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Emerging Trends in Plant Pathology

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To the Legend....

(Dr. Y. L. Nene)

Foreword

Plant pathology as a discipline of agricultural science has played pivotal role over the years in understanding plant diseases and in mitigating losses through cultural and technological innovations. This is despite the disease scenarios that kept on evolving and changing due to biotic, abiotic and edaphic factors. The plant pathologists have eventually contributed significantly towards food security and in ameliorating the livelihood of farmers across the globe. The science of plant pathology has been an innovative and ever-emerging discipline in its scope, importance and technologies. There have been many such innovations and advancements in each and every aspect of plant pathology starting from the identification of the pathogen, underlining the molecular mechanism of pathogenicity and resistance and also the management strategies. With the commencement of the concept of sustainable agriculture, plant disease management has become more important and has shifted from the traditional chemical-based to more eco-friendly integrated disease management strategies with more focus on the biocontrol and other green technologies. The latest innovations in the field of detection and diagnosis, host resistance, disease forecasting and plant biotechnology have helped us in better management of the diseases, but challenges are still many more.

This first edition of *Emerging Trends in Plant Pathology* edited by K. P. Singh, B. K. Sarma and Shamarao Jahagirdhar provides a comprehensive description and highlights of the latest innovation and trends in the field of plant pathology and allied fields. The focus is on understanding both the basic and applied aspects of plant pathology and plant disease management. I hope the book would be of special interest to both academics and professionals, working in the fields of plant pathology, microbiology, biotechnology and plant breeding, as well as the plant protection sciences. This book is a comprehensive reference for all those curious to understand the latest advancements in their field of specialization.

I congratulate the editors and contributors for their dedicated effort to bring out such a classic reference book for the scientific fraternity.

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Preface

The science of plant pathology is essential for reliable food production through management of plant diseases. Dynamics in the evolution of new races of plant pathogens and changes in the global climatic scenario have made plant pathology an ever-emerging discipline in its scope, importance and technologies. During the past decade tremendous advancements and innovations took place in every aspect of plant pathology starting from identification of pathogens to their management. Additionally, with commencement of the concept of sustainable agriculture, plant disease management has become more important and disease management strategies have shifted from the traditional chemical based to more eco-friendly strategies. Further, advancements in molecular biology studies have armed the researchers to develop newer strategies for plant disease management. Therefore, recently more focus has been on the applications of biocontrol agents, development of transgenic cultivars, plant genome editing to other green technologies. Recent innovations in the field of detection and diagnosis of plant diseases, host resistance, disease forecasting and plant biotechnology have helped in developing strategies to manage the diseases better and address the challenges still on the way.

In this first edition of *Emerging Trends in Plant Pathology* we have compiled chapters to reflect on the recent trends and innovations in the field of plant pathology. Emphasis was given to understanding both basic and applied aspects of modern tools and techniques developed for detection and diagnosis of plant diseases that has helped identifying pathogens associated with a disease in a very short time. Quicker detection and diagnosis leads to identification of many new and emerging plant pathogens, and it is of great help in designing effective management strategies against them. The book has therefore also focused on the host-pathogen systems at molecular level without considering the hosts and their pathogens as separate entities. Chapters were also compiled to elaborate our understanding on host resistance to plant pathogens and the mechanisms of actions of *R* and *Avr* genes of the host and pathogen, respectively. Additionally, plant diseases and their epidemics are highly influenced by environmental conditions and crop microclimate. The book also includes chapters covering broad overviews of the recent advancements in disease forecasting, remote sensing, GIS and GPS applications that help accurate prediction of plant diseases and thereby saving crop losses from pathogens. The book also highlights the developments in the area of biological control of plant pathogens and use of microbial consortium which have received much attention in the past decade due to promotion in organic farming and sustainable agriculture globally. Further, the new-generation fungicides are considered far more eco-friendly and very effective at low concentration and are highly target specific. In this book, we have also focused on advancements in the use of secondary metabolites from microbes and novel plant extracts as eco-friendly pesticides. We also compiled chapters on the use of transgenics, cisgenics and genome editing that are being increasingly used for plant disease management.

This book is very timely in providing essential and comprehensive source materials, as it includes most relevant areas on emerging trends in plant pathology and their role in crop protection.

