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Plant Disease Detection Technology Assessment

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OAK RIDGE National Laboratory

ORNL IS MANAGED BY UT-BATTELLE LLC FOR THE US DEPARTMENT OF ENERGY

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Biosciences Division

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ABBREVIATIONS

AK9	agricultural canine
APHIS	USDA Animal and Plant Health Inspection Service
AQI	Agricultural Quarantine Inspection
AQIM	Agriculture Quarantine Inspection Monitoring
ATR	attenuated total reflectance
BVOC	biogenic volatile organic compound
CBP	United States Customs and Border Protection
CBPAS	United States Customs and Border Protection Agriculture Specialist
ddPCR	Digital Droplet PCR
DHS	United States Department of Homeland Security
EAN	Emergency Action Notification
eDNA	Environmental DNA
ELISA	enzyme-linked immunosorbent assay
E-nose	Electronic Nose
FAV-D	DHS S&T Food, Agriculture, and Veterinary Defense Program
FDA	United States Food and Drug Administration
FTIR	Fourier transform infrared
GC-MS	gas chromatography–mass spectrometry
HSI	hyperspectral imaging
ITS	internal transcribed spacer
LAMP	loop-mediated isothermal amplification
LFIA	lateral flow immunoassays
LWIR	long-wave infrared
ML	machine learning
MWIR	mid-wave infrared
NARP	National Agricultural Release Program
NDVI	normalized difference vegetative index
NIR	near-infrared
ORNL	Oak Ridge National Laboratory
PCR	polymerase chain reaction
POE	port of entry
PPQ	USDA APHIS Plant Protection and Quarantine
PTR-MS	proton-transfer-reaction mass spectrometry
PTR-ToF-MS	proton-transfer-reaction time-of-flight mass spectrometry
RBS	risk-based sampling
RGB	red, green, and blue
RPA	recombinase polymerase amplification
SWIR	shortwave infrared
ToBRFV	*Tobamovirus Tomato brown rugose fruit virus*
TRL	technological readiness level
USDA	United States Department of Agriculture
UV	ultraviolet
VNIR	visible near-infrared
VOC	volatile organic compound

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EXECUTIVE SUMMARY

Visual inspections by US Customs and Border Protection agriculture specialists have identified approximately 20,000 regulated, quarantined pests each year in agricultural products entering the United States. Most of these pests identified in the Agriculture Quarantine and Inspection (AQI) program are insects.

Many pathogens are difficult to detect in agricultural products, particularly in the early stages of infection on live plants. New technologies can help to detect plant pathogens and the diseases that they cause. This technology assessment was performed for the US Department of Homeland Security Science and Technology Directorate (DHS S&T) through the Food, Agriculture, and Veterinary Defense (FAV-D) program to identify emerging technologies that could address this hard problem. These emerging technologies differ in their diagnostic sensitivities and specificities, as well as in their measurement time and training requirements. New instruments that detect volatile organic compounds characteristic of plant disease or pathogens could provide a less invasive inspection method. Dogs, which can successfully detect many concealed agricultural products, have also been trained to detect some plant pests and pathogens. Simple immunological tests offer sensitive and specific detection of many pathogens at the point of use. Advanced imaging methods that use AI to sort fruits and vegetables and recognize anomalies at high speeds could be used in cooperation with exporters to improve food quality and reduce pests at the point of origin. Advances in nucleic acid–based detection methods that have become gold standards for confirmatory diagnostics are now making those methods available for faster, point-of-use detection. These new methods should be developed in the context of USDA APHIS AQI operational requirements, which apply risk-based sampling protocols to protect agriculture, natural resources, and export market access while facilitating commerce and passenger transit.

1. INTRODUCTION

Plant pests and diseases reduce the global yield of major crops by more than 20% [1]. Losses may increase due to the spread of plant pathogens caused by trade, movement of people and commodities, and domestic and global threats, including climate change [2-5]. The increasing frequency of plant disease pandemics illustrates this risk [6]. Furthermore, the increased volume of fruits, vegetables, and nuts imported into the United States raises the likelihood of plant pests entering the United States and spreading to economically significant domestic crops [7-9]. For example, citrus canker was introduced in Florida and was eradicated several times in the 20th century before the current epidemic of *Xanthomonas axonopodis* pv. *citri* emerged in the early 1990s and spread so widely as to make eradication impossible [10]. The Karnal bunt fungal disease (*Tilletia indica*) was probably introduced to the United States on imported wheat in the 1980s before spreading to wheat fields in the Southwest in the 1990s [11]. The use of unregulated antifungal agents to control plant disease in some countries increases the risk of importing antimicrobial-resistant, generalist pathogens to the United States, where they could cause environmentally transmitted disease [12; 13]. Preventing the spread of plant pests and diseases is a priority for the 185 parties that have signed the International Plant Protection Convention [14].

Plant pests are defined in statute to include a broad range of insects, microorganisms, protists, and parasitic plants that can injure, damage, or cause disease in plants or plant products (Appendix B). In practice, *pests* may be understood to mean invasive insects and larvae that are readily detected due to their size, colors, and movement. However, this assessment effort focuses on the detection of pathogens in phytosanitary inspections that can cause serious plant diseases, an area that has received less attention. Specifically, this work assessed technology with the potential to detect regulated and quarantined or novel plant pathogens and the diseases that they cause in imported agricultural products that are relevant to the US Department of Homeland Security (DHS) mission to protect against cross-border threats.

To prevent the importation and spread of plant pests, advanced technology is required to rapidly detect plant pathogens and their vectors. Current methods, which rely on manual inspections of samples from agricultural shipments, may be inadequate to detect emerging diseases [9]. Most plant pathogen detection methods were developed to diagnose diseases on agricultural plants, focusing particularly on leaves [15]. Therefore, detection methods for pathogens that are transmitted on agricultural products have been developed mainly to promote food safety or consumer acceptance. The diverse nature of plant pathogens complicates detection, but advances in plant pathology, spectroscopic instrumentation, imaging, and AI could foster technology development to expedite inspections; detect high-risk, emerging pathogens; facilitate identification; and reduce costs. A National Academies of Sciences, Engineering, and Medicine report identified technology for the "early and rapid detection and prevention of plant and animal diseases" as a major agricultural research goal for the next decade [16]. This technology assessment identifies opportunities to develop and deploy advanced plant pest detection systems that could address cross-border threats without impeding trade.

2. BACKGROUND

Phytosanitary regulations in the United States are enforced by several federal and state organizations. The US Department of Agriculture's (USDA's) Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ) program implements phytosanitary standards under the International Plant Protection Convention that protect US agriculture and natural resources from invasive pests. Domestic phytosanitary responsibilities can be delegated to state-managed programs [17]. PPQ currently regulates 6,919 quarantined pests under the Plant Protection Act [18]. However, the Homeland Security Act of 2002 (Public Law 107-296) transferred most physical agricultural inspection functions performed upon entry to the US from USDA to the US Department of Homeland Security's (DHS) Customs and

Border Protection (CBP) [19; 20]. APHIS/PPQ and CBP collaborate to inspect agricultural imports through the Agricultural Quarantine and Inspection (AQI) program and enforce regulations that protect against foreign pests and diseases [9]. APHIS/PPQ performs biosurveillance, develops pest risk assessments, and establishes quarantines. CBP inspects most agricultural product imports to detect pests or contraband. If an APHIS/PPQ identifier confirms an actionable pest, then CBP issues an Emergency Action Notification (EAN) to alert trade entities of non-compliance. A joint strategic plan outlines priorities for both agencies, including applying risk-based methods to efficiently use inspection resources to identify actionable pests while expediting trade and passenger movement [21]. In FY23, CBP intercepted 38,173 quarantine-significant pests among 882,387 agricultural cargo inspections [22]. In addition to performing agricultural inspections, CBP enforces regulations on customs duty, contraband items (including drugs, weapons, and currency), biological threats, and human smuggling while facilitating cross-border trade.

Take the following example to contextualize the international flow of commerce: on a daily basis in fiscal year 2022, approximately 869,000 passengers and pedestrians and over 91,000 truck, rail, and sea containers carrying goods worth approximately $9.2 billion entered the United States through 328 US land, sea, and air ports of entry (POEs) [23]. The United States imported agricultural products worth more than $180 billion in 2023 (USDA GATS). The largest agricultural exporters to the United States include Mexico, Canada, Europe, South America, Latin America, and East Asia. CBP enforces cross-border regulations at US POEs for imported agricultural products. Enforcement locations include the major land border district in Laredo, land borders in Nogales and Otay Mesa, and airports and seaports in New York, Long Beach, Philadelphia, Wilmington, Houston, Miami, and Laredo [24]. Each POE receives a different mixture of agriculture products and consequently pests, which can vary seasonally. Cut flower imports are one example of the dynamic operating environment in which efficient and accurate inspection of agricultural products is required (Figure 1). PortMiami imports the majority of cut flowers into the United States. More than 1.3 billion flowers were imported to the US in 2022 for Mother's Day, and 1,514 significant pest interceptions were completed [25]. According to data from DAT Freight & Analytics, during the first week of February 2022, the load volume for refrigerated trucks—the type of truck that carries flowers—increased 77% compared to the week before. In the six weeks leading up to February 14th, around 500 truckloads of roses leave Miami every day.

Figure 1. Cut flower inspections for pests. (Left) Flowers inspected at the Miami Seaport. Credit: D. Graham (ORNL). (Right) CBP Agricultural Specialists inspecting cut flowers at Dulles airport. Credit: Anthony Guas (CBP).

Commercial shipments arriving at POEs are usually preceded by documentation, which CBP uses to plan agricultural inspections based on background information and APHIS guidance. For example, the Border Cargo Release Program, the National Agricultural Release Program (NARP), and its risk-based sampling (RBS) program successor provide dynamic inspection criteria for specific agricultural products. NARP, introduced in 2007, identifies low-risk commodities imported from Mexico that are inspected at a reduced rate. The National Cut Flower Release Program reduces inspections of certain high-volume, low-risk flower imports. If shipments are not automatically released, they may be inspected by CBP Agriculture Specialists to screen for pests. At sea ports, ISO steel shipping containers 40 ft in length may be subject to tailgate inspections portside or complete offloading and examination at a cargo warehouse. Ports are noisy, high-traffic areas with varying temperature, humidity, and precipitation and limited access to power or electronic communications. At air cargo facilities, perishable products may be unloaded in refrigerated warehouses where produce is staged for inspection. Finally, at land borders, trailers may be directed to a warehouse for unloading and inspection, and rail cars can be inspected at depots. In addition to performing agricultural inspections for pests, CBP also inspects shipments for contraband, often assisted by x-ray scanning technology or canines trained to detect concealed drugs or explosives.

Phytosanitary inspections have several objectives. First, they verify that the cargo is correctly documented. Second, they screen for regulated pests using plans similar to the acceptance sampling techniques developed for product quality control [26], whereby a statistically determined selection of products are inspected to infer the quality of the entire batch. Third, APHIS/PPQ can use samples and records to identify new and emerging pests as part of an ongoing biosurveillance initiative. This data may inform future regulations or changes in inspection frequency.

When cargo is opened for inspection, Agriculture Specialists use their training and experience to look for live pests, including evidence of plant disease. This visual inspection can be aided by hand magnifier lenses or loupes. If insect pests are detected, then they are collected using forceps or a fine paint brush and either transferred to a vial filled with alcohol or frozen, if insect colors must be preserved for identification by APHIS/PPQ identifiers [27]. This is a presumptive pest identification [28]. If these insects are common, non-actionable, and subject to the limited Cargo Release Authority granted by USDA to experienced CBP Agriculture Specialists, then the cargo may be directly released. Otherwise, the vials are transported to a USDA facility, where entomologists will examine them and determine whether the pest is quarantine-significant and actionable (confirmatory identification). At some POEs that are distant from USDA facilities, pests may be photographed, and the image electronically transferred to USDA, instead. The World Trade Bridge POE has photomicroscopes with a digital imaging capability to document pests (Figure 2). Digital images may be sent to USDA entomologists for pest identification, particularly from sites distant from USDA facilities or outside normal operating hours. In FY22, PPQ National Specialists performed 77% of identifications based on digital images [29].

Figure 2. Microscope and stored digital image of an insect from the World Trade Bridge, Laredo, TX. Credit: D. Graham (ORNL).

Pests are categorized as non-actionable (i.e., non-reportable or reportable) or actionable. If no pests are identified or submissions are determined to be non-actionable, then cargo is conditionally released from the port, warehouse, or import lot. Alternatively, when an actionable violation is detected due to a prohibited pest, an EAN is generated by CBP, and the cargo can be re-exported, destroyed, or directed to a licensed fumigation facility for treatment.

