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Everything Is Faster: How Do Land-Grant University–Based Plant Diagnostic Laboratories Keep Up with a Rapidly Changing World?

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plant health, pathogen detection, diagnostics, plant pathology, extension, test method

Abstract

Plant diagnostic laboratories (PDLs) are at the heart of land-grant universities (LGUs) and their extension mission to connect citizens with research-based information. Although research and technological advances have led to many modern methods and technologies in plant pathology diagnostics, the pace of adopting those methods into services at PDLs has many complexities we aim to explore in this review. We seek to identify current challenges in plant disease diagnostics, as well as diagnosticians' and administrators'

perceptions of PDLs' many roles. Surveys of diagnosticians and administrators were conducted to understand the current climate on these topics. We hope this article reaches researchers developing diagnostic methods with modern and new technologies to foster a better understanding of PDL diagnosticians' perspective on method implementation. Ultimately, increasing researchers' awareness of the factors influencing method adoption by PDLs encourages support, collaboration, and partnerships to advance plant diagnostics.

INTRODUCTION

Most land-grant universities (LGUs) have been supporting plant diagnostic laboratories (PDLs) for the past 50 years (and some for much longer) (13) because they recognize the need to provide this service to their state plant-related professionals as well as the general public. The PDLs deliver more than pathogen identification. They provide an evaluation of the plant's health and any management options specific to the client, plant, and situation. As part of LGUs' extension service, PDLs have always strived to make their services widely available to all types of clients with all kinds of plant problems at a price point affordable to noncommercial clients (6).

This review was prepared by seven diagnosticians and two administrators who work with PDLs across the nation. In this review, we focus not only on diagnostics but also on the diagnosticians. Diagnosticians are critical in ensuring that PDLs remain relevant to their LGUs and their clients. Technological advancements have brought changes to the ways clients and diagnosticians communicate with one another and the tools available to diagnosticians to diagnose plant problems. The art of diagnostics is based in knowing the appropriate tools to adopt in the PDL and choosing the appropriate tools to utilize for each sample. This review dissects and discusses diagnostics as a combination of technology, tools, and decision-making processes.

PLANT DIAGNOSTIC LABORATORIES AND THE LAND-GRANT MISSION

The Morrill Acts of 1862 and 1890, and later the 1994 Equity in Educational Land-Grant Status Act, established LGUs to teach agriculture, military tactics, the mechanical arts, and classical studies to working-class citizens (3). LGUs were to provide access to education and research with direct relevance to agricultural and industrial workers to improve their daily lives. In 1887, the Hatch Act established federal funding to create Agricultural Experiment Stations to promote research within each LGU. The mission of LGUs was further expanded to include cooperative extension services by the Smith-Lever Act of 1914. Extension systems serve as a conduit between the LGU and the citizens of its state. Extension personnel collect information about citizens' needs and problems, which determine the priorities for university research. In turn, the extension system provides educational resources that communicate research findings and how they can be applied to improve lives (24).

The first land-grant PDL was established in 1888 at the Connecticut Agricultural Experiment Station. Most LGU extension systems began supporting a plant diagnostic lab between 1950 and 1980. These labs' missions varied, but most were created to support their states' plant professionals. The labs were generally focused on diagnosing plant diseases and other problems, training students and county extension agents in diagnostics, documenting new disease problems, and serving as the public face for plant pathology departments (13). Today, there are more than 70 diagnostic laboratories that provide the citizens of their state with research-based, unbiased pathogen detection, pest identification, and diagnoses of plant problems.

LGUs: land-grant universities

PDLs: plant diagnostic laboratories

Table 1 Monetary and in-kind support reported by plant diagnostic laboratory administrators included salary, funds for materials, operations support, and dedicated laboratory or office space

Support type	Number of institutions with an affirmative response[a]	Percentage of administrators that indicated monetary support[b]				
		<$50,000	$50,001–$100,000	$100,001–150,000	$150,001–$200,000	>$200,000
Salary	18	55.6% (10)	16.7% (3)	5.6% (1)	11.1% (2)	11.1% (2)
Materials and supplies	9	77.8% (7)	11.1% (1)	0.0% (0)	0.0% (0)	11.1% (1)
Operations support	12	83.3% (10)	8.3% (1)	0.0% (0)	0.0% (0)	8.3% (1)
Dedicated lab space	20	NA	NA	NA	NA	NA
Dedicated office space	19	NA	NA	NA	NA	NA

[a] 34 administrators responded to the survey question.
[b] The number of administrators is indicated in parenthesis.

To document the status and services of those labs in 2020, we surveyed the LGU PDLs and their administrators [department chairs, extension administrators, and integrated pest management (IPM) coordinators]. Our survey had three primary objectives: (*a*) assess the perceived and reported levels of support for PDLs by their clients and administrators; (*b*) determine how often diagnosticians working in an LGU PDL provide other support activities (teaching, extension, student advising, research) for their institution; and (*c*) identify methods commonly used in PDLs and the factors influencing their use and adoption. To find details on the survey methodology and survey questions, please refer to the **Supplemental Material**.

Supplemental Material >

IPM: integrated pest management

PLANT DIAGNOSTIC LABORATORY SUPPORT AND SUSTAINABILITY

Understanding and support of the PDL mission by LGU administrators are critical to the lab's long-term sustainability as PDLs adapt to the rapidly changing landscape of diagnostic and communication methods and technology. Although LGU PDLs receive external funding, most rely on in-kind support such as laboratory space and salary support from their home institutions (**Table 1**). Other sources of funding include sample fees and external contracts and grants.