Pantnagar, Uttarakhand, India — Krishna P. Singh
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Dr. Birinchi Kumar Sarma is a Professor in the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India. A specialist in legume pathology and genomics, he completed his post-doctoral research at the University of California, Davis, USA. He was a Visiting Researcher at Dalhousie University, Canada, under the Shastri Indo-Canadian Institute – FTI Award. He was also made an Associate of the National Academy of Agricultural Sciences, New Delhi. He has published over 85 research papers, edited 3 books and filed 5 patents. He also serves as editor for the international journals *PLOS ONE* and *Frontiers in Microbiology*.

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Emerging Plant Diseases: Research Status and Challenges

1

Dipannita Mitra

Abstract

Plant diseases result in significant crop destruction thereby inadequate food supply and lead to economic and post-harvest losses in the agricultural production sector throughout the world. Early detection of plant diseases and pathogens is important for maintaining sustainability for the economy of the agricultural sector. The prevention of plant disease and pathogens during the early stages aids in plant health control and yield improvement. It is also crucial to analyze the disease spread in plants for overcoming the issues related to physiological and biological states in crop protection. This chapter reviews the research status of the various emerging plant diseases responsible for a large amount of crop destruction every year all over the world and the challenges that the agricultural sector face to overcome this problem.

Keywords

Plant pathogen disease · Symptomatic stage · Remote sensing · Flow cytometry

1.1 Introduction

Agriculture plays a dominant part in the worldwide economy and is the main source of food, fiber, fuel, timber, income, and employment, thus maintaining socioeconomic stability. The major threat to agriculture is nationwide crop losses due to pathogen-induced plant diseases, which is considered to be a primary challenge for the whole scientific community. The branch of plant pathology thus largely focuses

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on increasing the fundamental understanding of host-pathogen interactions to detect plant diseases and mitigate crop losses and enhance the total agricultural yield.

It was estimated in 1994 that worldwide crop losses due to plant diseases ranged from 9.7 to 14.2% of the total yield; the figure is different in modern days due to the emergence of new pesticides with a varied target range (Zadoks 1996). But, the ability of these target pathogens to gradually develop resistance to frequently used pesticides has allowed diseases to remain persistent and is proving to be a big threat in today's world (Strange and Scott 2005). A recent article in the Food and Agriculture Organization (FAO) of the United Nations reported that there has been a loss of about 20 to 40% of the global crop production due to pest infestations.

"Each year, plant diseases cost the economy around $220 billion and the invasive pathogen around US$70 billion" as per the FAO reports. This huge amount of losses deprives more than 800 million of the total worldwide population of adequate consumable food (http://www.fao.org/news/story/en/item/1187738/icode/).

The fact that plant pathologists should be concerned with the need to minimize losses due to endemic diseases correlates with the introduction of new foreign pathogens resulting from the globalization of plants and plant products (Mack et al. 2000).These plant pathogens can rapidly spread from an infected or diseased plant to a healthy plant. The microorganisms that cause plant diseases include fungi, viruses, nematodes, bacteria, and mycoplasmas (Lucas et al. 1992).

Plant diseases can be defined as any deviation from their healthy state with symptoms and disorders mainly on their shoots, leaves, flowers, fruits, stem, and roots. Diseased plants produce poor yields in terms of both quantity and quality. Losses in the total yield in a plant can occur from the seed to the harvesting stage. Quality of a plant product is attenuated when diseased spots or blotches are present; thus monitoring plant health and detecting the pathogens early in the plant life cycle is essential to reduce the chances of disease spread and also to facilitate effective management practices.

It is a well-known fact that vegetable crops represent an important economic segment of the total global economic production. But, gradually reoccurrence of crop diseases has played havoc toward the society and for total agronomical growth. For example, the famous famine in Ireland which was started in Europe during 1845 as an epidemic was caused by the late blight of potato by *Phytophthora infestans* (Mundt et al. 2009). Similarly, plant pathogens threaten other food crops globally including citrus, banana, and grapes. In southwestern Europe, a region known for its grape cultivation, a disease caused by phytoplasmas called *Flavescence doree* is widespread and is a major cause of annual economic losses (Martinelli et al. 2016).