During FY23, 87,519 pest interceptions were submitted to USDA, and 38,173 (44%) were determined to be quarantine significant. CBP issued 18,574 EANs for pests in FY23, along with 15,414 EANs for other agricultural products that did not meet entry requirements [30]. Between the years of 1984 and 2000, the majority of pests intercepted by USDA were insects, and 13.1% were plant pathogens [31]. The list of 6,919 PPQ-regulated pests currently includes 582 fungi, 27 viruses, 26 bacteria, and 18 oomycetes; most of the remaining pests are insects [18]. There is no prescribed time limit for inspections, although importers, brokers, and CBP staff all share a goal of expediting trade and releasing cleared shipments as soon as possible.

US regulations prohibit international passengers arriving at air, sea, or land ports of entry from bringing many agricultural products into the US to prevent the introduction of pests [31]. In contrast to commercial and permitted agricultural imports, these items are prohibited regardless of their pest status. Almost 2.5 million passengers entering the US had agricultural inspections in FY23, resulting in 755,636 Quarantine Materials Interceptions and 7,395 penalties being issued for undeclared prohibited agricultural items [30]. CBP uses a multi-layered security strategy to deter and intercept contraband, including passenger education, declaration forms, amnesty programs to discard prohibited items, interviews, baggage screening, and fines and seizure. CBP Agricultural Specialists and agricultural canine (AK9) teams screen passengers and their baggage using non-intrusive techniques (Figure 3). X-ray scanning systems, including common backscatter detection and 3D computed tomography, produce detailed images of baggage to identify undeclared agricultural products. Trained beagles and Labrador Retrievers in AK9 teams are sensitive detectors for both meats and fruits and ambassadors for CBP agricultural

programs [32]. Subsequent intrusive inspections can result in confiscation of prohibited items, which may be checked for pests for USDA identification and disposition. Pest identification is not required for product confiscation or passenger processing. Although there is no time limit for passenger screening, all parties value rapid screening. New technologies that detect contraband and augment current methods could expedite passenger screening and increase interdictions of prohibited items.

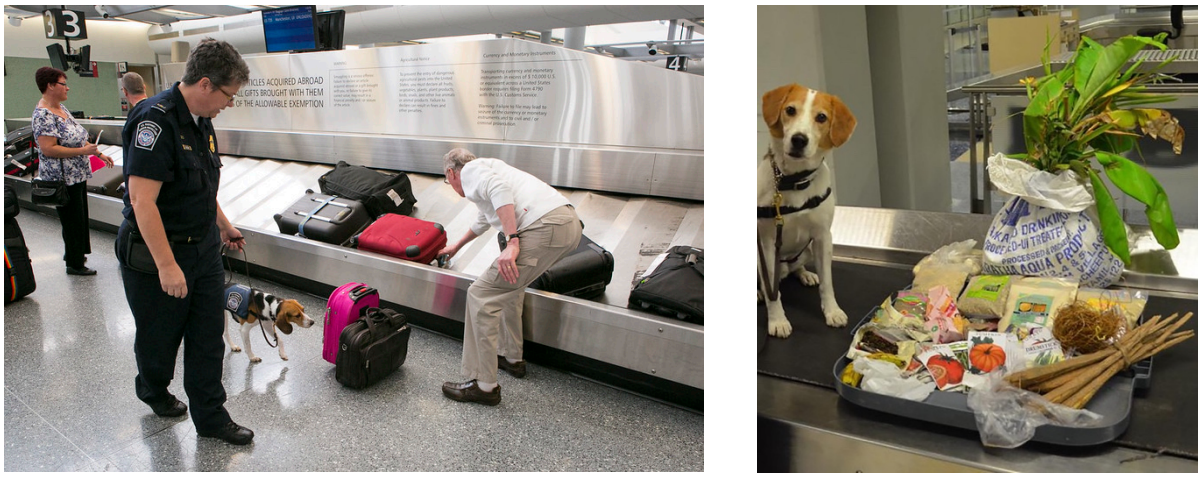

Figure 3. Agricultural canine teams screen international luggage to detect prohibited meats and agricultural products. Left, AK9 at the Philadelphia Airport. Credit: James Tourtelotte (CBP). Right, AK9 with seized plants and seeds at Dallas Fort Worth International Airport. Credit: CBP.

3. AVAILABLE DETECTION TECHNOLOGIES

Phytosanitary inspections currently use several technologies, which are described in this section, to identify concealed agricultural products imported by passengers and pests imported in agricultural cargo. These methods rely on visual inspection due to the diversity of plants, plant products, and pests in imports. Commercial traps for pests and spores have long been used to survey agricultural pests but could be adapted to survey agricultural cargo during transport. Immunological and molecular diagnostics based on validated confirmatory tests have been commercialized and adapted to low-cost, point-of-use devices that could be used for rapid presumptive tests to identify pests. These intrusive methods rely on risk-based sampling protocols to facilitate commerce and passenger transit.

However, non-intrusive inspection methods complement current physical inspections. X-ray imaging is becoming ubiquitous at POEs because of its capacity for rapid inspections that detect many types of contraband. Agricultural canine teams rely on trained dogs' excellent sense of smell to detect concealed meats, plants, and produce through non-intrusive inspections. Some canines can also detect many plant diseases and pathogens, although those dogs are not currently used to screen imported products.

3.1 VISUAL INSPECTION

APHIS/PPQ identifiers use visual taxonomic characterization as the primary tool for identifying both plant pests and diseases [33]. The International Plant Protection Convention defines an inspection as an "official **visual** examination of plants, plant products, or other regulated articles to determine if pests are present or to determine compliance with phytosanitary regulations" [34]. CBP Agricultural Specialists can detect small pests in agricultural products removed from their packaging, aided by simple magnifiers

and a white background. Because collection and confirmatory identification by APHIS/PPQ experts are required for proper quarantine actions, visual inspections of agricultural imports are required. However, strong light and good vision are required to spot miniscule insects and larvae. Pests obscured from view will not be detected. Therefore, specialists shake, sieve, or cut some agricultural products to identify concealed pests (Figure 4). Specialists usually rely on prior knowledge of pests expected in particular agricultural crops from known locales to rapidly screen for pests. The identification and classification of pests or disease by APHIS/PPQ experts also support biosurveillance objectives.

Figure 4. A CBP Agricultural Specialist performs a tailgate inspection of a shipping container to screen for pests in bulk grains. Credit: Jerry Glaser (CBP).

Specialists can also identify pests or symptoms of plant disease on agricultural products, particularly in advanced stages of infection. At early stages of disease, symptoms may be obscure and difficult to detect. Under ideal conditions of 550 ft candle illumination with an 18% gray background, an inspector could detect 95% of 150 µm particles 95% of the time [35]. These results may translate well to insect pest detection, whereby dark, moving insects or larvae are detected under bright lights. However, most plant pathogens cannot be directly visualized without a microscope (< 100 µm), and similar disease symptoms could be caused by abiotic factors or non-actionable pests. Accurately detecting plant disease requires both acute visualization and specific recognition of disease symptoms that are caused by a regulated plant pathogen in an agricultural product [36].

Visual inspection may not be sufficient for the presumptive identification of disease-causing plant pathogens. For example, *Tobamovirus Tomato brown rugose fruit virus* (ToBRFV) is a quarantine pest that infects tomato and pepper plants, causing a mosaic pattern on leaves, reducing yield, and causing plants to produce blotchy fruit with wrinkled (rugose) surfaces [37; 38]. In 2019, APHIS/PPQ issued import restrictions on tomato and pepper plants and materials infected with ToBRFV [39]. Symptoms can be difficult to observe in fruit from plants infected late in the growth cycle [40]. Culling symptomatic fruit prior to industry certification is permitted by USDA, increasing the chances of infected fruit importation. Imported, infected fruit was sold at retail stores despite the import order, presumably because visual detection is difficult [41]. APHIS/PPQ has deployed ImmunoStrips® at POEs to perform presumptive immunological tests for symptomatic fruit, recognizing the limitations of visual inspections [42]. Limitations of visual detection and identification may be more problematic for future pathogens that can be readily transmitted to domestic farms [43].

Accurate visual inspections require excellent visual acuity and controlled environmental conditions [44]. For consistency in human visual inspections, eye examinations should result in 20/20 near-focus visual acuity, and a color blindness test must be passed as well [35]. Intensive training and experience are required to detect pests or diseases. Environmental conditions such as lighting, noise, and temperature affect human performance, in addition to organizational and management factors such as shift duration, workload, management support, training, feedback, engagement, and incentives.

3.2 SAMPLING

Sampling methods are integral to programs that inspect less than 100% of products, although they are not detection techniques. Because it is not practical to inspect each item in a shipment with intrusive methods, the International Standards for Phytosanitary Measures describes statistical and non-statistical methods for selecting samples in shipments [45]. Statistical methods can be used to estimate the risk that pests are present but not detected in a shipment with some level of confidence. For example, an APHIS/PPQ Risk Management plan may require using a hypergeometric table to randomly select a specified number of boxes from a shipment for inspection [45; 46]. Similar probability sampling strategies have been used since 1929 for acceptance sampling of products [26].

Non-random (non-probability) sampling is operationally relevant to detect pests when experience or separate information helps prioritize sampling. In contrast to simple random sampling, in which each member of a population has an equal chance of being selected, non-random sampling selects members based on other factors [47]. For example, convenience sampling selects the most accessible members, such as products near the door of a shipping container. Judgmental sampling relies on a specialist's expertise to select members most likely to contain a pest. For example, an Agricultural Specialist examines edges of packaging materials to detect wood-boring and hitchhiking pests [48; 49]. CBP Agricultural Specialists frequently find insect pests on the floors of shipping containers or trailers [50]. Alternatively, CBP officers may inspect a particular pallet for contraband based on non-intrusive x-ray inspections. Snowball sampling examines members close to other members (acquaintances). An Agricultural Specialist could examine other peppers from a case that contains a pepper with visible pest damage to detect a live pest. Although non-random sampling is more difficult to incorporate into risk management plans, it can be an efficient and logical method of detecting pests using the experience of specialists [51].

A third sampling approach is the potential adoption of Bayesian methodology into the sampling strategy. This would require synthesis of external data sources that could be used to calculate a prior, which would then be used in a Bayesian analysis to calculate the probability of a pest's presence. For instance, in the case of a shipment arriving from a location where a known outbreak exists, parameters such as the quantity and duration of the outbreak, quarantine and safety protocols at the point or origin, and en route data derived from onboard sensors could all be used to calculate a prior probability of the presence of a pest. This prior can then be incorporated into the sampling algorithm to determine which shipments to inspect. Forecasting models could also be used to predict invasive pest and pathogen risks [52]. Such an effort would require coordination with shippers and with other authorities at the ports of origin and would require a fairly extensive data integration effort. However, it is possible given the current state of technology and could serve to maximize the efficiency and efficacy of the sampling strategy if aligned with policies.

Although sampling expedites inspections, facilitates trade, and supports APHIS/PPQ pest surveillance objectives, it does not necessarily allocate resources to prioritize detection and interception of high-risk and emerging pests [53]. Since 1987, APHIS/PPQ has used RBS at land border POEs to direct inspection resources to high-priority commodities [54]. The National Agriculture Release Program (NARP) is a science-based program that uses historical data to adjust the frequency of inspections of high-volume fruit and vegetable shipments with low rates of quarantine pest interceptions. The Agriculture Quarantine Inspection Monitoring (AQIM) program provides data and statistical analyses that support dynamic changes to RBS plans [55]. Future RBS programs will continue to optimize sampling and inspection methods to improve efficiency [56; 57].

Statistical sampling methods usually assume that a shipment is homogeneous—that is, a box selected randomly from a shipment has the same probability of carrying pests as any other box in the shipment.

However, shipping containers, trailers, and rail cars often contain multiple lots of agriculture products—produced by different farms and packed at one or more processing facilities. These lots may have different levels of pest infestation and may need to be sampled independently [45]. Whereas some commodities, such as cut flowers, are sampled on an individual lot basis [27], other commodity shipments are treated as homogeneous. Therefore, random sampling for inspection may not always detect pests at the desired confidence level.