More than 90% of LGU administrators surveyed felt that their PDL helps fulfill the land-grant mission and provides a necessary service (**Figure 1**). However, fewer diagnosticians (76%) felt their administrations valued their activities and services and only 46% felt their administrations actively promoted their service to clients (**Figure 2**). Only 67% of diagnosticians

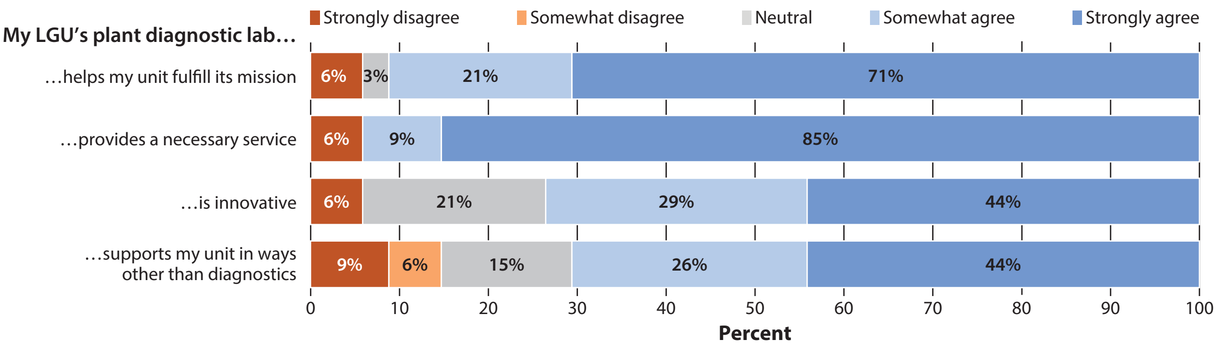

Figure 1

Survey responses of 39 land-grant university (LGU) administrators regarding the value they place on plant diagnostic labs at their home institutions.

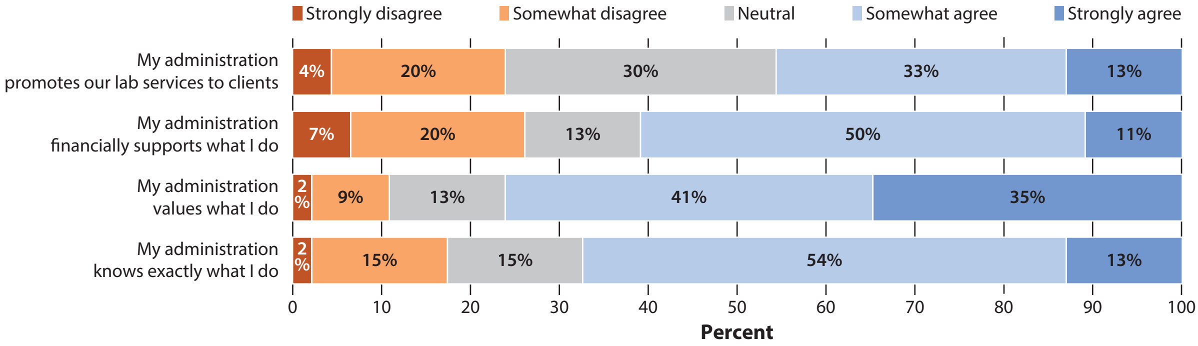

Figure 2

Survey responses of 46 diagnosticians assessing their perceived level of understanding and support by their land-grant university administration.

felt that their administration had knowledge of the PDL's services, and 61% reported that their lab received financial support from their home institution. These results indicate an opportunity to improve communication between administrators and their PDLs to promote and support diagnostic services.

Many PDLs also indicated that they receive grant support from the United States Department of Agriculture–National Institute of Food and Agriculture (USDA-NIFA) through the National Plant Diagnostic Network (NPDN) and/or IPM programs. Through cooperative agreements for equipment, supplies, and salaries, the NPDN supports at least one lab in every state and American Samoa, Guam, Puerto Rico, and the US Virgin Islands. Thirteen of the fifteen IPM coordinators surveyed indicated that they provide either monetary support or space for their diagnostic lab or personnel. Financial stability and the ability to sustain a PDL are vital factors in a lab director's consideration of whether to adopt new technologies and how they should be implemented in the lab.

THE NATIONAL PLANT DIAGNOSTIC NETWORK

In 2002, the NPDN was established as a USDA response to biosecurity assessments after the terrorist attacks of September 11, 2001. Built on the existing LGU extension system, the USDA funded NPDN, formally linking PDLs in every state to ensure the availability of highly trained diagnosticians and robust communications during detections of high-impact diseases and pests (35). Currently, more than 70 labs and over 140 diagnosticians participate in the NPDN (34), allowing diagnosticians to enhance their services for their local clientele and ensure they are better prepared to aid in regional and federal outbreak detection and management through partnerships with regulatory agencies (36).

Specialized regulatory plant diagnostic laboratories are also supported by 24 state departments of agriculture, nine of which are also affiliated with (but not funded by) the NPDN. These regulatory laboratories are focused mainly on diagnostics to assist in regulating the movement of plant material to prevent the spread of insects and pathogens while facilitating domestic trade and export of agricultural products. In states without a regulatory diagnostic lab, the LGU PDL usually performs both extension and regulatory functions.

The NPDN's National Data Repository (NDR), a database of the network's diagnostic records, has become fundamental to the operation of the NPDN by supporting the development of standardized libraries of taxonomically correct host plant names, pathogens, pests, and a set of

USDA-NIFA: United States Department of Agriculture–National Institute of Food and Agriculture

NPDN: National Plant Diagnostic Network

NDR: National Data Repository

generally accepted terms for many abiotic problems, enabling aggregation of diagnostic data from across the network. Since 2004, nearly 2.3 million diagnoses have been entered. These records have become informative for epidemiological research and trade negotiations. Researchers, regulators, and extension specialists request these records to undergird research, inform pest regulations, and serve as a basis for extension publications. This trove of information is highly useful to the diagnosticians by providing a continuously updated index to pests and pathogens found on specific plants and alerting them to potential new threats coming from outside their state (20).

BEYOND THE DIAGNOSIS: THE MANY SERVICES OF A PLANT DIAGNOSTIC LABORATORY

Services offered by a PDL depend primarily on the clientele's needs and are directly proportional to the support the lab receives. In states with robust county-based extension systems, the public is encouraged to contact local county extension staff to help solve plant problems. Those that cannot be readily diagnosed at the county level are forwarded to the LGU PDLs. This filtering mechanism ensures that university labs typically see the most challenging problems.