Similarly, ready-to-eat salads like bagged salads have gained popularity throughout the world especially in Europe since their introduction in the early 1980s, marking opportunities for the fresh food industry. As this industry is growing, the number of new diseases is growing in parallel. The past review indicates that these seasonal salads are grown massively in a highly dense region in five to six cycles annually in the same farms (Fig. 1.1); thus it lacks crop rotation and also sometimes a shortage of applicable fungicides causing the growth of many fungal diseases, e.g., downy mildew of basil and *Fusarium* wilt of lettuce (Farr and Rossman 2019).

Fig. 1.1 Multitunnel cultivation of lettuce and wild rocket in southern Italy with five to six production cycles per year. (Source: Gullino et al. 2019)

Other examples of major economic losses due to plant diseases are soybean rust which is mainly a fungal disease in soybeans, but it was reported that by removing 20% of the infection, the farmers could make a profit of $11 million (Roberts et al. 2006). It was also estimated that the crop losses due to pathogen infection in the United States could be attributed to non-native or foreign plant pathogens, e.g., chestnut blight fungus, Dutch elm diseases, and Huanglongbing citrus diseases (Pimentel et al. 2005; Sankaran et al. 2010).

Plant diseases can be spread over a larger area of cultivation land with time through the accidental introduction of vectors or through plant materials. The other route of the spread of plant pathogens can be through ornamental plants that act as hosts. Ornamental plants are always in increasing demand and are sold worldwide before these diseases could be detected.

Recent technology has made it possible to consume crops that are produced on foreign land. Thus, international import-export has resulted in new kind of plant diseases, where a very low level of seed contamination can result into rapid emergence of new diseases in a totally new geographic areas, thus resulting into severe crop losses; it can affect the biological equilibrium of that region and sometimes also start an epidemic (Gullino et al. 2019). The globalization of agricultural products has resulted in many new resistant strains of pathogens which causes diseases that are tough to detect. If these new strains migrate to a healthy area of cultivation, the crops may not be able to resist these new pathogen strain infestations, similarly like *P. infestans* in the 1840s. The problem of plant diseases is a very challenging factor in developing countries because of their limited resources to fight these pathogens through scientific research. Lack of proper resources makes the developing nations unable to efficiently identify the disease causal organisms and detect and mitigate the symptoms for crop yield loss.

In this chapter, we have focused mainly on the emerging plant diseases and strains, the risk they possess in the successful cultivation of crops worldwide, the possibility to detect these strains at an early stage by using traditional and innovative

Fig. 1.2 Aerial image of olive groves in Puglia in Italy showing olive trees infected by *Xylella fastidiosa* (left). (Source: https://www.theguardian.com/world/2019/sep/09/deadly-olive-tree-disease-spreads-france)

detection methods, and lastly the challenges that plant disease management faces in the modern era. These challenges need to be addressed through science-based cooperation on a global scale at a scientific and political level (Fig. 1.2).

1.2 Emerging Plant Diseases and Their Research Status

Our knowledge of global crop losses due to plant-pathogen infestations is very limited. There is an increase in emerging pathogen strains, and with that integrated disease management should evolved as well. In recent years, there has been a great spike in types of plant pathogens and strains especially in imported plants and plant products. Changes in climate conditions have also resulted in various new plant diseases. For example, according to recent news, the Animal and Plant Health Agency (APHA) reports some new plant diseases and new pests with their symptoms and also gave information about the countries where they can be found. Figure 1.3 and Fig. 1.4 show some examples of emerging plant diseases and plant pests.

