot-infected tomatoes, which take place upon pathogen proliferation on the fruit, but also for healthy tomatoes (Fig. 2). Such spectral changes very likely reflect metabolomic activity of the fruit itself or bacteria living on or in the fruit.

**Raman Spectroscopy (RS)** is a modern analytical technique that provides information about molecular vibrations and, like IR, the structure of the analyzed specimen [43,44]. In contrast to IR, Raman spectra are generated by collection of inelastically scattered photons from samples of interest. Additionally, water has a very small Raman cross-section, allowing for spectral acquisition in live biological systems such as cells and tissues [34,45]. Raman instrument design can enable unique applications: addition of a telescope can increase the spectra collection range to over 60 meters [46,47].

These advantages make RS one of the most promising tools in agriculture for detection and identification of plant diseases.

### 3.1. Raman-based detection and identification of fungal diseases

Recently, Farber and Kurouski showed that RS can be used to detect and identify several different fungal pathogens on maize kernels [48]. For this study, the researchers used a hand-held Raman spectrometer equipped with a 1064 nm laser. Farber and Kurouski found that spectral changes associated with *Fusarium* spp., *Diplodia* spp., *Aspergillus niger*, and *A. flavus* diseases were observed in lignin (1600 and 1633 cm⁻¹), a structural molecule important for a hard cell wall, carotenoids (1523–1547 cm⁻¹), pigments that gives kernels their gold color, proteins that are identified by their characteristic amide peptide bond (1640–1670 cm⁻¹), and carbohydrates, including monomeric sugars (1115, 1082, 1052, 1043, and 1024 cm⁻¹) and starch (1153, 938, and 864 cm⁻¹) (Fig. 3). With lignin, maize kernels showed almost no band after *Fusarium* proliferation, while these band intensities were lower in *A. niger* and *A. flavus*. The intensity of this band did not change in *Diplodia*-infected kernels. This shows pathogen-specific spectroscopic responses to disease in plants. The researchers utilized orthogonal partial least-squares discriminant analysis (OPLS-DA) to demonstrate that RS has high accuracy of plant disease detection and identification. It was found that coupling of OPLS-DA with RS allowed for 100% accurate detection and identification of these four pathogens on maize kernels.
This work was followed by Egging and co-workers who showed that this spectroscopic approach could be extended on detection of fungal diseases on wheat and sorghum grain [49]. It was found that in the spectra of ergot-infected wheat, the amide I band had two maxima at 1650 and 1667 cm$^{-1}$. These bands were not evident in the spectra of healthy wheat or the black tip-infected wheat. This observation indicated that ergot infection in the wheat may be associated with expression and deposition of $\alpha$-helical and $\beta$-sheet proteins.

In the same work, Egging and co-authors also investigated whether the proposed spectroscopic approach could be used for investigating disease development. The researchers collected spectra from mold-infected sorghum seeds at different stages of disease progression, including healthy, middle and late stage grain. They observed a decrease in intensities of lignin, carotenoids and carbohydrates vibrational bands in healthy, middle, and late stage progression of mold on sorghum seeds.

### 3.2. Raman-based detection of pests in beans

Many pests or pathogens of plants live within their hosts. To address whether RS can detect these hidden threats, Sanchez and colleagues studied *Callosobruchus maculatus*, an insect pest of cow peas [50]. These insects lay their nearly invisible eggs on the surface of the peas; upon hatching, the larvae burrow into the seeds and consume them before emerging as adults. One breeding pair of insects can quickly destroy a store of cow peas, so rapid detection of the larvae is essential. The researchers scanned cow pea seeds containing the insect larva at different stages of development. It was shown that RS could detect spectroscopic peaks specific to the insect larvae and their excrements within the peas. Using PLS-DA, Sanchez and co-workers demonstrated that not only could the healthy and infected peas be distinguished from one another, but also the developmentally early and late stage larvae with high accuracy. These results together suggest that Raman is not limited to pests and pathogens on the surface of plants, but also within them.

### 3.3. Raman-based detection and identification of bacterial and viral diseases

Sanchez and co-workers recently showed that RS in combination with multivariate statistical analysis enables highly accurate detection of HLB on early and late stages as well as nutritional deficiency (ND) on orange and grapefruit citrus trees [51]. The accuracy of detection of Raman-based diagnostics of healthy vs. HLB infected vs. nutritional is ~98% for grapefruit and ~87% for orange trees, whereas the accuracy of early vs. late stage HLB infected is ~85% for grapefruits and ~94% for oranges.

The researchers observed significant increase in the intensity of 1601–1630 cm$^{-1}$ bands, which has been assigned to lignin (Fig. 4). The intensities of these bands in Raman spectra collected from grapefruit leaves with late stage HLB infection appeared to be higher compared to the spectra of early stage HLB-infected leaves. In contrast, the intensities of these bands in Raman spectra collected from orange leaves with late and early stage HLB were nearly identical. The increase in the intensities of these bands is even more pronounced in the spectra of both grapefruit and orange leaves that have ND symptoms. Thus, one can envision that ND may have a unique vibrational fingerprint related to lignin that is distinctly higher from Raman signatures of both healthy and HLB-infected leaves.

This work made two groundbreaking discoveries in diagnostics of plant diseases. It showed that RS can 1) distinct biotic (diseases) and abiotic (ND) stresses on plants and 2) enable pre-symptomatic diagnostics of HLB with high accuracy.