Most PDLs are staffed by a diagnostician whose primary expertise is plant pathology. About half of diagnostic labs have additional experts within their lab or at their LGU who can diagnose and provide management information for insect, nematode, or weed problems for clients. PDLs also reach out to diagnostic labs within their region for assistance when diagnosing samples for which they lack the expertise or equipment to diagnose, as well as to other extension services for management recommendations appropriate for their client and situation (8).

Laboratory diagnosticians do more than identify the cause of the problem; they also tailor management recommendations to the client, setting, and disease stage. Because the labs are integrated within their institutions, there is often a close relationship between the diagnosticians and the faculty who perform field research. This creates a seamless and intentional conduit for research outcomes to support unbiased, science-based disease and pest management guidelines in the clients' diagnostic reports. For many clients of the diagnostic labs, this interaction may be the only direct contact they have with the university and falls squarely within the LGU mission.

Plant Diagnostic Laboratories as Part of the Land-Grant University Extension Mission

Each PDL strives to meet their clientele's unique needs, which may be drastically different between regions of the United States because of pest and plant host ranges, common crop types, and agricultural practices that affect pest pressure. As a result, the resources and expertise available to the labs may vary widely (33). Most LGU laboratory diagnosticians work closely with extension faculty within a plant health–related department. A majority of diagnosticians (78%) provide faculty with reports of disease and insect pests that impact their specialty. In turn, diagnosticians rely on extension faculty expertise to assist with diagnosis and provide current management recommendations.

In states with limited extension personnel or the ability to cover all areas, diagnosticians often fill in the gaps by taking a more prominent extension role. They lead workshops and give presentations (87%) or author newsletter articles, videos, and blogs (76%), among other activities (**Figure 3**).

Communication is integral to the extension mission. The heart of a PDL is the ability to communicate effectively with its clientele. A diagnostician must be able to communicate the diagnosis and management methods in a way that is understandable and actionable by the

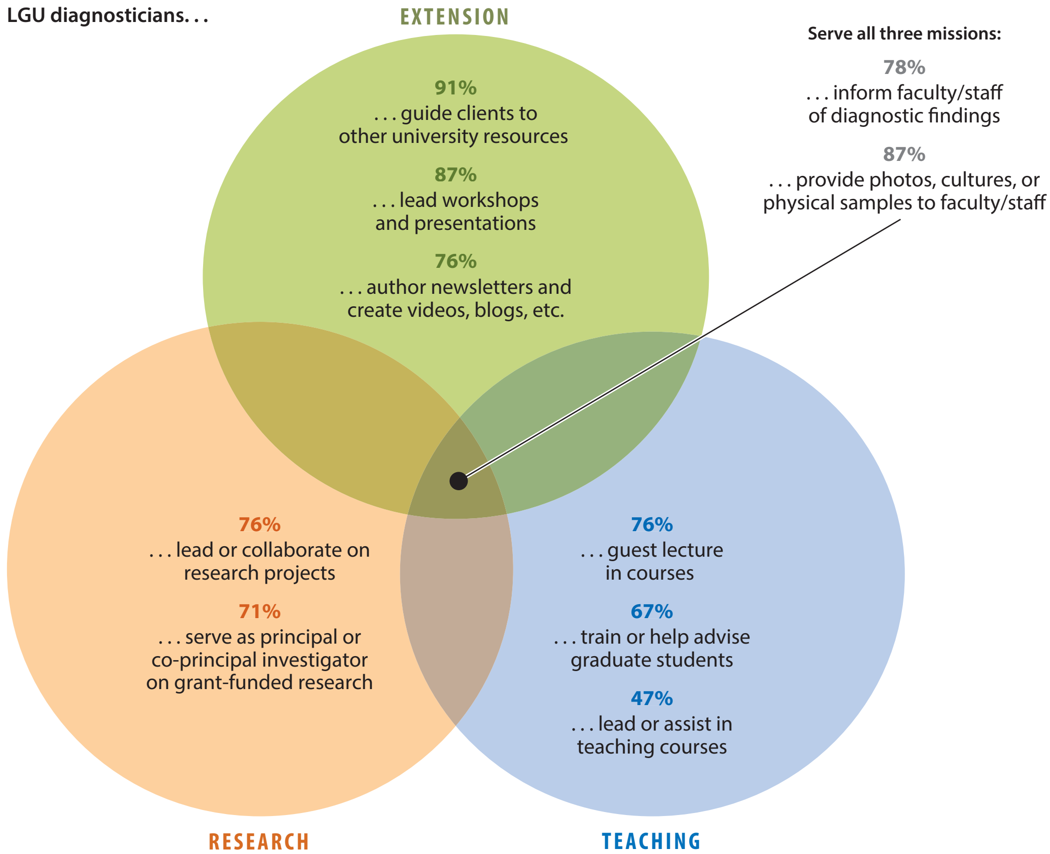

Figure 3

Ways in which land-grant university (LGU) diagnosticians ($n = 45$) support the extension, research, and teaching missions of their land-grant institutions.

Laboratory information management systems (LIMS): databases for recording and reporting sample information. Examples of widely used systems include Plant Diagnostic Information System (PDIS 2009 version 2.0) and PClinic databases (Teaspoon Software, LLC)

client. When university-based PDLs were being organized in the 1950s, the primary means of communication was often face-to-face discussions with the farmer or grower. This interaction was usually followed by mailed plant material or phone calls.

As email replaced other slower means of reporting and communication, diagnostic labs began organizing records in spreadsheets or rudimentary relational databases. The majority of labs now utilize one of several comprehensive laboratory information management systems (LIMS) to record data, export data to the NDR, track billing, and send reports to clientele. Labs also utilize various tools to reach clients with new information about lab services and plant problems, including websites, newsletters, and social media. Tools like Facebook, Instagram, and Twitter facilitate interaction with the public, other labs, and specialists in advertising extension events and programming and increasing lab visibility.