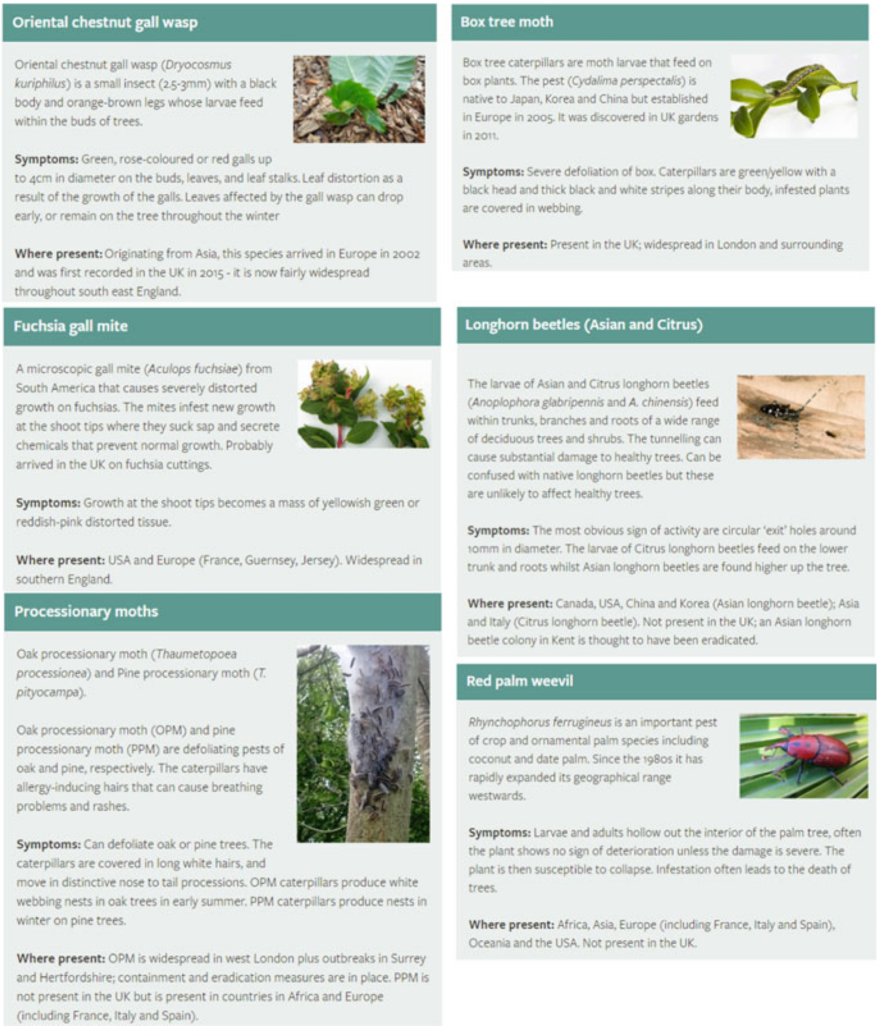
Recent survey articles published by British Broadcasting Corporation (BBC) and *The Guardian* report the massive outbreaks caused by *Xylella fastidiosa* in European countries like Italy, France, Germany, and Spain, wiping out entire olive groves and thus causing major economic losses in these countries. This disease is also called olive quick decline syndrome and is believed to affect more than 350 plant species. This disease has also spread in the vineyards in the north and south of America. It was first detected in Puglia in Italy in the year 2013, but now it has spread almost in every corner of Europe (https://www.theguardian.com/world/2019/sep/09/deadly-olive-tree-disease-spreads-france).

The European Commission suspects it could threaten olive gardens throughout the world and thus has sought crop protection actions against the spread of *Xylella*. To help mitigate the problem, the Royal Horticultural Society of the United Kingdom has come up with some new principles for future-proofing UK gardens: (1) All imported semi-mature trees will be held for 12 months before planting them, (2) evaluation of plant health risk will be monitored according to the criteria

Fig. 1.3 Emerging new plant diseases, symptoms, and regions where they are present. (Source: https://www.rhs.org.uk/science/plant-health-in-gardens/protect-your-garden/new-pd-risks)

provided by the Royal Horticultural Society, and (3) this society will generate a list of suppliers who meet these specified criteria.

Other major factors in causing various new plant diseases are the climate changes resulting from natural and human activities. Climate change is the result of the increasing amount of global trade, agricultural system modifications, and changes in the consumer lifestyle; the global circulation of the crop also affects the global circulation of new pathogens and diseases.



Oriental chestnut gall wasp

Oriental chestnut gall wasp (*Dryocosmus kuriphilus*) is a small insect (2.5-3mm) with a black body and orange-brown legs whose larvae feed within the buds of trees.