Dynamic RBS plans will continue to be deployed to improve inspection efficiency. However, new pest and pathogen detection technology could change the assumptions about detection probability, which could reduce the number of samples required. Non-intrusive or less-intrusive inspection technology could detect pests and pathogens in aggregate shipments and potentially estimate the pest infestation level. Under current USDA regulations, pests must be isolated and sent to USDA staff for confirmation, so new sampling methods may be required to efficiently locate pests for a confirmatory identification based on a presumptive identification made using advanced techniques.

3.3 CANINES

Dogs have proven their ability to detect concealed agricultural products for USDA and CBP, working alongside Agricultural Specialist handlers in AK9 teams (Figure 5). USDA first trained Labrador Retrievers in 1979 to conduct mail and baggage inspections in controlled areas [58]. The Beagle Brigade, established by USDA in 1984 at Los Angeles International Airport, succeeded in both detecting plant and animal products and publicizing agricultural import restrictions. Larger Labrador Retriever dogs began working in cargo facilities again in 2001. Adopted dogs are trained at APHIS PPQ's National Detector Dog Training Center in Newnan, Georgia, for 10 to 13 weeks [59], and they require continuous training on target scents to maintain skills. Dogs are trained to smell fruit, vegetable, and meat products, with greater than 85% success rates. Trained dogs can detect 85 odors. AK9s at airports may spend four hours per day sniffing passengers' baggage, generally working for 30 minutes at a time. In Australia, a biosecurity detector dog program has been operating for 30 years, and dogs average 9,000 biosecurity risk items detected during their careers [60]. A dog's career in an AK9 team may last 6 to 8 years before retirement.

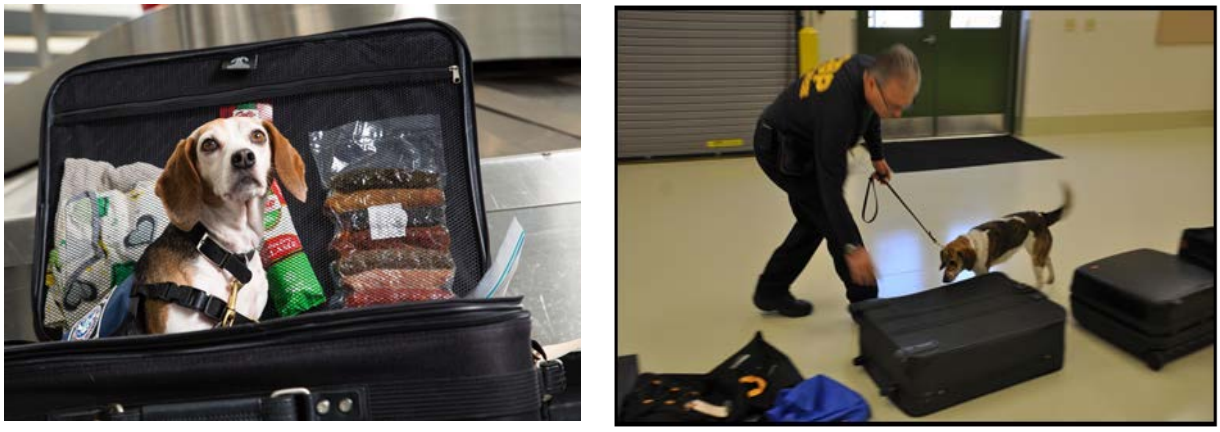

Figure 5. Beagles identify meat and agricultural products in luggage. (Left) An AK9 sits in luggage containing contraband agricultural products. Credit: Glenn Fawcett (CBP). (Right) A CBP Agricultural Specialist canine team inspects a closed bag. Credit: CBP.

Detector dogs have a renowned ability to detect scents at low concentrations and to distinguish scents with high specificity. Canines have an elaborate nasal airway, high neuronal density, and hundreds of millions of olfactory receptors that enable them to detect some volatile organic compounds (VOCs) at low parts per trillion concentrations [61; 62]. By sniffing targets and sensing multiple VOCs, dogs may integrate signatures to detect scents with high specificity [63]. Detector dog training includes both positive reinforcement (using food) for target scents and no reinforcement (withholding rewards) for non-targets or distractor scents like processed foods, candy, and perfumes. Dogs are usually trained to report detecting a target scent by alerting—sitting or placing paws on the object. The dogs alert the same way, regardless of which target scent they detect. Detector dogs have long been used by US law enforcement agencies for the presumptive detection of controlled substances, creating substantial precedent and case law for their evidentiary use in prosecutions [64].

Dogs can also detect VOCs released by pathogens and disease processes [65; 66]. They have accurately detected cancer disease states in human stool and breath samples [67; 68]. Many animal pathogens produce VOCs that dogs can smell [69; 70]. German shepherds detected gypsy moth egg masses in 1976 [71], and dogs from three breeds detected lanternfly egg masses in 2021 [72]. Recently, dogs have also been trained to detect plant pathogens with significant economic significance (Table 1). Eleven dogs detected Asian citrus canker caused by *X. citri* pv. *citri* in seedlings, fruit orchards, packing houses, and even isolated bacterial cultures [73]. The dogs detected *X. citri* infections with a good sensitivity (73%) and specificity (99%). The detector dogs usually required less than a minute to detect their targets and alert.

Table 1. Canine Detection of Plant Pathogens and Diseases

Plant pathogen	Dogs	Accuracy
Bacteria		
Xanthomonas citri pv. *citri* (Asian citrus canker lesions on fruit)[a]	11	0.98
Candidatus Liberibacter asiaticus (huanglongbing, HLB of citrus)[b]	10	0.99
Raffaelea lauricola (laurel wilt disease)[c]	3	0.994
Bretziella fagacearum (oak wilt)[d]	2	
Clavibacter michiganensis (Bacterial Ring Rot)[e]		ND[f]
Viruses		
Plum pox virus (PPV)[g]		0.99
Potato virus Y[e]		ND
Squash vein yellowing virus (SqVYV)[h]		ND
Tomato chlorotic spot virus (TCSV)[h]		ND
Tomato spotted wilt virus (TSWV)[h]		ND
Tomato yellow leaf curl virus (TYLCV)[h]		ND
Protozoa		
Plasmodiophora brassicae (clubroot)[i]	2	0.90
Fungi		
Heterobasidion root disease[j]	7	0.70
Heterobasidion parviporum, *Cronartium flaccidum* and *Peridermium pini*[k]		ND

Sources:
[a.] [73]
[b.] [74]
[c.] [75]
[d.] Cornell (unpublished, 2020)
[e.] Nose Knows Scouting (unpublished)
[f.] ND, Not determined.
[g.] Gottwald et al. (unpublished)
[h.] F1K9 (unpublished)
[i.] Alberta Agriculture and Forestry (unpublished, 2019)
[j.] [76]
[k.] Lapland University of Applied Sciences & Agricultural Resources (unpublished, 2022)

Detector dogs efficiently identified a variety of plant diseases caused by viruses, bacteria, fungi, and protists (Figure 6). They have also been trained to detect insect pests, including coconut rhinoceros beetle and Mediterranean fruit fly with 90% proficiency [77], as well as spotted lanternfly and Japanese beetle. Many of these studies require further validation in field tests or relevant work environments, using relevant agricultural products, additional dogs, distractor scents, and a large number of negative samples. AK9s require substantial resources, continual training, feeding, care, and exercise. They typically work for brief periods and are well-suited for scheduled inspections such as the arrival of an international flight. Their skills cannot be transferred to new dogs, and each dog may recognize a different combination of VOCs that comprise the target scent of the pathogen or disease. Despite these limitations, the success of detector dogs provides strong evidence for VOC signatures of many plant pests and diseases.

Figure 6. Detector dog training to detect plant pests and diseases. Credit: USDA

3.4 TRAPS

Traps for insects and fungal spores are simple tools for the collection of airborne pests. However, it is unclear who would pay the costs associated with the purchase and installation of traps in containers or trailers.

3.4.1 Insect Traps

Insect traps are widely used in agriculture and have a long history of innovation [78; 79]. They are a mainstay for the APHIS fruit fly exclusion and detection program [80]. Sticky traps covered with glue detect flying pests at low cost (Figure 7). Mechanical insect traps have been available since the 18th century and continue to evolve. Modern insect traps are equipped with smart monitoring solutions to allow insect counting and monitoring from a remote terminal. One example of a remote monitoring solution is the iSCOUT® product from Pessl Instruments GmbH (Weiz, Austria), which includes an insect-specific pheromone, feeding lure, or color trap; a digital camera; solar panel power source; and modem.

Figure 7. Pest traps. (Left) Yellow sticky trap for Asian citrus psyllid sampling in lime trees. Credit: David Bartels (USDA APHIS). (Right) Aerial spore trap. Credit: Kylle Roy (USGS).

A trapping device would be ineffective for real-time detection at POEs but could be integrated into the overall pest detection strategy. For instance, a container or truck full of produce could be outfitted with a sticky trap or smart trap by a regulated vendor at the packing plant. The trap could be collected and analyzed at the POE to provide a profile of the entire cargo in a container. Alternatively, a smart device could be integrated with networking and GPS capabilities to enable continuous data transmission and location tracking to the POE while the shipment is en route. Upon arrival at the POE primary inspection, a verification could be used to alert the Agricultural Specialist that the sensor has arrived and give a simple report. For loads requiring further inspection, machine learning (ML) algorithms could be deployed to quantify and characterize the contents of the trap prior to arrival at the secondary screening station, and deliver actionable metrics (number of insects, probable species) to the secondary screener [81]. Traps may not effectively sample larvae and immature insects, and they may not collect effectively in refrigerated or frozen shipping containers.

3.4.2 Fungal Spore Traps

In 2001 to 2003, *Phakopsora pachyrhizi,* the causal agent of Asian soybean rust, was first detected in South America, where it caused up to 90% yield loss, destroying the Brazilian soybean industry [82]. In 2003, Ray Schneider from Louisiana State University first detected this fungus in the continental United States [83]. He developed the ionic spore trap to capture rust spores through the air current, which was then collected on a membrane, which then can be washed and used for molecular confirmation. Since this technology emerged, several other spore-trapping devices have been implemented to collect spores from diseased fields and confirm their identity through molecular techniques [84; 85].

Traps have also been used to detect plant pathogens [86]. Spores of *Botryosphaeriaceae* spp. were trapped in California vineyards [87]. This fungus causes canker disease and dieback in many plants, including grapevines. Glass microscope slides coated with petroleum jelly were placed in the vineyard for

one week. After collection, spores were eluted into water and plated on agar growth medium for morphological identification and counting. A commercial version of this impactor particle collector with a vacuum pump was designed by Hirst for continuous collection over one week and is currently manufactured by Burkard Manufacturing (Hertfordshire, UK) [88]. An ionic spore trap was developed by Maverick PDM using electrostatic deposition to capture fungal spores [89]. Impaction, virtual impaction, cyclone, and electrostatic volumetric spore samplers have been used to survey airborne pathogens in many air samples [86; 89; 90]. Similar to insect traps, particle collectors could be deployed in sealed shipping containers during transport to survey dispersed pathogens. Compared to conventional sample-based inspections, this method could survey an entire shipping container and could be used to do so in a less intrusive manner. This technology requires that the pathogen be suspended in aerosols or droplets for collection. However, fruits, vegetables, and flowers are often frozen, chilled, or packaged for distribution before importation, which would limit dispersal. Furthermore, a separate method of detection is required to identify pests and pathogens captured by the collector.

3.5 X-RAY AND GAMMA RAY IMAGING

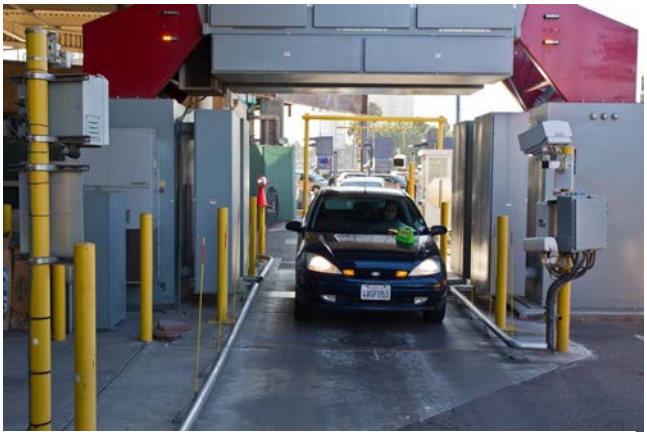

Figure 8. A car passes through an x-ray scanner at the land border in San Ysidro, CA. Photo credit: Josh Denmark (CBP).