Changes in technology have increased the capability of PDLs to communicate and share an array of information with their clientele. Widespread access to high-speed internet connections and smartphone technology has improved the quality and quantity of photos, videos, and other information provided with samples compared to printed photos and lengthy letters that were previously necessary. Images and videos can provide valuable information concerning symptoms, patterns, sites, and environmental conditions. The use of images can greatly impact the selection of diagnostic procedures to perform, what kind of sample material is recommended for proper testing, and what further information may be needed.

Pathogens can rarely be seen or confirmed via images submitted by clients, so diagnostics without a sample is not ideal. In 2020, as LGUs implemented various safety measures in response to the SARS-CoV-2 (COVID-19) pandemic, many PDLs pivoted to increased photo-only diagnoses while limiting the number of personnel in labs. Labs that did not initially offer photo-only diagnostic services began to implement pilot programs to continue to provide plant diagnostic assistance to stakeholders. These efforts focus on developing online image submission tools (L. Miles, personal communication) or adapting their LIMS to automate client photo submission (M.A. Hansen, personal communication). From February through October 2020, there were 7,460 photo-only reported diagnoses for all PDLs reporting diagnoses to the NDR. In contrast, that number was 4,672 for the same period in 2019 and 4,816 in 2018, demonstrating the success of these and other efforts. Diagnosticians continue to adapt to changing methods to share information and strive to meet clients' needs and innovate in the process.

Eyes on emerging threats. PDLs hold a crucial position at the forefront of new disease detection. When a diagnostician recognizes that something new is causing a problem, they feed the information back into the regulatory and extension systems for their delimitation, regulation, identification, and management expertise. Additionally, because the diagnosticians are often the first to see something new, they are an essential part of research and teaching efforts, performing the assays to confirm pathogenicity, determining the species of an organism, and/or teaching other scientists how to isolate and manipulate the organism.

Diagnosticians and regulatory officials work closely to keep each other informed about unusual detections. For example, in 2015, corn tar spot (caused by *Phyllachora maydis*) was detected for the first time in the United States by a diagnostician at the PDL at Purdue University (18). After confirmation by the USDA national mycologist, the PDL worked together with the Purdue University Extension to quickly inform state and USDA regulatory officials, the diagnostic community, and farmers about this new disease.

Teaching and Training in the Plant Diagnostic Laboratory

The experienced diagnostician is an invaluable part of their university department because they are generalists, with expertise across many organisms and hosts. Training the next generation of plant scientists is important, and the diagnostic lab is a place of broad experiential learning that cannot be duplicated in a single research or extension lab. Indeed, 47% of diagnosticians report they lead or assist in teaching undergraduate or graduate courses (**Figure 3**). The wide-ranging experiences of a diagnostician provide a level of richness to teaching programs that few others can provide, and 67% of diagnosticians report using their expertise to provide individualized training or advising to graduate students (**Figure 3**). Their laboratories often house equipment, cultures, and samples that allow both undergraduate and graduate students the opportunity for practical hands-on training beyond the specialized and focused work of a degree program or lab course. Finally, 76% of diagnosticians also provide guest lectures in courses for allied departments covering diagnostics, emerging diseases, or specific areas of interest (**Figure 3**). Although a diagnostic lab's primary mission is to diagnose plant problems, decisions related to supporting the purchase and maintenance of equipment and deployment of new techniques are often greatly influenced by how these can serve a dual purpose for research and teaching.

PROFESSIONAL DEVELOPMENT

NPPLAP: National Plant Protection Laboratory Accreditation Program, Animal and Plant Health Inspection Service (APHIS), US Department of Agriculture (USDA)

Polymerase chain reaction (PCR): amplification of short DNA segments conducted at multiple temperatures, requiring the use of a thermal cycler

In addition to being heavily involved in teaching the next generation of plant health specialists in LGU classrooms, diagnosticians frequently develop and provide education for their fellow diagnosticians to keep up with changing pests, techniques, and outreach methods. The NPDN provides funds that allow diagnosticians to develop and attend professional development workshops. In 2019, NPDN prioritized the development of online training for diagnosticians (currently being developed) to maximize their time and available funding while creating an avenue for a wider audience than is possible with in-person workshops.

Over the past decade, USDA-NIFA, through grant guidelines, has encouraged the integration of extension and research projects to ensure that the research products find application for US citizens as quickly as possible. To fulfill these new requirements, grant recipients have increased training opportunities in new diagnostic methods as an extension component of many research projects that develop diagnostic methods. Additional training opportunities in methods and taxonomy have been developed by NPDN members and partners and have been offered at the American Phytopathological Society (APS) and NPDN national and regional meetings. Since 2003, 646 diagnosticians have participated in capacity-building training in 14 USDA–Animal and Plant Health Inspection Service (APHIS)-funded molecular technique topics (K. Snover-Clift, personal communication). These training sessions were targeted at increasing capacity for the detection and diagnosis of regulated organisms. The NPDN–state–federal partnership that supported these trainings also encouraged individuals to become certified through the National Plant Protection Laboratory Accreditation Program (NPPLAP) to carry out specific testing of three regulated pathogens: *Plum pox virus*, *Candidatus* Liberibacter asiaticus/americanus, and *Phytophthora ramorum* (23). More than 70 diagnosticians have obtained training in *P. ramorum* identification, a skill set that was in high demand in 2019 and 2020 during a regulatory event that involved multiple states and thousands of samples (27, 34; data from the NDR, used with permission of the NPDN Executive Team).

Plant Diagnostic Laboratories Support the Research Mission of Land-Grant Universities

Constant access to fresh disease samples allows PDLs to provide data and other resources that contribute to research. In fact, 87% of diagnosticians reported providing either photos, isolates, or physical samples to faculty or staff at their LGU, and 76% report leading or collaborating on research projects (**Figure 3**). The benefit to the researcher and lab is the accumulation of diverse isolates and strains that can further improve diagnostics and management.