Symptoms: Green, rose-coloured or red galls up to 4cm in diameter on the buds, leaves, and leaf stalks. Leaf distortion as a result of the growth of the galls. Leaves affected by the gall wasp can drop early, or remain on the tree throughout the winter

Where present: Originating from Asia, this species arrived in Europe in 2002 and was first recorded in the UK in 2015 - it is now fairly widespread throughout south east England.

Fuchsia gall mite

A microscopic gall mite (*Aculops fuchsiae*) from South America that causes severely distorted growth on fuchsias. The mites infest new growth at the shoot tips where they suck sap and secrete chemicals that prevent normal growth. Probably arrived in the UK on fuchsia cuttings.

Symptoms: Growth at the shoot tips becomes a mass of yellowish green or reddish-pink distorted tissue.

Where present: USA and Europe (France, Guernsey, Jersey). Widespread in southern England.

Processionary moths

Oak processionary moth (*Thaumetopoea processionea*) and Pine processionary moth (*T. pityocampa*).

Oak processionary moth (OPM) and pine processionary moth (PPM) are defoliating pests of oak and pine, respectively. The caterpillars have allergy-inducing hairs that can cause breathing problems and rashes.

Symptoms: Can defoliate oak or pine trees. The caterpillars are covered in long white hairs, and move in distinctive nose to tail processions. OPM caterpillars produce white webbing nests in oak trees in early summer. PPM caterpillars produce nests in winter on pine trees.

Where present: OPM is widespread in west London plus outbreaks in Surrey and Hertfordshire; containment and eradication measures are in place. PPM is not present in the UK but is present in countries in Africa and Europe (including France, Italy and Spain).

Box tree moth

Box tree caterpillars are moth larvae that feed on box plants. The pest (*Cydalima perspectalis*) is native to Japan, Korea and China but established in Europe in 2005. It was discovered in UK gardens in 2011.

Symptoms: Severe defoliation of box. Caterpillars are green/yellow with a black head and thick black and white stripes along their body, infested plants are covered in webbing.

Where present: Present in the UK; widespread in London and surrounding areas.

Longhorn beetles (Asian and Citrus)

The larvae of Asian and Citrus longhorn beetles (*Anoplophora glabripennis* and *A. chinensis*) feed within trunks, branches and roots of a wide range of deciduous trees and shrubs. The tunnelling can cause substantial damage to healthy trees. Can be confused with native longhorn beetles but these are unlikely to affect healthy trees.

Symptoms: The most obvious sign of activity are circular 'exit' holes around 10mm in diameter. The larvae of Citrus longhorn beetles feed on the lower trunk and roots whilst Asian longhorn beetles are found higher up the tree.

Where present: Canada, USA, China and Korea (Asian longhorn beetle); Asia and Italy (Citrus longhorn beetle). Not present in the UK; an Asian longhorn beetle colony in Kent is thought to have been eradicated.

Red palm weevil

Rhynchophorus ferrugineus is an important pest of crop and ornamental palm species including coconut and date palm. Since the 1980s it has rapidly expanded its geographical range westwards.

Symptoms: Larvae and adults hollow out the interior of the palm tree, often the plant shows no sign of deterioration unless the damage is severe. The plant is then susceptible to collapse. Infestation often leads to the death of trees.

Where present: Africa, Asia, Europe (including France, Italy and Spain), Oceania and the USA. Not present in the UK.

Fig. 1.4 Emerging new plant pests, symptoms, and region where they are present. (Source: https://www.rhs.org.uk/science/plant-health-in-gardens/protect-your-garden/new-pd-risks)

Plants and pathogens not only interact in isolation but also with the environment according to the “disease triangle” concept, for instance, for a disease to occur by a pathogen, a specific favorable environment is required (Fig. 1.5). Various environmental conditions affecting plant disease development include water, temperature, light, soil quality, wind speed, CO_2 concentration, and others. Though we know that plants have evolved various sophisticated defense mechanisms like PAMP-triggered immunity (PTI), effector-triggered immunity (ETI), RNA interference (RNAi), and hormonal regulation via abscisic acid, jasmonic acid, and ethylene, but new studies have shown that environmental conditions can gradually modulate these defense