CBP uses fixed and mobile x-ray imaging for non-intrusive inspections to detect contraband and concealed products at most POEs; one such installation is shown in Figure 8 [91]. At the World Trade Bridge in Laredo, CBP uses a drive-through multi-energy x-ray portal system for secondary inspections of trucks [92]. The 2020 Securing America's Ports Act (Public Law 116-299) required a plan to scan all commercial and passenger vehicles as well as rail freight entering the US using large-scale non-intrusive inspections. X-ray imaging is also used to screen some international mail and passenger luggage in secondary inspections at airport and land border POEs. For most applications, operators are looking for differences in x-ray scattering in the images or reconstructions that correspond to objects of different densities that may be undeclared or illicit—including narcotics, currency, weapons, and destructive devices. Low-energy gamma radiation imaging systems are also used to inspect truck and container shipments and offer better contrast [93; 94].

X-ray imaging systems often detect contraband fruit and meat products in baggage (Figure 9). The Australian Department of Agriculture, Fisheries and Forestry collects 3D x-ray images of biosecurity risk items, which could be used for ML to train future automated screening systems. However, systems designed to scan vehicles and baggage usually lack sufficient resolution to identify plant pests or pathogens.

Figure 9. Non-intrusive airport baggage inspection by x-ray scanner. (Left) Baggage x-ray scanner with automatic tag reader system at Brisbane International Airport. (Right) Remote screening room for 3D x-ray scans at Melbourne International Airport. Credit: Australia Department of Agriculture, Fisheries and Forestry [95].

At a smaller scale, x-ray computed tomography has been used to detect pests in fruit [96; 97] and for quality inspection of agricultural products [98; 99]. Both insects and fungi have been detected in wheat kernels using soft x-ray methods [100; 101]. A micro-focus x-ray source, which was deployed for the automated inspection of fruits to detect insect infestation and damage, used image processing algorithms to distinguish fruits with insect eggs from undamaged fruit with 97% sensitivity, 99% specificity, and 98% accuracy [102]. Advances in ML and pattern recognition using AI could improve this system. Recent studies developed a soft x-ray line sensor system with deep learning tools to identify with 98% accuracy Hass avocados with internal rot caused by fungal infection [103; 104]. Although x-ray systems may not detect plant pathogens directly, they can detect disease damage to agricultural products with automated workflows that are less intrusive than current inspection methods. Damage from both insect pests and microbial pathogens could be detected. These high-resolution x-ray systems differ from the larger-scale non-intrusive inspection systems currently used to screen imports.

3.6 IMMUNOLOGICAL AND MOLECULAR DIAGNOSTICS

Culture-based methods to identify plant pathogens have been progressively replaced by molecular diagnostics to detect known pests and disease [105]. Methods and tools developed for human health diagnostics and environmental surveillance have been adapted for plant pathology. Serological or immunological methods, which typically use antibodies to recognize proteins or cell walls of pests, have been used in plant pathology laboratories since the 1960s [106]. Nucleic acid–based methods, including hybridization, targeted sequence amplification, and genome sequencing, have grown rapidly since the 1970s and have flourished since the introduction of the polymerase chain reaction (PCR) method in the 1980s [107; 108]. Molecular diagnostics typically combine extraordinary sensitivity with high specificity to detect markers of known pathogens [109]. However, these methods may not distinguish live pests from active infections nor dead pests from treated agricultural products. Most molecular methods are performed in laboratories for confirmatory identification, but this work focuses on technologies that can be applied at the point of use (i.e., on-site, close to the agricultural field or POE) for presumptive identification [110-112].

3.6.1 Immunological Assays

Antibodies can be developed to bind specifically to fragments of plant pests. These antibody immunochemicals can be validated for specificity, sensitivity, and selectivity using an enzyme-linked immunosorbent assay (ELISA), the gold standard for immunological assays. ELISA is cost-efficient, quick, boasts a low chance of false positives, and is the most frequently used technology for all types of plant pathogens (i.e., bacteria, fungi, oomycetes, and viruses) [113; 114]. Proficiency testing for laboratory analysts using an ELISA method to detect bacterial ring rot in potatoes produced acceptable results in more than 90% of tests, demonstrating good reproducibility [115]. However, standard ELISA methods are difficult to execute outside of a laboratory.

Figure 10. Lid and base of a lateral flow immunoassay device, showing sample pad, conjugation pad, test and control lines on nitrocellulose, and an absorption pad. Credit: Wikimedia Commons, Secretlondon, 2021. CC BY-SA 4.0 DEED.

Lateral flow immunoassays (LFIA) translate antibody detection methods to a point-of-use device that is low-cost, rapid, and simple to use [116]. LFIAs were widely distributed to screen nasal swabs for SARS-CoV-2 infection during the COVID-19 pandemic. Plant, agricultural product, or pathogen samples must be sampled by swab or homogenization and then dispersed in a buffered solution. Several drops of the solution are applied to the disposable LFIA device, and results are observed by eye or camera after 10 to 20 minutes (Figure 10). LFIA tests have been commercialized for plant pathogen detection, and several examples that detect PPQ quarantine pests are shown in Table 2. APHIS PPQ has used lateral flow assays at POEs to screen for viral and oomycete pathogens that are difficult to detect by visual inspection.

Table 2. Representative LFIA Tests to Detect PPQ Quarantine Pests

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
Tomato brown rugose fruit virus (ToBRFV)[a]	100%	100%
Cucumber green mottle mosaic virus (CGMMV)[b]	100%	100%
Plum pox virus (PPV)[c]	100%	100%
Ralstonia solanacearum[d]	100%	87.2%
Phytophthora spp.[e]	87.6%	82.9%

Sources:
[a.] ImmunoStrips® (Agdia) [117]
[b.] ImmunoStrips® (Agdia) [118]
[c.] ImmunoStrips® (Agdia) [119]
[d.] Pocket Diagnostic [120]
[e.] Foresight Diagnostics [121]

The convenience of LFIA detection can be tempered by technical limitations of the devices. Immunological tests require a specific antibody and a reliable source. Antibody development and validation could take a long time, delaying test development for emerging pathogens. Only a small number of samples and a small quantity of product can be screened. These tests can yield false negatives if the pathogen has not quantitatively developed in infected tissue. Additionally, these assays are effective only if the sample is infected with a pathogen: if non-infected tissue is used and produces a negative result, then it may not be indicative that all the products in a container are disease free. Usually, only a few symptomatic plants are tested, which can bias estimates of disease. Sample preparation is critical because other components of the sample could interfere with testing (matrix effects). For some families of pathogens with genetic and structural diversity, it may not be possible to develop a single antibody

reagent that detects all of the target pathogens (i.e., low sensitivity). Alternatively, closely related microbes that are not quarantined could cross-react to give a false detection (i.e., low specificity). Although LFIAs are considered rapid diagnostic tests, they still require 5 to 15 minutes to develop in addition to sample processing time. Agriculture specialists may not be available to read the tests within a fixed period if they are immediately assigned another task at a busy site. Faster tests or automation technology could mitigate this problem.

3.6.2 Targeted Nucleic Acid Detection

Nucleic acids (DNA or RNA) that are unique to plant pests have long been targets for molecular detection [107]. Hybridization of isolated nucleic acids with labeled DNA probes identified many pathogens and plant diseases in the laboratory. Since the 1980s, PCR has accelerated molecular detection and substantially increased sensitivity by amplifying nucleic acids to form DNA fragments that can be characterized by sizing or sequencing. These gene targets, sometimes called *barcoding regions*, have been used for two decades to identify plant pathogens to the genus or species level, such as bacteria, fungi [122], oomycetes [123; 124], and viruses. Because the barcoding region is useful in identifying these pathogens, quick analysis can be conducted for specific pathogens by designing nucleic acid amplification techniques such as digital droplet PCR (ddPCR), quantitative PCR, loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA) [15]. Most nucleic acid–based detection systems include three steps: extraction, amplification, and detection [125]. Several companies have built and marketed portable microfluidic devices for point-of-use nucleic acid extraction, purification, and detection—including BioFire Diagnostics (Salt Lake City, UT), Abbott Rapid Diagnostics, Cepheid (Sunnyvale, CA), Sony, GenePOC (Quebec, Canada), and Roche Molecular Diagnostics [110; 126]. However, these molecular diagnostic systems were developed to analyze human clinical samples for infectious diseases and have not been optimized to detect plant diseases.

Isothermal nucleic acid amplification methods lower the barriers to point-of-use molecular diagnostics by performing assays at low, fixed temperature (reduced power consumption) and by amplifying lower-quality DNA samples (reducing purification costs and robustness) [15; 111]. LAMP methods can be performed in less than 30 minutes to produce a visible color change with a signal that can be quantified using a smartphone camera or integrated sensor. A real-time LAMP assay detected *Xylella fastidiosa* with 70–90% diagnostic sensitivity and 97–100% specificity [127]. Viruses also can be detected using LAMP [128], including a reverse transcription LAMP assay that detected several strains of plum pox virus [129]. Both LAMP and ddPCR methods have been shown to provide better analytical sensitivity than PCR to detect *Phytophthora infestans* in potato tubers [130]. LAMP devices are readily manufactured [131] or commercially available, such as the BioRanger (Diagenetix) with portable batteries or Genie® instruments from OptiGene. Assay reagents and validations are often developed in-house. Another isothermal method, RPA, has become competitive with LAMP. An RPA assay was used for the multiplex detection of *Botrytis cinerea*, *Pseudomonas syringae*, and *Fusarium oxysporum* [132]. A related method used toehold-mediated strand displacement to detect RNA from wheat rust using field-deployable colorimetric paper and a smartphone [133].

Downsides to these molecular techniques include longer analysis times, sensitivity to genetic variation [134], and false positive results. Reagents are highly specific for individual pathogens, although multiplex methods have been developed to assay for several pathogens at the same time. Control reactions are usually required to identify cross-contamination and inhibitors of the polymerases used in the reactions. Some test kits require cold storage, which could limit point-of-use testing. Due to their high costs for instrumentation and consumables, these devices are more commonly used to detect human diseases than plant diseases [135]. Molecular diagnostics could be completed at the point of origin (with supervision) before shipment or during transit.

4. EMERGING DETECTION TECHNOLOGIES

This section evaluates unconventional technologies that could be used to detect plant pathogens and diseases on agricultural products. Detection, the presumptive identification of a pest by CBP Agricultural Specialists, must be confirmed by APHIS/PPQ experts who identify and classify pests. If the sensitivity of detection technology outpaces confirmatory identification technology, then improvements in detection efficiency may not increase pest interceptions or expedite commerce. Therefore, new technologies that improve specificity as well as sensitivity could benefit both CBP and APHIS/PPQ operations.

4.1 AUTOMATED IMAGING

As an alternative to visual inspection, optical systems can automatically image light reflected by plants or agricultural products to detect disease symptoms. In addition to the light spectra visualized by humans, automated systems can detect light reflected in the non-visible wavelengths, such as infrared and ultraviolet. Such systems can automatically process the digital images and apply pattern recognition algorithms augmented by AI or ML to spot diseases [136]. Similar to visual inspection, inspection via cameras offers views of plant surfaces or pests that are exposed to and reflect light. However, automated imaging systems can detect a broader range of light (including near-infrared (NIR) and thermal), evaluate more wavelength bands (hyperspectral imaging, HSI), and process images without fatigue. When incidents of a disease in agricultural products are low or the disease causes variable changes in products, it may be difficult to collect sufficient images to train AI/ML systems. Emerging tools, such as few shot learning and ML for anomaly detection could address this problem [137].

Automated systems are currently used to assess food quality and safety as well as to recognize, grade, and sort foods in real time [138; 139]. For instance, a high-throughput optical sorter was used to image up to 8,800 kg of wheat kernels per hour with 675 nm light, detecting and rejecting kernels infected with Karnal bunt (*T. indica*) with 100% sensitivity when 8% of the kernels were discarded [140]. These instruments can also perform non-destructive analysis of fruits and vegetables for insect pest infestations [97]. Software developed and trained to detect anomalies on agricultural products can be easily transferred to new systems, scaling capacity quickly.

The following sections discuss variations on this theme: multi-spectral imaging, HSI, and thermal imaging. Equipment using each class of imaging technology has been commercialized for automated food quality assessment and sorting. This application typically requires high diagnostic sensitivity and specificity for disease states but may not distinguish among pathogens.