For example, research led by the Oregon State University Plant Clinic resulted in the development of detection methods for gall-inducing bacteria (32). In 2002, a client inquired about testing a large number of plants for crown gall (commonly caused by *Agrobacterium tumefaciens*). However, the symptoms looked typical for leafy galls and shoot proliferations caused by *Rhodococcus fascians*. The routine methods used to confirm *R. fascians* at the time [isolation, purification, biochemical profile, polymerase chain reaction (PCR), 16S sequencing, and bioassay on indicator hosts] were time-consuming, taking 2–4 weeks from sample arrival to diagnosis. A collaboration between the diagnosticians and researchers resulted in the development of a rapid and specific diagnostic test that also addressed *R. fascians* population variability (28).

Other examples of collaborative problem-solving for diagnostic deployment include detection and discrimination of the laurel wilt pathogen *Raffaelea lauricola* (11), soybean rust pathogens *Phakopsora pachyrhizi* and *Phakopsora meibomiae* (18), and *Rose rosette virus* (5) as well as technology transfer to develop diagnostic services, such as molecular detection of geminiviruses (21), potyviruses (2), and Palmer amaranth (26).

Adopting Modern Laboratory-Based Diagnostic Methods

In the past decade, pathogen detection methods have swiftly advanced and diversified. Diagnosticians frequently face the challenge of deciding which technologies and specific tests to adopt. Traditional plant diagnostics methods, such as visual examination of symptoms and signs, pathogen isolation (culturing), tissue incubation, and microscopic observation, continue to be essential and commonplace in PDLs. In our survey, we asked diagnosticians to indicate which modern methods they use and the reason(s) for why they employ each method. We found that laboratories frequently use serological [ELISA (enzyme-linked immunosorbent assay) and LFDs (lateral flow devices)] and molecular techniques (PCR, sequencing, etc.) to detect pathogens and help diagnose plant problems (**Table 2**). Data uploaded to different LIMS showed a 43% increase in serological diagnostic methods employed from 2015 to 2019 compared to 2010 to 2014 [J. Dizon (Plant Diagnostic Information System), personal communication; T. Powell (PClinic Databases, Teaspoon Software), personal communication]. There was a 61% increase in molecular methods used when comparing these two timeframes. However, many labs have yet to adopt molecular methods despite their interest (**Table 2**).

To learn more about how diagnostic methods are adopted and become routinely used in PDLs, we asked diagnosticians to select reasons for using or not using a method (**Supplemental Material**). We performed a hierarchical agglomerative cluster (HAC) analysis to identify patterns in the use of methods and factors influencing their use or adoption (1, 14). The HAC was conducted using Euclidean distance and run with the psych, cluster, matrix, and gplots packages in R statistical software (7, 22, 29, 39).

Supplemental Material >

Table 2 Percentage of plant diagnostic laboratories (PDLs) that use modern diagnostic methods and of those that want to adopt them, determined by the diagnostician survey

Method	Percentage of PDLs that use the method[a]	Percentage of PDLs that want to adopt the method[b]
LFD	85% (40)	0% (0)
ELISA	72% (34)	38% (5)
PCR	60% (28)	68% (13)
qPCR	49% (23)	79% (19)
Bioassay	40% (19)	11% (3)
RT-PCR	38% (18)	38% (11)
Multiplex PCR	36% (17)	30% (9)
Sequencing	36% (17)	20% (6)
Biochemical	21% (10)	16% (6)
LAMP	9% (4)	42% (18)
RPA	9% (4)	21% (9)
Next-gen sequencing	4% (2)	29% (13)

[a]The value in parentheses indicates the number of labs that use each method out of 47 responses.
[b]The value in parentheses indicates the number of labs that want to adopt each method. The percentage was calculated by dividing the number of labs that want to adopt the method by the number of labs that do not currently use the method.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; LAMP, loop-mediated isothermal amplification; LFD, lateral flow device; PCR, polymerase chain reaction; qPCR, quantitative or real-time PCR; RPA, recombinase polymerase amplification; RT-PCR, reverse transcription polymerase chain reaction.
See **Supplemental Tables 1–3**.