It should be noted that Vallejo-Perez and co-workers also attempted to demonstrate RS-based detection of HLB in 2016 [52]. In that study, the researchers observed only changes in the background of spectra collected from HLB-positive and healthy leaves, whereas no specific bands or relative changes in intensities of vibrational bands have been observed. Background-based spectral changes may not be reliable and specific for confirmatory HLB diagnostics. The work by Sanchez and co-authors revealed that both HLB and ND had specific vibrational fingerprint in the Raman spectra of citrus leaves. These spectral differences enable highly accurate detection and identification of HLB and ND.
In 2017, Yeturu and co-workers investigated the possibility of RS-based detection of Abutilon mosaic virus (AbMV) on Abutilon, a flowering shrub found in the tropics [53]. Infection by the virus manifests as yellow spots, or mosaic patterns, on the leaves of the plant. The researchers observed a continuous decrease in the intensity of all vibrational bands in the Raman spectra collected from the plants infected with the virus. Statistical analysis performed by the authors showed that the differences in intensities between the spectra was 99%, confirming that they were able to detect the virus before the symptoms appeared. At the same time, such approach should be taken with a grain of doubt the main symptom of AbMV is the yellow mosaic pattern on the leaves. It is possible that a coherent decrease in the intensity of all bands could represent the change in the leaf color rather than be associated with the viral disease itself. This evidence suggests that RS based diagnostics could be considered only if relative change in band intensities has been observed. These illustrate issues with Raman-based detection, primarily related to fluorescence and absorbance. Different color patches will have different fluorescent backgrounds and absorb different amounts of light, thus changing the spectra. These factors should be considered in Raman analysis of plant tissue.

3.4. Surface-Enhanced Raman Spectroscopy (SERS)-based detection of mycotoxins

In 1977, Van Duyne discovered that Raman scattering can be amplified up to 10^8 by coherent oscillations of an electron cloud at the surface of nanoparticles [54]. This phenomenon, known as surface-enhanced Raman spectroscopy (SERS), allows for the single-molecule detection and therefore has been broadly utilized for detection of toxins associated with fungi [55–57]. Additionally, SERS has been applied to related problems such as detection of environmental pollutants and identification of bacteria [58,59].

While RS and IR investigate primarily bulk-volume samples of plant tissue for spectroscopic evidence of infection, SERS can detect toxic metabolites produced by a pathogen directly; however, these toxins must first be extracted from a sample, meaning that this method is often invasive and destructive. This significantly restricts utilization of SERS directly in the field. Additionally, fluctuations of the signal intensities, which is typical for SERS, remain the major drawback of this spectroscopic approach [60]. Nevertheless, Wu and co-workers demonstrated that using silver nanorod (AgNR) array substrates SERS-based detection of aflatoxin (AF) B1, B2, G1 and G2 could be achieved [61]. They determined the limits of detection (LODs) reach 5 × 10^-5 mol/L for AFB2, 1 × 10^-4 mol/L for AFB2, and 5 × 10^-6 mol/L for both AFG1 and AFG2 in bulk solution. Lee and co-workers used SERS coupled to various methods of multivariate statistical analysis to detect aflatoxin in maize at concentrations of 0–1206 µg/kg [62,63]. Zhao and co-workers proposed to use aptamer-coated Ag@Au core–shell (CS) nanoparticles (NPs) for double detection of ochratoxin A (OTA) and aflatoxin B1 in maize meal [64]. This enabled significant improvement in the LOD, which has been found to be as low as 0.006 ng/mL for OTA and 0.03 ng/mL for AFB1.

Galarreta and co-workers developed aptamer-based SERS microfluidic sensor for the detection of OTA [65]. A thiol-modified aptamer of OTA was immobilized onto the 2D SERS platform enabling detection of OTA. Additionally, Li and co-workers constructed similar aptamer–based SERS chip for the ultrasensitive determination of AFB1 in spiked peanuts [66]. Presence of AFB1 in the sample, released AFB1 aptamers from the hybridization duplex strands of AFB1 aptamers and their partial complementary strands. Next, this aptamer was immediately hybridized with hairpin DNA on the Au film of chip surface. After this, the hairpin DNA was hydrolyzed by exonuclease III at a restriction site, leaving short single-stranded DNA to capture Raman signals via hybridization on Au film and releasing complementary DNA for recycling. Although this approach is very sophisticated, it demonstrated high sensitivity and good selectivity for the detection of AFB1 enabling detection of AFB1 in the range from 1 × 10^-6 µg/L to 0.4 × 10^-6 µg/L. Thus, demonstrating that such aptamer-SERS sensing chips could provide an important application for more rapid and portable determination of mycotoxins. Development of more robust and reliable SERS substrates with high enhancement factors should increase sensitivity to toxins. Additionally, development of capture layers would tremendously increase SERS specificity [60].

4. Conclusions

This review critically discussed spectroscopic methods, as well as modern molecular and imaging methods, used for plant disease diagnostics. It showed a great potential of both RS and IR for confirmatory, non-invasive and non-destructive detection and identification of plant diseases. The review also revealed advantages and disadvantages of RS and IR, as well as other spectroscopic techniques such as SER and VIS/NIR-TM and RM spectroscopy, for the plant disease diagnostics. One can envision that a decrease in the cost of portable spectrometers and development of Raman telescopes, which are capable of a standoff spectral acquisition, will foster direct practical applications of RS in plant pathology and other areas of agriculture.

Acknowledgments

The authors are grateful for the financial support of Agrilife Research at Texas A&M and acknowledge the Governor’s University Research Initiative (GURI) grant program of Texas A&M. GURI Grant Agreement No. 12-2016, M1700437.

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