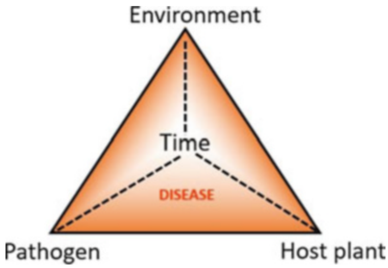


Fig. 1.5 Disease triangle showing four dimensions responsible for plant diseases, pathogen, host plant, environment, and time. (Source: http://www.ucanr.org/blogs/blogcore/postdetail.cfm?postnum=28845)

mechanisms (Couto and Zipfel 2016; Wu et al. 2019). For example, high humidity condition interferes with ETI-associated mechanism; thereby the response to *C. fulvum* Avr4 and Avr9 effectors by tomato CfR proteins is reduced when air humidity reaches more than 95% (Wang et al. 2005). Since tropical climates are prevalent in most developing countries, plant diseases are more common, causing a great part of economic loss. In contrast, cold temperate reduces the chances of rapid disease spread.

To solve the problems related to the emerging plant diseases, pathogen exclusion through the plant quarantine must be the first step to combat food security in both developing and developed countries. Other methods that need to be implemented should be intercropping and crop rotation methods, use of pesticides, adequate knowledge of the molecular mechanisms of these pathogen-host interactions, and knowledge about post-harvest protection. The classically accepted phenomenon of host-pathogen interactions now will no longer be relevant for these emerging plant diseases. Thus, we must thrive to improve the traditional detection methods and focus more on new approaches for these new pathogen strains. During the last 100 years, accuracy and precision in the detection of plant diseases were based solely on the traditional methods; however, these methods are too slow and ineffective and thus need to be improved and updated.

Maintaining genetic variability in crop plants is also of major importance for better crop yield. Future breeding programs for new improved varieties of crops must incorporate the growth and biotic and abiotic resistance variability, which should favor plant immunity and not the pathogen virulence. These features are found mostly in wild-type relatives of cultivated crops and possess combined abiotic and biotic resistance over a long time. But this is not the case in the modern crop plant variety. With the help of genome-wide association study (GWAS) analysis method and various marker-assisted selection methods, these features can be introduced into the cultivated variety of plants.

1.3 Overview of Disease Detection Methods and Their Ability to Combat Emerging Diseases

Plant pathologists define "plant disease monitoring as detection i.e. deviation from a healthy state of plants to stressed state, identification i.e. diagnosis of symptoms for various diseases, and quantification i.e. measurement of disease severity for e.g. reduced chlorophyll content or reduced leaf area," etc. (Mahlein et al. 2012).

After the onset of plant disease symptoms, there are many methods applied to detect the presence of diseases, for example, two main methods used are enzyme-linked immunosorbent assay (ELISA) which is based on proteins produced by the pathogen and polymerase chain reaction (PCR), based on specific DNA sequences of the plant 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 the availability of these techniques, there is always a demand for fast, sensitive, and effective methods for the detection of plant diseases caused by varied plant pathogens. According to Sankaran et al. (2010), disease detection techniques can be broadly classified into two main groups: direct and indirect methods (Fig. 1.6).

Among the direct approaches, molecular methods and serological methods provide essential tools for accurate plant disease detection. Although DNA-based molecular methods and serological methods have improved plant disease detection, they are sometimes not very reliable, especially at the asymptomatic stage.

Other modern methods based on nucleic acid and protein analysis have been proven to be more efficient in plant disease detection (Martinelli et al. 2015). The main conclusion from a review by Martinelli et al. (2015) states