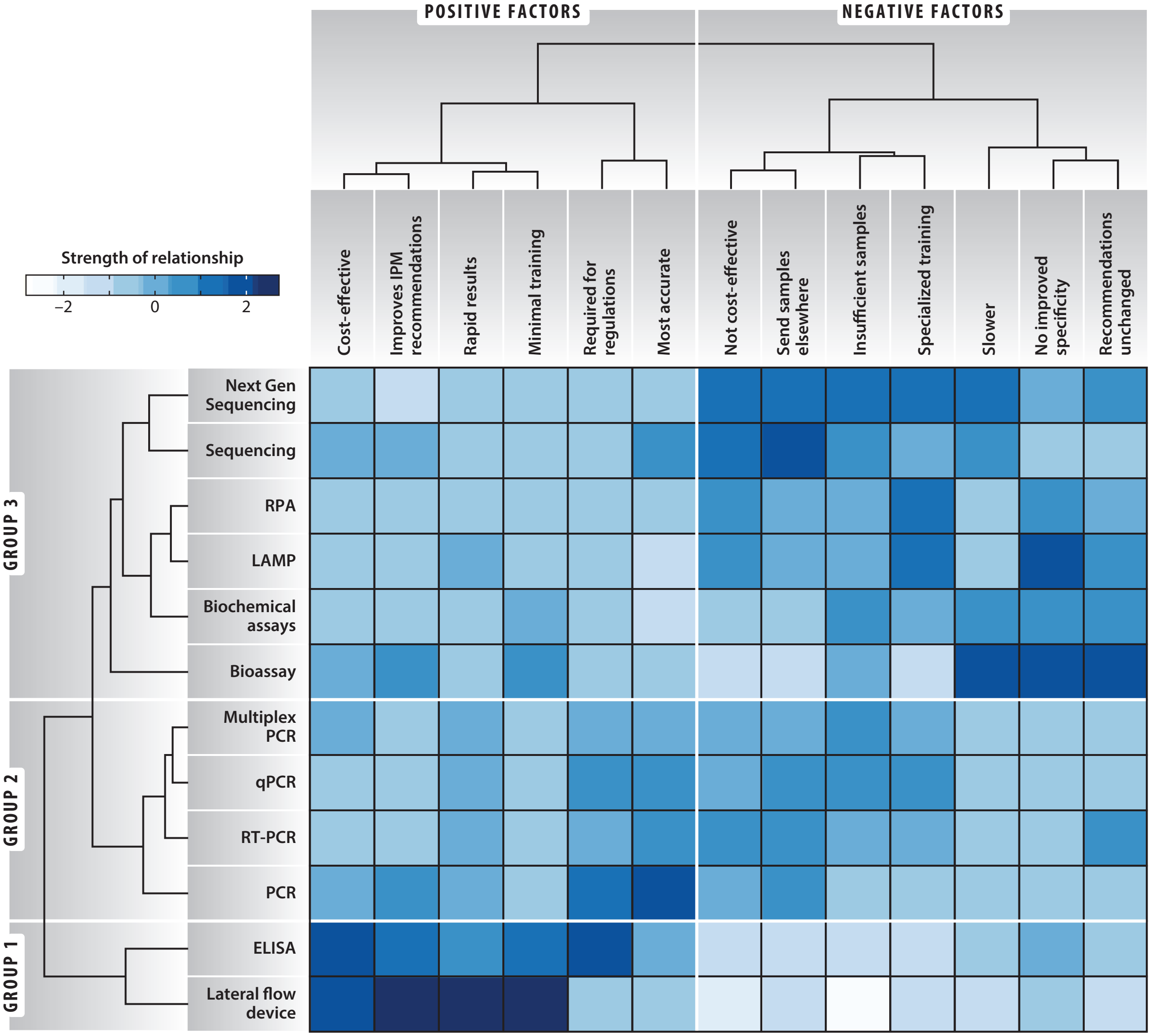

Figure 4

Cluster analysis showing patterns in the factors influencing diagnostician use or adoption of methods. Groups indicate that the reasons for adopting or not adopting are similar. Factors considered by respondents are included at the top of the figure, with positives, i.e., factors considered beneficial to clients or the plant diagnostic laboratories (PDLs), on the left and negatives, i.e., factors not considered beneficial to clients or the PDL, on the right. The key indicates the strength of the relationships. White indicates a strong negative relationship and dark blue indicates a strong positive relationship. Abbreviations: ELISA, enzyme-linked immunosorbent assay; IPM, integrated pest management; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction; qPCR, real-time or quantitative PCR; RPA, recombinase polymerase amplification; RT-PCR, reverse transcription polymerase chain reaction.

Three primary groups of methods were identified based on why diagnosticians did or did not adopt the method in their PDL (**Figure 4**; **Table 2**). Group 1 (ELISA and LFD) was most frequently used because the methods were cost-effective, rapid, easy to use, and perceived to improve IPM recommendations and met regulatory concerns. Group 2 (PCR-based methods) was used mostly for its accuracy and time-to-results and because it met regulatory requirements. Reasons for not using methods in Group 2 were primarily that another lab offered them, the lab did not receive enough samples, the methods were not cost-effective, and they required specialized knowledge. Group 3 includes a subgroup of sequencing methods and another composed of recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), and biochemical assays. Group 3 methods were less frequently used because they were perceived to be slow, required specialized training or equipment, were not cost-effective, or could be performed in another lab.

Overall, the survey results indicate that the decision to use or adopt a new method depends on the following factors also discussed in the literature: (*a*) advantages to the diagnostic process (10), (*b*) method objective and effectiveness (9), (*c*) required knowledge or training, (*d*) required resources and/or infrastructure, (*e*) cost, (*f*) impact on disease management (30), and (*g*) regulatory concerns.

Recombinase polymerase amplification (RPA): DNA amplification at a constant temperature (isothermal) that uses a strand-displacing polymerase

Loop-mediated isothermal amplification (LAMP): uses primers (up to 6) designed to create "loop" structures as strand-displacing polymerase amplifies short DNA segments

Advantages to the Diagnostic Process

The primary reasons a laboratory might adopt a new method include speed, specificity, sensitivity, and ease of use, but, most importantly, they must provide a measurable benefit to the client (10). For example, deploying a rapid LFD to detect a group of viruses is both faster and easier than staining and identifying virus inclusion bodies, although the specificity may not be as good. If the client only needs to know if a virus is present rather than a specific identification, this more rapid method allows them to take any necessary management action more quickly and avoid increasing plant losses. However, if a client making a regulatory decision requires specificity in the identification, a PCR test that can detect a species or subspecies is preferred even if it takes more time and expertise. PDL diagnosticians adopt and use methods that they believe have measurable benefits to their client, even if those methods complicate the diagnostic process, as long as benefits outweigh the drawbacks.

Method Objective and Effectiveness

The method's objective within the diagnostic process is defined by its scope, otherwise known as fitness for purpose. Diagnosticians often use several methods to reach an accurate disease diagnosis based on their initial observations. An appropriate method is determined by multiple factors, including its overall effectiveness, the level of specificity needed, cost-to-value ratio, and the growers' concerns or requests. A method's effectiveness can be determined by its sensitivity and specificity. Sensitivity refers to the limit of detection, i.e., the smallest amount of the target pathogen the method can accurately detect. Specificity is the ability to identify and distinguish the target pathogen from closely related and unrelated organisms (9, 12). Methods with high rates of false negatives or false positives or requiring secondary confirmation testing are less likely to be adopted for routine diagnostic work. Validation data are critical for increasing confidence in a method's fitness for the purpose by providing further evidence of its accuracy, precision, and robustness (9). It is important to note that less-specific assays that can test for groups of pathogens, such as virus families or specific genera of fungi or bacteria, can be effective and valuable methods for screening purposes, and results may be enough to provide general management recommendations.