4.2 MULTI-SPECTRAL IMAGING

Multi-spectral imaging is a widely deployed technology in crop monitoring today. Digital cameras that capture three spectral bands in the visible range, red, green, and blue (RGB), are ubiquitous on mobile phones and sophisticated photography equipment. More advanced systems include multiband imagers, such as the MicaSense (Seattle, WA) product line. The RedEdge-P product includes RGB bands as well as NIR and red edge (near thermal) bands. These instruments are commonly mounted on unmanned aerial vehicles and used to fly over field to detect crop anomalies. The advantage of multi-spectral over traditional RGB (three-band) imagery is the ability to calculate vegetation indices. One of the most common indices is the normalized difference vegetation index (NDVI). This is used to quantify the greenness of vegetation. NDVI is useful for assessing changes in overall plant health. A yellow or mottled leaf color may indicate the presence of a pathogen. The photochemical reflectance index is commonly measured to estimate leaf photosynthetic radiation use efficiency. Indices tuned to specific pests have

been developed, and this is an area of active research. Most often, the vegetation indices are combined with traditional RGB data processing—for instance, counting leaf spots—to ascertain the presence and severity of a disease. Many fungal diseases can be accurately detected and sometimes quantified using this technology. Most multi-spectral imaging systems for disease detection analyze plant leaf images [141; 142].

Multi-spectral image analysis has long been used to identify animal pests, including insects. The USDA APHIS Remote Pest Identification Program facilitates pest identification by sharing RGB images of pests from POEs with National Identification Services specialists across the country to confirm identification of quarantined pests and identify first-in-nation pests. The Australian Government's Department of Agriculture, Water and the Environment partnered with the Commonwealth Scientific and Industrial Research Organisation to develop a mobile phone application used by biosecurity officers that uses AI to identify brown marmorated stink bugs, along with 45 different related species [143]. Deep learning methods of AI are well suited to train models to classify images of pests [144]. Applications for smartphones are readily available to analyze RGB images, including Google Lens, Picture Insect, Smart Identifier, and Seek. However, taxonomic classification accuracy depends on the image quality (Figure 11) [145].

Figure 11. Representative smartphone applications that identify plant pests or diseases using multi-spectral (RGB) images.

Citrus fruits have been inspected using optical imaging for more than a decade because many diseases produce unique peel lesions (Figure 12) [138]. Fungi on the fruits have been detected using fluorescence from UV illumination [146] and Fourier transform infrared (FTIR), the preferred method of infrared spectroscopy [147]. ML and AI methods, including convolutional neural networks, have recently been used to develop classifiers that operate with high accuracy. A deep learning model that combined neural network with long short-term memory network models identified diseased citrus fruit in images with 98% accuracy [148]. This model classified multiple diseases with high accuracy, including canker disease (97%), scab (95%), melanosis (99%), greening (97%), and black spot (97%).

Figure 12. Imaging plant disease on fruit. (Left) Citrus greening caused by Huanglongbing (HLB); (Center) citrus blackspot caused by the fungus *Phyllosticta citricarpa;* and (Right) *cucumber green mottle mosaic virus* (CGMMV). Photo credits: USDA APHIS/PPQ.

Disease detection in potato tubers has also benefitted from optical imaging and pattern recognition. A deep convolutional neural network detected four disease classes in RBG images of potato tubers with 96% accuracy [149]. Another convolutional neural network system produced the user application 'ScabyNet' to detect common scab on tubers from RBG images [150]. TOMRA Systems (Asker, Norway) has commercialized food sorting and grading equipment that collects multispectral images of fruit and uses deep learning methods to detect visible defects [151; 152]. For example, the TOMRA Spectrim X series powered by the LUCAi® grading system equipment detects >95% stem-related imperfections in apples, processing thousands of multi-channel fruit images per second. The TOMRA Neon optical grader processes up to 500 blueberries per second, removing >90% of green and red berries. NEWTEC (Odense, Denmark) manufactures optical sorting machines for potatoes, using automated imaging and ML to identify surface defects on tubers. However, the food processing industry does not require specificity for plant disease detection. It is concerned with classifying a product as either positive or negative for disease and damage to determine whether a food is consumable or saleable. Therefore, this equipment prioritizes sensitivity and speed.

4.2.1 Hyperspectral Imaging

HSI provides a high level of detail with respect to the light that an object reflects, potentially improving the ability to detect plant disease compared to that offered by multi-spectral imaging [153]. HSI splits a wide spectrum of light into narrow bands. HSI differs from multi-spectral imaging in that the bands are contiguous. Hyperspectral instrumentation can span the entire electromagnetic spectrum; however, the most common HSI instruments cover the spectra from approximately 400 to 2500 nm. Most HSI instruments cover only a portion of that range. The lower end, 400–1000 nm, is commonly referred to as *visible and near-infrared* (VNIR), whereas the 1000–3000 nm range is commonly referred to as *shortwave infrared* (SWIR). A few companies manufacture mid-wave infrared (MWIR) instrumentation (3000–5000 nm), but this is less common.

Hyperspectral instrument architectures are categorized into spatial scanning, spectral scanning, and non-scanning architectures. In a push broom spatial scanning architecture, the camera moves across the frame line by line to obtain the spectra. In whiskbroom architecture, the aperture is reduced to a single pixel. These architectures require stabilized mounts for accuracy. They are often used in aerial photography or for applications where the object is moving, such as a scenario in which items are moving along a conveyor belt. In spectral scanning, the entire scene is captured at once, but only one band at a time is recorded. This is similar to how a regular camera works. The hyperspectral effect is achieved by exchanging filters to remove various areas of the light spectrum. Spectral scanning requires that both the camera and the objects being scanned remain fixed. Movement by either instrument or subject causes distortion in the resulting image. In a non-scanning architecture, the entire dataset—the hypercube—is

captured at once. This modality is by far the most accurate and energy-efficient, as a single snapshot can capture all the information. Moreover, image acquisition is quicker, and smearing effects are reduced. However, the manufacturing costs and computational infrastructure required to process this type of imagery are currently quite high.

Hyperspectral, narrow-band multispectral, and thermal imagery acquired at a high spatial resolution can be used to detect disease symptoms before they are visible, allowing growers to differentiate between infected plants and plants that may be affected by confounding environmental stresses. Often, such pathogen stress produces diverse symptoms (detectable responses of the plant)—for example, those similar to water stress or nutrient deficiency. This is because infection by certain vascular pathogens restricts water and nutrient flow through the crop xylem. Test results from high-resolution remote-sensed data (e.g., Worldview) indicate that disease incidence can be detected with overall accuracies ranging from 0.63 to 0.83 and *kappa* coefficients (κ) ranging from 0.29 to 0.68. However, detection of the early stages of disease with multispectral satellite data has been shown to be poorer, with κ values of 0.22–0.45, compared with κ values of 0.3–0.69 obtained from hyperspectral data. The addition of thermal-based crop water stress indicators to the satellite data is shown to improve the overall detection accuracies by 10–15%. The results indicate that early detection of disease symptoms in the field is more achievable using hyperspectral and thermal data rather than common commercial multispectral high-spatial-resolution data. In general, it can be concluded that choosing and combining appropriate sensors for each plant–pathogen system and measuring with sufficient spatial resolution can enable specific and accurate measurements of above-ground signatures and symptoms of plant disease.

All modalities and architectures of HSI have been applied to biological problems with varying levels of success [154]. The massive datasets produced by HSI require computer algorithms or AI/ML methods to accurately classify disease states. Examples of the powerful combination of HSI and AI/ML analysis to detect disease have been reported for grapefruit, wheat, and potatoes. A convolutional neutral network model classified hyperspectral images of grapefruit with eight different peel diseases with 99.8% accuracy [155]. HSI combined with a support vector machine classifier identified *Fusarium* head blight with 78–100% accuracy from 3 to 30 days post-inoculation [156]. This exceeded the accuracy of classifiers based on infrared thermography or chlorophyll fluorescence imaging. Sensitivity of detection was approximately 67% in a related study [157]. HSI was also used to detect Zebra chip disease in potato tubers caused by the bacterium *Candidatus Liberibacter solanacearum* using partial least squares discriminant analysis with 92% accuracy [158]. HSI data from VNIR–SWIR wavelengths were used to identify tubers with internal defects and demonstrated 96% classification accuracy using linear discriminant analysis [159].

Commercial systems have been developed to sort and grade fruits and vegetables using HSI for nondestructive analysis. NEWTEC has developed and commercialized a pushbroom 900–1700 nm hyperspectral imaging system with a high-frame-rate camera to sort fruit, although few operational details about its data analysis workflow have been published [160]. Hyperspectral SWIR imaging with classifier analysis improved disease detection in onion bulbs, which can have healthy outer surfaces despite internal disease [161; 162].

4.2.2 NIR Spectroscopy and Imaging

Organic molecules absorb NIR light in characteristic vibrational absorption bands between 800 and 3000 nm wavelengths (12,500 to 3,333 cm^{-1} wavenumbers). These features are common in protein and carbohydrate molecules in plant cell walls. Light in the 700 to 900 nm wavelengths can penetrate up to 10 mm into fruits, providing more insight into tissue than that possible with shorter-wavelength visible or longer-wavelength NIR radiation used in nondestructive imaging [163]. Therefore, NIR imaging can

reveal new information about biomarkers in diseased plant products that could improve the sensitivity and specificity of detection. FTIR with attenuated total reflectance (ATR) measurements can detect chemical signatures reflected from the surface of samples. Raman infrared spectroscopy uses an infrared laser to excite molecules and then measures infrared light scattered at other wavelengths. Both methods can be nondestructive, although sample preparation may improve imaging. Results are compared to spectral libraries to identify tested materials. Although pure fungal cultures also have characteristic NIR signatures [164], only diseased agricultural products are considered in this section. Both FTIR and Raman spectroscopy are commonly used by CBP and other law enforcement officers for the presumptive identification of hazardous chemicals and illicit drugs in less than one minute [165].

FTIR spectroscopy is the most common and familiar method of NIR analysis. Example applications to disease detection include studies on guava plants, tomato fruit, and potato tubers. An FTIR–ATR signature of leaves from guava plants infected by a root-knot nematode was reported but has not yet been validated for diagnostics [166]. A study of sour rot infection in tomato fruit used FTIR–ATR methods to classify diseased fruits with 83%, 97%, and 83% sensitivity for damaged, early infection, and late infection samples, respectively [167]. Specificities were 78%, 92% and 96% for the same groups of samples. Finally, FTIR was used to identify spectral biomarkers on the surface of tubers that correctly identified potatoes infected by the fungus *Colletotrichum coccodes* [168].

FTIR spectrometers and NIR imaging systems are commercially available, although the computer software and applications in detecting disease in plant products are still in development. Portable and handheld FTIR systems are sold by Agilent, Bruker, Thermo Scientific, Smiths, and Mistra [169]. Limitations of FTIR technology include its high sensitivity to water, which can interfere with plant product analysis. In an ATR mode, FTIR measures surface properties and may not detect changes inside agricultural products. NIR imaging can also detect insect eggs and larvae inside agricultural products using non-destructive techniques [170; 171].

Raman spectroscopy measures the Raman scattering of infrared light after a sample surface is excited by an intense laser [172]. It is less sensitive to water than FTIR methods. Infections by *Candidatus Liberibacter* spp. that cause citrus greening disease in sweet oranges produced spectral anomalies that were detected by portable Raman spectroscopy with 86.9% sensitivity and 91.4% specificity [173]. Raman HIS was used to detect *Acidovorax citrulli* infections in watermelon seeds with approximately 75% sensitivity, although further studies with more samples will be required to validate the method [174]. Pankin et al. compared FTIR and Raman spectroscopy for the detection of *Fusarium* fungal infections of oat grains [175]. The FTIR classification method detected diseased grains with 75% to 95% sensitivity and specificity, whereas the Raman classification method was more accurate, with 95% to 100% sensitivity and 100% specificity. Most studies using Raman spectroscopy to detect plant disease have focused on leaf imaging, and some disease states can also be detected using conventional RGB imaging [176]. Some spectral signatures may be generic responses to biotic and abiotic stresses, which will require further validation [177]. Due to the requirement for a spectral signature for a presumptive identification, Raman spectroscopy and imaging will be most useful for targeted identification of diseased plant products based on other information about the shipping consignment. Finally, Raman spectroscopy cannot be used for dark samples that fluoresce. Portable Raman spectroscopy systems are manufactured by Cobalt Light, Rigaku, Thermo Scientific, and B&W Tek. They are marketed for hazardous chemical and controlled substance characterization, and some are in use by CBP officers.

4.2.3 Infrared (Thermal) Imaging

Long-wave infrared (LWIR) and thermal imaging technologies have a long history in pest detection, particularly to detect insects. This technique detects concealed termites, hornets, rodents, and other

macroscopic pests [178]. Thermal imaging may also be useful to detect pests and hitchhikers in wood packaging materials and shipping containers. CBP uses thermal imaging to detect heat signatures of people and concealed weapons through non-intrusive inspections [179].