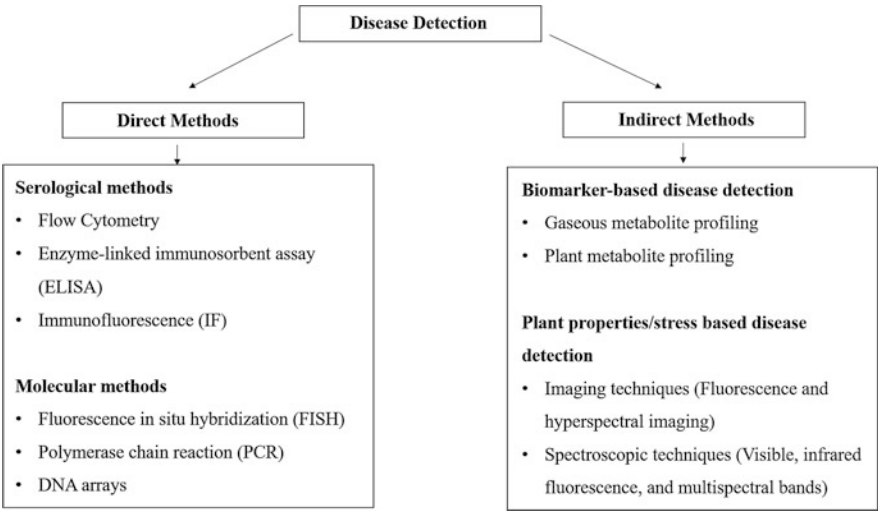


Fig. 1.6 Methods of Plant disease detection. (Source: Sankaran et al. 2010)

(1) novel sensors based on the analysis of host responses, for example, differential mobility spectrometer and lateral flow devices, deliver much more reliable and instantaneous results and can effectively detect early infections directly in the field; (2) secondly, biosensors based on phage display and biophotonics can also detect infections very fast although they can be also integrated with other systems; and lastly (3) remote sensing techniques coupled with spectroscopy-based methods allow high spatialization of the results, these techniques can prove to be very effective as a very reliable, sensitive and rapid preliminary identification of primary infections. These tools in the long run help plant disease management and complement serological and DNA-based molecular methods.

According to plant pathologists, serological and PCR-based methods are the commonly used methods to confirm plant disease detection, but volatile and biophotonic sensors provide rapid and more effective results and may be used to identify infections at asymptomatic stages. Remote sensing technologies are also very efficient tools to spatialize diagnostic results and thus provide agriculture more sustainability and safety, avoiding expensive use of pesticides for crop protection (Martinelli et al. 2015).

1.3.1 Molecular Methods for Disease Detection

Molecular methods for plant disease detection are well established. It was reported that the sensitivity of the molecular techniques for detecting bacteria ranged from 10 to 10^6 colony-forming units/mL (Lopez et al. 2003). The most commonly used molecular techniques for plant disease detection are fluorescence in situ hybridization (FISH) and PCR. As shown in Fig. 1.6, the other most commonly used methods include immunofluorescence, flow cytometry, FISH, and DNA microarrays.

Another categorization of the molecular methods can be nucleic acid-based, i.e., (1) DNA-based methods like FISH and the PCR variants nestedPCR (nPCR), cooperativePCR (Co-PCR), multiplex PCR (M-PCR), real-time PCR (RT-PCR), and DNA fingerprinting. (2) RNA-based methods include reverse transcriptase PCR, nucleic acid sequence-based amplification (NASBA), and AmpliDet RNA (Martinelli et al. 2015; Lopez et al. 2003). In the PCR method, the DNA of the pathogen is extracted, purified, and amplified. Then it is used for gel electrophoresis which if shows a specific band confirms the presence of the plant pathogen. The concept of molecular detection methods is based on the specific design of oligonucleotides and probes. Target sequences for molecular detection methods can be obtained from the National Center for Biotechnology Information (NCBI, Bethesda, MD, USA).

DNA fingerprinting is another molecular genetic method for plant pathogen detection, where unique patterns are identified in the DNA of the plant pathogen samples also called polymorphisms. This method was first described by Alec Jeffreys in 1984. The various DNA fingerprinting methods use either PCR or restriction fragment length polymorphism (RFLP) and sometimes both to target specific areas of DNA. Apart from these methods, DNA diagnostic microarrays are being used for plant pathogen detections.

1.3.2 Serological Assays

In the ELISA-based method of plant disease detection, the microbial protein associated with a specific disease is injected into an animal that produces the antibodies against these microbial proteins, better known as antigens. These antibodies are then extracted from the animal and are tagged with a fluorescence dye and enzymes and are used for the detection of host-target interactions. ELISA method was first used in the 1970s and is so far the most widely used immunodiagnostic technique because of its efficiency and specificity. ELISA is also a highly sensitive method, but its sensitivity depends on the samples and the volume of samples, for example, bacteria can be detected only at 100 cfu mL – 1 (Schaad et al. 2002, 2003).