Traditional diagnostic methods (e.g., culturing, microscopy, tissue incubation) are cost-effective methods for routine diagnosis for morphologically identifiable and commonplace pathogens, but incubating or culturing to obtain microscopically identifiable structures is not fast and is limited to specific identifications and easily culturable or identifiable organisms. If symptoms or signs are cryptic or if the pathogen is fastidious, a decision tree is employed by the diagnostician to determine whether advanced methods are needed to identify the pathogen. For example, diagnostic labs seldom utilize culturing for *Xylella fastidiosa* because of the potential for contamination and false negatives involved in extended incubation times. Serology was the primary detection method for *X. fastidiosa* before thermal cyclers became more common in PDLs. The use of PCR and real-time or quantitative PCR (qPCR) to detect *X. fastidiosa* has dramatically increased since 2015, whereas ELISA use has decreased during that same time period (**Figure 5*a***).

In most cases, new or advanced methods augment traditional ones rather than replace them. The diagnostic procedure performed by PDLs to detect *Bretziella fagacearum*, the causal agent of oak wilt, was initially almost exclusively culturing. However, the pathogen's successful isolation can be compromised by the host species and high temperatures, which can fluctuate during plant sample transit (40). Molecular methods have been increasingly utilized for the detection of *B. fagacearum* since 2014 to complement culturing because of the potential for false negatives (**Figure 5*b***). Corroborating evidence from multiple methods is often useful and, at times, necessary to provide an accurate diagnosis.

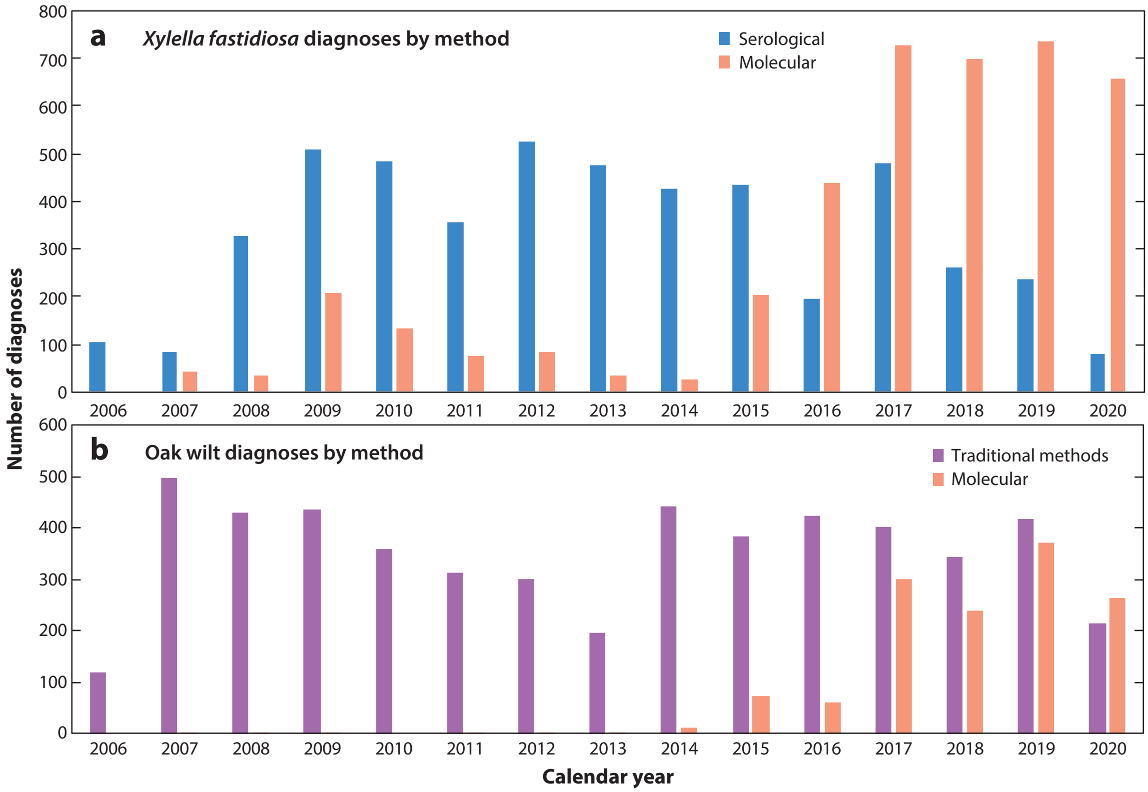

Figure 5

Examples of diagnostic methods that have changed over time. (*a*) Serological and molecular detection of *Xylella fastidiosa* from 2003 to present. (*b*) Traditional and molecular techniques used to diagnose oak wilt (*Bretziella fagacearum*) from 2004 to the present. Both charts show the addition of molecular methods, which increase sensitivity and confidence in the diagnosis. These data are from the National Data Repository (NDR) used with permission of the National Plant Diagnostic Network (NPDN) Executive Team.

Required Knowledge or Training

In most cases, the selection of a specific diagnostic method assumes prior knowledge of the potential causes of the disease in question, including symptom or pathogen recognition (38), as well as working knowledge of method availability, specificity, and appropriateness. Knowing when to use a specific method is as important as having the capability to perform it. The adoption of new methods can be hindered by the lack of training (**Figure 4**) or experience required to perform the assay, analyze data, or interpret results. Methods that require highly specialized equipment or highly technical skills further serve as a potential barrier to widespread adoption in PDLs.

Serology: antibody-based method for the detection of target proteins, including ELISA (enzyme-linked immunosorbent assay) and LFDs (lateral flow devices)

Real-time or quantitative PCR (qPCR): amplification is monitored using fluorescent dyes or probe technology and specialized thermal cyclers

Required Resources and/or Infrastructure

It is essential to consider whether the PDL has access to the resources necessary to implement a method successfully (10). These resources include test controls, biological collections, reference standards, databases, software licenses, equipment, and permits. High-throughput or rapid methods may require access to clean space, freezers (−20°C to −80°C), automated/robotic liquid handlers, or PCR thermal cyclers that may not be owned or readily available to diagnosticians. The value of the method for diagnostics may depend on whether it optimizes the use of currently available resources, requires the acquisition of specialized equipment, or even requires significant changes in established policies or procedures. An additional consideration is the availability of personnel hours to deploy a new method, aside from specialized training. A more sensitive or specific method may take more hands-on time to complete compared to culturing or other traditional methods. It would need to be evaluated for overall efficiency, performance under lab-specific conditions (verification), and personnel time usage. If the technique is expected to replace another, this type of improved efficiency must be considered. If the PDL is maxed out using current methods, it is difficult to make the time to verify a new method even if adoption will ultimately be beneficial to the PDL.