Objects both reflect LWIR from illumination and emit thermal energy to dissipate heat. Plant and agricultural product temperatures are usually close to those of the ambient environment, so sensitive detectors are required to resolve temperature differences of only a few degrees. LWIR imaging and terahertz spectroscopy are often used to assess water movement in plants, through root uptake and leaf transpiration through stomata. Diseases caused by fungi and oomycetes can interfere with transpiration, enabling plant disease diagnosis by thermal infrared imaging [180] as well as terahertz time-domain spectroscopy [181]. Thermal imaging has also been used to detect pests in stored grains due to animal respiration [182] and damage to guava fruit caused by disease [183]. A study of this technology on apples showed that it successfully detected a decrease in temperature 48 hours after inoculation with a variety of fungal pathogens [184]. Thermal imaging identified the fungus *Fusarium solani* on heated tubers with 98.5% accuracy [185]. Although LWIR hyperspectral sensors exist, they are normally used only when very precise temperature measurements are required.

This technology could be deployed to quickly scan incoming cargo for signs of spoilage, thus indicating the presence of a pathogen. To maximize the efficacy of this effort, continuous monitoring of the temperature from the time of packing to the time of inspection would be desirable. If continuous monitoring is not possible, then a minimum baseline reading for such monitoring could be taken before packing and at inspection. Finally, if scans can be performed only at the POE, then differential analysis of the temperature gradients within a scan may be effective at pinpointing spoilage, thus targeting the efforts of visual inspections. Handheld thermal LWIR imaging equipment is commercially available from Teledyne FLIR, Fluke, Seek Thermal, etc., with many more manufacturers of fixed cameras in a $2 billion+ annual market. Plugin thermal cameras are also available for smartphones, with lower resolution. These thermal methods may have limited specificity and could be difficult to apply in refrigerated shipping containers.

Temperature differentials measured using thermal imaging are frequently less than 1 °C, requiring controlled conditions to accurately detect disease states [186]. Pulsed-phase thermography could be more sensitive to detect apple defects. In this method, apples are heated with a thermal pulse, and the temperature decay is measured by thermal imaging to detect surface and near-surface defects [187]. However, heating may not be acceptable or cost-effective for high-volume analyses of agricultural imports. Similar to commercial equipment that sorts and grades fruit using multispectral imaging, thermal imaging systems lack specificity for plant diseases.

4.3 BIOGENIC VOLATILE ORGANIC COMPOUND DETECTION

Plants produce thousands of biogenic volatile organic compounds (BVOCs) [188]. BVOCs are organic compounds that evaporate under normal conditions and are produced by biological systems. Terpenes (e.g., isoprene and α-pinene) emitted from leaves are the most abundant BVOCs, mixing with other gases to convey plant health status. Diseased plants release different BVOCs than those released by healthy plants [189], and bacteria, fungi, and oomycetes pathogens produce different BVOCs (e.g., butanone and pentanone) [190; 191]. Together, this BVOC profile is the basis for canines to detect diseases, discussed previously. The scents used for AK9 training could inform the development of mass spectrometry or electronic nose systems. Similarly, BVOC profiles detected by these systems could be used to advance AK9 training in the future.

4.3.1 GC-MS and PTR-MS

BVOCs have traditionally been collected by adsorption, followed by thermal desorption and qualitative or quantitative analysis by gas chromatography–mass spectrometry (GC-MS) [192]. Manufacturers of portable GC-MS systems include 908 Devices (Boston, MA), FLIR, and Smiths Detection. Many more companies produce GC-MS systems for fixed installation in laboratories, including Agilent, Shimadzu, and Thermo Scientific. This method was used to detect pine seedlings infected by the fungal pathogen *Fusarium circinatum*, which release characteristic sesquiterpenes that are different from BVOCs released during infection by other *Fusarium* species [193]. Other species of *Fusarium* that infect wheat also emit signature BVOCs [194]. This method can be used to detect viral disease states as well: GC-MS was used to identify BVOC signatures of pepper yellow leaf curl virus infecting chili plants [195]. Insect pests such as bed bugs [196] produce other characteristic mixtures of BVOCs.

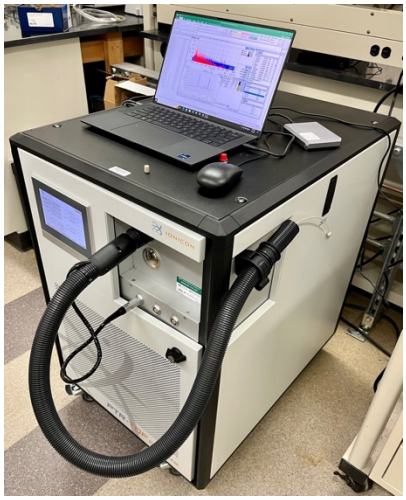

Figure 13. A proton-transfer-reaction mass spectrometer analyzes a profile of VOCs in less than one second.

This sensitive GC-MS method has been augmented by movable, fast (100 ms), and high resolution (>6000 m/Δm) methods such as proton-transfer-reaction mass spectrometry (PTR-MS), ion mobility mass spectrometry, and FTIR gas analyzers that enable real-time measurements of many compounds. PTR-MS systems are manufactured by IONICON Analytik (Insbruck, Austria), and field-deployable ion mobility spectrometry systems are produced by Smiths Detection (Hemel Hempstead, UK), Leidos, Bertin Environics (Mikkeli, Finland), and Bruker. Portable FTIR gas analyzers are manufactured by Gasmet Technologies (Vantaa, Finland) and Bruker. BVOC analysis can be non-intrusive and rapid, which expands its utility as a screening tool for presumptive detection.

The recent development of proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS) offers an alternative approach via direct intake of air samples for the detection of BVOCs in food and agricultural products (Figure 13) [197; 198]. The sample analysis time for the PTR-ToF-MS is on the order of seconds, compared to 20–30 min per sample for traditional VOC-detecting instruments such as the GC-MS. Despite this technology only recently becoming commercially available, numerous uses have been reported for agriculture pathogen detection that span grains, vegetables, and ornamental crops (refer to Table 3 below for examples). Though this technology is promising, multiple considerations and possible constraints must be addressed before its deployment for diagnostic purposes. First, a standardized sampling method must be developed. Numerous approaches exist for designing chambers and cuvettes for plant VOC sampling [199]. How extension to large transport containers can be achieved is an open question. Second, BVOC profiles differ among plant tissues and change during the course of pathogen infection. Third, data analysis workflows are still being developed, including classification systems that can translate peak data into accurate predictions of disease states. Classification does not require identifying the chemical responsible for each observed feature, and the absence of a signal for a compound can also inform the fingerprint [200]. At present, specific models of plant disease state BVOC profiles must be developed for each host–pathogen pair.

ML and AI methods have proven valuable in developing tools to classify the disease state of plants and plant products based on complex BVOC profiles. Convolutional neural networks have been frequently used to extract features from the profiles and to develop predictive models [137]. Linear discriminant analysis was used to classify apples infected with two different bacteria based on BVOC profiles measured by PTR-MS [201]. This method showed 100% sensitivity and 86% specificity for *Erwinia*

amylovora and 83% sensitivity and 83% specificity for *P. syringae* (Table 3). Fungal volatiles produced during growth on grains and cereals have been extensively characterized, including alcohols, aldehydes, esters, and furans [202]. These fingerprints may be sufficient for presumptive detection of fungal disease but would require confirmatory detection using another method to establish whether the pest is actionable. Another study of *Fusarium* spp. fungal infections on wheat or rice grains classified pathogens with 99% sensitivity and 99% specificity using PTR-MS analyses [203].

Table 3. Plant Diseases and Pathogens Detected Using BVOC Markers

Crop	Disease	Pathogen	Representative BVOC markers
Bacteria			
Potato[a]	Brown rot	*Ralstonia solanacearum*	3-methylbutanoic acid 2,2,3,4-tetramethylpentane 2,3,4-trimethylhexane 4-methyl-2-propyl-1-pentanol
Potato[a]	Ring rot	*Clavibacter michiganensis* subsp. *sepedonicus*	2-propanol 3-methyl-3-buten-2-one toluene
Tomato[b]	Bacterial speck	*Pseudomonas syringae* pv. *tomato*	Hydroxylated monoterpenes 3-Hexenyl esters Isoprenoid chlorides
Apple[c]	Fire blight	*Erwinia amylovora*	2-Ethoxy-2-methyl-propane 3-Hexenal 2-Hexenal
Apple[c]	Blossom blight	*P. syringae* subsp. *syringae*	2,4,4-Trimethyl-1-pentene
Fungi			
Small grain cereals[d]	Fusarium head blight	*Fusarium* spp.	Acetylene Formaldehyde Pentanal Xylene
Raspberry[e]	Gray mold	*Botrytis cinerea*	N.D.

Sources:
[a.] [204]
[b.] [205]
[c.] [201]
[d.] [203]
[e.] [200]

4.3.2 Electronic-Nose Sensors

Although PTR-MS or GC-MS methods can be used both to measure BVOC profiles and analyze agricultural products, BVOC profiles and markers can also be used to design sensors using electronic nose (E-nose) technology or other portable detectors [206]. E-noses contain arrays of gas sensors with readout circuits and signal-processing electronics [207; 208]. Rather than identifying individual chemical components of a scent, E-noses integrate signals across sensors using classical algorithms or AI/ML. They have been extensively applied to measure food quality [209]. Cellini et al. used two commercial E-noses to detect *E. amylovora* infections of apples with 75% sensitivity and specificity [201]. An E-nose was used to distinguish pure cultures of fungi and oomycetes [210]. A graphene oxide–based sensor array functionalized with chemical ligands was used to sense BVOCs from tomato plant leaves associated with *P. infestans* infection [211]. E-nose measurements are usually completed within a few minutes.

The versatility, low weight, ease of use, short measurement time, and relatively low cost of E-nose devices makes them attractive frontline tools. However, compared to other BVOC analytical methods, E-noses can have low analytical sensitivity as well as susceptibility to interference by temperature and humidity, which could limit their application for non-intrusive presumptive diagnostics [212; 213]. Several companies manufacture and distribute electronic nose instruments with proprietary sensors to detect VOCs. These include Sensigent (Baldwin Park, CA), Alpha MOS (Glen Burnie, MD), MUI Robotics (Nontaburi, Thailand), Electronic Sensor Technology (Newbury Park, CA), Plasmion (Augsburg, Germany), and the eNose Company (Zutphen, The Netherlands).

As an alternative to electronic gas sensors, colorimetric indicators can be used in arrays to detect soluble gases associated with plant disease. Li et al. used ligand-functionalized nanoplasmonic materials to detect volatiles associated with *P. infestans* infections of tomatoes [214]. A smartphone was used to record and quantify color changes in the chemical sensors, resulting in 95% sensitivity and 100% specificity of detection. Nanomaterials can also be used in immunological and nucleic acid–based diagnostics, with the potential for hybrid diagnostic tests in the future [215].

4.4 TARGETED NUCLEIC ACID SEQUENCING

The amplification and sequencing of marker genes or specific loci have revolutionized taxonomic classification in the laboratory. For example, many fungi can be identified by DNA extraction and amplicon sequencing with a fungal barcoding nrDNA region called the *internal transcribed spacer* (ITS) *region* [216]. However, detecting rust fungi using ITS is ineffective due to intraspecific and interspecific variation [134; 217]. Following the introduction of portable nucleic acid sequencers produced by Oxford Nanopore Technologies and Illumina (San Diego, CA), sequencing has become available as point-of-use technology. A Mobile And Real-time PLant disEase (MARPLE) diagnostics pipeline was developed for targeted sequencing of 242 genes to identify individual strains of wheat yellow rust pathogen (*Puccinia striiformis* f.sp. *tritici*) within 48 hours [218]. Targeted sequencing at the point of use may be an invaluable tool to identify new plant pathogens or strains—even if the cost, complexity, and speed make these methods unattractive for routine detection.

4.4.1 Environmental DNA (eDNA) Analysis

DNA isolated from soil, water, plant material, and air is collectively referred to as *environmental DNA* (eDNA). This emerging technique, first described in 2009, is capable of being deployed to monitor entire biological communities for organisms of interest. Marker genes or barcodes are amplified and sequenced from the environmental sample to characterize the community composition—a hybrid of targeted and untargeted nucleic acid analysis. It has been suggested that eDNA sampling from air, water wash, crop surfaces, etc. could be adopted at border control points to reduce instances of cross border outbreaks [219]. Researchers at the University of Canberra have noted the applicability of portable eDNA monitoring for protecting Australia from grain pests such as the Khapra beetle [220].