Antibodies against many viruses and bacteria have been developed and are being used in numerous ELISA methods for plant disease detections globally, but due to the fact that they might show cross-reactivity, monoclonal antibodies using hybridoma technology have been developed which are more specific to the target (Nolasco et al. 2002; Holzloehner et al. 2013). To date, both polyclonal and monoclonal antibodies are available and are being used for various plant disease detections by ELISA. Pathogens like viruses, bacteria, and fungi can now be detected using these specific antibody techniques such as western blots, immuno-binding assays, and serologically specific electron microscopy (SSEM) (Alarcon et al. 1990; Caruso et al. 2002; Serological methods for detection and identification of viral and bacterial plant pathogens. A laboratory manual 1990).

However, these molecular and serological techniques have some limitations, for instance, they are time-consuming, require an elaborate sample preparation procedure, are labor-intensive, and require specific reagents to detect each specific pathogen. Also, sometimes the concentrations of seed samples, soil, water, and pathogen are below the sensitivity limit, thus hindering efficient detection by these methods.

Another issue is the occurrence of false negatives and false positives due to the degradation of target DNA sequence or poor quality of the reagents (Louws et al. 1999). Lastly, the cost of equipment, reagents, sample preparation, etc. used in molecular detection methods are very high making it less popular for most agriculture-based industries. Thus, spectroscopic techniques can be potential alternative methods for the rapid detection of new plant pathogens.

1.3.3 Spectroscopic and Imaging Techniques

Recent research developments focus on automated nondestructive methods of plant disease detection that will act as an efficient tool for disease monitoring on a large scale. With the advancement of spectroscopic methods, the detection of plant diseases has simplified. Many spectroscopic and imaging techniques have been studied for the detection of the early and late stages of plant diseases. Some of the methods include fluorescence imaging, infrared spectroscopy, fluorescence spectroscopy, visible spectroscopy, nuclear magnetic resonance spectroscopy, etc. Spectroscopic methods can either be based on imaging or non-imaging techniques and help in crop disease monitoring because of their potential, flexible, and cost-effective role as operational instruments.

The most common imaging-based spectroscopic approaches include fluorescence spectroscopy where the fluorescence from the object of study is measured after being excited with an ultraviolet spectrum. To monitor plant stress and physiological states in plants and to monitor nutrient deficiency in plants, usage of laser-induced fluorescence is very popular (Belasque et al. 2008; Cerovic et al. 1999). Imaging spectroscopy was used and has proved to be very effective for wheat kernels for *Fusarium* head blight disease and also for weed infestations (Delwiche and Kim 2000; Okamoto et al. 2007).

Non-imaging spectroscopy methods are based on optical properties of leaf pigments, chemical components, and structural features. These spectra collected are then used for various remote sensing detection methods; this method has been used to detect winter wheat yellow rust, aphid infestation, curl mite infestation, etc. (Jacquemoud and Ustin 2001; Stilwell et al. 2013; Yuan et al. 2014; Zhang et al. 2014).

1.3.4 Other Innovative Detection Methods

During the past few years, many novel approaches were developed which are rapid, inexpensive, efficient, and reliable, for example, lateral flow microarrays (LFM) using an easily visualized colorimetric signal (Carter and Cary 2007). Metabolomics is used as well to detect plant metabolites from primary and secondary metabolism for various plant pathogens (Ibanez et al. 2014; Martinelli et al. 2016).

Volatile compounds emitted by plants for their growth, defense, and survival purposes can also be used as biomarkers to detect plant diseases in volatile compound profiling using gas chromatography-mass spectrometry (GC-MS) (Cardoza et al. 2002).

Biophotonics is also an emerging technique that has been developed for efficient plant pathogen disease detection. The main concept of this technology is based on the molecular detection of probe-target interactions based on specific peptide sequence recognition where the probe-target complex is identified using ELISA. This method uses proteins as probes, increasing the possibility of multiple epitopes for a single target present resulting in a cross-reaction. To mitigate this problem, probe size is reduced to obtain more specificity and sensitivity via various biosensors (Goulart et al. 2010).