Cost

Diagnosticians may avoid or delay using new methods if the monetary costs associated with adopting, validating, and incorporating them into routine lab use are too high. Lab managers must weigh these expenses against any potential benefits, such as obtaining faster or more precise results. Some tests may have too few requests to justify developing and maintaining the ability to perform the test. Most diagnostic labs serve a wide-ranging clientele base, receive many types of plants, and diagnose a very diverse collection of diseases and plant problems. Those methods that have application across multiple crops, save time, or reduce costs associated with a commonly encountered pathogen or group of pathogens offer the greatest promise for adoption in the lab.

Any additional costs incurred with the adoption of new tests must be weighed against the ability to recover those costs through fees or justify the costs within an existing pool of resources. Labs that charge testing fees can potentially pass some or all of the costs associated with advanced testing on to the client, so the cost of specific methods must be within the clients' financial tolerance. Methods with a high-cost threshold will likely see low numbers of requests, will be used only for specific circumstances, or may be provided at no charge at the discretion of the lab director, all of which can vary by the commodity and state where they are offered. According to the survey, lack of samples and cost-effectiveness are primary reasons diagnosticians do not adopt particular new methods and technologies.

Impact on Disease Management

Determining how the results of a new method will influence disease management is key to its implementation in a diagnostic lab that serves extension clientele (30). It is crucial to choose the most appropriate method to reach an accurate diagnosis. A more specific method may be necessary when diagnosing a sample with a disease of economic or regulatory significance or when disease management is predicated on limited, particular options available to the grower. Updated methods may be necessary for accurate monitoring of pest or pathogen spread to evaluate risk or the need to deploy management tools. Methods providing more specificity may not change the disease management if the crop is beyond the point of effective management, if no cost-effective options exist, or if group-level pathogen identification results in the same management recommendations. The appropriateness of a method is also determined in part by its cost and the economic impact of management. Therefore, the diagnostician must decide which methods to use based on their hypothesis and experience and weigh diagnostic specificity against the potential to manage the disease effectively for each situation.

Regulatory Plant Pathology and Other Considerations

When a PDL fulfills a regulatory role in their state they may be required to use protocols to determine the species, race, or even biovar of a pathogen. Industry clientele may need to use new, available molecular testing to rule out regulated viruses from propagative material. Labs that serve ports and inspection stations may need highly specific tests to identify morphologically cryptic pathogens or test asymptomatic host material. These considerations may encourage a lab to adopt a new method. Many LGU diagnosticians prioritize adopting specific methods when needed to provide support to their state department of agriculture, process regulatory samples, or issue phytosanitary certificates for export.

FUTURE DIRECTIONS

Protecting plant health is foundational to ensuring human and environmental health. An important aspect of safeguarding plant health is quickly identifying pathogens and responding appropriately. In this review, we illustrate that diagnostics is much more than a test in a lab. Protecting plant health with accurate diagnostics means we need diagnosticians who instruct the next generation of plant health practitioners, teach each other new techniques, collaborate in research, and reach out to the stakeholders of their state with vital information.

Although the development of diagnostic methods has accelerated in recent decades, the adoption of new methods by PDLs is limited by the many factors discussed in this article. Delays in adopting new technologies and protocols are also a common occurrence in other disciplines, including seed testing (25), veterinary medicine (16, 19), and human pathology (15, 17, 31, 37). The development of new technologies and methods can provide diagnosticians with tools to more quickly identify pathogens, with improved specificity and sensitivity, and often enable accurate diagnosis of diseases that were previously impractical to confirm. The ever-expanding and increasingly complex toolbox of diagnostic methods presents a challenge to diagnosticians to stay abreast of the science and employ the best methods to meet client needs.

A concerted effort to overcome method adoption challenges should focus on collaboration among researchers and diagnosticians to develop and deploy fit-for-purpose methods that fulfill the needs and challenges of diagnosticians, PDLs, and their stakeholders. New methods should be cost-effective, accurate, properly validated, and feasible within the typical PDL's normal operations. The evaluation process and metrics described in this manuscript should encourage thoughtful discussion of method development, validation, verification, and cost analyses to ensure sustainable technological advancement and adoption.

The support of national programs such as NPDN and IPM, as well as local contributions from home institutions and industry, sustains diagnostic labs in every state, bringing the science of the land-grant institutions to the people they serve. Organisms and technologies shift, but the primary mission of the LGU PDLs has not changed in a hundred years. Indeed, PDLs may be able to better serve their clientele now than in the past, thanks to technological advances in both diagnostics and communications. Responses from administrators at LGUs indicate opportunities to better engage, promote, and support the many ways PDLs contribute to the LGU mission. Diagnosticians are integral members of their LGUs, fulfilling far more roles than sample diagnosis alone. To continue the history of service of each PDL while expanding their services, it is vital to recognize the value of the diagnostician and their sense of commitment to the diagnostic process and support them accordingly (4).

PDL diagnosticians and administrators must make strategic decisions about which technologies to adopt. It is imperative that we not get distracted by the technology to the detriment of the diagnostic process. Regardless of the technologies on the horizon, diagnostic advancements must continue to support accurate diagnoses that inform disease management and future prevention. This decision-making process is affected by external and internal forces, including funding, expertise, and needs, which may change from year to year. However, the process itself does not change; as new technological advances come to the PDLs, they will engage in similar decision-tree activities, working out how best to serve their stakeholders and partners by using the latest science and most appropriate methods.

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