4.5 UNTARGETED NUCLEIC ACID DETECTION

The targeted methods to detect nucleic acids described above provide excellent sensitivity and specificity for individual pathogens. Many new formats for targeted molecular diagnostics have been proposed but not yet validated [221]. However, these methods may be too specific for a presumptive diagnostic test. They may fail to detect emerging pathogens or pathogens that have mutated sequences. Untargeted methods that do not rely on lists of known pathogens or sequences have become increasingly popular in clinical diagnostics [222]. These methods isolate DNA, RNA, proteins, or metabolites from samples and analyze the complex mixtures by sequencing or mass spectrometry. One study conducted in Kenya

demonstrated the applicability of untargeted analysis using a low-cost portable sequencing technology to detect plant viruses and pathogens on-site at a farm [223]. A pipeline has also been developed to identify plant pathogens from metatranscriptomic sequencing using RNA-seq technology [224]. The resulting datasets are ideal for biosurveillance because they are more likely to identify unknown, emerging, or unexpected pathogens and disease than other methods [108]. Untargeted methods may be more expensive, take longer to process samples and prepare libraries, require complex data analysis pipelines, and provide lower sensitivity than targeted analyses.

4.6 BIOSENSORS

Many of the immunological and nucleic acid–based techniques described above are time-consuming and require complex instruments and expertise. Consequently, many of them are better suited for confirmatory diagnostics in a laboratory than rapid, presumptive diagnostics in the field. Therefore, there is a strong interest in developing new biosensing systems for detecting plant pathogens with high sensitivity and specificity at the point of use [225]. Biosensors for detecting plant pathogens are based on biological recognition using different receptors (e.g., antibodies, DNA probes), along with optical and electrochemical techniques for reporting the output signals [225].

4.6.1 Lab-on-a-Chip Electrochemical Biosensors

Electrochemical biosensors, which combine microfluidic approaches with electrochemical biosensing, represent affordable, portable, and easy to use devices for food pathogen detection [226]. Lab-on-a-chip electrochemical biosensors feature quick detection time (ranging from a few minutes to 3 hours), accurate detection (with limits of detection as low as 4 colony forming units per mL whole bacteria, or 60 copies when the biorecognition element is genome-based), and relatively high-throughput (ability to combine multiple sensors on one platform to detect multiple pathogens at the same time) [226]. A rapid assay for *X. fastidiosa* using a lab-on-a-chip device with specific antibodies detected the pathogen spike on plant leaves at lower concentration than an ELISA method [227]. A microneedle system with a LAMP amplification platform and smartphone camera sensor was used to detect tomato spotted wilt virus RNA in tomato plant leaves [228]. This portable system detected virus-infected plants 5 days after infection with 98% sensitivity for lab-inoculated samples, 100% sensitivity for field samples, and 100% specificity for both samples. By combining extraction, purification, and measurement activities in a single device, these systems could provide a robust platform for field-based detection with high specificity.

5. OPERATIONAL REQUIREMENTS FOR DETECTION TECHNOLOGY

New technology to detect plant pathogens and disease must fit the needs of phytosanitary inspection organizations if it will be used to screen imports. Passenger and commercial cargo screening have different objectives and operational requirements in the US, as described above. This section considers how detection technology could be used at US POEs to inspect agricultural imports to complement or replace current methods.

CBP has successfully integrated several types of detection technology into daily operations across major POEs. In 2018, CBP was required to increase chemical screening devices to interdict illegal drugs by the INTERDICT Act (Public Law 115-112). This new federal law included appropriations. As of October 2020, CBP deployed 390 handheld electronic devices, including FTIR and Raman spectrometers, at POEs [165]. Additional field-testing kits have been deployed, including color changing test kits and fentanyl test strips. In keeping with a multi-layered approach, the above-cited illegal drug detection platforms are legally categorized as presumptive. Presumptive tests can be sensitive but less specific, and thus small

amounts of the substance can be detected [28]. The positive presumptive tests support further investigation to include additional searches and temporarily detaining of cargo. Confirmatory testing within a certified laboratory is utilized within the criminal legal system, a much more rigorous verification of the presence of an illegal chemical. This model presents a layered detection system for detecting illegal drugs with technology. These rapid, presumptive detection technologies complement targeting information, primary screening, and visual inspections at secondary screening to improve detection of cross-border threats.

New technologies to detect plant pathogens and disease in agricultural products at US POEs would enhance the joint CBP–USDA AQI program [21]. Plant disease detection technology may have limited value if highly technical, specialized laboratory equipment is coupled with an intensive labor requirement. A similar model to implementing the successful chemical detection technology may be developed with plant disease detection technology. Utilizing technology that quickly identifies the presence of an agricultural pathogen is the first screening or presumptive test. However, plant pathogen detection differs from illegal drug detection due to the diversity of bacteria, fungi, viruses, and oomycetes that colonize plants. Most of these microorganisms do not significantly burden the plants, affect food quality, or impede agriculture. Therefore, most microorganisms are not regulated as quarantined pests. If a highly sensitive test detects microorganisms that cannot be readily classified by USDA identifiers or result in an EAN, then commerce could be slowed. Detecting dead pests or agricultural product damage without active disease would not be useful for the AQI program. Therefore, a strategy of applying a presumptive test with high sensitivity to detect disease at the expense of specificity for regulated pests may not be productive [229].

Useful tests for plant disease must balance sensitivity and specificity for regulated pests during the development and validation processes (i.e., high accuracy). This will not significantly increase the delay in moving agricultural products through the inspection process. Depending on the initial test results, further investigation with additional technology may be needed. Some of the rapid technologies described herein could prove useful for confirmatory identification by USDA staff, as well. In summary, working with existing technology implementation models could result in the development and adoption of one of the previously described technologies.

Technology used for the presumptive detection of plant pests would need to provide results quickly and address stakeholders' strategic priorities and the logistical constraints summarized in Table 4 [21]. Environmental conditions at POEs can have a degrading effect on the technology utilized to detect plant diseases. For example, air pollution is a significant concern at port facilities. Mobile sources such as combustion engines at ports release pollutants, including particulate matter, nitrogen oxides (NO_x), sulfur oxides (SO_x), VOCs, and air toxics [230]. Along with high humidity, salt water at seaports has a degrading effect on electronic components. Operating temperatures will vary substantially between open air inspections during the summer at the southern US border and among measurements performed in refrigerated warehouses or containers. Plant disease detection technology deployed at a POE will have to be hardened to withstand the environment, and testing under relevant operating conditions will be required.

Operations must also consider power, training, reliability, and cost of any new detection technology. Agricultural specialists may not have access to electrical line power during inspections: battery-operated devices may be preferred. Training to use a new instrument and demonstrate proficiency could be a significant labor cost, depending on the technology and its human interface. The reliability of instruments and access to support or repair services is essential to maintain usability and build trust in the technology. Finally, costs of acquisition, maintenance, calibration, and consumables directly affect capacity and capability. Although it is premature to estimate the costs of the emerging technologies described herein, the development and commercialization process should consider foreseeable expenses.

New detection technology should consider the operational policies and priorities at the POEs and AQI program. Non-intrusive inspection methods are strongly preferred. These methods are faster, safer for Agriculture Specialists, and more acceptable to shippers and passengers. Transitioning from current intrusive visual inspections to mostly non-intrusive inspections would be a significant improvement in current screening processes. The time required to complete a measurement is also an important factor. There is no prescribed time limit for inspections, although importers, brokers, and CBP staff all share a goal of expediting trade and releasing cleared shipments as soon as possible. Versatile technology that can be used to detect a broad range of plant pests (including insects, nematodes, and microorganisms) would be preferred. Technology that can also detect other contraband or hazardous materials such as illegal drugs, contraband meats, wood packaging pests, undeclared agricultural products, weapons, or hazardous materials could be integrated into the CBP inspection workflow at POEs and would gain acceptance more quickly.

Table 4. Factors to Evaluate Potential Plant Pathogen and Disease Detection Technologies

Factor	Description
TRL	The technology readiness level measures the technical maturity of a system based on its demonstrated capabilities [231].
Validation	Detection methods that have been tested independently from the developer and tested in multiple environments are more likely to meet operational performance requirements [232].
Cost	Lower costs of equipment purchases, consumables, and lifecycle operations facilitate acquisitions to deploy capabilities at scale [233]. Total costs can be difficult to estimate at early stages of development.
Time	Short times to produce actionable results are preferred for perishable agricultural products and trade facilitation.
Intrusiveness	Non-intrusive inspections are usually preferred. Compared to intrusive methods, these methods are usually faster, reduce labor costs, and have higher acceptance from passengers, importers, and brokers.
Sensitivity	Presumptive tests should have high diagnostic sensitivity, the percentage of samples with pests that test positive [28; 234].
Specificity	Confirmatory tests must have high diagnostic specificity, the percentage of samples without pests that test negative [28; 229]. High specificity in presumptive tests will reduce the number of unnecessary referrals for identification and will expedite commerce.
Versatility	Methods that can be used to detect a broad range of high-risk and emerging pests or other contraband will identify multiple threats to cross-border commerce [21; 235].
Interferences	Components of a sample, cargo, or inspection environment that reduce the accuracy of tests will interfere with detection.
Data analysis	Methods that provide a clear and reproducible readout of pest detection are preferred.
Training	Detection methods that can be easily learned and executed without operator errors will be easier to adopt.
Ruggedness	Devices that resist damage and are easily repaired or replaced are more likely to be used consistently.
Temperature	Methods that can be performed onsite, in a full range of outdoor temperatures and refrigerated warehouses are preferred.
Humidity	Methods that can be performed onsite, in all US climate zones and refrigerated warehouses are preferred.
Power	Tailgate inspections at seaports and full-offload inspections in import lots may not have access to fixed electrical power. Battery-powered devices may be preferred for some applications.
Electronic communications	Seaport and remote inspection locations may not have access to secure, reliable wireless or cellular networks. Self-contained devices may be preferred.
Security	Detection results could be sensitive, and devices should have strong cyberphysical security.
Stakeholder acceptance	Safe technology that can be easily explained to travelers and importers will improve the stakeholder experience [235]. Technology that addresses both CBP and USDA goals may promote acceptance [21].

6. TECHNOLOGY OPTIONS

Two classes of new plant pathogen detection technologies described herein could be deployed to address the major operational requirements for phytosanitary inspections of imported agricultural products. Immunological diagnostic methods have proven successful for both animal and plant health, with increasing acceptance of point-of-use technologies that are rapid and inexpensive. BVOC detection methods have lower stages of technological readiness, but they have promising characteristics of rapid detection with lower intrusiveness than that of current inspections and tremendous versatility in detecting a broad range of pathogens, plant diseases, insect pests, and contraband materials.

Table 5 compares nine classes of detection technologies, including five currently used in agricultural pest inspections. Current inspection methods that rely on visual detection by Agriculture Specialists are intrusive and could be less sensitive for detecting plant pathogens than insect pests. AK9 teams are currently used to screen mail and luggage for undeclared agricultural products. A new generation of canines that detects pests would need to be trained: it would be difficult to desensitize current AK9s to the agricultural products that are ubiquitous in import lots. X-ray methods will continue to be used to detect contraband at POEs, although it is unlikely that they can detect regulated pests with high specificity. Traps are limited to aerosolized particles such as spores, with significant interference and a requirement for a separate method to detect collected pathogens. Traps must be pre-deployed, requiring engagement with upstream packers and shippers.

Automated imaging lacks specificity for many diseases and may not fit AQI program requirements. The tools currently reject a substantial quantity of products due to physical or environmental damage, or pests not subject to EANs. However, automated imaging combined with AI/ML methods of deep learning and pattern recognition could be adopted by packing companies and distributors to grade and sort agricultural products, improving quality and adding value for sales. USDA foreign commodity preclearance programs and trust fund agreements could be used as frameworks for collaborative engagement. For example, an agreement between the Mexican avocado industry and APHIS for importing commercial consignments of Hass avocados combines agriculture oversight by the National Plant Protection Organization of Mexico with biosurveillance, product cleaning and grading, and secure transport to the US [236]. Most costs would be paid by growers, packers, and exporters.

Nucleic acid–based detection will likely remain the gold standard for confirmatory diagnosis of plant diseases. Improvements in targeted analysis will continue to reduce costs for point-of-use diagnostics. However, nucleic acid extraction requirements, matrix interferences, and amplification requirements of current methods require more time than other methods. These methods could detect nucleic acids from dead or inactive pathogens due to their low limits of detection. The high specificity and sensitivity of nucleic acid–based tests will become even more useful for USDA identifiers and biosurveillance of emerging pathogens.

Antibody-based immunological methods have been validated and marketed to detect agricultural pathogens. Barriers to using them for routine pathogen detection include costs and total time required for testing. These methods can be highly specific, which may limit their utility for detecting multiple pathogens. Multiplexing, performing multiple immunoassays at the same time, could make these tests more attractive. Current immunoassays use antibody reagents from animals, which take time to develop and scale manufacturing. Advances in using libraries of nanobodies, selective peptides, and aptamers to rapidly develop assays could make these rapid tests available quickly to detect and exclude emerging pathogens before they become established in the US.

Automated BVOC detection could replicate the demonstrated successes of canines identifying scents from agricultural products and pests. Advances in the analytical sensitivity of mass spectrometry and the robustness of electronic noses create new opportunities for non-invasive detection. New AI/ML classifier tools can automate data analysis and report compositions in real time. Time requirements for BVOC analyses are decreasing, as traditional separation systems—like gas chromatography—are no longer required to deliver samples to modern ionization systems and mass analyzers. Key areas for the development of BVOC detectors include developing specific BVOC profiles of plant diseases, validating the accuracy of detector–classifier systems, addressing the limit of detection issues for less sensitive sensors like e-Nose devices, and mitigating the effects of temperature, humidity, and gas contaminants on detector performance in operational environments. These systems could also be used to detect agricultural contraband (including meats), illicit drugs, hazardous chemicals, and explosives.

Table 5. Relative Performance of Detection Technologies

Factor	Visual	Canine	Traps	X-ray	Immunological	Nucleic Acid	BVOC	Automated Imaging	Advanced Nucleic Acid
Time	5 min	1–5 min	(1–5 min)	2–5 min	10 min	20 min	1–20 min	2–5 min	2 days
Intrusiveness	High	Med	Med	Low	High	High	Med	High	High
Sensitivity	Med	High	Med	Low	Med	High	Med-High	Med	Low
Specificity	Med	Med	Low-High	Low	Med	High	Low	Low	High
Versatility	High	Med	Low-High	High	Med	High	Med	High	High
Interferences	Med	Med	High	Low	Med	Med	Med	Med	Med
Data analysis	Med	Low	N/A	Med	Low	Med	Med	Med	Med
Automation	Low	Low	Med-High	High	Med-High	Med-High	High	High	Med-High
Training	Med	Med	Low	Med	Low	Med	Med	Med	High
Ruggedness	N/A	Low	High	Low	High	Low	Med	Med	Med
Temperature	Low	Low	Med	High	Med	Low	High	High	Med
Humidity	Low	Low	Med	Med	Low	Med	Med	Med	Med
Power	N/A	N/A	Med	High	Low	Med	Med-High	Med	High
TRL[a]	M[b]	M[b]	M[b]	3	M[b]	M[b]	4	4	3

[a.] The technology readiness level (TRL) indicated for the most advanced application of a technology to detect a plant pathogen or disease. Applications to detect a new pathogen may be at a lower TRL.

[b.] Mature, the technology is commercially available or fully implemented.

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APPENDIX A. OBJECTIVES, SCOPE, AND METHODOLOGY

A.1. OBJECTIVES

This project assessed the state of technology to identify opportunities for the United States Department of Homeland Security (DHS) to develop and deploy advanced plant pathogen detection systems. The assessment could inform future research and development activities to demonstrate applications of the technology, test commercial systems, or refine specifications for sensor technology. This project was funded by the U.S. DHS Science & Technology Directorate (S&T) through the Food, Agriculture, and Veterinary Defense (FAV-D) program.

The project adapted and applied the GAO Technology Assessment process [237]. This process includes Initiation, Design, Message Development, and External Review phases. The initiation phase engaged DHS S&T staff and stakeholders to focus the scope of the assessment to address mission requirements. The design phase included initial research, identification of technology options, and consultations with CBP stakeholders. The message development phase collected information about the capabilities and technology readiness level (TRL) of commercial and pre-commercial pest detection technology. Finally, the external review phase solicited and addressed feedback from DHS stakeholders, external subject matter experts, and USDA APHIS stakeholders in the AQI program.

A.2. SCOPE

This technology assessment focused on methods to detect plant pathogens and disease. We further focused on technology relevant to the mission space of DHS components. For example, US Customs and Border Protection (CBP) performs agricultural inspections on passengers, cargo, and international mail at US ports of entry (POEs) and could test new detection technology in future campaigns.

This study did not evaluate technology to detect insect, nematode, or invasive plant pests. Human and animal pathogen detection was also not included.

A.3. METHODOLOGY

This technology assessment was based on international published literature, publicly available materials, and discussions with subject matter experts and stakeholders. We did not test any technology or independently reproduce reported tests in this project. Names of commercial products and companies that manufacture detection technology are included to illustrate the feasibility and TRLs of detection methods. Usage does not imply endorsement of a commercial product or service.

Our DHS S&T Program Manager shared insights into CBP agriculture inspections based on a visit to the New York and New Jersey Port Authority. We conducted virtual interviews with CBP Program Managers, Supervisors, and Agriculture Specialists from the Office of Field Operations. We also visited CBP Agricultural Inspection operations at the Miami Port of Entry, including the Miami Seaport and Miami International Airport (MIA). At the MIA complex, we toured bonded commercial cargo warehouses with agricultural imports, the Miami International Mail Facility, and the Agricultural Inspection screening area for passengers at MIA. In-person meetings with CBP managers and Agriculture Specialists provided outstanding context to understand current inspection procedures, workload, operational requirements, and opportunities.

Subsequently, we visited two POEs overseen by the Laredo Field Office to observe agriculture inspections at a land border POE. We toured the import lot at the Hidalgo/Pharr Bridge, near Hidalgo, TX. We also toured the import lot at the commercial World Trade International Bridge, the passenger Juarez-Lincoln bridge, and the Laredo International Railway Bridge, all near Laredo, TX. Discussions with CBP staff at these sites complemented Miami discussions, with a different profile of agricultural imports and different operational requirements.

A literature search was performed using internet search engines and reference databases during the period of September 2023 to March 2024. Unpublished reports and web pages were identified by internet searches and references from relevant materials. Citations of key papers and references from those papers were used to identify relevant examples of detection technology applications. Research scientists at ORNL provided expert guidance on strengths and limitations of specific detection methods.

A virtual meeting with external subject matter experts was held on February 9, 2024, to gather feedback on draft findings and to identify technology best suited for the phytosanitary inspections of agricultural products. These experts from academia and agriculture extension services provided broad insight into basic research on pathogen detection as well as pragmatic information about the challenges of rapidly identifying pathogens in plants and agricultural products.

Finally, a virtual meeting with USDA Animal and Plant Health Inspection Service Plant Protection and Quarantine staff was organized to gain a deeper understanding of the APHIS perspectives on pest and pathogen detection by CBP Agriculture Specialists and subsequent identification by USDA identifiers. This discussion helped to shape our description of operational requirements for detectors.

APPENDIX B. DEFINITIONS

Accuracy (Diagnostic)
The proportion of correctly classified samples based on the target characteristic.

$$Accuracy = \frac{\#\ true\ positives + \#\ true\ negatives}{total\ population} \times 100$$

Article
Any material or tangible object, including a living organism, that could harbor living plant pests or noxious weeds. The term includes associated articles such as soil and packaging. Source: 7 C.F.R. § 330.

Biogenic volatile organic compound (BVOC)
Organic chemical compounds that evaporate under normal indoor atmospheric conditions of temperature and pressure [238] that are produced by biological systems.

Contaminating pest
A pest that is carried by a commodity, packaging, conveyance or container, or present in a storage place and that, in the case of plants and plant products, does not infest them. Source: ISPM5 [34].

Economically significant domestic crops
Agricultural field crops that were the largest contributors to the U.S. economy in 2022 included corn, soybeans, wheat, cotton, rice, peanuts, sorghum, oats and barley. Production of fruit (grapes, apples, strawberries and oranges), tree nut (almond, walnut and pistachio) and vegetable (tomatoes and potatoes) also had high horticultural value. Source: USDA ERS.

Emergency action
A prompt official operation undertaken to prevent the entry, establishment or spread of a pest in a new or unexpected situation not addressed by existing phytosanitary measures. Source: ISPM5 [34].

Insect
Any of the numerous small invertebrate animals generally having the body more or less obviously segmented, for the most part belonging to the class insecta, comprising six-legged, usually winged forms as for example, beetles, bugs, bees, flies, and to other allied classes of arthropods whose members are wingless and usually have more than six legs, as for example, spiders, mites, ticks, centipedes, and wood lice. Source: 7 U.S.C. § 136

Living
Viable or potentially viable. Source: 7 C.F.R. § 330.

Plant
Any plant (including any plant part) for or capable of propagation including trees, tissue cultures, plantlet cultures, pollen, shrubs, vines, cuttings, grafts, scions, buds, bulbs, roots, and seeds. Source: 7 C.F.R. § 330.

Plant disease
Physiological damage caused by living organisms (called pathogens), such as fungi, bacteria, viruses, nematodes, phytoplasmas, protozoa, and parasitic plants; and by nonliving agents, such as air pollutants, nutrient imbalances, and unfavorable environmental factors. Source: adapted from the American Phytopathological Society.

Plant pathogen
A disease-producing organism or biotic agent. Source: D'Arcy et al. [34; 239].

Plant pest
Any living stage of any of the following that can directly or indirectly injure, cause damage to, or cause disease in any plant or plant product: A protozoan, nonhuman animal or insect, nematode, parasitic plant, or microorganisms (bacterium, fungus, virus or viroid, infectious agent or other pathogen), or any article similar to or allied with any of the foregoing. Sources: 7 C.F.R. § 330 and 7 U.S.C. § 136.

Plant product
Any flower, fruit, vegetable, root, bulb, seed, or other plant part that is not included in the definition of plant; or any manufactured or processed plant or plant part. Sources: 7 C.F.R. § 330, [34].

Point-of-use diagnostics
Tests performed near the area of plant growth or agricultural inspections to provide immediate actionable information. These simple tools are analogous to point-of-care tests for clinical diagnostics. Source: FDA.

Quarantine pest
A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled. Source: ISPM5 [34].

Regulated non-quarantine pest
A non-quarantine pest whose presence in plants for planting affects the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party. Source: ISPM5 [34].

Selectivity (analytical)
Extent to which the method can determine a particular compound in the analyzed matrices without interference from matrix components. Source: Bioanalytical Method Validation Guidance for Industry, FDA [240; 241].

Sensitivity (analytical)
Lowest analyte concentration in the matrix that can be measured with acceptable accuracy and precision (i.e., lower limit of quantification). Source: Bioanalytical Method Validation Guidance for Industry, FDA [240; 241].

Sensitivity (diagnostic)
A test's capability to yield a positive result when the targeted characteristic is present –the effective accurate pathogen detection level. Source: Caldwell et al. [229].

$$Sensitivity = \frac{\#\ observed\ positives}{\#\ true\ positives + \#\ false\ negatives} \times 100$$

Specificity (analytical)
Ability of the method to assess, unequivocally, the analyte in the presence of other components that are expected to be present (e.g., impurities, degradation products, matrix components, etc.). Source: Bioanalytical Method Validation Guidance for Industry, FDA [240; 241].

Specificity (diagnostic)

A test's ability to correctly yield a negative result when the targeted characteristic is not present [229].

$$Specificity = \frac{\#\ observed\ negatives}{\#\ true\ negatives + \#\ false\ positives} \times 100$$

Surveillance
An official process which collects and records data on pest presence or absence by survey, monitoring or other procedures. Source: ISPM5 [34].

Technology readiness level (TRL)
A type of measurement system used to assess the maturity level of a particular technology. TRLs are a compendium of characteristics that describe increasing levels of technical maturity based on demonstrated (tested) capabilities. Sources: NASA and GAO [231]

Vector
A living organism (e.g., insect, mite, bird, higher animal, nematode, parasitic plant, human) able to carry and transmit a pathogen and disseminate disease. Source: D'Arcy et al. [239]

Visual examination
Examination using the unaided eye, lens, stereoscope or other optical microscope. Source: ISPM5 [34].

APPENDIX C. CONTACTS

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